Microbiology

is the study of small living things. Generally this means living things that are too small to see without the use of a microscope. These life forms are called microorganisms or microbes. Microorganisms include bacteria, archaea , viruses, protozoa (single-cell eukaryotes like amoeba), microscopic fungi and yeasts, and microscopic algae (plant-like organisms). Microorganisms were discovered over three hundred years ago and it is thought that many new microbes have yet to be discovered. Microbiology is a wide area of science that includes bacteriology, virology, mycology, phycology, parasitology, and other branches of biology.

History

- In 1676, Anton van Leeuwenhoek observed bacteria and other microorganisms , using a single-lens microscope of his own design.

- In 1796, Edward Jenner developed a method using cowpox to successfully immunize a child against smallpox. The same principles are used for developing vaccines today.

- Following on from this, in 1857 Louis Pasteur also designed vaccines against several diseases such as anthrax, cholera and as well as pasteurization for food preservation.

- In 1867 Joseph Lister is considered to be the father of antiseptic surgery. By sterilizing the instruments with diluted carbolic acid and using it to clean wounds, post-operative infections were reduced, making surgery safer for patients.

- In the years between 1876 and 1884 Robert Koch provided much insight into infectious diseases. He was one of the first scientists to focus on the isolation of bacteria in pure culture. This gave rise to the germ theory, a certain microorganism being responsible for a certain disease.

- A major milestone in medical microbiology is the Gram stain. In 1884 Hans Christian Gram developed the method of staining bacteria to make them more visible and differentiable under a microscope. This technique is widely used today.

- In 1929 Alexander Fleming developed the most commonly used antibiotic substance both at the time and now penicillin.

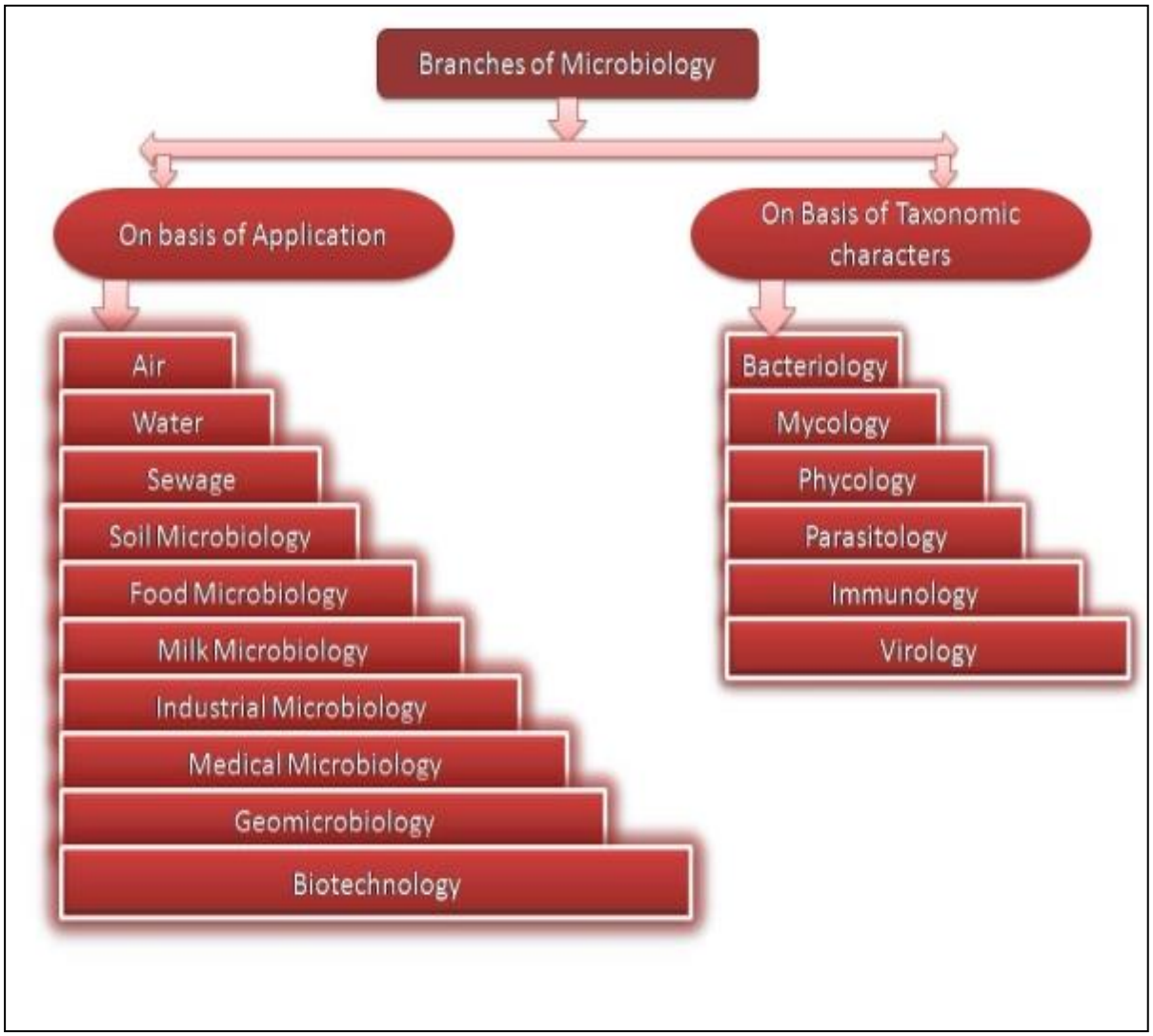
- DNA sequencing, a method developed by Walter Gilbert and Frederick Sanger in 1977. caused a rapid change the development of Vaccines , medical treatments and diagnostic methods. Some of these include synthetic the first genetically vaccine in 1986 for hepatitis B.

Branches of Microbiology

Micro-organism are present everywhere in nature and this micro-organism has a great effect on other life form like human being ,plants and animals in several ways. The effect of micro-organism to the environment may be desirable or undesirable .These bacteria show wide range of activity and diversity. On basis on taxonomic characters and application of micro-organism . according taxonomic characters divided into the following branches **:**

1. Bacteriology

Bacteriology is a branch of Microbiology that deals with study of Bacteria. This Bacteria are prokaryotic, unicellular in nature .Their mode of multiplication is by Binary fission .Bacteria can be parasitic or can be free living in atmosphere .The nuclear material is not bound to nuclear membrane .This bacteria may be motile or non-motile .They may vary in their shape like rod, spiral or cocci and they may be aerobic, non-aerobic or facultative anaerobic in nature. On the basis of mode of nutrition some bacteria may have autotrophic or hetrotrophic mode of nutrition.



2.Mycology

Mycology is a branch of microbiology that deals with study of Fungus. This fungus cells are eukaryotic in nature the nuclear material is surrounded by Chitin or cellulose or both. This fungal cells are non-photosynthetic and chemoorganotrophic in nature .These fungal cells are divided into two types and that is yeast and molds.

- **Yeast** -The yeast cell may occur in single cell or pseudomycelium form. The mode of reproduction is by budding or by Spore formation. Yeast are also know as Ascomycetes and this yeast cells may be oval, rod or spherical in shape.
- **Molds**-The molds grow in form of multi-cellular filamentous structure called as hyphae. They can reproduce by both means of sexual and asexual mode of reproduction.

3. Phycology

Phycology is a branch of microbiology that deals with the study of algae.They are photosynthetic,eukaryotic,and multi cellular organism.

4. Parasitology

It is a branch of biology that deals with the study of parasites. This branch mainly include the study of three major group of bacteria parasitic protozoa, parasitic worms, and arthropods. In the relationship between host and parasite is also studied. This parasites may be unicellular or multi-cellular. This parasites are mainly responsible for causing infection in humans and animals.

5.Immunology

Immunology is a branch of microbiology that deals with the study of Immune system of all organism specially human being and animals. In this branch of microbiology the relationships between host body ,pathogen and immunity is studied.

6. Virology

This branch of microbiology deals with the study of Viruses .Viruses are very small ultra-microscopic in nature and they are visible through electron microscope. Viruses are metabolically inert and are completely dependent on host cell for replication .Viruses are capable to infect all types of cells from a bacteria to a human .It contain only one type of nucleic acid that is either DNA or RNA.

Structure of Bacteria

The general structure of bacteria component from the following:

1. **Capsule .**
2. **Cell wall.**
3. **Cytoplasmic membrane.**
4. **Cytoplasm.**
5. **Nucleus**.
6. **Ribosomes.**
7. **Flagella .**
8. **Pili.**
9. **Spore.**

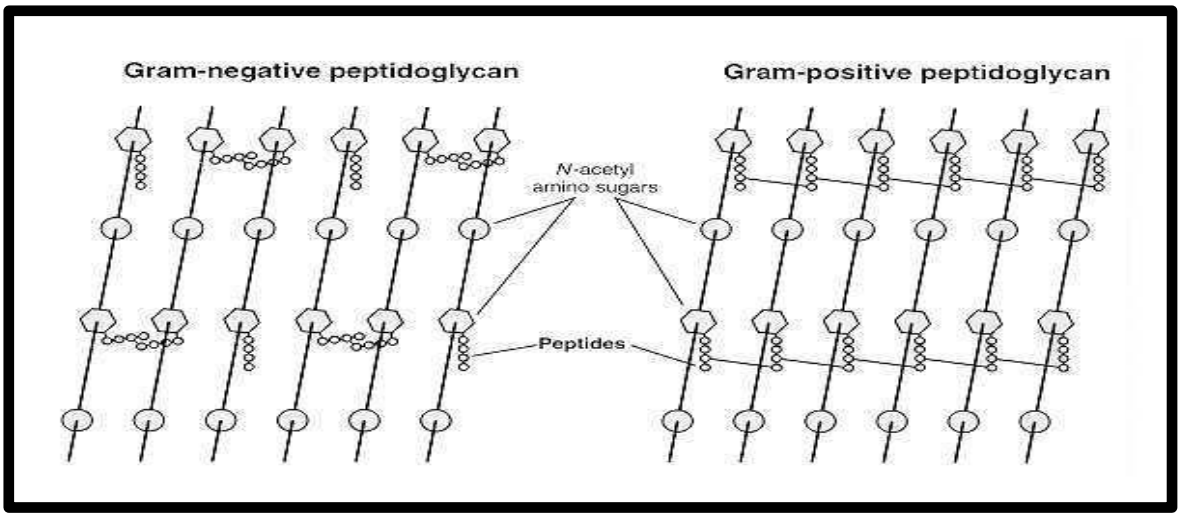
1. Capsule

it is gelatinous secretion of bacteria which get organized as thick coat around the cell wall it may be composed of polysaccharide e.g *klebsella* or polypeptide e.g *bacillus anthrax* or hyaluronic acid e.g *S. pyogenes*

a. protect the cell wall
b. having capsule means that the bacteria were more virulent due to the capsule protect the bacteria from immune response.

2. Cell wall

The layers of cell envelope lying between the cytoplasmic membrane and the capsule are referred to collectively as cell wall.
account 20% of the total dry weight of the cell. Only mycoplasma bacteria lack the cell wall
a. give the shape of bacteria
b. rigid structure, protect the bacterial cell against physical and chemical treatment.
c. freely permeable
d. important in cell division
e. important in classification of bacteria
Both Gram-positive and Gram-negative bacteria possess cell wall peptidoglycans are unique to prokaryotic organisms and consist of a glycan backbone of muramic acid and glucosamine (both N-acetylated), and peptide chains highly cross-linked with bridges in Gram-positive bacteria (e.g., *Staphylococcus aureus*) or partially cross-linked in Gram-negative bacteria (e.g., *Escherichia coli*).



Diagrammatic representation of peptidoglycan structures

Lysozyme enzyme cause lysis of the bacteria they act by splitting cell wall mucopeptide linkages when lysozymes act on gram positive **.**

Protoplast is formed consisting of cytoplasm membrane and contents with gram negative bacteria the result **Spheroplast.**

3. Cytoplasmic membrane

The bacterial membrane is composed primarily of protein and phospholipid (about 3:1). It performs many functions, including biosynthesis, and energy transduction.

a. selective permeability contain (permease) enzyme play important role in passage through membrane

b. biosynthesis, and energy transduction.

c. cell growth

4. Cytoplasm

The cytoplasm it is gel-like in consistency and includes the prokaryotic chromosome, ribosomes, vacuole and Constituents of cytoplasm include the suspension of organic and in organic compound in viscous watery solution

The cytoplasm also lacks organelles such as mitochondria, Golgi apparatus or endoplasmic reticulum.

5.Nucleus

absence of nuclear membrane and the chromosome in bacteria is typically a single, closed circle DNA .some bacteria possess smaller extra chromosomal pieces of DNA called **plasmids** confer certain properties like toxigenecity , drug resistance and hydrocarbons breakdowns but not essential for the life of the cell. Transmitted from cell to another one by

a. during binary fission
b. conjugation
c. bacterial phage
d.

6. Ribosomes: The ribosomes is composed of termed 70 S (Svedberg units) divided in two smaller units to 50s and 30s. They are the sites of protein synthesis.

7. Flagella

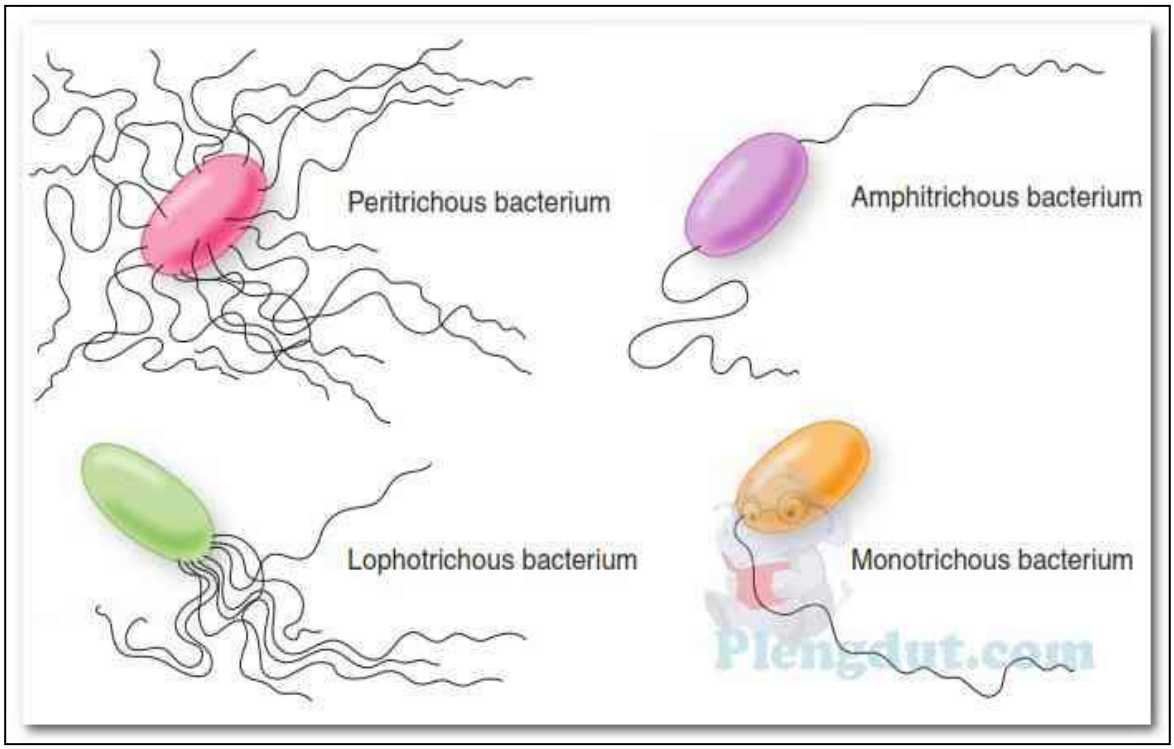
Almost all motile bacteria possess flagella as the organ of locomotion composed of protein called flagellin. Such bacteria tend to move towards or away from the source of stimulus. These stimuli can be chemicals (chemotaxis), light (phototaxis)
Flagella arrangements are:

1- Monotrichous – a single flagellum at one pole (also called polar flagellum) E.g. Vibrio , pseudomonas .

2- Amphitrichous – single flagellum at both poles. E.g. Spirilla.

3- Lophotrichous – two or more flagella at one or both poles of the cell E.g. Bartonella.

4- Peritrichous- completely surrounded by flagella E.g. *E. coli*
Other mechanisms of bacterial locomotion include gliding and motion by axial filament contraction.



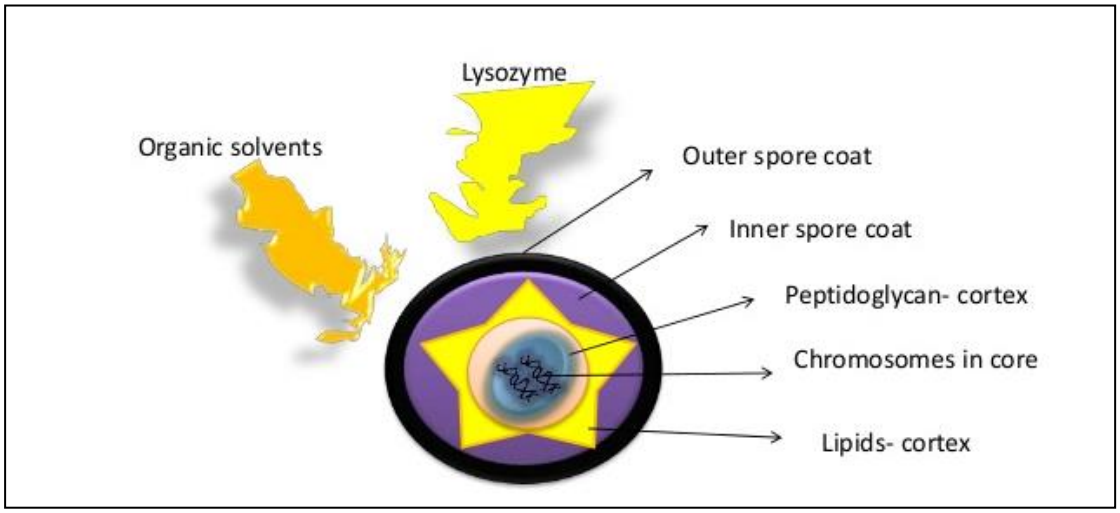
Typical arrangements of bacterial flagella

8- Pili (fimbriae)

They are filamentous short thin straight hair it is smaller than flagella at as sex pili and used for adhesion on the surface.

9- Spores

They are highly resistance dormant state of bacteria found in certain genera e.g bacillus and clostridium they make survival under unfavorable condition like drying, freezing, heating and toxic chemical the location of spore central or sub terminal or terminal.



Structure of spore

Morphology of Bacteria: (Shape of Bacteria)

Bacteria can be classified into four bases shape as under.

1- Single Round or spherical form cocci like *Micrococcus*

a. Diplococci two cocci adhering to each other like *Neisseria*
b. Chain like Streptococci if fission continues while they remain attached forming chains
c. Cluster (random clumps) like Staphylococci cell division not in one plane and daughter cells remain attached in irregular cluster.
d. *Peptococcus tetracocci* aggregate of 4 cocci
e. *Sarcinacocci* aggreate of 8 cocci

2- Rod form –bacilli: the bacilli are not forms as many grouping like *Bacillus* and *Salmonella*.in some of organism's length approximates the width these are called coccobacilli e.g. *B.pertussis* .

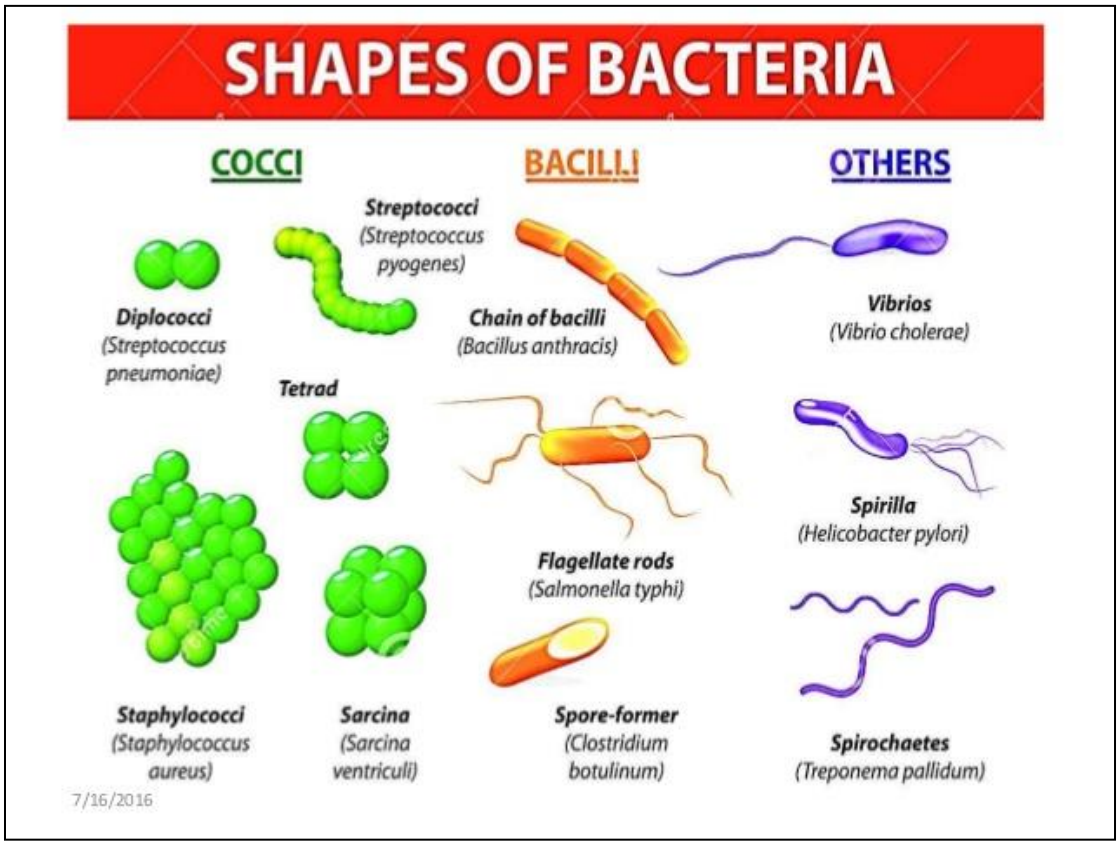
3- Curved of shaped bacilli, comma shape for ex: *vibrio* spp. as *vibrio cholera*.

4. Spiral form: *Spirochete*, *Treponemia*.

5. Chines letter arragment : corynebacteria .

6. Branching filamentous : actinomycets .

7. Don't possess stable morphology : mycoplasma bacteria lack cell wall they are oval or rounded bodies with interlacing filaments.



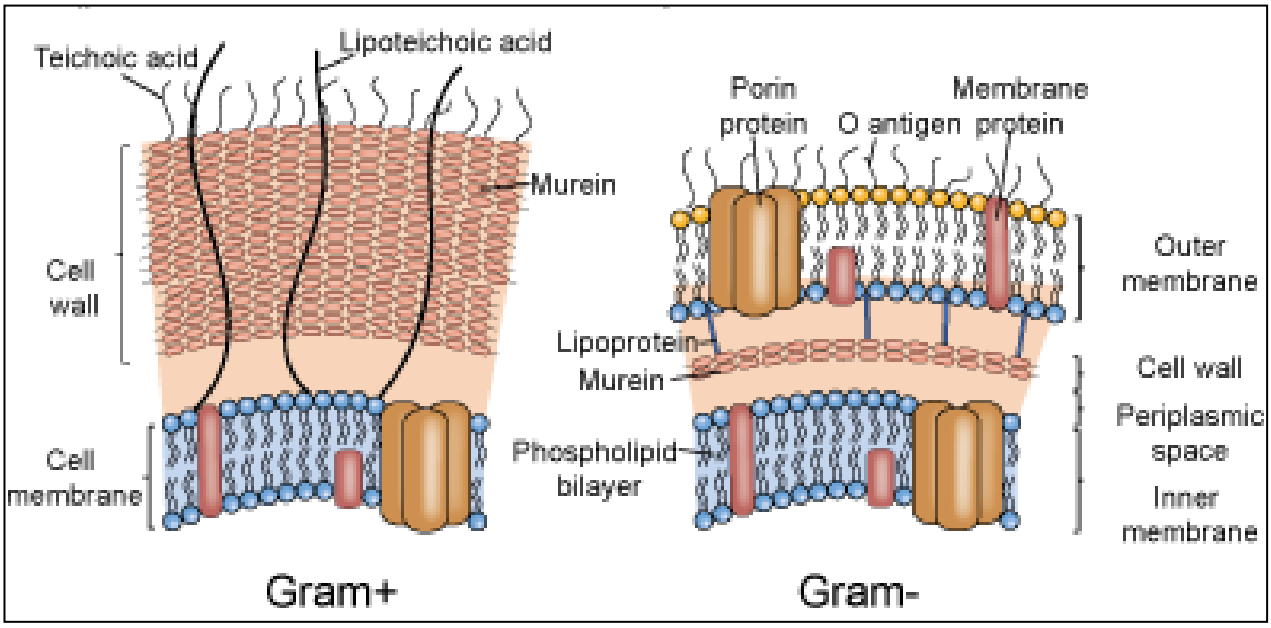
Morphology of bacterial shapes

Gram Positives and Gram Negatives: Key Differences

1. Gram positive bacteria have simpler, but thicker walls, with a relatively large amount of peptidoglycan. The walls of Gram negative bacteria are thinner and have less peptidoglycan but are more complex in structure.

2.An outer membrane on the Gram positive cell wall contains **Teichoic acids** are ploy phosphate polymers bearing a strong negative charge They are strongly antigenic and An outer membrane on the Gram positive cell wall envelope contains **Lipoteichoic acids** as membrane teichoic acids are polymers of amphiphitic glycophosphates with the lipophilic glycolipid and anchored in the cytoplasmic membrane. They are antigenic, cytotoxic and adhesions (e.g., *Streptococcus pyogenes*). on the Gram positive cell wall envelope contains **lipopolysaccharides** (LPS) These are endotoxic substances responsible for making Gram negative organisms more threatening .

Gram negative	Gram positive
1- The walls of Gram negative bacteria are thinner and have less peptidoglycan but are more complex in structure and thickness10-15 mμ .	1- Gram positive bacteria have thicker walls with relatively large amount of peptidoglycan and thickness 15-23 mμ
2-Lipid High 15-20 %	2-Lipid Low 2-4%
3- An outer membrane on the Gram negative cell wall contains **lipopolysaccharides (LPS)**	3- An outer membrane on the Gram positive cell wall contains **teichoic acids and Lipoteichoic acids**



Comparison of the thick cell wall of Gram-positive bacteria with the comparatively thin cell wall of Gram-negative

Requirement of Bacteria

Physiology of Bacteria

If an individual organism is to survive it must be able to react to changes in its environment. It must be able to feed, respire and must be able to reproduce. **Metabolism** refers to all the biochemical reactions that occur in a cell or organism and divided into **Anabolism** is the (building up) - synthesizes large molecules from smaller or precursor components, usually requiring energy in the process and **Catabolism** is the (breaking down) - a series of destructing chemical reactions that break down complex molecules into smaller units, and in most cases releasing energy in the process.

Nutrition of Bacteria

Bacteria can be divided into groups based on their nutritional requirement in two different ways.
a. how they obtain their **energy**?
b. how they obtain the **carbon** needed for synthesis of all organic molecules?
So:-
1. Some bacteria obtain energy from **sunlight** through the agency of pigments. These are called ***phototrophs***
2. Some bacteria are able to grow with **inorganic molecule like Co_2** as the main source. These are called ***autotrophs*.**
3. Most of the human pathogenic bacteria require to be supplied with **organic carbon molecules**. These are called ***heterotrophs*.**

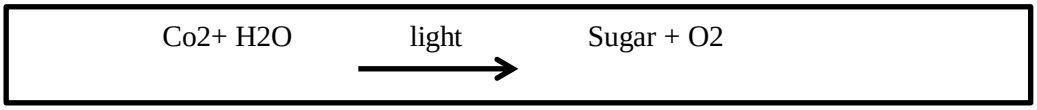
Autotrophic Bacteria

These are bacteria which are able to synthesize their own organic food from inorganic substances. They use carbon dioxide for obtaining carbon and utilize hydrogen sulphide (H2S) or ammonia (NH3) or hydrogen (H2) as the source of hydrogen to reduce carbon. These bacteria can be distinguished further into two types as follows:

Photoautotrophic Bacteria

The photoautotrophic bacteria possess photosynthetic pigments in membrane bound lamellae (or thylakoids) and utilize solar energy. The bacterial photosynthesis is different from that of green plants since here water is not used as a hydrogen donor. Hence oxygen is not released as a byproduct. For this reason, the process is described as an oxygenic photosynthesis. Photosynthetic bacteria include the green purple bacteria and the cyanobacteria

$$CO_2 + H_2S \xrightarrow{\text{light energy}} \text{sugars} + \text{sulphur} + \text{water}$$



Chemoautotrophic Bacteria

These are bacteria which manufacture organic compounds from inorganic raw materials utilizing energy liberated from the oxidation of inorganic substances. Following are the common types of chemo autotrophic bacteria.

1. Nitrifying bacteria which derive energy by oxidizing ammonia into nitrates. Eg: Nitrosomonas, Nitrobacter.

$$NH_4^+ + 2O_2 \rightarrow NO_2 + 2H_2O + \text{energy}$$

2. Sulphur bacteria which derive energy by oxidizing hydrogen sulphide to sulphur. Eg: Thiobacillus, Beggiatoa.

$$2H_2S + O_2 \rightarrow 2S + 2H_2O + \text{energy}$$

3. Iron Bacteria which derive energy by oxidizing ferrous ions into ferric form. Eg: *Ferrobacillus, Gallionella*.

The Main Forms of Energy Capture

	General group	Subgroup (s)	Energy source	Carbon source
1	Autotroph	Photoautotroph chemoautotroph	Light Inorganic Substances	CO_2
2	Heterotroph	Photo heterotroph like heliobacteria chemoheterotrophic like human, pathogenic bacteria	Light Organic compounds	Organic compounds

Factors effecting growth of bacteria

1. Physical factors

A. The requirement of Oxygen and carbon dioxide:

1- Obligate aerobes:

Need oxygen to grow. E.g. *Pseudomonas aeruginosa.*

2- Obligate anaerobes: not need oxygen. Some are killed by free oxygen. E.g. *Clostridium sp.*

3- Microaerophiles: means little-air- loving. Need large amount of CO2 and trace amounts of oxygen. E.g. *Treponema pallidum (syphilis).*

4- Facultative Anaerobe (Facultative aerobe or facultative): can use oxygen if available, but can survive without it too. E.g. *Escherichia coli.*

B. pH: there are three groups of microorganisms according to pH.

1- Neutrophiles: Most bacteria grow between pH 7.2-7.6.

2- Acidophiles: these grow in an acidic pH(1-5) like lactobacillus.

3- Alkalophiles: these grow optimally under alkaline conditions pH (8-9.5) like vibrio.

C. Temperature: there are (3) groups of microorganisms according to temperature

1- Psychrophilic: grow at an optimal temperature of 0 to 20° C. like water bacteria.

2- Mesophiles: They grow at optimal temp between of (30-40) these include bacteria producing disease.

4- Thermophiles Which have an optimal temperature of (55-75)°C like bacillus.

D- Moisture:

All actively metabolizing bacteria generally require some water in their respective environments.

E. Osmotic pressure : Bacteria are usually resistant to changes in osmotic pressure, 0.5% Nacl is add to almost all cultural media to make isotonic.

F. radiation:

Various forms of radiant energy, such as gamma rays or ultraviolet radiation can cause mutations and /or kill bacteria. Some organisms have protective pigments or enzymes that can repair radiation-caused DNA damage.

2- Chemical factors

A- Nitrogen

- In amino acids, proteins
- Most bacteria decompose proteins
- A few bacteria use N_2 in nitrogen fixation

B- Sulfur

- In amino acids, thiamine, biotin
- Some bacteria use SO_4^{-2} or H_2S

C- Phosphors

- In DNA, RNA, ATP, and membranes
- is a source of phosphorus PO_4

d- Carbon

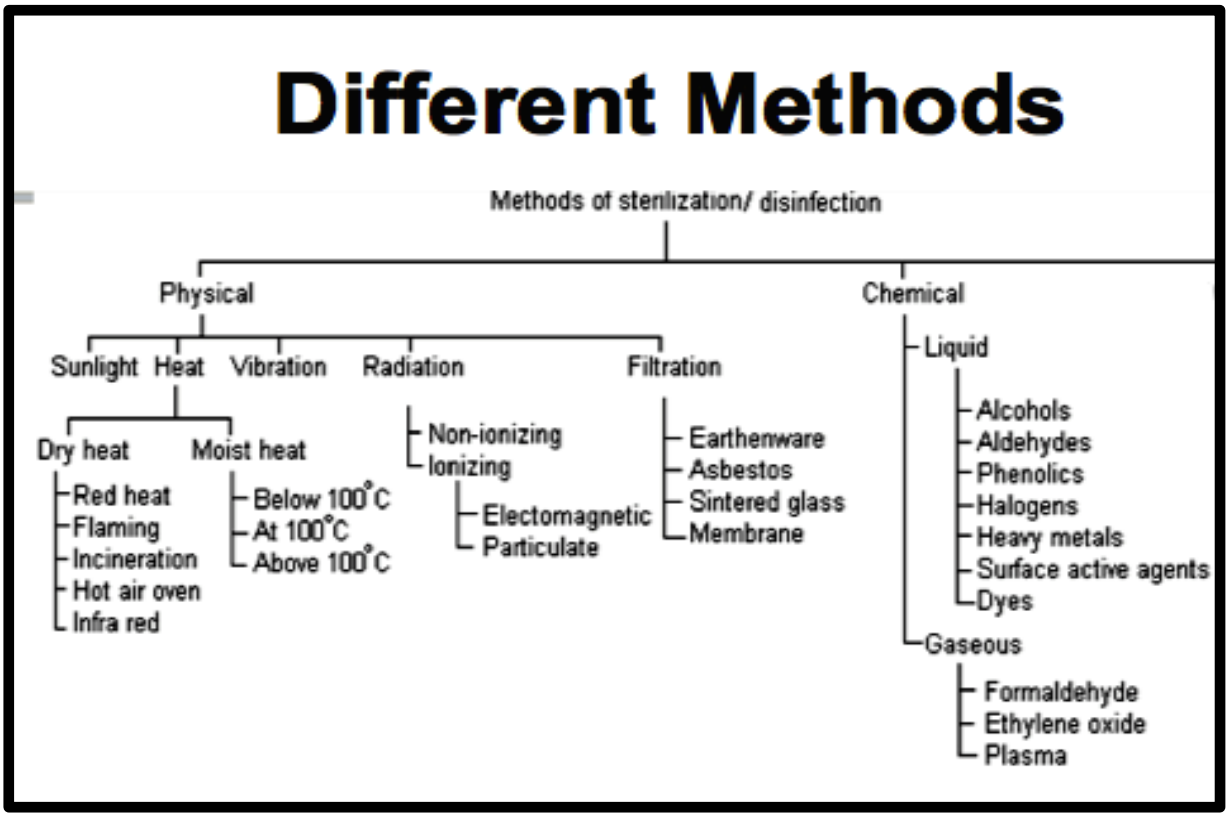
- Structural organic molecules, energy source
- Chemo heterotrophs use organic carbon source

Functions of Some Common Vitamins in Microorganisms		
Vitamin	**Functions**	**Examples of Microorganisms Requiring Vitamina**
Biotin	Carboxylation (CO2 fixation) One-carbon metabolism	*Leuconostoc mesenteroides* (B)
Cyanocobalamin (B12)	Molecular rearrangements One-carbon metabolism—carries methyl groups	*Lactobacillus* spp
Folic acid	One-carbon metabolism	*Enterococcus faecalis*
Lipoic acid	Transfer of acyl groups	*tetrahymena* spp
Pantothenic acid	Work at coenzyme A—carries acyl groups	*Morganella morganii*
Pyridoxine (B6)	Amino acid metabolism (e.g., transamination)	*Lactobacillus* spp.
Niacin (nicotinic acid)	carry electrons and hydrogen atoms	*Haemophilus influenzae*
Riboflavin (B2)	carry electrons and hydrogen atoms	*Caulobacter vibrioides*
Thiamine (B1)	Aldehyde group transfer	*Bacillus anthracis*

Sterilization and Disinfection

Sterilization: is defined as the process of elimination for all the living microorganisms, including vegetative bacterial or spore state are killed.

Disinfection: is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects. Disinfection can be achieved by physical or chemical methods. Chemicals used in disinfection are called disinfectants. Not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out.



Physical Methods of Sterilization

1-Sunlight: The activity of sunlight is mainly due to the presence of ultra violet rays in it. It is responsible for spontaneous sterilization in natural conditions. The sunlight is more effective in killing germs due to combination of ultraviolet rays and heat.

2-Heat: Heat is considered to be most reliable method of sterilization. Heat acts by oxidative effects as well as denaturation and coagulation of proteins. Heat is divided into two type :

1. Dry heat

A . Red heat: it is used to sterilize metallic objects by holding them in flame till they are red hot e.g. inoculating wires, needles, forceps, spatulas ... etc.

B. Flaming: The article is passed over flame without allowing it to become red hot e.g. mouth of culture tubes, flasks, glass slid.

C. Incineration: This is excellent method for rapidly destroying material e.g. experimental dead animals and pathological materials... etc.

D. Hot air oven: This the best method for sterilizing all glass, Petri dishes, test tubes, flask, pipettes, scalpels, scissors.

E. Infra- red radiation: To sterilize metal instruments and glass syringes.

2. Moist heat

1- At temperature below 100 °C

a. pasteurization of milk temp. Employed is either 65°C for 30 min or 72 for 15-20 sec.

2- At temperature of 100°C

a. Tyndallization: Which medium is placed at 100°C for (20-30) min each on 3 successive days? Used for sterilizing media containing sugars.

b. Boiling: For needles and instruments boil in water for (10-30) min. is sufficient to sterilize

c. Steam at 100°C: Is used to sterilize culture media which may decompose if subjected to higher temp. Like (gelatin agar)

3- At temperature above 100°C: we used Autoclave to sterilized inoculated media.

3. Filtration: For sterilize the fluids that do not stand heating e.g. plasma, vitamins, carbohydrates solutions and antibiotics

4- Radiation:

a. ultraviolet radiation and lamps: It is chief bactericidal factor present in sunlight kill the germs .

b. X-rays and other ionizing radiation: They are useful for sterilization of disposable material like disposable syringes and adhesive dressing ... etc.

5- **Ultrasonic and sonic vibrations**: They are bactericidal causing mechanical agitation and rupture of bacteria.

B- Chemical methods

1- Acids and alkaline: They are in inhibitory to the grow of bacteria. *Microbacteria* are more resistant to acid than alkaline;

2- Metallic ions: $Hgcl_2$ and $AgNo_3$ prevent the growth on many bacteria in concentration less than 1 ppm.

3- Inorganic anions: They are much less toxic to bacteria such as fluoride inhibits many enzyme of bacteria.

4- Halogens: Iodine is used chiefly for skin –chlor for water.

5- Oxidizing agents: They are weak antiseptic e.g. H_2O, potassium permanganate.

6- Formaldehyde: It is useful in sterilization bacteria vaccine also is highly lethal (irritant water soluble) to kill all kinds of microorganisms and spores.

7- Phenol group: It is used for sterilizing surgical instrument; Lysol and cresol are used generally in 3% solution.

8- Soap and detergents: Bacteriostatic for G+ and some acid fast organism.

9- Alcohol: Ethyl alcohol is most effective in 70% solution than 100% alcohol. It doesn't kill spores.

10- Dyes: Gentian violet... are active against G+ bacteria.

11- Aerosols and gaseous disinfectants: SO_2 , chlorine, and formalin vapor have been used as gaseous disinfectant –propylene glycol is powerful disinfectant.

ANTIBIOTICS & THE BASES OF CHEMOTHERAPY

Chemotherapeutic agents: antimicrobial agents of synthetic origin useful in the treatment of microbial or viral disease. Examples: sulfonilamides, isoniazid…

Antibiotics: antimicrobial agents produced by microorganisms that kill or inhibit other microorganisms.

*Antimicrobial agents divided into classes in the type of **action**:-

Bacteriostatic drugs: - inhibit the microbial growth e.g tetracycline, chloramphenicol .

Bactericidal drugs: - kill the bacteria have lethal action e.g penicillin's, cephalosporin .

The range of bacteria or other microorganisms that are affected by a certain antibiotic are is expressed as its **spectrum of action**. Antibiotics effective against prokaryotes which kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to be **broad spectrum** . If effective mainly against Gram-positive or Gram-negative bacteria, they are **narrow spectrum**. If effective against a single organism or disease, they are referred to as limited spectrum.

Kinds of Antimicrobial Agents and their site and mechanisms of modes of action :

1. **Cell wall synthesis inhibitors**: Cell wall synthesis inhibitors generally inhibit some step in the synthesis of bacterial peptidoglycan. Generally they exert their selective toxicity against bacteria because human cells lack cell walls.e.g Beta lactam antibiotics the products of two groups of fungi, Penicillium and Cephalosporium, The beta lactam antibiotics inhibit the last step in peptidoglycan synthesis, the final cross-linking between peptide side chains.

Semisynthetic penicillin's :- (6-aminopenicillanic acid) which can be modified chemically by the addition of side chains increased spectrum of activity like Amoxycillin and Ampicillin or inhibits beta lactamase enzymes like Methicillin

Bacitracin: is a polypeptide antibiotic produced by Bacillus species It prevents cell wall growth.

2. **Cell membrane inhibitors**: disorganize the structure or inhibit the function of bacterial membranes. The integrity of the cytoplasmic and outer membranes is vital to bacteria, and compounds that disorganize the membranes rapidly kill the cells, like Polymyxin, produced by Bacillus polymyxis , Polymyxin bind to membrane phospholipids and thereby interfere with membrane function

3**. Protein synthesis inhibitors**: have an specificity for 70S ribosomes, like aminoglycosides antibiotics are products of Streptomyces species and are represented by streptomycin, kanamycin, erythromycin, chloramphenicol and gentamicin. These antibiotics exert their activity by binding to bacterial ribosomes and preventing the initiation of protein synthesis.

4.**Effects on Nucleic Acids:** Some chemotherapeutic agents affect the synthesis of DNA or RNA, can block the growth of cells , like Nalidixic acid and Rifampin.

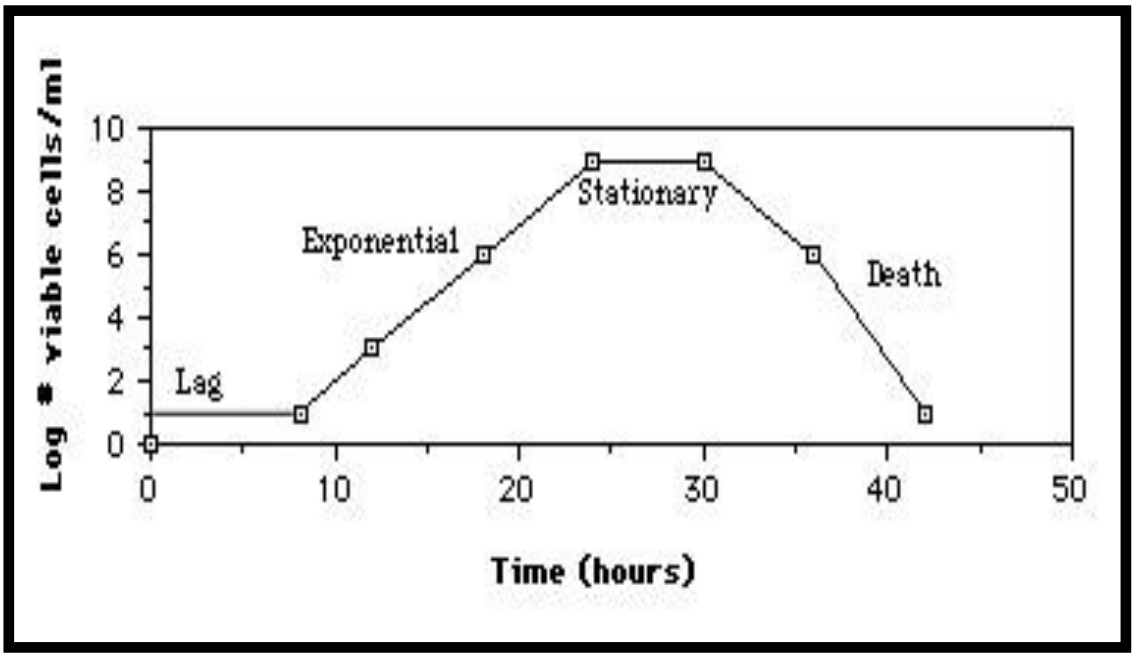
bacterial growth curve

- The **bacterial growth curve** represents the number of live cells in a bacterial population over a period of time.
- **Lag Phase:** This initial phase is characterized by cellular activity but not growth. A small group of cells are placed in a nutrient rich medium that allows them to synthesize proteins and other molecules necessary for replication. These cells increase in size, but no cell division occurs in the phase.

- **Exponential (Log) Phase:** After the lag phase, bacterial cells enter the exponential or log phase. This is the time when the cells are dividing by binary fission and doubling in numbers after each generation time. Metabolic activity is high as DNA, RNA, cell wall components, and other substances necessary for growth are generated for division. It is in this growth phase that antibiotics and disinfectants are most effective as these substances typically target bacteria cell walls or the protein synthesis processes of DNA transcription and RNA translation.

- **Stationary Phase:** Eventually, the population growth experienced in the log phase begins to decline as the available nutrients become depleted and waste products start to accumulate. Bacterial cell growth reaches a plateau, or stationary phase, where the number of dividing cells equal the number of dying cells. This results in no overall population growth. Under the less favorable conditions, competition for nutrients increases and the cells become less metabolically active. Spore forming bacteria produce endo spores in this phase and pathogenic bacteria begin to generate substances (virulence factors) that help them survive harsh conditions and consequently cause disease.
- **Death Phase:** As nutrients become less available and waste products increase, the number of dying cells continues to rise. In the death phase, the number of living cells decreases exponentially and population growth experiences a sharp decline. As dying cells lyses or break open, they spill their contents into the environment making these nutrients available to other bacteria. This helps spore producing bacteria to survive long enough for spore production. Spores are able to supports life.

Pathogenicity



Infection: The few microbial species that are able to invade and then damage tissue and cause disease are called pathogenic infection. The capacity of a microorganism to cause disease is referred to as pathogenicity.

Some microorganisms cause a single characteristic disease, e.g. *Clostridium tetani*, which causes tetanus. Other microorganisms can cause a wide range of different diseases. For example, *Staphylococcus aureus* can cause skin infections such as abscesses and wound infection, pneumonia and osteomyelitis, other microorganisms are able to cause disease only in individuals with impaired defenses and are called opportunistic pathogens.

Stages in the Development of Infection

1- Acquisition

2- Adhesion to host cells Penetration of cells.

3- Damage to tissues

4- Spread to other tissue

5- Resolution or death

Source of Infection

1- Man is himself a common source of infection from a patient or carrier. Healthy carrier is a person harboring pathogenic organism without causing any disease to him.

2- Animals: Infection diseases transmitted from animals to man. Zoonosis may be bacteria (e.g. rickettsia).

3- Insects: The disease caused by insect are called arthropod borne disease. Insects like mosquitoes, lice that transmit infection are called vector.

4- Some vector may act as reservoir host (e.g. ticks in spotted fever).

5- Soil: Soil may serve as some of parasiting infection like round worm and hook worm. Spore of tetanus bacilli remain viable in soil for a long time.

6- Water: Cholera vibrio, infective hepatitis virus, guinea worm may be found in water.

7- Food: Contaminated food may be a source of infection. Presence of pathogens in food may be due to external contamination (e.g. food poisoning by staphylococcus).

Methods of Transmission of Infection

1- Contact: gonorrhea, trachoma.

2- Inhalation: Influenza, tuberculosis.

3- Infection: Cholera (water) food poisoning (food dysentery).

4- inoculation organism : rabies (dog)

5- Insects: Act as mechanical vector (dysentery and typhoid by house fly) or biological vector (malaria) of infection disease.
6- Genital: syphilis, Trichomonas.
7- Laboratory infection: Infection may be transmitted during procedure like injection.

Bacterial toxins

Toxins: are substances released by microbial cells, which by damaging or destroying specific tissues are responsible for some or all of the disease processes. **There are two types of toxin.**

1- Exotoxins are usually protein enzymes secreted by bacteria into their local environment. They may be transported in the bloodstream and cause damage in parts of the body remote from the site of infection..
2- Endotoxins: are lipopolysaccharides contained in the outer cell membrane of Gram –negative bacteria. They do not have enzymatic activity but have profound systemic effects on the host, including:
1- Induction of high fever.
2- Reduction in blood pressure and disruption in coagulation causing bleeding into the tissues.

Virulence

The term virulence is used to **describe the degree of pathogenicity a microorganism to cause disease**. Virulence depends on the microorganisms' ability to invade, multiply in and damage the host and is mediated by factors in both the host and the microorganism. The ability of a microbe cause disease depends on some factors that:

1- Assist adhesion (e.g. fimbriae, slime).
2- extracellular enzymes (e.g. Urease ,protease , Hemolysin).
3- Protect against the immune system (e.g. capsules).
4. Determine toxin production.

Staphylococcus

General Characters:

1- They are G +, Ovoid or spheroidal, Non motile arranged in groups usually form irregular clusters the cluster formation is due to cell division occurring in three planes with daughter cell tend to remains in close proximity , facultative anaerobic.
2- On the culture media they form colonies and produced pigment White, yellow or golden yellow in color their hemolytic capacity is variable.
3- They are **catalase positive, oxidase negative**, ferment glucose, and have teichoic acid in their cell walls.

Classification:

A- On the basis of **pigment** production 3 types of staphylococci are identified.

1- *Staphylococcus aureus* produce golden yellow colonies and are pathogenic.
2- *Staphylococcus albus produce* white colonies and are nonpathogenic.
3- *Staphylococcus citreus* produce lemon-yellow colonies and are nonpathogenic.

B- On the pathogenicity based on the synthesis of the **Coagulase-positive** strains:

1- Pathogenic species Coagulase-positive strains like *Staphylococcus aureus* (CPS)

2- Non pathogen species Coagulase-Negative strains like Staphylococcus *epidermides* (CNS)

B- Presence of hemolysis. While others don't have any hemolysis, *Staphylococcus aureus* has Beta hemolysis.

There are at least three Staphylococci species of clinical importance:
1. *Staphylococcus aureus* is the most pathogenic for humans.

2. *Staphylococcus epidermidis*, which is part of the normal flora and is of low pathogenicity grown on the skin.

3. *Staphylococcus saprophyticus* which can cause urinary tract infections,.

Some Biochemical test for Staphylococcus

The type of test	***Staphylococcus aureus***	***Staphylococcus albus***	***Staphylococcus citreus***
Catalase test	+	+	+
Coagulase test	+	-	-
Hemolysis test	Beta on (Blood agar)	None	None
Color differences in colonies on Mannitol salt agar	Gold – yellow	White	lemon to yellow

***Staphylococcus aureus* :**

Biochemical reaction:-
They ferment number of sugar production acid and no gas (glucose , lactose , sucrose , maltose , mannitol) .

Culture characterization:
Nutrient agar
After incubation colonies are pigmented golden yellow (size from 2-4 mm) circular, convex, opaque with entire edge, pigment production enhanced when 1 % glycerol mono acetate or milk is incorporated in medium.

Blood agar:
A wide zone of β- haemolysis (clear zone) is produced around colonies.

Egg yolk medium: the organism produce Shiny, black colonies surrounded by an inner opaque (lipase reaction) halo and clearing zones (protease reaction)

Mannitol salt agar: the media contain 7- 10 % Nacl, Yellow colonies; may have yellow halo around colonies.

Toxins

1. Toxic epidermal is caused blistering skin lesions in neonates and young
2. Toxic shock syndrome toxin (TSST) The toxin causes a range of symptoms including fever, diarrhea, TSST can be fatal. and can also be caused by *Streptococcus pyogenes*.
3. Haemolysin: *Staph. aureus* produces at least 3 types of haemolysin known as Alfa, beta and gamma. Beta haemolysin haemolysis rabbit and sheep red cell rapidly.
4. Leucocidin: Leucociden is closely associated with delta lysis damage polymorphonuclear leucocyte.
5. Enterotoxin: The toxin is responsible for infection of Staph. food poisoning, vomiting an diarrhea within 6 hours of taking contaminated food

Various enzymes and protein produced by staphylococci

Product	**Physiological action**
B-lactamase	Breaks down penicillin
Catalase	Converts hydrogen peroxide into water
Coagulase	Binds complex structure called staphylothrombin in which a clot is formed
DNase	Destroys DNA
Lipase	Break down Lipid molecules
Protease	Break down proteins

Clinical Infection

Staphylococci can cause many forms of infection include:

A. **Cutaneous lesions**: Pustules are common in acne,

B. **Deep infection**: Acute osteomyelitis, tonsillitis, pharyngitis, sinusitis, pneumonia, pulmonary abscess.

C. **Staphylococcal food poisoning**: It is results when food contaminated with enterotoxin produced by Staphylococci is consumed e.g. meat, fish, milk and milk products. Diarrhea and vomiting set in within 6 hours of taking contaminated food.

Treatment

The antibiotic of choice for the treatment of ***S. aureus*** infection is Cloxacillin, or Erythromycin if the patient is allergic to penicillin.and Methicillin-resistant many strains of ***S. aureus*** (MRSA) or used vancomycin .

Streptococcus

General characters:

They are Gram positive cocci arranged in chains, non-motile and non-spore former. Facultative anaerobic, Catalase negative. They require media enriched with blood, serum for their growth. They are important human pathogens causing pyogenic infection with a characteristic tendency to spread. They are also responsible acute rheumatic fever and glomerulonephritis

Classification of streptococcus: Several system of classification have been ;

A. Morphology

1- Morphological classification: Attempt to classify streptococci in to long chain (pathogenic strain) and short chain (nonpathogenic) forming cocci.

B. Cultural character

1- Obligate anaerobe (***peptostreptococci*).**

2- Facultative anaerobes (***Streptococcus)***

C. Biochemical reaction

Fermentation is used in differentiating different species of streptococci e.g. Mannitol is fermented by enterococci

D. **Classification of Streptococci Based on Hemolysis on Blood Agar**;

- **α - hemolysis**

Partial hemolysis

Green discoloration around the colonies

e.g. non-group able streptococci (*S. pneumonia* & *S. viridans*)

- **β-hemolysis**

Complete hemolysis

Clear zone of hemolysis around the colonies

e.g. Group A & B (*S. pyogenes* & *S. agalactiae*)

- **γ-hemolysis**

No lysis

e.g. Group D (Enterococcus spp)

E. Antigenic structures

Classification based on C- carbohydrate antigen of cell wall using to know the antibodies dividing in to Lancefield group. A,B,C,D,.

***Streptococci pyogenes* (Group A):**

Is one of the most important bacterial pathogens. The different serotypes produce:-

1- Structural components:- **M protein** that help retard phagocytosis. It attaches to mucous membranes anywhere in the body. M-protein helps inhibit phagocytosis, **Lipoteichoic acid & F protein** helps in adhesion which acts to camouflage the bacteria

2- **Extracellular enzymes** that break down host molecules. Like C5a peptidase cleaves a potent neutrophil chemotaxin called C5a, The C5a peptidase is necessary to minimize the influx of neutrophils in early infection as the bacteria are attempting to colonize the host's tissue

3- **Streptokinase**, enzymes that activate a host-blood factor that dissolves blood clots.

4-**Toxin production**:

1. Pyogenic toxin : dividing into group A and B the pyogenic toxin A cause the toxic shock syndrome and pyogenic toxin B caused necrotizing fasciitis
2. Pyrogenic toxins: that stimulate macrophages and helper T cells to release cytokines and generate the fever
3. Hemolysis: They are toxic substances. Divided in two types:

a. Streptolysin O, which is oxygen مستقرlabile, heat labile, strongly antigenic. beta-hemolytic property
b. Streptolysin S is oxygen stable, heat stable, non-antigenic. Beta-hemolytic. A potent cell poison affecting many types of cell including neutrophils, platelets, and sub-cellular organelles
4. Erythrogenic toxin is heat stable toxin. In small doses if injected caused scarlet fever.

Diseases:

1. Pharyngitis (tonsillitis or Strep. Throat)

Is the primary site of invasion causing sore throat. It may be localized in tonsils (tonsillitis) or may involve pharynx (pharyngitis) with vomiting and fever.

2. **Skin infection**

It may cause infection of skin e.g. wound, burns, lymphangitis.

3. Scarlet Fever (Strep. throat with rash)

S. pyogenes produce streptococcal pyrogenic exotoxins originally called erythrogenic toxins, are produced by lysogenic strains of streptococci. Cause skin allergy and rash

4. Pyoderma-confined, pus-producing lesion that usually occurs on the face, arms, or legs.

5. Necrotizing fasciitis

Known as **flesh-eating disease** or **flesh-eating bacteria syndrome** Deep infection of the subcutaneous tissue. Characterized by extensive destruction of muscle and fat, Systemic toxicity, multi-organ failure, and death (50%) are classic, Fasciitis must be treated not only with aggressive drug therapy, but also surgical debridement of necrotic tissue is necessary.

6. Others

Bronchi-pneumonia and Septicemia

Autoimmune Diseases (Infections)

A. Rheumatic Fever

It is a chronic disease of the heart and valves. Swollen and aching joints and fever are typical. Sometimes chest pain and myocarditis also present.

B. Acute Glomerulonephritis

Inflammation of the kidney tubules (also it is not a result of direct bacterial attack). It is caused by autoimmune cross-reaction following Strep. skin and /or infection. The antibodies attack the kidney tubules.

- **Laboratory diagnosis:**

Hematological investigations:

1- Total leucocyte count may show considerable increase.
2- Differential leucocyte count show increase in neutrophil count.
3- Erythrocytes sedimentation rate (ESR) is raised especially in rheumatic disease.

Bacteriological method: Most important specimens are throat swab, nasopharyngeal swab, pus swab, sputum, cerebrospinal fluid, blood etc...

a. Smear from above material after Gram's staining show Gram positive cocci arranged in chains.
b. Culture: Specimen is cultured on blood agar or crystal violet blood agar media with loop.

After overnight incubation at 37°C colonies are studied. These are small (pin point), raised colonies showing beta haemolysis.

Skin test is known by the name Dick test.

Dick test: It is done to find out susceptibility of a person to scarlet fever. 0.2 ml erythrogenic toxin is injected intradermal on the fore arm and same amount of heated inactivated toxin on the other forearm. A bright red rash appears within 6 hours and becomes maximum in 24 hours and this fades away. Control forearm doesn't show any reaction. A positive reaction means no immunity to scarlet fever.

Lancefield group B streptococci (*Streptococcus agalactiae*)

Streptococcus agalactiae: is responsible for mastitis in cow. It may be present in human throat and vagina of a proportion of women as commensal. As a result, vertical transmission to the neonate may occur either in utero or during delivery and may cause puerperal infection, septicemia, meningitis and ulcerative endocarditis etc...

Lancefield groups C streptococci (Streptococcus equisimilis)

Streptococcus equisimilis: They are isolated from horses and cows. They may produce streptolysin O and fibrinolysin. The organisms have been isolated from puerperal infection, cellulitis, wound and scarlet fever.

Lancefield groups D streptococci (*Enterococcus faecalis*)

Enterococci these bacteria, previously known as group D non-hemolytic streptococci the most common species, *E. faecalis* and *E. faecium*, are part of the normal flora of the lower intestine but can cause infections of the urinary tract infection, surgical wounds and soft tissue, endocarditis and septicemia

Streptococcus pneumonias

This is alpha-hemolytic streptococcus that characteristically occurs in pairs. Pathogenic species produce complex polysaccharides, which form a capsule around the cell. *S. pneumoniae* frequently colonizes the upper respiratory tract asymptomatically. *S. pneumoniae* cause pneumonia meningitis, otitis media sinusitis and bacteremia.

CAMP test

This test (named for its discoverers) It is an acronym for ((Christie Atkins Munch-Petersen)) tests for presence of the CAMP factor protein that is produced by Group B Streptococci. The CAMP factor, in combination with the hemolysis produced by Staphylococcus aureus, produces a dramatically increased zone of hemolysis.

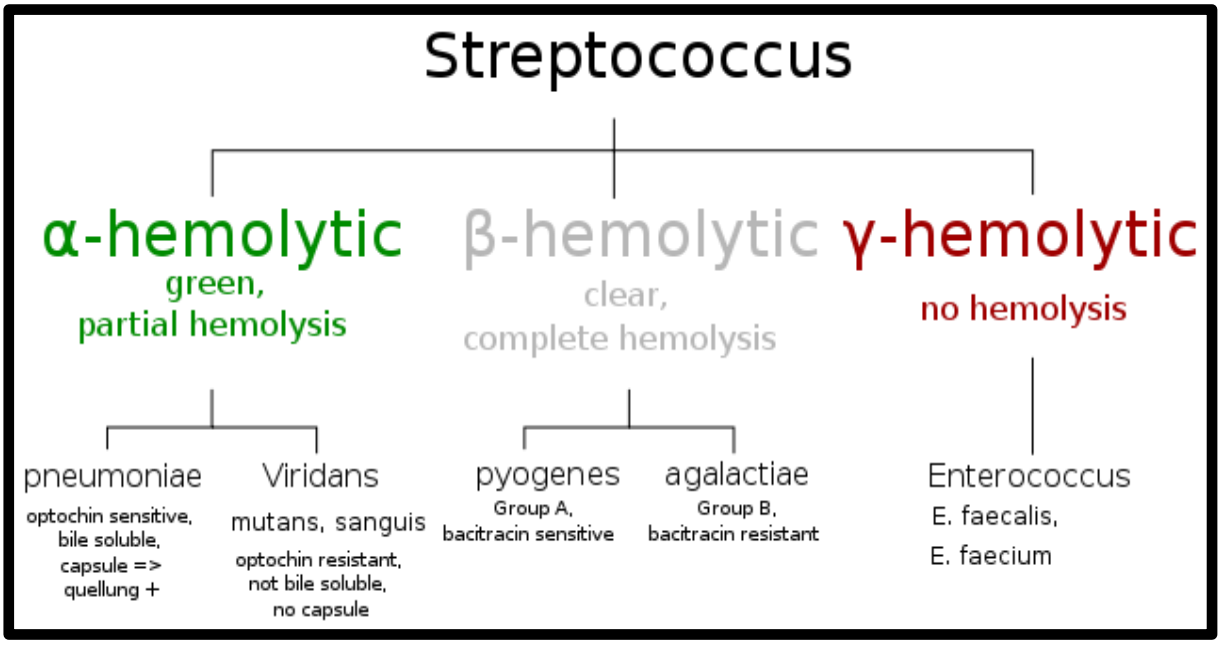
Results

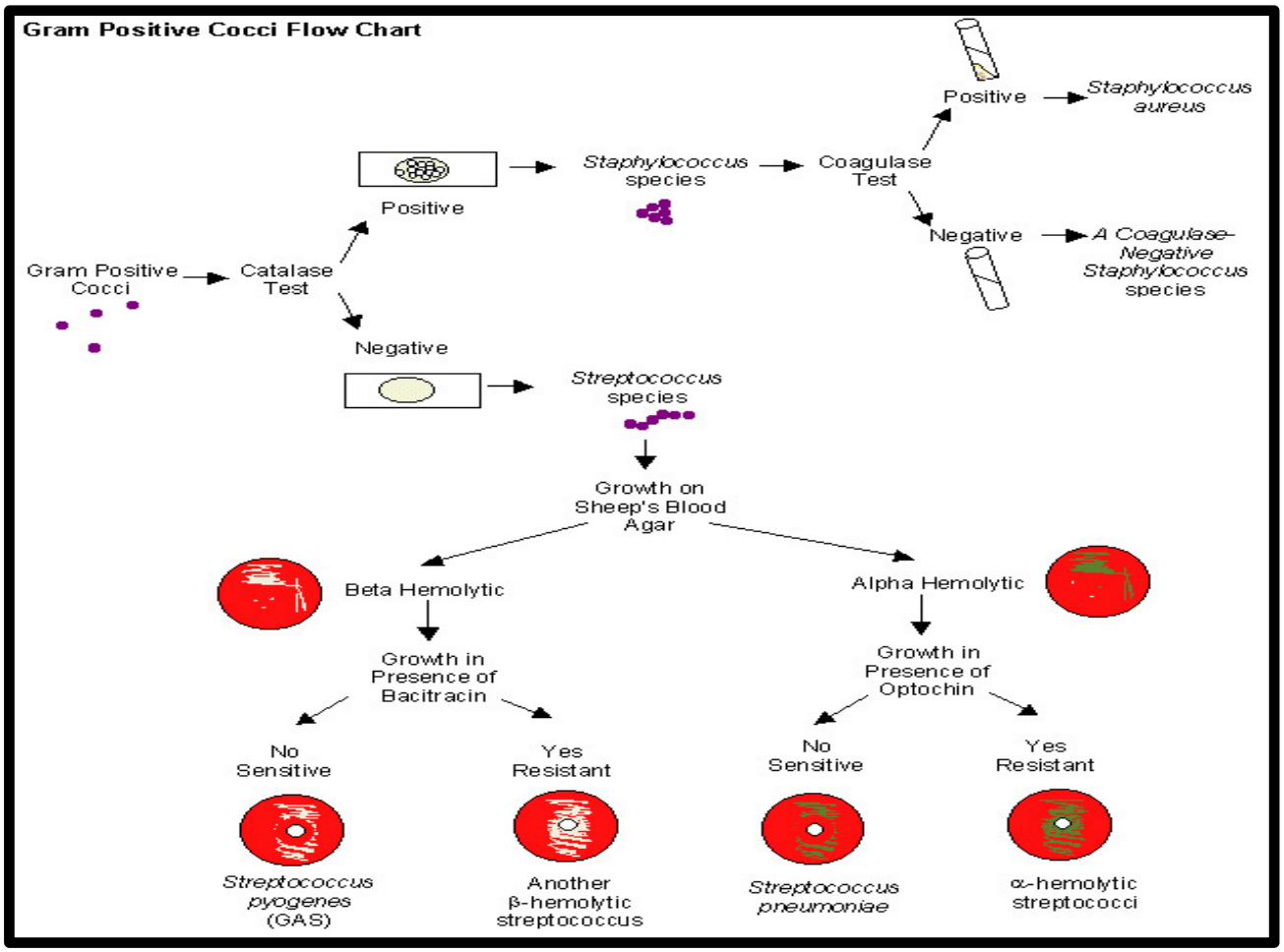
+ = expanded zone of hemolysis where unknown organism grows near Staphylococcus aureus

- = No expansion of hemolysis where unknown organism grows near Staphylococcus aureus

Classification of common streptococcal Pathogens

Biochemichal Classification	**Serological classification**	**Hemolysis pattern**	**Bacitracin sensitivity**	**CAMP test**
S. pyogenes	group A	Beta (complete)	Susceptible	Negative
S. agalactiae	group B	Beta	Resistant	Positive
S. equisimilis	group C	Beta	-	-
Enterococcus faecalis	group D	Non hemolytic	-	-
S. pneumonias	No group	Alpha hemolytic	-	-





Differentiation between Gram-Positive cocci

Corynebacterium

General character:

They are G+ ve nonacid fast and non-motile bacilli accruing in palisade club-shaped. Non capsulated, the bacilli are arranged in pairs or groups. Bacilli form various angles with each other like V or L. This is called chines letter arrangement. *Corynebacterium diphtheriae* is the pathogenic member and it shows metachromatic granules on Albert stained smear. It produces powerful exotoxin. Toxigenic strains are lysogenic for one of a family of corynebacteriophages that carry the structural gene for diphtheria toxin, *tox.*

Culture

1- Corynebacteria grow slowly, even on enriched media.
2- In terms of nutritional requirements, all need biotin to grow.
3- Some strains also need thiamine and PABA (Para amino benzoic acid).
4- Corynebacterium grows slowly, even on enriched media. The bacteria grow on Loeffler's serum medium and/or on blood tellurite agar (BTA) or Potassium tellurite agar (selective medium) aerobically at 37C for 24 hrs.).
5- They form small creamy grayish colonies with a granular appearance, mostly translucent, but with opaque centers, convex, with continuous border.
6- Their color tends to be yellowish white in Loffler's medium. In (BTA) it can form grey to black colonies it act as selective media inhibiting the growth of other organism.

Habitat

Corynebacteria species occur commonly in nature in the soil, water, plants, and food products. The non-diphtheriroid *Corynebaterium* can even be found in the mucosa and normal skin flora of humans and animals.

Classification:

There are four biotypes subspecies recognized.

Corynebacterium diphtheriae

Corynebacterium mitis

Corynebacterium intermedius

Corynebacterium gravis

Gravis	**intermedius**	**mitis**
Morphology: Short uniform with few granules	Long irregularly with poor granulation	Long curved with prominent granules
Colony : Daisy head colony	Frog egg colony	Poached egg colony
Consistency Brittle not emulsify	Between gravis and metis	Soft , butyrous and emulsify easily
Haemolysis : Is variable	Non haemolysis	Haemolytic
Glycogen and starch fermentation : positive	Negative	Negative
Antigenic structure : 13 types	4 types	40 types

Role in disease

Main article: Virulence and toxin production of C. Diphtheria

The most notable human infection is diphtheria; the symptoms of diphtheria include pharyngitis, fever, swelling of the neck or area surrounding the skin lesion, hypoxia due to airway obstruction by the pseudo membrane. The toxin is distributed to distant organs by the circulatory system and may cause paralysis and congestive heart failure

Diphtheria toxin is synthesized which **must be cleaved into two chains to work**. A is active chain, B mediates entry an N-terminal fragment A (catalytic domain), and fragment B (trans membrane and receptor binding domains). Fragment A catalyzes the NAD^+-dependent ADP-ribosylation

of elongation factor 2, thereby **inhibiting protein synthesis in eukaryotic cells and kill susceptible cell**. **Fragment B binds to the cell surface receptor and facilitates the delivery of fragment A to the cytosol, the toxigenecity of diphtheria depends upon presence of bacteriophage and iron concentration need 0.1 mg optimum concentration for production 0.5 mg inhibits toxin production.**

Laboratory diagnosis:

Bacteriological investigations:

1- Smear examination: Gram staining show thin G +ve bacilli showing chines letter arrangement Albert stain is done for the demonstration of metachromatic granular

2- Culture: The swab is inoculated on loeffler slope blood tellurite media and blood agar plate, the serum slop shows growth in 6-8 hours. Smear is stained with Albert stain and we may find bacilli with metachromation granules – blood tellurite plate may be incubated for at least 2 days.

3-**Schick test** the test is a simple procedure. A small amount (0.1 ml) of diluted (1/50 MLD) diphtheria toxin is injected intradermal into the arm of the person. If a person does not have enough antibodies to fight it off, the skin around the injection will become red and swollen, indicating a positive result. This swelling disappears after a few days. If the person has an immunity, then little or no swelling and redness will occur, indicating a negative result

4- **Elek's test** for toxogenecity is used to determine whether the organism is able to produce the diphtheria toxin or not. The test procedure

Sterile filter paper impregnated with diphtheria antitoxin is imbedded in agar culture medium. Isolates of *C diphtheriae* are then streaked across the plate at an angle of 90° to the antitoxin strip. Toxigenic *C diphtheriae* is detected because secreted toxin diffuses from the area of growth and reacts with antitoxin to form lines of precipitin.

Immunity:-

1. **Active immunity :**

Formal toxoid adjuvant vaccine such as alum precipitated toxoid

(ATP) , purified alum precipitated toxoid (PAPT) , toxoid antitoxin floccules (TAF) are available and (diphtheria, tetanus, pertussis**) (DPT)**

Given at older and adult children due to produce hypersensitivity given at 6 month a booster dose is given at 18 month at another at 5 years.

2. **Passive immunity :**

This is an emergency case given antitoxin (anti diphtheric serum) subcutaneously

Diphthroids

Diphthroids are non-pathogenic found on mucous membrane, oral cavity and genitalia

1. **C hofmanns** is found in human throat
2. **C xerosis** is found in skin as normal flora
3. **C acne** is found in acne pustule

Diphtheria	**Diphthroids**
Habitat *Corynebacteria* species occur commonly in nature in the soil, water, plants, and food products	can even be found in the mucosa and normal skin flora of humans and animals.
Morphology : 1. Gram positive and thin 2. Metachromatic granules are more 3. Pleomorphism present 4. Chines letter arrangement	 1. Gram positive short and thick 2. They are less or absent 3. Little Pleomorphism 4. No such arrangement is seen
Ferment glucose only	Ferment glucose and sucrose
Pathogenic	Non pathogenic
produced powerful toxin	Not produced toxin

***Mycobacterium tuberculosis* (MTB)**

General character: They are slender, curved rods, acid fast (resistant decolonization by acid and alcohol) hence Ziehl-Neelsen staining, (acid fastness is due to mycolic acid) they are classified as acid-fast Gram – positive bacteria due to their lack of an outer cell membrane. aerobic and requires high levels of oxygen, non-motile, non-capsulated and non-spore forming , The cell wall contains complex waxes , glycolipids and peptidoglycolipids (primarily mycolic acid) , Growth is generally slow(generation time 14-15 days) colonies appear in 2-6 weeks . They don't grow on ordinary media. They require enrichment of media with egg albumin e.g. Lowenstein Jensen's medium. Can remain inside the host in dormant state and reactive later

Classification of mycobacteria

A- Tubercle bacilli
1- *M. tuberculosis* (human)
2- *M. bovis* (cattle)
3- *M. avium (avian*)
B- Lepra bacilli
1- *M. leprae*
2- *M. leprae murium*
C- Mycobacteria causing skin lesion.

Virulent Mechanisms of TB

TB mechanism for cell entry

- The tubercle bacillus can bind directly to mannose receptors on macrophages via the cell wall-associated mannosylated glycolipid (LAM)

TB can grow intracellular

- Effective means of evading the immune system
- Once TB is phagocytosed, it can inhibit phagosome-lysosome fusion (lack of immediate host immune response)
- alveolar macrophage ingests TB bacillus; bacillus sits in phagosome; phagosome normally incorporates proton-ATPase into membrane leading to decreased pH and acidification within phagosome; acidified phagosome then normally fuses with cell lysosome, exposing organism to lysosome's toxic enzymes

BUT MTB prevents insertion of proton-ATPase into phagosome so phagosome never gets acidified and never merges with lysosome) perhaps by modulating the activity of a membrane proton pump

- TB can remain in the phagosome or escape from the phagosome (Either case is a protected environment for growth in macrophages)

Virulent mechanisms of TB

- Slow generation time Immune system cannot recognize TB, or cannot be triggered to eliminate TB

High lipid concentration in cell wall

- accounts for impermeability and resistance to antimicrobial agents
- Accounts for resistance to killing by acidic and alkaline compounds in both the inracellular and extracelluar environment

- Also accounts for resistance to osmotic lysis via complement deposition and attack by lysozyme

Virulent Factors of TB

Antigen 85 complex

- It is composed of proteins secreted by TB that can bind to fibronectin.
- These proteins can aid in walling off the bacteria from the immune system

Cord factor

- Associated with virulent strains of TB
- Toxic to mammalian cells and be directly cytotoxic to macrophages and to be an inhibitor of PMN migration

Pathogenesis of the M TB

Droplet nuclei are inhaled, and are generated by talking, coughing and sneezing

TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages. The primary site of infection in the lungs, known as the "Ghon focus", is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe. Tuberculosis of the lungs may also occur via infection from the blood stream. This is known as a Simon focus and is typically found in the top of the lung.

Tuberculosis is classified as one of the granulomatous inflammatory diseases. Macrophages, T lymphocytes, B lymphocytes, and fibroblasts are among the cells that aggregate to form granulomas, with lymphocytes surrounding the infected macrophages. The granuloma prevents dissemination of the mycobacteria and provides a local environment for interaction of cells of the immune system. Bacteria inside the granuloma can become dormant, resulting in latent infection. Another feature of the granulomas is the development of abnormal cell death (necrosis) in the

center of tubercles. To the naked eye, this has the texture of soft, white cheese and is termed caseous necrosis.

If TB bacteria gain entry to the bloodstream from an area of damaged tissue, they can spread throughout the body and set up many foci of infection, all appearing as tiny, white tubercles in the tissues. This severe form of TB disease, most common in young children and those with HIV, is called miliary tuberculosis. People with this disseminated TB have a high fatality rate even with treatment (about 30%).

Lab- diagnosis: Diagnosis is based on:

A .Symptoms

- Producing cough, chest pain, night sweats, fatigue, fever

B. TB tests

- Tuberculin Skin Test (purified protein derivatives) (PPD)
- Blood Tests
- Chest X-Rays

C. Diagnostic microbiology

- Sputum smear – acid-fast bacilli (AFB) test

Bacillus

They are rod shaped, spore forming classified in two groups:

a. Aerobic Bacillus

b. Anaerobic Bacillus.

Aerobic bacillus are G +ve , non-motile, spore bearing bacilli occurs in chains. They are thick with truncated or convex ends. Bacillus anthracis is the only pathogenic species causing Anthrax, bacillus subtilus are opportunist and Bacillus cereus may produce food poisoning.

Bacillus Anthrax

It remains in parasitic form in cattle and sheep. Infection in man is the result of an accidental contact with infected animal.

Morphology:

They are non-motile, Capsule made of glutamic acid, non-acid fast, G+ve measuring 1 - 1.2µm in width x 3 - 5µm in length they may be arranged singly or in short chains. The entire chain may be surrounded by capsule-

capsule is poly particle. In culture the bacilli are arranged end to end in chains.

Cultural character:

They are aerobic growing at optimum temp -37°C (range being 12°C. The optimum temp- for spore formation is 25°C to 30°C. Growth may occur on ordinary media.

Nutrient broth:

There may be turbidity or no turbidity.

Agar plate:

Colonies are irregular round, 2-3 mm in diameter greyish, white.

Blood agar:

The colonies are non haemolytic.

Gelatin stab:

The characteristic inverted tree appearance is seen with slow liquefaction starting from the top.

Selective medium (PLET):

It consist of polymyxin, lysosyme, ethylene diamine tetra acetic acid (EDTA) and thallous acetate added to heart infusion agar. It is used to isolate anthrax from mixture of spore bearing bacilli.

Pathogenesis: disease takes one of three forms, depending on the route of infection. **Cutaneous anthrax** results from infection through skin lesions **this exotoxin** causes localized tissue necrosis, evidenced by a painless round black lesion with a rim of edema. This lesion is called a "malignant pustule" because without antibiotic therapy.

Gastrointestinal anthrax results from ingestion of spores, usually in infected meat *Bacillus anthracis* matures and replicates within the intestine, where it releases its exotoxin. The exotoxin causes a necrotic lesion within the intestine. Patients present with vomiting, abdominal pain, and bloody diarrhea.

pulmonary anthrax (woolsorter's disease) results from inhalation of spores. The spores are taken up by macrophages in the lungs and transported to the lymph nodes where they germinate. Mediastinal hemorrhage occurs resulting in mediastinal widening (enlarged area around and above the heart seen on chest radiograph)

The **toxins** and the **capsule** are the primary virulence factors of the anthrax bacillus. Virulent strains harbor two large plasmids: pX02 codes for the capsule and pX01 codes for the exotoxin. The **anthrax toxin** is complex, consisting of **three protein** components: I, II, and III. Component I is the **edema factor (EF)**, component II is the **protective antigen (PA)** and component III is the **lethal factor (LF)**. Each component is a thermo labile protein. EF and LF gain entry into target cells by competitively binding with PA that has a membrane translocation function. These three components act synergistically to produce the toxic effects seen in anthrax. **protective antigen (PA) reacts with host cell tissue receptors where it is proteolytically activated to form heptamer so that it binds LF and EF to allow entry of LF and EF into the host cells via endocytosis Components. EF converts ATP to cAMP, Increases cAMP levels over 1,000 fold , Impairs neutrophil function, and Edema (Alters water homeostasis) and LF cleaves MAPKK mitogen-activated protein kinase kinases (MAPKKs) at its N terminus , Disrupts pathways involved in cell growth & maturation , Increased synthesis of tumor necrosis factor-α & interleukin-1β , Macrophage lysis and More cells infected with bacteria & toxin cause Septic shock and death**

Death probably results from high levels of bacteria secreting LF toxins in blood

Lab- diagnostic

1- Gram stain (G+ve bacilli from exudate)
2- Ordinary media: grow on nutrient and blood agar and PEA agar (prepared by adding 0.3% phenylethyl alcohol to brain-hear infusion agar.
3- Animal inoculation (guinea pigs or mice).
4- Serological test: (Ascoli's precipitation test).
5- Motility test: differentiate between anthrax bacilli (non- motile) and non- pathogenic bacilli (motile).
6- Gelatin stab. Shows on inverted fur tree appearance

Clostridium

The genus clostridium consists of G+ve , anaerobic, spore forming, spindle- rod shape and highly pleomorphic bacilli, spores are winder than bacillary bodies. The genus contains bacteria causing 3 major diseases of man, **Clostridium tetani cause tetanus**, ***Clostridium perfrigens*** **cause gas gangrene and *Clostridium Botulinum* cause food poisoning**. Some pathogens. E.g. ,*clostridium welchiiano* are found normally in human and animal intestine

Clostridia are motile with peritrichouse flagella, except, *C. welchii* and *C. tetani* type VI . and *C. butryricm* are capsulated while others are not so pathogenic Clostridia form powerful exotoxins. The gas gangrene clostridia are toxigenic and in revise causing even septicemia.

Clostridium perfringens

It is normal inhabitant of the large intestine of man and animals. It is found in feces and contaminates of skin

Morphology:

It is an plump G+ve bacillis with straight parallel sides, rounded or truncated ends about 4-6 μX1.

They may occur singly or in chains. It is pleomorphic- filaments and involution forms are common. It is capsulated a non-motile spores are central or sub terminal.

Antigenic structures:

C.perfrigens is differential into 6 types (A, b, c, d, e, f) on the basis of toxin produced by the strains. Toxins are antigenic and antitoxic sera are routine typing of strain.

Biochemical reaction:

Glucose, maltose, lactose and sucrose are fermented with production of acid and gas. In litmus milk they produce acid with gas. Milk is disrupted due to vigorous production of gas. This is called storm clot. Indole is negative and H_2S is formed abundantly.

Toxin:

C.perfrigens produces at least 12 distinct toxins besides many other enzymes and biological active soluble substances according to kind and amount of toxins produced. The most important toxin **(alpha or lecithianse toxin)** that destruction lecithin (phospholipid) material found in cytoplasmic membrane of the cell and cause **gas gangrene**, which is necrosis, putrefaction of tissues, and gas production due to this bacteria production H2S gas .The gases form bubbles in muscle (crepitus) and the characteristic smell in decomposing tissue. After rapid and destructive local spread (which can take hours), systemic spread of bacteria and bacterial toxins may result in death.

Clostridium tetani

Morphology:
It is slender-long, slightly curved, G+ve. Occurring singly or in chain. It shows considerable variation in length. It is non-capsulated and motile. Spores are spherical, terminal and bulging giving the bacilli **tennis rackets** or **drumstick** appearance. *C. tetani* is found as spores in soil or in the gastrointestinal tract of animals.*C. tetani* produces a tow biological toxin, **Tetanolysin** which causes **lysis of RBCs** and **tetanospasmin,** and is the causative agent of **tetanus** Tetanospasmin (Neurotoxin) released in the wound is absorbed into the circulation and reaches the ends of motor neurons all over the body. Toxin blocks inhibitory impulses, by interfering with the release of **neurotransmitters** so will be cause **Spastic paralysis** or cause disease know **lock Jaw**

Antigenic
Eleven strains of *C. tetani* have been identified, which differ primarily in flagella antigens and in their ability to produce tetanospasmin the genes for toxin production are encoded on a plasmid which is present in all toxigenic strains, and all strains that are capable of producing toxin produce identical toxins.

Immunity

Active Immunization:
Usually two injections 1 ml each of tetanus toxoid or the older (diphtheria, tetanus, pertussis) **(DPT)** vaccine is given intramuscularly at the interval of 6 weeks. Third in action is given after 6-12 months. A full course of immunization confers for 10 years.

Passive Immunization:
It is emergency procedure to be used only on. It is by giving anti tetanus serum (ATS). Intramuscularly as early as possible after wounding.

Neisseria

General character:
They are G-ve, aerobic, non-sporulation, non-motile, oxidase positive cocci arranged in pairs kidney-bean shape (diplococcic). Grown on Thayer-martin media or chocolate agar media. The genus contains about 30 species. Two important pathogens are:

a. *N. meningitidis* inhabits the human nasopharyns responsible for meningitis
b. *N. gonorrhea* normally colonizes mucosal surfaces. Humans are the only host and transmission is via sexual contact *responsible for the disease gonorrhea*

- There are four types of N. gonorrhea based on the presence of fimbriae; T1, T2, T3 and T4.
- *N. meningitidis* is serotyped by the antigenic character of its capsular polysaccharide; several groups including A,B,C, 29E, W-135 and Y are recognized

Biochemical reactions:

They are catalase and oxidase +ve. Glucose and maltose are fermented producing acid and no gas.

The virulence of Pathogenesis:

- Fimbriae (pili) are very important of the gonococcus to attach to host cells. *N. gonorrhoeae* lacking fimbriae are a virulent.
- A substance called Protein I makes up 66% of the outer membrane protein of *N. gonorrhoeae*. This protein is antigenic and is used as the basis of some serological tests.
- *N. gonorrhoeae* produce cytotoxic substances that damage ciliated epithelial cells in fallopian tubes; the LPS endotoxin may be partly responsible.
- *N. gonorrhoeae* also produce an extracellular protease that cleaves a proline- threonine bond in immunoglobulin IgA. This causes loss of antibody activity.
- The virulence of *N. meningitidis* is associated with its antiphagocytic capsule.
- The meningococcal LPS is as toxic as *Escherichia* or *Salmonella* and causes suppression of leukotriene B4 (a chemo kinetic/chemotactic factor) synthesis in PMNs.

Pathogenesis of *N. gonorrhea*:

Gonorrhea is usually acquired by sexual contact. Gonococci adhere to columnar epithelial cells, penetrate them, and multiply on the basement membrane. Adherence is facilitated through pili and opa proteins. Gonococcal lipopolysaccharide stimulates the production of tumor necrosis factor, which causes cell damage . **In male** it start as acute urethritis with mucous purulent discharge ,dysuria (painful urination) and Epididymitis . **In female** it star as Petechiae (small, purplish, hemorrhagic spots) , Pustules, Tenosynovitis (inflammation of tendon sheath) , Septic arthritis, Cervicitis; Vaginitis; Pelvic Inflammatory Disease (PID) and Disseminated Gonococcal Infection (DGI)

Pathogenesis of *N. Meningitidis* :

Neisseria Meningitidis is non-motile and is transferred among people via direct contact with bodily fluids in which the bacteria has inhabited

N. Meningitidis can be the cause of three major diseases. These three are **nasopharyngitis**, **meningococcal septicemia**, and **meningococcal**

meningitis. Nasopharyngitis is usually a very short illness and sometimes there aren't even any symptoms. If the *N. Meningitidis* bacteria colonize in the nasopharynx and spread into the blood stream, the disease becomes known as Meningococcal Septicemia. If the **bacteria** may enter the central nervous system causes meningitis.

Organisms are internalized into phagocytic vacuoles, avoid intracellular killing , Replicate intracellular and migrate to sub epithelial space where excess membrane fragments are released Hyper production of endotoxin (lipid A of LOS) into surrounding environment

most clinical manifestations including diffuse vascular damage (e.g., endothelial damage, vasculitis (inflammation of vessel walls), thrombosis (clotting), disseminated intravascular coagulation (DIC)

Diagnosis:

A. ***N. gonorrhea***

- microscopically in **purulent urethral discharge** sample
- **Susceptible to drying and cooling**, so **immediate culture** of specimen onto **pre-warmed selective** (e.g., modified Thayer-Martin, Martin-Lewis agars) **and non-selective media** (chocolate blood agar) with **moist atmosphere containing 5% carbon dioxide**
- **Some strains inhibited** by vancomycin (in many selective agars) and toxic substances like fatty acids and trace metals in protein hydrolysates and agar found in nonselective media

B. ***N. Meningitidis***

- **Microscopically cerebrospinal fluid (CSF)** sample
- Transparent, non-pigmented no hemolytic colonies on **chocolate blood agar** with **enhanced growth in moist atmosphere with 5% CO_2**
- Oxidase-positive
- Acid production from glucose and maltose but not from other sugars

Haemophilus

General characters:

They are small (cocobacilli) , pleomorphic, non-motile, Gram negative bacilli , Facultative anaerobic, Fastidious bacteria (mostly) , forming Satellite Phenomenon on blood agar , that are parasitic on man or animal belonging to the *Pasteurellaceae* family , Transmitted via respiratory droplets, or direct contact with contaminated secretions , Normal flora of the human respiratory tract and oral cavity , requires hemin (factor X) and NAD+ (factor V) Chocolate agar for growth species are:

H. influenzae

H. haemolyticus

H. parainfluenaze

H. ducreyi : found only in humans during disease (not normal microbial flora) sexually transmitted cause ulcerative genital infection (Chancroid) Syphilitic lesions

Clinical characteristics

H. influenzae

Major virulence factor is polyribitol phosphate capsule (PRP)

- enhance resistance to phagocytosis
- Serologic typing based on antigenic characteristics
- Six capsule types: a, b, c, d, e, or f
- Type b is the most commonly associated with
- Serious human infection
- **Infections are often systemic and life-threatening:**

  Meningitis, epiglottitis, cellulitis, Otitis media, Endocarditis with bacteremia, septic arthritis, pneumonia and Brazilian purpuric fever

  Also produce factors that promote attachment to human epithelial cells

***Heamophilus influenzae* is the most important pathogen and have been subdivided according to:**

1. Serotypes according to capsular antigens (a through f, the most important **type b**) non typable (if they lack a capsule)
2. Biotypes according to biochemical properties (**biotypes I** through VIII) according to indole production, urease activity, and ornithine decarboxylase activity

3. Bio groups according to clinical purposes (**bio group** *aegypticus* is the most important clinically)

Virulence and Pathogenesis:

- Transmitted via respiratory droplets, or direct contact with contaminated secretions
- Type b H influenzae colonizes the nasopharynx, and may penetrate the epithelium and capillary endothelium to cause bacteremia. Meningitis may result from direct spread via lymphatic drainage or from hematogenous spread
- Capsule contains polyribitol phosphate (resist phagocytosis and complement-mediated lyses)
- Lipopolysaccharide, low molecular weight glycopeptide in cell wall (impair ciliary function)
- Pili and nonpilus adhesions (maintain adhesion to mucosal membrane)
- Lipopolysaccharide lipid A (meningeal inflammation)
- produce neuraminidase and IgA proteases produced by bacteria play important role in invasion

Immunity:

- Active immunization with **Conjugated PRP Vaccine** prevent most ***H. influenza*** type b infection
- **Conjugated Vaccine:** PRP conjugated to protein carrier induces protective immunity (carriers may include: **diphtheria toxoid, tetanus toxoid or meningococcal OMP)**

Bordetella

Small, Gram-negative bacteria, aerobic cocco-bacilli. Highly fastidious, grown on **Bordet-genou agar**

Contains three medially important species human pathogens:

- *B. pertussis*
- *B. Para pertussis*

  Colonizes the respiratory tract causing Whooping Cough (Pertussis)
- *B. bronchoseptica* causing respiratory disease in human and kennel cough and atrophic rhinitis in dogs and pigs
- Others in mammals, and in birds

B. hinzii,

B. avium

Cause respiratory disease in poultry

Pathogenesis and Virulence factor:

- **Adhesions**

a. Filamentous hemagglutinin (FHA)
b. Pertactin
c. Fimbria

It attaches to cilia by adhere to galactose residues of the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract.

FHA can actually decrease phagocytosis affect cell mediated immunity by inhibiting cytokine responses

- **Toxins**

Pertussis toxin is unique important in colonization, the disruption of host cell signaling pathways, and immune evasion and also histamine release

Adenylate cyclase toxin is an invasive toxin that is also important in the disruption of signal transduction pathways; in addition it also plays a role inhibition of natural killer cell activity. Inhibition of host cell phagocytic cell it is damage tracheal cell local edema and damage (hemolysis)

Tracheal cytotoxic toxin (TCT) is a disaccharide tetra peptide that is derived from the cell wall this toxin has been observed to cause the killing and sloughing off of ciliated cells in the respiratory tract, all of which could lead to mucus accumulation and coughing.

The destruction of the ciliated epithelial cells in a *B. pertussis* infection is thought to be due to the production of nitric oxide by non-ciliated, mucus secreting and an increase in body temperature through the simulation of interlukin-1

Dermo necrotic toxin is a heat stable toxin that induces inflammation, vasoconstriction, and dermonecrotic lesions around colonies of B. pertussis in the respiratory tract this toxin also affects the regulation of cell growth and division systems Heat-labile toxin may also be involved in tissue damage during infection

Lipopolysaccharide associated with the surface of the bacteria and has potent endotoxin activity

it secretes toxins that lead to the death of these epithelium cells, a decrease in ciliary beating, and an accumulation of mucus and cell debris that triggers coughing

Vaccine

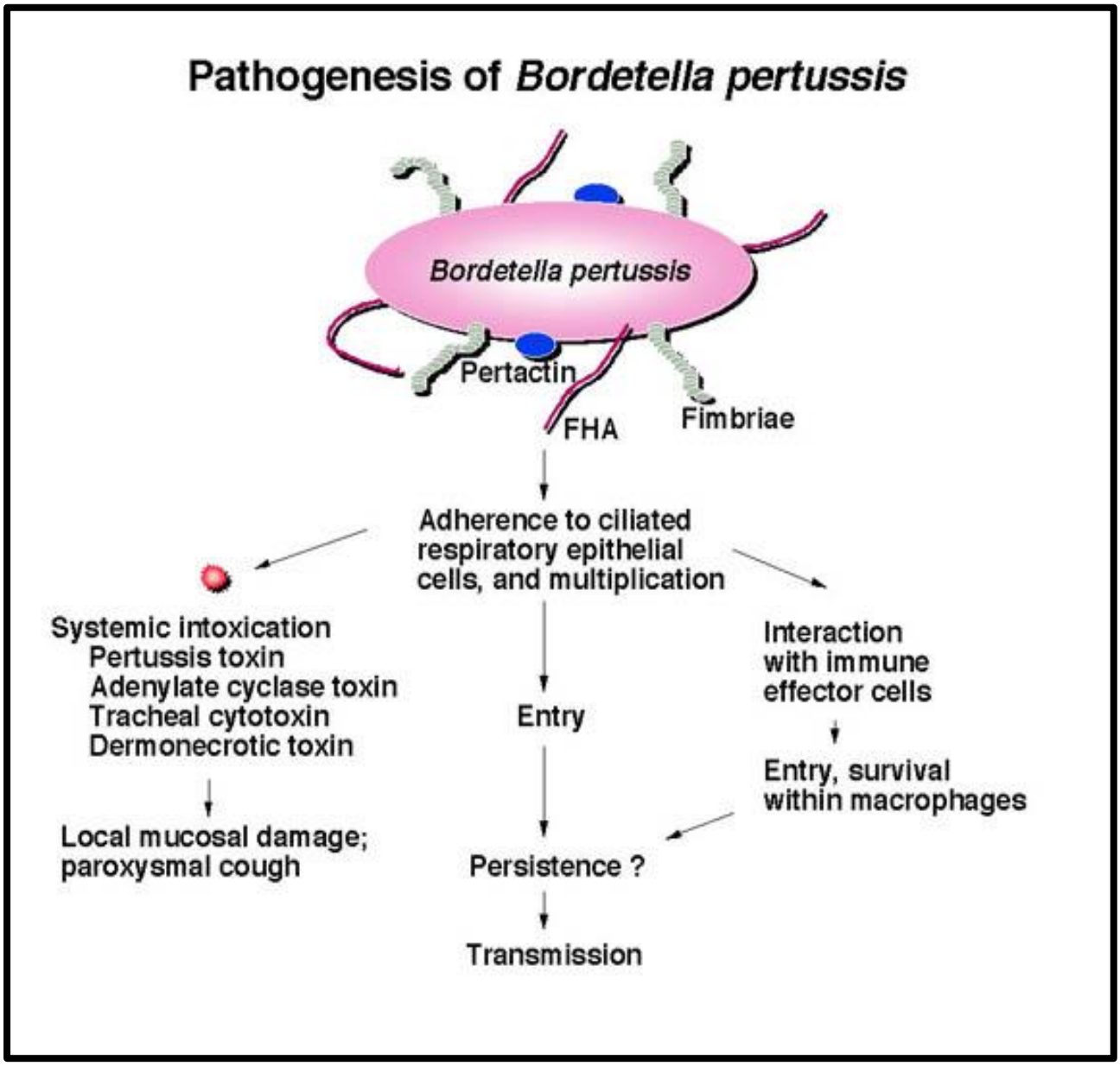
- killed bacterial cell suspension -DTaP vaccine (classic)
- Vaccine- induced immunity wanes after five to ten years
- a cellular vaccines
- Multicomponent a toxoids vaccines - DTP

Recommended Diphtheria and Tetanus Taxoids, and Pertussis Immunization Schedule for Children in the United States

Dose	Age (months)	Product*
1	2	DTaP
2	4	DTaP
3	6	DTaP
4	12 to 18	DTaP
5	4 to 6 years	DTaP

* DTaP (diphtheria and tetanus toxoids, and acellular pertussis vaccine) is the preferred vaccine for all doses in the immunization, including completion of the series in children who have received ≥1 dose of whole-cell DTP vaccine. Whole-cell DTP of whole-cell DTP-*Haemophilus* type b combinations are acceptable alternatives to DTaP. The fourth dose of DTaP may be administered as early as 12 months of age, provided at least 6 months have lapsed since the third dose and the child is considered likely to return at 15 to 18 months of age.

Table 1


Pathogenesis of *Bordetella pertussis*
Bordetella pertussis
Pertactin
FHA
Fimbriae
Adherence to ciliated respiratory epithelial cells, and multiplication
Systemic intoxication
Pertussis toxin
Adenylate cyclase toxin
Tracheal cytotoxin
Dermonecrotic toxin
Local mucosal damage; paroxysmal cough
Entry
Persistence ?
Transmission
Interaction with immune effector cells
Entry, survival within macrophages

Enterobacteriaceae

General character:

They are G-ve rods, All motile with peritrichate flagella except *Shigella* and *Klebsiella* , non-spore forming, non-acid fast, All ferment sugar with or without formation of gas Inoculation on MacConkey'sor Eosin-Methylene Blue (EMB)agar differentiates family members by lactose fermenting ability, All reduce nitrates into nitrites form, catalase positive, All are oxidase negative, facultative anaerobes, normal part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites Enterobacteriaceae members is

- *Escherichia, Salmonella, Shigella, Klebsiella, Proteus, Enterobacter, Yersinia*, etc.

Virulence and Antigenic Factors of Enterobacteriaceae

- Ability to colonize, adhere, produce various toxins and invade tissues
- Some possess plasmids that may mediate resistance to antibiotics
- Many enterics possess antigens that can be used to identify groups
- O antigen – somatic, heat-stable antigen located in the cell wall
- H antigen – flagellar, heat labile antigen
- K antigen – capsular, heat-labile antigen K antigen is called the **Vi** (virulence) antigen in *Salmonella typhi*.

Taxonomy of *Enterobacteriaceae*:

- Differentiation is based on biochemical reactions and differences in antigenic structure
- Over 30 genera and 120 species
- More than 95% of clinically significant strains fall into 10 genera and less than 25 species

Clinical Manifestations

A. Some members of the *Enterobacteriaceae* are **true pathogens**

- *Salmonella* spp.
- *Shigella* spp.
- *Yersinia* spp.
- Certain strains of *Escherichia coli*

B. Most members of the *Enterobacteriaceae* are **opportunistic** or cause secondary infections of wounds, the urinary and respiratory tracts, and the circulatory system

Types of Infectious Disease

- **Intestinal (diarrheal) infection**
- **Extra intestinal infection**

  Urinary tract infection (primarily cystitis and prostatitis and pyelonephritis)
  Respiratory (nosocomial pneumonia)
  Wound (surgical wound infection)
  Bloodstream (gram-negative bacteremia)
  Central nervous system (neonatal meningitis)
  Spontaneous bacterial peritonitis (Usually in patients with liver ailments)
  Endocarditis (Vascular endocardia surface inflammation)

Escherichia coli

It lives in human or animal intestine. Detection of *E. coli* in drinking water is taken as evidence of recent pollution with human or animal excreta. Wide range of infection including meningitis, , urinary tract, wound, bacteremia and **Gastrointestinal Infections include**:

- **Enteropathogenic (EPEC)** – Diarrhea with large amounts of mucous without blood or pus occurs along with vomiting, malaise and low grade fever.
- **Enterotoxigenic (ETEC)** – "traveler's diarrhea"; watery diarrhea without blood
- **Enteroinvasive (EIEC)** – produce dysentery with bowel penetration types produce disease resembling shigellosis in adults and children
- **Enterohemorrhagic (EHEC) serotype 0157:H7 they have Shiga-like toxins and also called STEC (shigella toxin E.coli)** – associated with hemorrhagic diarrhea and hemolytic-uremic syndrome (HUS)
- **Enteroaggregative (EaggEC)** – cause diarrhea by adhering to the mucosal surface of the intestine; watery diarrhea

Biochemical reaction:

It ferment lactose, glucose, sucrose, maltose and mannital with acid and gas. Indol and methyl red is positive (v-p) and citrate is negative. Urease is not hydrolysed. H_2S is not produce.

- **Virulence factors**

- **Toxins**
- **Enterotoxins** – produced by enterotoxigenic strains of ***E. coli*** **(ETEC)**. Causes a movement of water and ions from the tissues to the bowel resulting in **watery diarrhea**. There are two types of enterotoxin:

LT – is heat labile LT is composed of two types of subunits. One type of subunit (the B subunit) binds the toxin to the target cells via a specific receptor that has been identified as Gm1 ganglioside. The other type of subunit (the A subunit) is then activated by cleavage of a peptide bond and internalized. It then catalyzes the ADP-ribosylation (transfer of ADP-ribose from nicotinamide adenine dinucleotide [NAD]) of a regulatory subunit of membrane-bound adenylate cyclase, the enzyme that converts ATP to cAMP. This activates the adenylate cyclase, which produces excess intracellular cAMP, which leads to hypersecretion of water and electrolytes into the bowel lumen.

- Increased cAMP alters the activity of sodium and chloride transporters producing an ion imbalance that results in fluid transport into the bowel.
- **ST – is heat stable** and binds to specific receptors to stimulate the production of cGMP with the same results as with LT . Those termed STa can stimulate intestinal guanylate cyclase, the enzyme that converts guanosine 5′-triphosphate (GTP) to cyclic guanosine 5′-monophosphate (cGMP). Increased intracellular cGMP inhibits intestinal fluid uptake, resulting in net fluid secretion.
- Both enterotoxins are composed of five beta subunits (for binding) and 1 alpha subunit (has the toxic enzymatic activity).

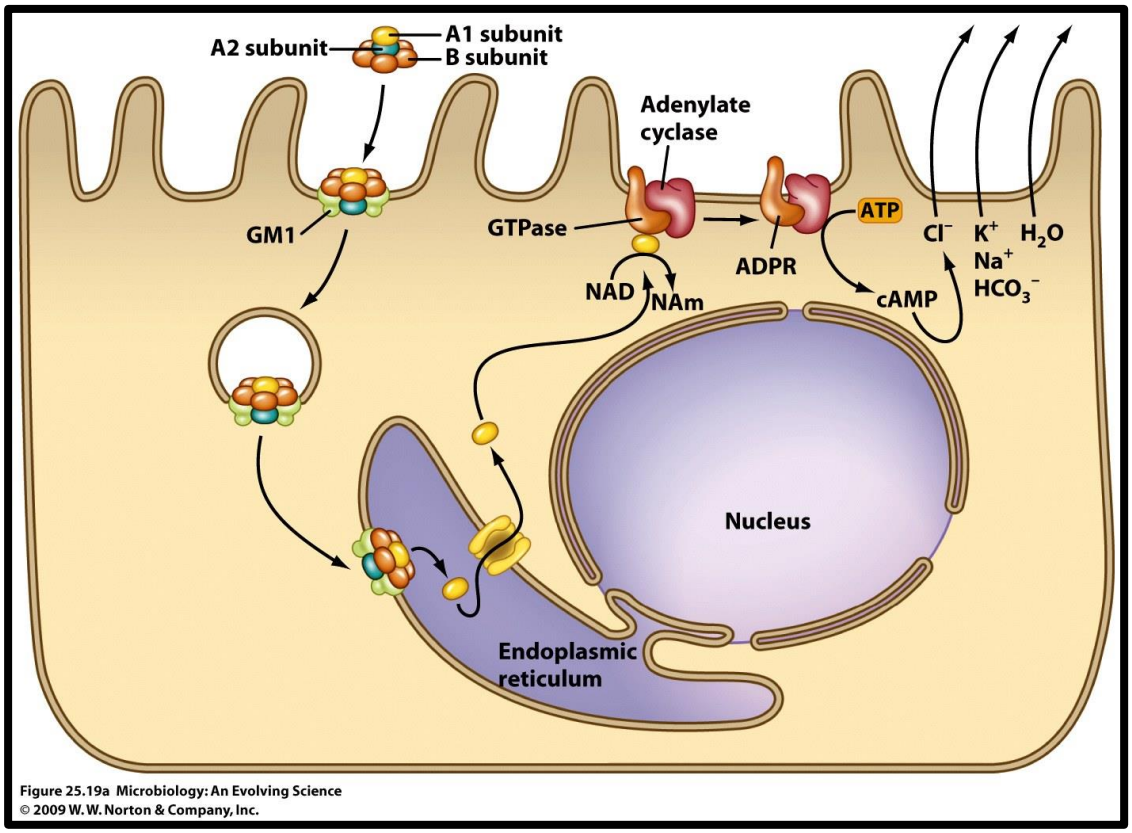
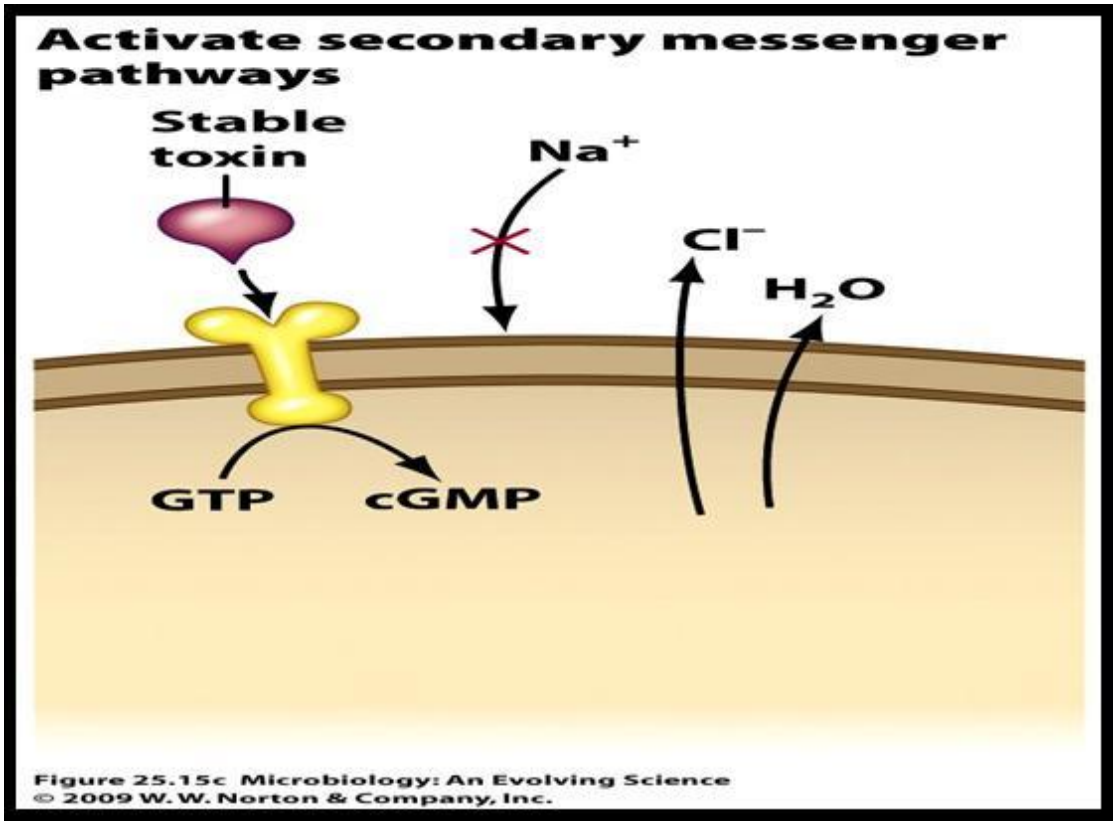


Figure 25.19a Microbiology: An Evolving Science



Klebsiella

They are found in the mucosa of upper respiratory tract intestine and genitourinary tract. They are non-motile, capsulated growing on ordinary media forming large mucous colonies of varying degree of stickiness. They have been classified into 3 species on the bases of biochemical reaction.

K. pneumoniae (ferments sugar)

K. ozaenae causes foul smelling nasal discharge.

K. rhinoscleromates causes rhinoscleroma

Biochemical reaction:

They form acid and gas from glucose (except proteus retigeri). They are indol and MR- ve and VP +ve. They are lactose fermenter. H_2S product. Citrate is +ve.

- **Virulence factors**
- Capsule
- Adhesions
- Iron capturing ability

Clinical significance

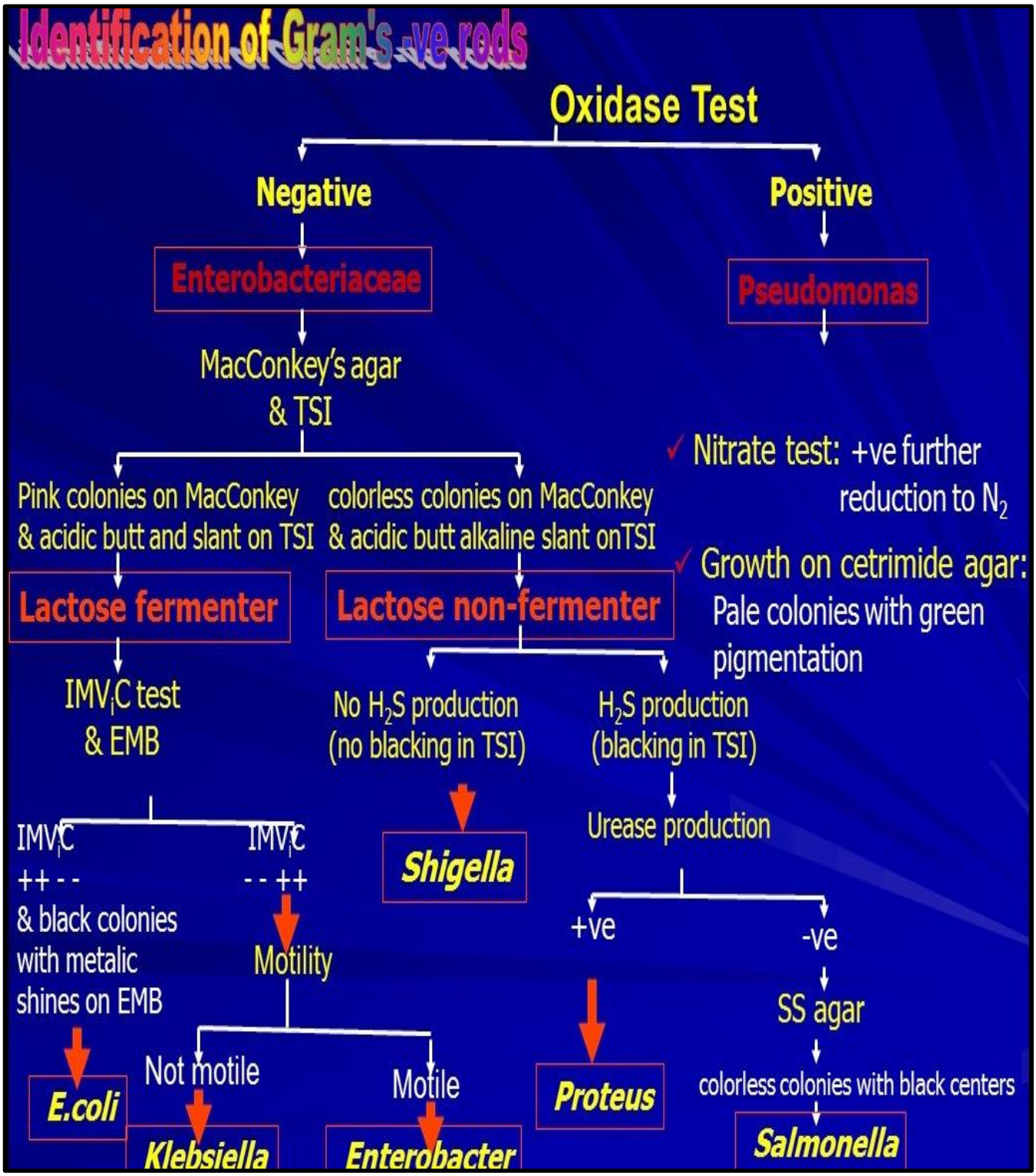
- Causes pneumonia, mostly in immunocompromised hosts.

- Permanent lung damage is a frequent occurrence (rare in other types of bacterial pneumonia)
- A major cause of nosocomial infections such as septicemia ,UTI and meningitis

	TSI	**Indole**	**MR**	**VP**	**Citrate**	**Urease**	**Motility**
E. coli	A/A/-	+ve	+ve	-ve	-ve	-ve	Motile
Citrobacter freundii	A/A/-	+ve	+ve	-ve	+ve	-ve	Motile
Klebsiella pneumoniae	A/A/-	-ve	-ve	+ve	+ve	+ve	Non motile

Enterobacter cloacae	A/A/-	-ve	-ve	+ve	+ve	+ve	Motile
Salmonella typhi	A/Alk/+	-ve	+ve	-ve	+ve	-ve	Motile
Shigella boydii	A/Alk/-	-ve	+ve	-ve	-ve	-ve	Non motile
Proteus mirabilis	A/Alk/+	-ve	+ve	-ve	+ve	+ve	Motile Swarwing

Summary of morphology, cultural characteristics, and biochemical reactions of *Enterobacteriaceae*


Identification of Gram's -ve rods
Oxidase Test
Negative
Positive
Enterobacteriaceae
Pseudomonas
MacConkey's agar & TSI
Nitrate test: +ve further reduction to N2
Growth on cetrimide agar: Pale colonies with green pigmentation
Pink colonies on MacConkey & acidic butt and slant on TSI
colorless colonies on MacConkey & acidic butt alkaline slant onTSI
Lactose fermenter
Lactose non-fermenter
IMViC test & EMB
No H2S production (no blacking in TSI)
H2S production (blacking in TSI)
IMViC ++ - - & black colonies with metalic shines on EMB
IMViC - - ++
Shigella
Urease production
+ve
-ve
Motility
SS agar
Not motile
Motile
colorless colonies with black centers
E.coli
Klebsiella
Enterobacter
Proteus
Salmonella

Genus *proteus*

General characters:

The *proteus* organisms are G-ve. Motile, aerobic. Most species are free-living in water, soil , sewage and All are normal intestinal flora. Non lactose fermenter, non-capsulated, non-spore forming. Pleomorphic , **It has a characteristic "swarming" pattern** (growth as ring revolved form similar to waves connection from center) and is an opportunistic pathogen of humans It is known to cause urinary tract infections and wound infections. The species are:

1- P. vulgarris
2- P. mirabilis
3- P. rettgeri
4- P. morganii

Clinical samples:

1- Urine
2- Stool
3- Sputum
4- Pus

Biochemical test

Proteus species do not usually non ferment lactose, but have shown to be capable lactose fermenters depending on the species in a triple sugar iron (TSI) test. It is oxidase-negative, but catalase- and nitrase-positive. Specific tests include urease positive and Only Enterobacteriaceae that makes phenylalanine deaminase tests. Produce powerful **urease**, which rapidly hydrolyzes urea to ammonia and carbon monoxide and the follow show the differentiation between coliform bacilli and proteus

TABLE 26-4 Differentiation of Coliform Bacilli and *Proteus* Found in Human Clinical Specimens

Organism	Motility	Lactose	Indole	Urease	H_2S	Other
Escherichia						
E coli	+	+	+	—	—	
Klebsiella						
K pneumoniae	—	+	—	+	—	large mucoid colonies
K oxytoca	—	+	+	+	—	large mucoid colonies
Enterobacter						
E aerogenes	+	+	—	—	—	some strains mucoid, LD+, AD—
E cloacae	+	+	—	d	—	LD—, AD+
E sakazakii	+	+	[—]	—	—	yellow pigment, LD—, AD+
E gergoviae	+	d	—	+	—	LD+, AD—
Pantoea						
P agglomerans	+	d	[—]	[—]	—	some strains yellow pigment, LD—, AD—
Serratia						
S marcescens [a]	+	—	—	—	—	some strains red pigment
S rubideae [b]	+	+	—	—	—	red pigment
Citrobacter						
C freundii	+	d	—	d	+	
C koseri [c]	+	d	+	d	—	
Proteus						
P mirabilis	+	—	—	+	+	"swarming" motility
P vulgaris	+	—	+	+	+	"swarming" motility

+ (≥ 90% strains positive), d (26-75% strains positive), [—] (11-25% strains positive), — (0-10% strains positive)
LD (lysine decarboxylase), AD (arginine dihydrolase)
[a] *S liquifaciens* group and *S ficaria* have same five reactions shown.
[b] *S fonticola, S odorifera,* and *S plymuthica* have same five reactions shown.
[c] *C amalonaticus* and other *Citrobacter* have same five reactions shown.

Pathogenesis and Virulence

Adherence factors

- **Fimbriae**- facilitate adherence and thus enhance the capacity of the organism to produce disease
- Proteus bacilli possess **somatic O and flagellar H antigens**

Inflammatory response

This rod shaped bacterium has the ability to produce high levels of urease. Urease hydrolyzes urea to ammonia (NH_3) and thus makes the urine more alkaline. If left untreated, the increased alkalinity can lead to the formation of crystals of struvite, calcium carbonate, and/or apatite. The bacteria can be found throughout the stones, and these bacteria lurking in the kidney stones can reinitiate infection after antibiotic treatment. Once the stones develop, over time they may grow large enough to cause obstruction and renal failure.

Symptoms

The first sign that you have a Proteus mirabilis urinary tract infection will often be in your urine. You may notice that your **urine is suddenly coming out darker or smellier than before. In some cases, you will even see blood in your urine**. Another sign of a urinary tract infection is the **increased urge to urinate, even if little or no urine comes out**. **You should also not be surprised if you feel a burning sensation when you do urinate**. It is common with most types of urinary tract infections.

Pathogenicity:

1- UT1
2- Pyogenic lesions like abscess, infection of wound, ear, and respiratory tract bedsores.
3- Infantile diarrhea by *P. morganii*

Treatments

Known antibiotics that *P. vulgaris* is sensitive to:
Ciprofloxacin
Ceftazidime
Netilmicin
Sulbactam or Cefoperazo
Meropenem
Piperacillin /tazobactam
Antibiotics should be introduced in much higher doses than "normal" when *P. vulgaris* has infected the sinus or respiratory tissues

Salmonella and Shigella

General characteristic:

Salmonella and *shigella* rod form, gram negative, non-spore forming facultative aerobic, catalase positive and oxidase negative, do not ferment lactose but which do ferment other carbohydrates, producing acid but not gas and *shigella* non motile but salmonella motile ,Antigenic structures is Somatic O and H (flagella) antigens and Vi antigen which is a capsular antigen but *shiegella* not have H antigen .they grown on S-S agar because this media contain bile salts and sodium citrate that inhibition the growth of gram positive bacteria and the others coliform bacteria and contain sodium thiosulfate and iron citrate for detection on H2S production so the salmonella colonies appeared with black precipitate because they positive and shigella without precipitation also contain reagent neutral red appeared red in acidic environment and blue in basic environment so the salmonella and shigella appeared blue colonies because they not fermentation lactose

Salmonella:

Salmonella typhi causes **typhoid fever**

S. paratyphi is caused **paratyphoid fever**

S. enteritidis caused **food poisoning**

S. choleraesuis, from swine, can cause severe blood poisoning in humans

S. gallinarum causes fowl typhoid

Salmonella as disease-causing agents

Salmonella infections are zoonotic and can be transferred between humans and non-human animals. Many infections are due to ingestion of contaminated food. A distinction is made between enteritis *Salmonella* and horny/paratyphoid *Salmonella*, where the latter- because of a special virulence factor and a capsule protein (virulence antigen) – can cause serious illness, such as *Salmonella enterica subsp*. *Enterica* server Typhi. *Salmonella* typhi. Is adapted to humans and does not occur in other animals.

Salmonella species are facultative intracellular pathogens that enter cells via macropinosomes.

Sources of infection

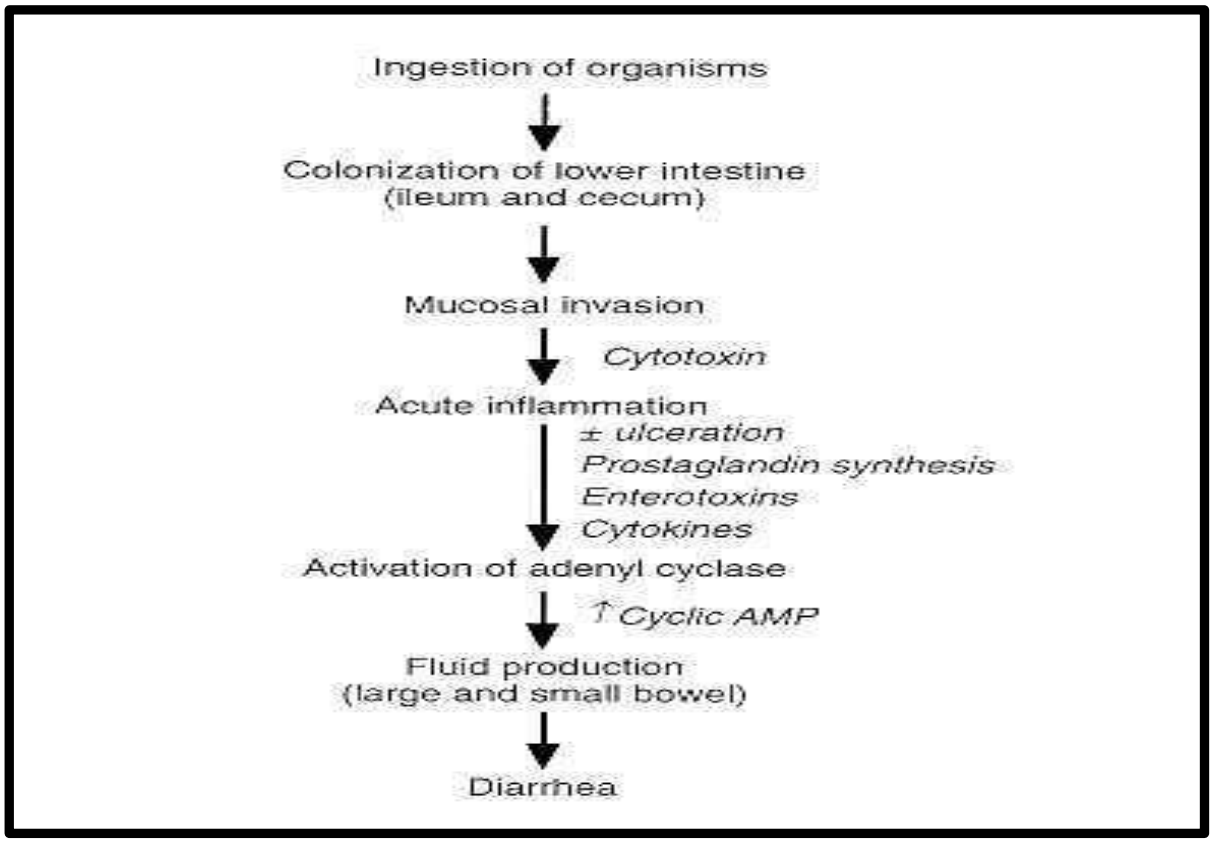
- Infected food, often gaining an unusual look or smell, then is introduced into the stream of commerce.
- Poor kitchen hygiene, especially problematic in institutional kitchens and restaurants because this can lead to a significant outbreak.
- Excretions from either sick or infected but apparently clinically healthy people or animals (especially endangered are caregivers and animals).
- Polluted surface water and standing water (such as in shower hoses or unused water dispensers).
- Unhygienically thawed fowl (the melt water contains many bacteria).
- An association with reptiles (pet tortoises, snakes, iguanas and frogs, but primarily aquatic turtles) is well described.
- *Salmonella* bacteria can survive several weeks in a dry environment and several months in water; thus, they are frequently found in polluted water, contamination from the excrement of carrier animals being particularly important. Aquatic vertebrates, notably birds and reptiles, are important vectors of *Salmonella*. Poultry, cattle, and sheep frequently being agents of contamination, *Salmonella* can be found in food, especially in milk, meats and sometimes in eggs which have cracks.

Virulence factors

- Endotoxin – may play a role in intracellular survival
- Capsule (for *S. typhi* and some strains of S. *paratyphi*)
- Adhesions – both fimbrial and non-fimbrial
- Type III secretion systems (**TTSS** or **T3SS**) also called **Injectisome** or **Injectosome**) is a protein appendage found in several Gram-negative bacteria. In pathogenic bacteria, the needle-like structure is used as a sensory probe to detect the presence of eukaryotic organisms and secrete proteins that help the bacteria infect them. The proteins are secreted directly from the bacterial cell into the eukaryotic cell, also known as "the host" cell and effector molecules – 2 different systems may be found:
- One type is involved in promoting entry into intestinal epithelial cells
- The other type is involved in the ability of *Salmonella* to survive inside macrophages so they can growth and replicated

Pathogenesis

Pathogenic salmonellae ingested in food survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. Invasion of epithelial cells stimulates the release of pro inflammatory cytokines which induce an inflammatory reaction. The acute inflammatory response causes diarrhea and may lead to ulceration and destruction of the mucosa. The **bacteria** can disseminate from the intestines to cause systemic disease. Three clinical forms recognize of salmonellosis: (1) gastroenteritis, (2) septicemia, and (3) enteric fevers.



Scheme of the Pathogenesis of *Salmonella* enter colitis and diarrhea

Food poisoning

Salmonella food poisoning is spread from the feces of infected people or animals through food or beverages. Common foods contaminated with *Salmonella* bacteria include undercooked eggs and poultry. *Salmonella* food poisoning results in irritation and inflammation of the digestive tract, which leads to symptoms such as abdominal pain, nausea, fever ,diarrhea and acute – gastroenteritis .

Immunity

1. (T.A.B) vaccine which contain (1000) million *salmonella typhi* , (500) million *salmonella paratyphi A* and *salmonella paratyphi B*
2. VICPS virulence antigen polysaccharides capsular vaccine

Shigella

All species cause bacillary dysentery Humans are only known reservoir

- *S. dysenteriae* (Group A)
- *S. flexneri* (Group B)
- *S. boydii* (Group C)
- *S. sonnei* (Group D)

Virulence factors

- Shiga toxin – is produced by ***S. dysenteriae*** and in smaller amounts by *S. flexneri* and *S. sonnei.*
- Acts to inhibit protein synthesis by inactivating the 60S ribosomal subunit by cleaving a glycosidic bond in the 28S rRNA constituents.
- This plays a role in the ulceration of the intestinal mucosa, it have three activates **neurotoxic , cytotoxic , enterotoxin**

Pathogenicity

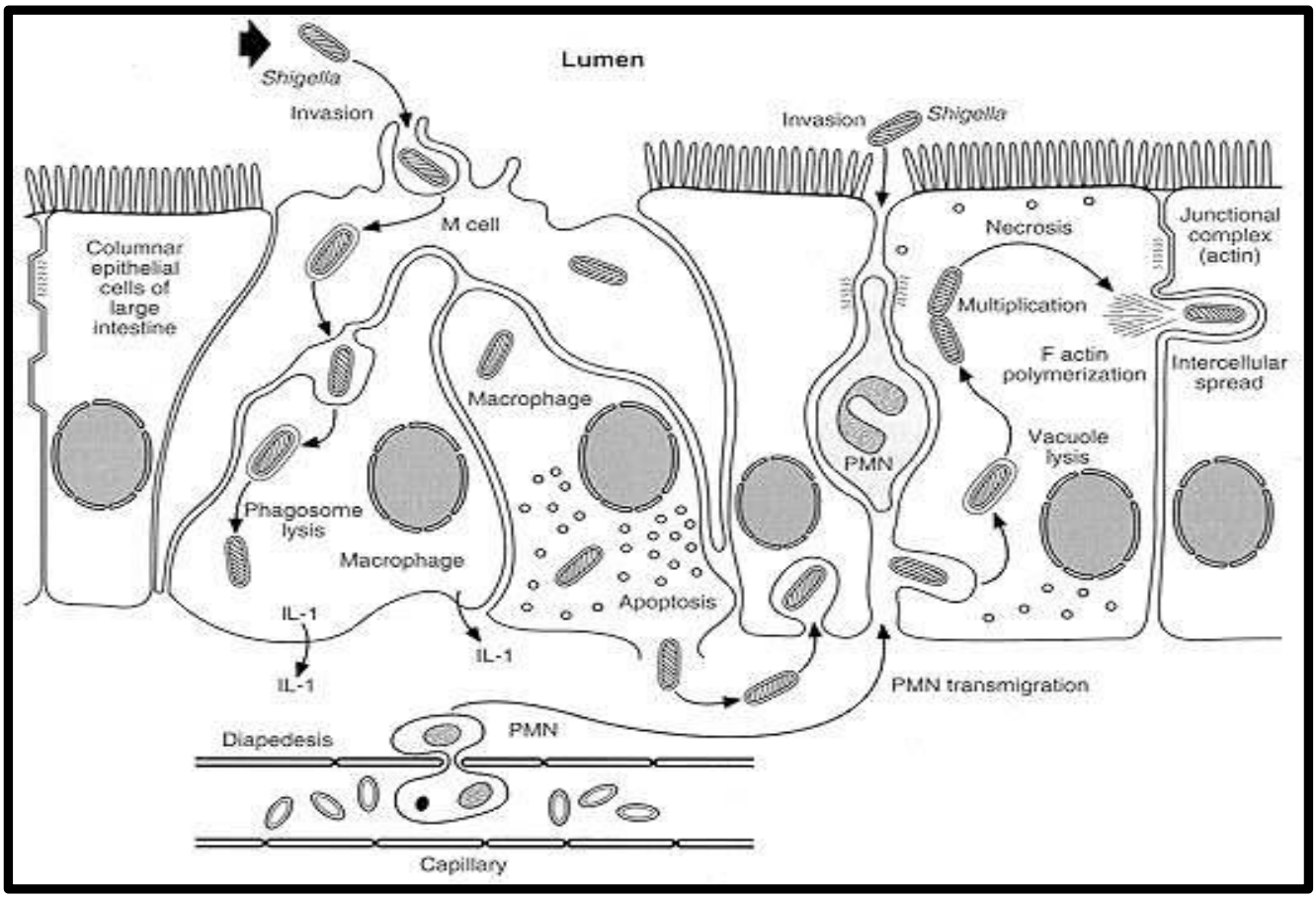
Also known as **Shigellosis**, **bacillary dysentery** or **Marlow Syndrome**, in its most severe manifestation, is a foodborne illness caused by infection by bacteria of the genus *Shigella*.

The organisms are initially ingested by membranous (M) cells that are associated with lymphoid micro follicles in the colon. After transcytosis through the M cell, the bacteria are deposited into the subepithelial space where they are phagocytosed by macrophages. The macrophage phagosome is subsequently degraded, and the intracellular shigellae cause release of IL-1 that evokes an influx of polymorphonuclear leukocytes (PMN). Eventually the infected macrophages undergo apoptosis (programmed cell death), and the bacteria are released onto the basolateral surface of adjacent colonic enterocytes. In addition, PMN transmigration through the epithelium disrupts tight junctions, allowing shigellae to migrate into the subepithelial space. The bacteria infect enterocytes by induced endocytosis, and the endocytic vacuoles are

subsequently degraded. The intercellular shigellae attach to actin in the enterocyte junctional complex, multiply, and spread to contiguous enterocytes by induced actin polymerization. Ultimately, the infected enterocytes die, and the resulting necrosis of the epithelium, in conjunction with the continuing inflammatory response, constitutes the lesions of shigellosis. Shigellosis has two basic clinical presentations**: (1) watery diarrhea associated with vomiting and mild to moderate dehydration, and (2) dysentery characterized by a small volume of bloody, mucous stools, and abdominal pain**

Treatment

Sulfonamides, ampicillin, tetracyclines and chloramphenicol.



Histopathology of acute colitis following peroral infection with *shigellae*

Genus: pseudomonas

General character:

Gram-negative, One or more polar flagella providing Rod shaped motility, slim layer (capsulated) , **grape like odor**, **Aerobic (non glucose fermenter and not reduce H2S) positive catalase test, positive oxidase test**.. Non–spore formingThey are mostly saprophytes being found in water, soil and Opportunistic Pathogens of Plants, Animals, and Humans These include *P aeruginosa*, *P putida*, *P cepacia*, *P stutzeri*, *P maltophilia*, and *P putrefaciens*. The two species, *P mallei* and *P pseudomallei*, produce specific human diseases: glanders and melioidosis. and they have Exotoxin like *C.diptheria* (ADP- ribosylate)

- **Production of pigments:** under iron-limiting conditions. *Pseudomonas* species may also produce additional types of siderophore pigment

– **Soluble blue**-coloured phenazine pigment called (**pyocyanin**)
– Some strains produce **red or black** colonies due to pigments termed **pyorubin** and **pyomelanin**, respectively
– *P. aerugnosa* produces **pyoverdin** (diffusible **yellow-green to yellow-brown pigm**
– **ent**) which, when produced with pyocyanin gives rise to green-blue colonies on solid media

Toxins

P. aeruginosa uses the virulence factor exotoxin A to ADP – ribosylate eukaryotic elongation factor 2 in the host cell, mush as the diphtheria toxin does. Without elongation factor 2, eukaryotic cells cannot synthesize proteins and necrosis. The release of intracellular contents induces an immunologic response in immunocompetent patients. In addition *P. aeruginosa* uses an exoenzyme, ExoU, which degrades the plasma membrane of eukaryotic cells, leading to lysis.

Pathogenesis

Pseudomonas causes nosocomial **infection burns contamination and chronic ulcers of skin**. It has been responsible for **infantile diarrhea**. **UTI** may persist for longer time may give rise to **septicemia lesion of**

eye, **otitis media**, **pulmonary empyema (Cystic fibrosis)**, **brain abscess and meningitis may occur**

Nosocomial distribution

Pseudomonas **biofilms**. A significant number of cells can produce exopolysaccharides known as biofilms. Secretion of exopolysaccharide such as alginate, makes it difficult for pseudomonads to be phagocytosed by mammalian white blood cells. Exopolysaccharide production also contributes to surface-colonising biofilms which are difficult to remove from food preparation surfaces. Growth of pseudomonads on spoiling foods can generate a "fruity" odor.

***Pseudomonas* has the ability to metabolize a variety of diverse nutrients**. Combined with the ability to form biofilms, **they are thus able to survive in a variety of unexpected places**. For example, they have been found in areas where pharmaceuticals are prepared. A simple carbon source, such as soap residue or cap liner-adhesives is a suitable place for them to thrive. **Other has the ability to lives on antiseptics**, such as quaternary ammonium compounds, and bottled mineral water. And also have the ability for **Antibiotic resistance.** For these causes and anther make the *Pseudomonas* flourishes in hospital environments

Hospital infections

Hospital infections	Details and common associations	High-risk groups
Pneumonia	Diffuse bronchopneumonia	Cystic fibrosis patients
Septic shock	Associated with skin lesion ecthyma gangerenosum	Neutropenia patients
Urinary tract infection	Urinary tract catheterization	
Gastrointestinal infection	Necrotizing enterocolitis (NEC)	NEC, especially in premature infants and neutropenic cancer patients
Skin and soft tissue infections	Hemorrhage and necrosis	Burns victims and patients with wound infections

Antibiotic resistance

Being Gram-negative bacteria, most *Pseudomonas spp.* are naturally resistant to penicillin and the majority of related beta-lactam antibiotics,

but a number are sensitive to piperacillin, imipenem, ticarcillin, tobramycin, or ciprofloxacin.
This ability to thrive in harsh conditions is a result of their hardy cell wall that contains **porins** (are beta barrel proteins that cross a cellular membrane and act as a pore through which molecules can diffuse. Unlike other membrane transport proteins, porins are large enough to allow passive diffusion) . **Their resistance to most antibiotics is attributed to efflux pumps**, which pump out some antibiotics before the antibiotics are able to act.
Pseudomonas aeruginosa is a highly relevant opportunistic human pathogen. One of the most worrying characteristics of *P. aeruginosa* is its **low antibiotic susceptibility**. This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes (e.g. *mexAB-oprM*, *mexXY*,) and **the low permeability of the bacterial cellular envelopes**. Besides intrinsic resistance, ***P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally-encoded genes**, or by the **horizontal gene transfer of antibiotic resistance determinants**. Development of multidrug resistance by *P. aeruginosa* isolates requires several different genetic events that include **acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes**. Hyper mutation favours the selection of mutation-driven antibiotic resistance in *P. aeruginosa* Some recent studies have shown phenotypic resistance associated to **biofilm formation** or to the emergence of small-colony-variants may be important in the response of *P. aeruginosa* populations to antibiotic treatment .and also the resistance come from **production enzyme or adding some chemical group that able to destroyed or alter the structure of the antibiotics so become an active or by alter the target site by mutation**

Genus: Vibrio

General character:

Gram-negative bacteria possessing a curved rod shape (comma shape), several species of which can cause foodborne infection, usually associated with eating undercooked seafood. Typically found in saltwater, *Vibrio* spp. are facultative anaerobes that positive for oxidase test and do not form spores. All members of the genus are motile and have polar flagella with sheaths, growing on alkaline media but not acidic like **TCBS (Thiosulfate Citrate Bile salts Sucrose) agar** and they have (H

flagella with O somatic antigen) . The most species Associated with Human Disease include :

V. cholerae (Rice water stool of cholera) like enterotoxic E.coli

V. parahaemolyticus

V. vulnificus.

***Vibrio spp.* (Family Vibrionaceae) Associated with Human Disease**

Species	Source of Infection	Clinical Disease
V. cholerae	Water, food	Gastroenteritis
V. parahaemolyticus	Shellfish, seawater	Gastroenteritis, wound infection, bacteremia
V. vulnificus	Shellfish, seawater	Bacteremia, wound infection, cellulitis
V. alginolyticus	Seawater	Wound infection, external otitis
V. hollisae	Shellfish	Gastroenteritis, wound infection, bacteremia
V. fluvialis	Seafood	Gastroenteritis, wound infection, bacteremia
V. damsela	Seawater	Wound infection
V. metschnikovii	Unknown	Bacteremia
V. mimicus	Fresh water	Gastroenteritis, wound infection, bacteremia
*V. furnissii**	Seawater	Gastroenteritis
*V. cincinnatiensis**	Unknown	Bacteremia, meningitis
*V. carchariae**	Seawater	Wound (shark bite)

Pathogenesis:-

Cholera is transmitted by the fecal-oral route. Vibrios are sensitive to acid, and most die in the stomach. Surviving virulent organisms may adhere to and colonize the small bowel, where they secrete the potent cholera enterotoxin (CT, also called "choleragen") (like ETEC). This toxin binds to the plasma membrane of intestinal epithelial cells and releases an enzymatically active subunit that causes a rise in cyclic adenosine 5^1-monophosphate (cAMP) production. The resulting high intracellular cAMP level causes massive secretion of electrolytes and water into the intestinal lumen. cholera is **watery diarrhea As more fluid is lost, stool changes to rice-water stools , Odor similar to fish odor and Speckled with mucus . Cholera toxin leads to profuse loss of fluids and electrolytes (sodium, potassium, bicarbonate) Hypokalemia (low levels of K in blood) ,increase in protein in the**

blood and decrease in urine and cause Cardiac arrhythmia and renal failure . Death attributable to Hypovolemic shock (due to abnormally low volume of circulating fluid (plasma) in the body) and Metabolic acidosis (pH shifts toward acid side due to loss of bicarbonate buffering capacity)

Diversity and evolution

Two serogroups of *V. cholerae*, **O1 and O139, cause outbreaks of cholera**. O1 causes the majority of outbreaks, while O139 – first identified in Bangladesh in 1992 – is confined to South-East Asia identified by 1) absence of agglutination in O group 1 specific antiserum; 2) by agglutination in O group 139 specific antiserum; and 3) by the presence of a capsule.

Many other serogroups of *V. cholerae*, with or without the cholera toxin gene (including the nontoxigenic strains of the O1 and O139 serogroups), can cause a cholera-like illness. Only toxigenic strains of serogroups O1 and O139 have caused widespread epidemics.

V. cholerae O1 has 2 biotypes, classical and El Tor, and each biotype has 2 distinct serotypes, Inaba and Ogawa. The symptoms of infection are indistinguishable, These organisms may be identified by agglutination in O group 1-specific antiserum directed against the lipopolysaccharide component of the cell wall and by demonstration of their enterotoxigenicity have only a mild illness. In recent years, infections with the classical biotype of *V. cholerae* O1 have become rare and are limited to parts of Bangladesh and India. Recently, new variant strains have been detected in several parts of Asia and Africa. Observations suggest that these strains cause more severe cholera with higher case fatality rates.

Prevention & Control

- Immunization

– Active Immunity induced by:

- attenuated *V. cholerae*
- Toxoid (not good antigen)

A. Oral

Sodium chloride (3.5 g/L)
+ Potassium chloride (1.5 g/L)
+ Rice flour (30-80g/L)

+ Trisodium citrate (2.9 g/L)

B.Intravenous (IV)

- Doxycycline or tetracycline (Tet resistance may be developing) of secondary value
- Preventing contamination of food and water e.g. boiling water, covering food
- Education

– Personal and domestic hygiene

Prevention of contamination of water supplies

Brucella and Yersinia

General characters:

—*Brucella spp* are small gram-negative aerobic coccobacilli lacking a capsule, flagella, endospores, or native plasmids. *Brucella* strains always catalase-positive; but oxidase and urease and H_2S production vary, some spp. require supplemental carbon dioxide for primary isolation. They are cause brucellosis which is zoonosis (transmitted from animal to man).**facultative intracellular pathogen**

Classification:

Three species of *brucella* medically important

1- *Brucella melitensis* is pathogen of goat and sheep first of all it was isolated from spleen of patient.
2- *Brucella abortus*. Is responsible for abortion in cows and buffaloes. It's infection is very common.
3- *Brucella suis* is natural parasite of pigs. Its infection is very infrequent.

Brucellosis in humans

Brucellosis in humans is usually associated with the consumption of unpasteurized milk and soft cheeses made from the milk of infected animals, primarily goats, infected with *Brucella melitensis* and with occupational exposure of laboratory workers, veterinarians, and slaughterhouse workers. Brucellosis induces inconstant fevers, sweating, weakness, anemia, headaches, depression and muscular and bodily pain.

The symptoms are like those associated with many other febrile diseases, but with emphasis on muscular pain and sweating. The duration of the disease can vary from a few weeks to many months or even years. In the first stage of the disease, septicemia occurs and leads to the classic triad of undulant fevers, sweating (often with characteristic smell, likened to wet hay), and migratory arthralgia and myalgia

Brucellosis in animals

Species infecting domestic livestock are *B. melitensis* (goats and sheep, *B. suis* (pigs), *B. abortus* (cattle and bison), *B. ovis* (sheep), and *B. canis* (dogs).

Brucellosis in cattle

The bacterium *Brucella abortus* is the principal cause of brucellosis in cattle. The bacteria are shed from an infected animal at or around the time of calving or abortion. Once exposed, the likelihood of an animal becoming infected is variable, depending on age, pregnancy status, and other intrinsic factors of the animal, as well as the amount of bacteria to which the animal was exposed. The most common **clinical signs of cattle infected with *Brucella abortus* are high incidences of abortions**, **arthritic joints and retained after-birth**. There are two main causes for spontaneous abortion in animals. **The first is due to erythritol, which can promote infections in the fetus and placenta. The second is due to the lack of anti-Brucella activity in the amniotic fluid**. Males can also harbor the bacteria in their reproductive tracts, namely seminal vesicles, testicles.

Brucellosis in dogs

The causative agent of brucellosis in dogs is *Brucella canis*. It is transmitted to other dogs through breeding and contact with aborted fetuses. Brucellosis can occur in humans that come in contact with infected aborted tissue or semen. The bacteria in dogs normally infect the genitals and lymphatic system, but can also spread to the eye, kidney. **Symptoms of brucellosis in dogs include abortion in female dogs and scrotal inflammation and orchitis (inflammation of the testicles) in males**. Fever is uncommon.

Yersinia pestis is a Gram-negative rod-shaped bacterium. It is a facultative anaerobe , bipolar staining (giving it a safety pin appearance). negative for urease, lactose fermentation, and indole but coagulase positive. that can infect humans and other animals. Human *Y. pestis* infection takes three main forms Is transmitted to humans by fleas found on rats :

pneumonic plagues : Centers in the lungs
septicemic plagues : Centers in the bloodstream
bubonic plagues : based on the lymphatic system

Virulence factor:

- Envelope antigen F1 inhibit phagocytosis
- Exotoxin and endotoxin

- Type III secretion system suppresses cytokine production and phagocytic killing
- plasmids carry a number of genes related to virulence

These virulence genes produce virulence factors that fall into four general categories:

- pPCP1 is a 9.5 kb plasmid that, among other things, encodes for Pla, which is an outer membrane plasminogen protease that primarily interferes with blood coagulation

- The pPMT1 plasmid includes the instructions for the capsular protein and a murine toxin, and has been hypothesized to have a role in resisting monocyte phagocytosis.

- The pCD1 bacteria which codes for the Type III secretion apparatus

- Another important factor in the avoidance of the immune system by *Y. pestis* is the apoptosis of T cells, which is the result of a complex signalling pathway. YopH, which is a phosphotyrosine phosphatase, is capable of causing a loss of potential in the mitochondrial membrane as well as internucleosomal DNA degradation and activating a caspase pathway to induce the mitochondrially regulated apoptosis of T cells.

Pathogenesis

Bacteria travel through the blood to the nearest lymph nodes
In lymph nodes, *Y. pestis* is ingested by fixed macrophages
Y. pestis is able to grow in inactivated macrophages and replicate
Elicits an inflammatory response (the bubo)
Bacteria from the bubo leak into the blood stream. (septicemic plague)
Lysis of the bacteria releases LPS, which causes septic shock
Eventually bacteria reach the lung, where they parasitize the lung macrophages (pneumonic plague)

pneumonic plagues :

- Arises from septic pulmonary emboli in bubonic plaque or inhalation of organism from infected individuals
- highly contagious

bubonic plagues:

- flea bites infected animal and then infected human

Symptoms

- rapidly increase fever
- regional buboes
- conjunctivitis
- lead to septicemia and death if not treated