Lec. One ; Historical Foundations Microbiology

Our current understanding of microbiology is the cumulative work of thousands of microbiologists; Robert Hooke (1635 – 1703) an Englishman was the first person to use a microscope to observe the first cell , he saw the cellular structure of plants described them as (small rooms) in cork around 1665 . His discovery led to the formulation of the cell theory, which states that cells are the basic unit of all living things.

He also saw fungi which he drew, because his lenses were of poor quality he was unable to "see" bacteria.

Anton van Leeuwenhoek (1673-1723) a Dutch linen merchant and self-made microbiologist was the first person to develop lens to view microbe and observed microorganisms in a drop of rain water , The original purpose of the microscopes was to examine cloth for flaws, but Leeuwenhoek turned them to other uses as well. He is considered the father of microbiology because he was the first person to observe microorganisms using a microscopes made by him and produce a correct descriptions of these organisms, although he was not the first to discover the microscope or to use magnifying lens, he was the first person to observe and describe microorganisms accurately. In 1668 he look at a sample of pond water rich in microbes and saw distinct shaped organisms. He made numerous microscopes and viewed everything he could. His best lens could magnify ~300-500 folds. He drew pictures of microbes and called them animicules (little animals). He used only

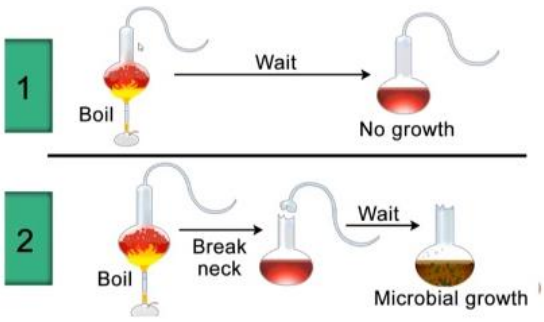
single lens and not the compound lens of the true microscopes we employ today. He wrote his observations to the Royal Society of London in 1676 and included numerous drawings.

The theory of spontaneous generation, is an early belief that some forms of life could arise from vital forces present in nonliving or decomposing matter was advanced by Aristotle who Suggested that mice could develop by spontaneous generation, This theory was disproved by Francesco Redi, (1626-1697) was first to present experimental evidence to refuse spontaneous generation by showing that maggots (fly larvae) do not arise from decaying meat (as others believed) if the meat is covered to prevent the entry of flies, He placed a piece of meat in three jars, one he left open, one he corked tightly and the third covered with a fine mesh gauze. Maggots fly only appeared in the open container that flies could reach to lay eggs on. , however, his results were not accepted by many who insisted that he only disproved spontaneous generation for macro organisms , maybe microbes were an exception., John Needham (1713-1781) supported the theory of spontaneous generation and

showed that nutrient solutions that boiled in flasks and then sealed could still develop microorganisms when cooled . Lazzaro Spallanzani (1729-1799) countered this with experiments demonstrating that if flasks sealed and then boiled had no growth of microorganisms, he found that microorganisms could only settle in a broth if the broth was exposed to the air, he also found that boiling the broth would sterilize it and kill the microorganisms. he also commented that external air might be needed to support growth he didn't disprove spontaneous generation he just proved that spontaneous generation required air.

John Tyndall (1820-1893) demonstrated directly that the growth of microbes in contaminated flasks was due to microbial cells from airborne dust particles, not from spontaneous generation and developed a method (tyndallization) to ensure sterilization of media through repeated boiling. Tyndall also provided evidence for the existence of heat-resistant forms of bacteria.

Louis Pasteur (1822-1895) exposed broth in flasks to the air via a curved tube that would not allow dust particles to come in contact with the broth. sterilized the broth beforehand , and left the flasks open to the air, no growth was observed because dust particles carrying organisms did not reach the medium, instead they were trapped in the neck of the flask , in this way Pasteur disproved the theory of spontaneous generation . Pasteur postulated and supported the germ theory of disease, which states that microorganisms are the causes of infectious disease and ended the long-held belief of spontaneous generation and proved the theory of biogenesis which stated that living things can only arise from living things , also Pasteur demonstrated that fermentation caused by microorganisms , invented pasteurization to preserve foods and developed vaccines



Robert Koch proved the germ theory of disease and demonstrated connection between single microbes and human disease also introduced the important

concept of aseptic technique to control the spread of disease agents . In 1876 he became interested in anthrax, a common disease of both the farmers and their animals. Using a microscope Koch always saw a large bacterium in the blood of cattle infected with anthrax and victims. Koch also found that he could transmit anthrax from one animal to another by taking a small sample of blood from the infected animal and injecting it into a healthy one causing the healthy animal to become sick. He thought that it might be the agent of the disease, he also found that he could grow the bacteria in a nutrient broth and if he injected the purified bacteria into healthy animals it will produce the disease, and when he examined the blood of the inoculated animals he re-isolates the same bacterium.

His procedure for defining the agent of any disease, called Koch's postulates . In addition Koch discovered the etiological agents of cholera, and tuberculosis . His studies, in combination of those of Pasteur's established the germ theory of disease. In general his contributions are ;

1. he showed the evidence that a specific germ (Anthrax bacillus) was the cause of a specific disease (spleenic fever in sheep)
2. He established that a specific germ can cause a specific disease and introduced scientific approach in Microbiology
3. He discovered *Bacillus anthracis* (Anthrax bacillus), Mycobacterium tuberculosis, and Vibrio cholerae.
4. He modified Ziehl-Neelsen acid fast staining procedure which was introduced by Ehrlich.
5. He devised the solid medium to grow the microorganism to get single colonies.
6. He introduced Koch's method to find out the efficacy of disinfectants
7. He established certain rules that must be followed to establish a cause and effect relationship between a microorganism and a disease.

They are known as Koch's Postulates

Dr. Walther Hess and his wife (Fanny Angelina) discussed the possibility of using agar-agar (a complex polysaccharide extracted from seaweed) to prepare microbial media.

The following characteristics of AGAR-AGAR make it almost perfect for the growth of microbes on solid medium:

(a) non-toxic to most microbes.

(b) only melts at 100^{o}C, but solidifies at about 45^{o}C (a temperature most bacteria can survive).

(c) nontoxic to other forms of life.

(d) stable to sterilization temperatures.

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

Edward Jenner was led to the discovery of immunization and to the elimination of pox from the earth. He observed that dairymaids that contracted a mild infection of cowpox seemed to be immune to smallpox , By 1796 he inoculated an 8-years old boy with cowpox and 8 weeks later inoculated the same boy with the pus from a pox lesion. The boy showed no effects of smallpox , others began to test his work and by 1803 it was an established medical procedure in England.

Paul Ehrlich (the father of chemotherapy) worked in Koch's lab. where he learned to study bacteria. While considering the differential staining phenomenon of different bacteria and different components of eukaryotic cells, he speculated that if a dye chemical could bind to one cell and not another or to one substance within a cell and not others, perhaps you could find chemicals that would selectively kill certain pathogens without harming the surrounding host cells. He search for a substance to cure syphilis, over many years he tested 100s of chemicals and finally in 1910 he found one, he named SALVARSAN that killed the syphilis organisms without killing the host. This discovery laid the ground for the discovery of antibiotics and other chemotherapeutic agents .

; BIOLOGICAL BACKGROUND & CLASSIFICATION

Prof. Dr . Hayder altee .

Microbiology is the study of organisms too small (i.e., microorganisms) to be clearly seen by the unaided eye it concern with their structure , reproduction , physiology , metabolism , classification …ect and how they interact with humans and environment. Microorganisms can be defined as living organisms so small that cannot be seen without the aid of a microscope . these include viruses, bacteria, archaea, protozoa, algae, and fungi

Some microbes (e.g., algae and fungi) are large enough to be visible, but are still included in the field of microbiology; it has been suggested that microbiology be defined not only by the size of the organisms studied but by techniques employed to study them (isolation, sterilization, culture in artificial media). Microorganisms of medical importance can be classified in to five groups (1) ; Bacteria (2); Rickettsia and Chlamydia (3) ; Viruses (4) ; Fungi (5); Protozoa

The cells of fungi & protozoa are similar in structure to those of higher plants & animals & these organisms are therefore known as eukaryotes ("true nucleus") , whereas bacteria , Rickettsia & Chlamydia are known as prokaryotes ("before nucleus") because their cells have a much simpler nuclear structure , do not have nuclear or other dividing membranes & have in their walls mucopeptide a substance not found in eukaryotic cells.

Prokaryotes

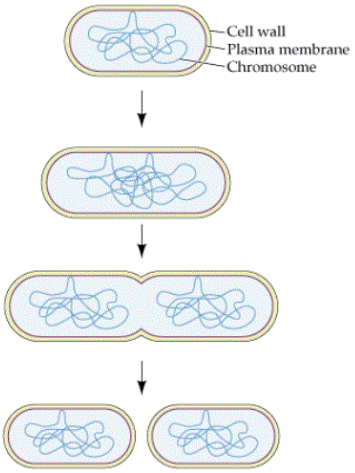
A prokaryote is a simple, unicellular organism that lacks an organized nucleus or other membrane-bound organelle.

Prokaryotes are almost unicellular although some species can aggregate into complex structures as part of their life cycle , these organisms are divided into two groups , the bacteria and Archaea

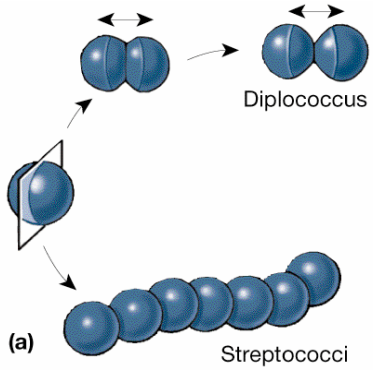
Bacteria ; unicellular organisms possess a prokaryotic type of cell structure, their DNA (usually circular) can be found throughout the cytoplasm rather than within a membrane-bound nucleus , individual cells are of order 0,5 – 1 micron broad by 0.5 – 8 micron long ,

the shape of bacterial cell is determined by it's rigid cell wall which also prevents it from swelling up & bursting as the results of osmosis .

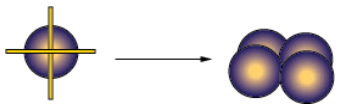
Reproduction is mainly by simple <u>binary fission</u> (an asexual process) one cell enlarging and then dividing into two approximately equal parts , some can grow at temperatures as low as 0 C others thrive in hot springs at temperatures of 90 C or more ,

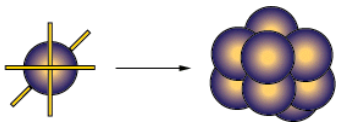


but most live between these two extremes . Bacteria cause a variety of chemical changes in the substances on which they grow , they can decompose many substances deposited in or on the land or sea . Some types can cause diseases in animals or plants , they are very widely distributed in nature

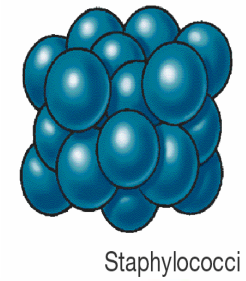


Bacteria differ in the shape. They exist in four major shapes: bacilli (rod shape), coccus (spherical shape), spirilla (spiral shape), and vibrio (curved shape). . Cells that are spherical are called cocci . These are commonly grouped together, if in





pairs they are referred diplococci . Repeated division in the same plane produces chains known as streptococci.


Staphylococci

division in two or three planes at right angles produces regular packets of four (known as tetrads) , eight (known as sarcinae) or more , and division without any definite orientation produces irregular clusters known as staphylococci .


Vibrio


Bacillus


Spirillum


Spirochete

The bacilli or rods are elongated cylindrical forms, Certain bacteria which resemble bacilli but are more curved are known as vibrios or comma bacilli .The spirochetes are corkscrew-like spirals . Some of these (e.g. the leptospirae) are tightly coiled , whereas others (e.g. the borreliae) have large open coils . Actinomycetes and other higher bacteria resemble fungi (eukaryotes) in forming branched filaments .

The mycoplasmas are an exception to the role that bacterial cells are encased in rigid cell walls . Their cells are essentially protoplasts and are smaller in size than other bacteria (0.25 micron) they can grow and reproduce like other bacteria , but their

lack of cell wall means that they are of variable shape and can survive only in isotonic conditions .

Archaea

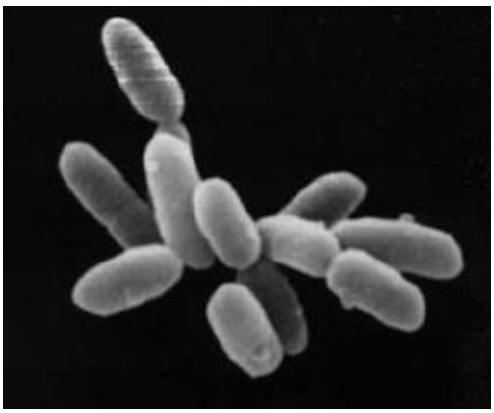
Archaea are prokaryotic cells adapted to extreme environmental conditions , it differ from true bacteria in their cell wall lack peptidoglycans , It contain a surface-layer proteins called S-layers . Based on their habitat, all Archaeans can be divided into the following groups:

methanogens (methane-producing organisms),

halophiles (live in salty environments),

thermophiles (live at extremely hot temperatures), and psychrophiles (live at cold-temperature) . all archaea are harmless .

<u>Rickettsiae and Chlamydia</u>



These organisms resemble bacteria in that they contain both RNA & DNA , have muramic acid in their outer coats , reproduce by binary fission and are susceptible to the action of antibacterial drugs that have no effect on viruses , on the other hand , with the diameters of only 0.25 – 0.5 micron they are nearer in size to viruses than to bacteria , and they are also virus-like in being

unable to reproduce except inside the cells of the host .

Protozoa

These are unicellular or multicellular organisms ,photosynthetic or non-photosynthetic , free living (harmless) or parasitic . Protozoa divided on their mode of locomotion to ;

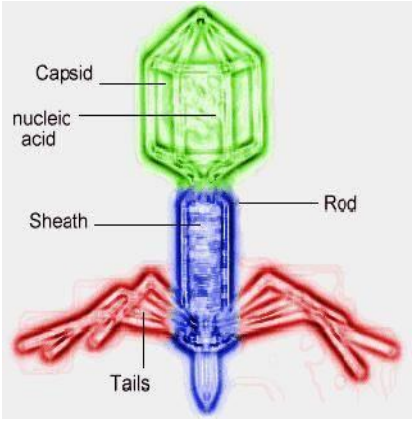
1) flagellates use their whip-like structure to move forward,
2) ciliates have tiny hair that beat to produce movement,
3) amoeboids have false feet or pseudopodia used for feeding and locomotion .
4) sporozoans are non-motile.

Algae

also called cyanobacteria or blue-green algae, are unicellular or multicellular eukaryotes that obtain food by photosynthesis. They live in water, soil, and rocks and produce oxygen and carbohydrates used by other organisms. It is believed that cyanobacteria are the origins of green land plants.

Fungi

These are unicellular or multicellular. Microscopic include yeasts and molds , non- photosynthetic , cell wall made of chitin , free living (harmless) or pathogenic .



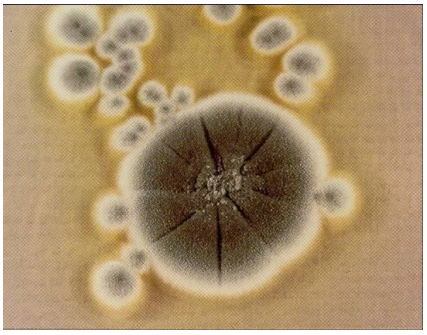
Viruses

Most of the viruses are smaller than any other known living organisms and are too small to be seen in the ordinary microscope they range in size from about 20 – 25 nm to 200 – 300 nm and exhibit several shapes .

The virus particle is called a virion not a cell and consists only of a nucleic acid core , the genome , packed within a protein coat (the capsid) . The nucleic acid found in a virus of any given type is either RNA or DNA but not both , viruses increase in number by replication inside the host cell .

DIFFERENCES BETWEEN PROKARYOTES/EUKARYOTES

Some distinguishing characters between prokaryotic and Eukaryotic cells



Character	Prokaryotic cells	Eukaryotic cells
1 - Cell wall (Peptidoglycan)	+	__
2 - Cytoplasmic region		
Ribosomes	70 S	80 S
Mesosomes	+	--
Mitochodria	--	+
Chloroplast	--	+ \ --
Golgi structure	--	+
Endoplasmic reticulum	--	+
Membrane bound vacuoles	--	+
3 - Nuclear membrane	--	+
4-Sexual reproduction	Rare	+

70S (S stands for Svedberg unit, a measure of size)
The Svedberg unit (S) offers a measure of particle size based on its rate of travel in a tube subjected to high g-force.
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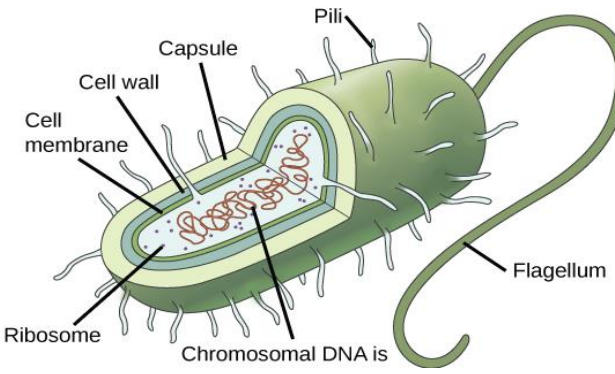
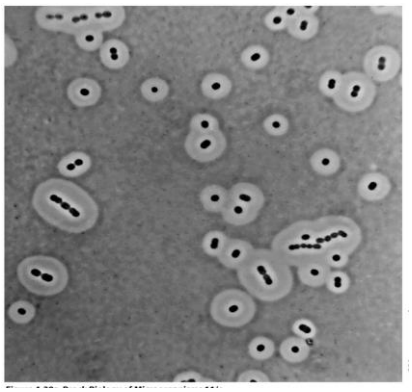
Lec. three ; BACTERIAL STRUCTURE

. Prof. Dr . Hayder altee



Examination of bacterial cell reveals various structural components; some of these are external to the cell wall while others are internal to it.



Capsule;

Some bacteria are surrounded by a gelatinous material (glycocalyx) that lies outside and in contact with the cell wall is called a Capsule, If the layer is thick and strongly adhered to the cell wall, it is called a capsule; if not, it is called a slime layer, it originates as a secretion from the cell membrane & excreted through the cell wall , Development of capsule is dependent on the existence of favorable environmental conditions such as presence of high sugar concentration, blood serum or growth in a living host.

1) ; it protect the cell from temporary drying .

2) ; provide protection against phagocytosis

3) ; it is also associated with virulence

4) ; serve as a means of adherence to surfaces .
5) ; may aid in the blocking of bacteriophage since receptors are components of the cell wall capsule thicker than the length of the tail of the phage (150 nm) would prevent the tail from coming in contact with the cell receptors .
the chemical composition of capsular materials differ in that some are polysaccharide (pneumococci and klebsiella) , some are polypeptide (Bacillus anthracis) or hyaluronic acid (Streptococcus pyogenes) & others are complexes of these.

Capsules have no affinity for dyes and so they are not seen in stained preparations Capsulated bacteria
are usually nonmotile as flagella remains unfunctional in the presence of capsule

Cell wall;

The cell wall encases the protoplast and lies immediately external to the Cytoplasmic membrane , it is 10 - 25 nm thick , may account 10 - 40 % of the dry weight of the cell . strong & rigid & this appears to be associated with muramic acid (also referred as Peptidoglycan) .
Cell Wall Function

1) porous being freely permeable and does not regulate the transport of substances into the cell .
2) it prevents the weak C.M. from bursting due to the high internal osmotic pressure of the protoplasm (5-25 atmospheres)
3) maintain the characteristic shape of the cell .
4) have a Role in division of bacteria
5) Offers resistance to harmful effect of environment
6) Contains receptor sites for phages and Provides attachment to complement .

Peptidoglycan or mucopeptide is the main structural component of the cell wall and is composed of alternating chains of N-acetylglucoseamine and N-acetylmuramic acid in equal amounts and cross linked by peptide chains consisting of 4 or 5 amino acid .

The significant differences in the cell wall between G^+ and G^{--} are

Character	G^+ cell wall	G^{--} cell wall
1- cell wall structure	Thick 15 - 80nm, single layer	Thin 10-15nm,triple layers
2- cell wall composition	Low in lipids1-4%, peptidoglycan account over	High in lipids 11-22%, peptidoglycan account about

	50% of dry weight. Teichoic acid present	10%, no Teichoic acid
3- suscceptibility to penicillin	More susceptible	Less susceptible
4- effect of basic dyes e.g., crystal violate on growth	Markedly retarted	Less retarted
5- nutritional requirements	Complex	Simple
6- resistance to physical disruption	More resistant	Less resistant

Cytoplasmic membrane

A cytoplasmic membrane surrounds the cytoplasm of all bacterial cells and are composed of protein and phospholipid , The phospholipids form a bilayer into which proteins are embedded, some spanning the membrane. The membrane carries out many

functions, including
the synthesis and export of cell-wall components, respiration, secretion of extracellular enzymes and toxins ,
it possess a selective permeability & control the passage of nutrients & waste products in & out of the cell either by passive or active transport

with the aid of permeases enzymes which plays important role in passage of selective nutrients. , it is 5 -10 nm thick & form 10 -20 of the dry weight , invagination of the C.M. form intracellular structures called mesosomes , Mesosomes are intracellular membrane structures formed by folding of the cytoplasmic
membrane. They occur more frequently in Gram-positive than in Gram-negative bacteria. Mesosomes present at the point of cell wall division are involved in chromosomal separation; at other sites they may be associated with cellular respiration , metabolism. transport , synthesis of cell wall , lipids , capsular materials & enzymes secreted in to the outer environment for digestion of the macromolecules nutrients .

Protoplasm

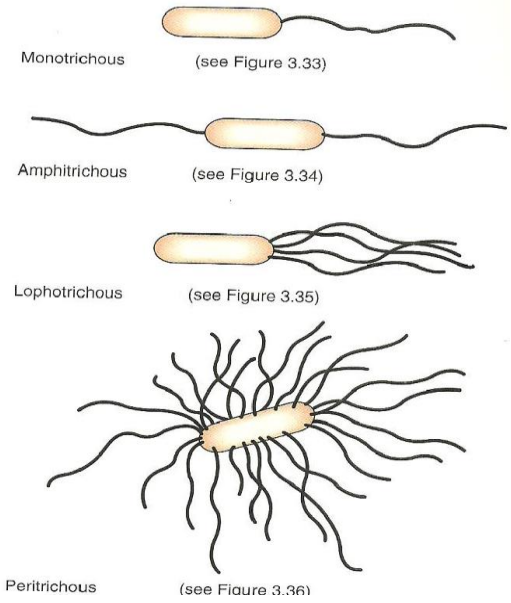
matrix composed primarily of water (90%) & proteins it is divided into;

(1) ; the cytoplasm; it is a viscous watery solution containing a variety of organic & inorganic solutes , it contains mesosomes , ribosome , nuclear material & other structures in a matrix containing various ions , amino acids , proteins ,enzymes & other organic substances essential to living matter , the cytoplasm is rich with R.N.A.(ribosomes) which are the site of protein synthesis.

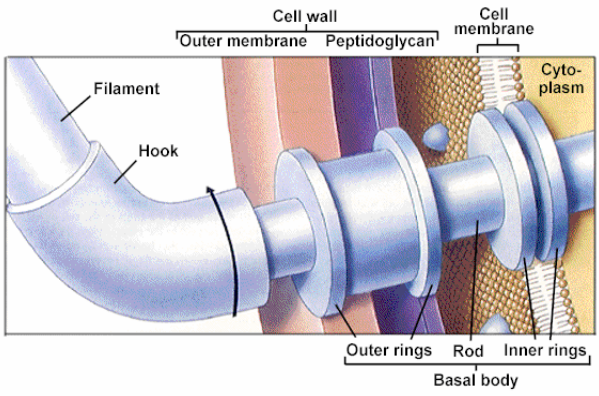
(2) ; Nuclear material ; The genetics information of prokaryotes occurs as a closed circle of double stranded D.N.A. known as chromosome it is not surrounded by a membrane so there is no differentiation into nucleus & cytoplasm .

Flagella

Bacterial flagella (singular; flagellum) are hair like helical appendages that protrude through the cell wall & responsible for motility. Their location on the cell varies depending on the bacterial species and may be polar at one end of the cell (Monotrichous) or lateral along the sides of the cell (Peritrichous) or, (Lophotrichous); a cluster of polar flagel and (Amphitrichous) ; flagella either single or clusters at both cell poles .

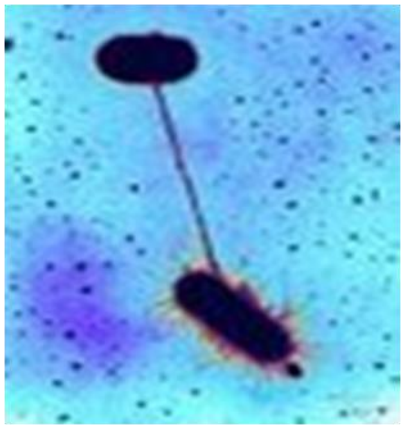


A flagellum is composed of three parts ; a basal body associated with the C.M. & cell wall , a short hook , & a helical filament which is usually several times as long as the cell. The chemical composition of the filament is a protein subunits known as Flagellin that make up the long filament.

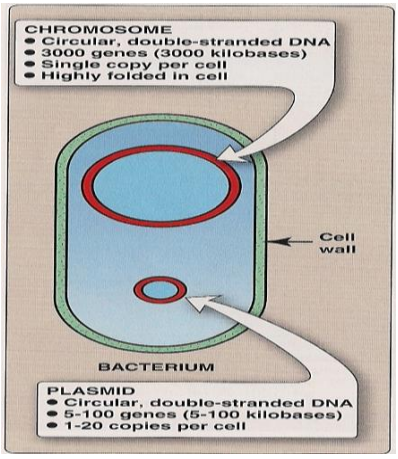


Pili (fimbriae (Latin for "thread" or "fiber,") **)**

Pili (singular ; pilus (Latin for "hair")) are hollow non helical filamentous appendages that are thinner , shorter & more numerous than flagella (about 200) and can be found covering the cell surface they do not function in motility since they are found in both motile & non motile bacteria , All pili are primarily composed of oligomeric pilin proteins ,



The pili contain chemical compounds called adhesins which allow the cell to bind and attach to specific receptors on various human tissues so pili play a major role in human infection. One type known as F pilus (sex pilus) serves as the port of entry of genetic material during **bacterial conjugation** . This form of pilus can be relatively long but is often found in few numbers, generally 1 to 6, protruding from the cell surface.



Plasmids

Extra-chromosomal DNA, usually present in multiple-copy number, which often code for pathogenesis factors and antibiotic resistance factors. Some forms are also involved in bacterial replication by coding for the production of sex pilus.

Spores

Endospores are A dormant, dehydrated, and non-reproductive structure produced by certain bacteria such as Bacillus & Clostridium, Sporosarcina, Thermoactinomyces & few other genera , the shape & location of endospores within the cell vary depending on the species & it is a good tool for bacterial identification , all spore formers are gram positive rods and some are of great importance in medicine causing such diseases as anthrax , gas gangrene , tetanus & botulism,

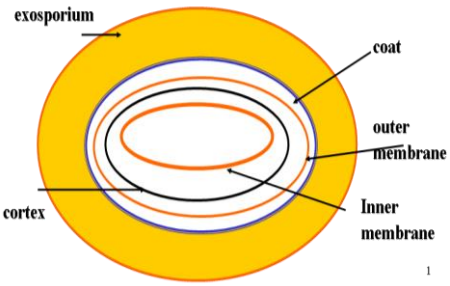
endospore is not a reproductive structure since one cell produce one spore in a process called sporulation & when appropriate stimulation it give rise to a single cell in a process known as germination,

spores can withstand extremes of pH , temperature , radiation, or other adverse physical conditions & this may be due to:

1) The low content of water

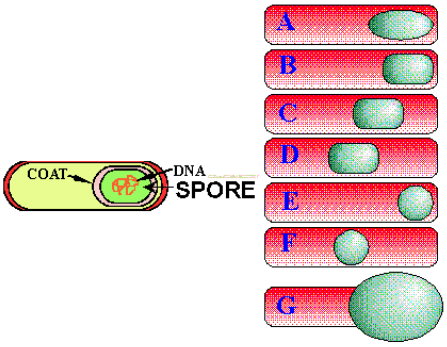
2) The presence of a large amount of calcium dipicolinate a substance found only in spores.

3) Spore coats & the thick cortex which composed of a special form of Peptidoglycan & an external lipoprotein & carbohydrate layer called an exosporium



4) Their very low metabolic & enzymes activity.

Sporulation occurs in response to adverse conditions such as starvation or exhaustion of limiting substances as at the end of log. Phase or early in the stationary phase , it develop from a portion of protoplasm near one end of the cell (the forespore) incorporates part of the nuclear material & acquires a thick covering layer the (cortex) & thin outer spore coat consisting of several layers , mesosomes seem to play a



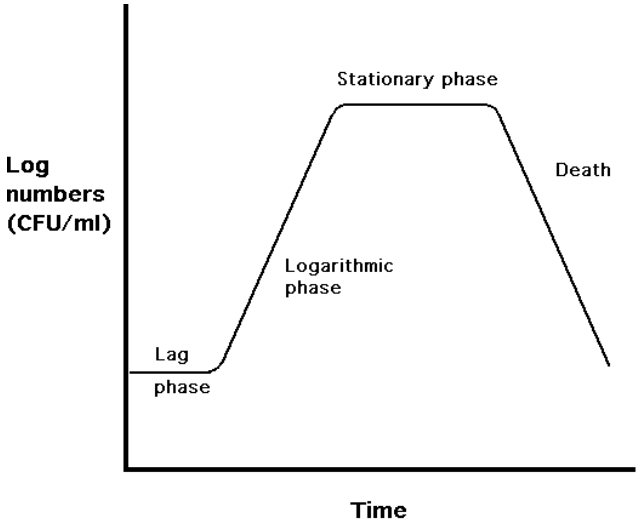
part in the development of endospores,

spores may be spherical , oval or elongated, occupying a terminal , sub terminal or central position & being narrow than the cell or broader & bulging it.

Lec. four; Growth & nutrition of bacteria

Prepared by ; Prof. Dr . Hayder altee

When an inoculum of cells is introduced into nutrient medium and incubated under appropriate conditions , the cells grow at a very rapid rate , this growth is normally associated with multiplication by binary fission (The process whereby a cell divides asexually to produce two daughter cells) it is important to realize the rapidity of growth , when a cell may double every 20 min.& if this rate is maintained for 24 hr. ,the progeny of a single cell would be about 1 X 10^{21} cells & would have a mass of about four thousand tons , but the condition used for culture never permit such a rate of multiplication for more than a short time because of an insufficiency of nutrients or of special growth factors. If the number of cells present at different times after inoculation is measured & plotted in relation to time , the resultant plot is referred to as batch culture & a typical growth curve has four main phases known as ;



(1) ; Lag. Phase ;

in this period cells increase in size & show marked metabolic activity & bacteria adapt themselves to the new growth conditions and maturing but do not divide so the depleted enzymes , metabolic intermediates , synthesis of RNA and other factors are build up . The length of the lag phase depends on the kind of bacteria, the age and size of the inoculum, the

nature of the medium from which they were taken, and the nutrients present in the new medium. .

(2) ; Logarithmic phase (log. Phase) ;

Exponential phase (sometimes called the log or logarithmic phase) is a period characterized by cell doubling. the cells divide at a constant rate , there is a linear relationship between time and the number of cells & bacteria have a high rate of metabolism & the actual rate of growth is directly related to the generation time (The time required for one cell to divide into two cells is called the generation time or doubling time or the time between two divisions) .

(3) ; Stationary phase ;

The exponential growth is no longer possible & the rate of multiplication decreases until it ceases and the cells pass into the stationary phase & reach a stable state of equilibrium between the rates of growth & death , the cessation of growth in this phase is mostly caused by the exhaustion of essential nutrients in the medium , accumulation of toxic waste products such as organic acids which can lead to the lowering of pH , at the onset of stationary phase ,many species of bacteria produce secondary metabolites i.e. natural products formed mainly or only by cells that have stopped dividing , they include many antibiotics & exotoxins , in spore forming species the sporulation occurs at the end of log. Phase & early in the stationary phase .

(4) ; Death or decline phase ;

After a variable period of time in the stationary phase ; the cells in a culture begin to die & become incapable of growth when transferred to a fresh medium & the causes of this death are various .

Nutritional requirements ;

All microorganisms share certain requirements for growth & this includes;

(1) ; a source of energy ; microorganisms which can utilize radiant energy or sunlight called phototrophs , while others which get energy from chemicals called chemotrophs (chemoorganotrophs if they derive energy from organic chemicals and chemolithotrophs if from inorganic chemicals) .

(2) ; a source of carbon ; are needed as an energy source (example ; glucose) and for building blocks , organisms obtain their sole carbon source from atmospheric CO_2 are autotrophs (inorganic carbon) & those obtain their carbon from organic compounds known as heterotrophs .

(3) a source of nitrogen commonly supplied as ammonia (NH_4) needed for amino acids and nucleotides some microbes fix a atmospheric nitrogen (N_2) and called (diazotroph) or from organic or inorganic nitrogen compounds , sulfur needed for amino acids, coenzymes , phosphorus needed for ATP, phospholipids, and nucleotides

(4) ; a source of elements such as sodium , potassium , calcium , magnesium , manganese ,iron , zinc , copper & cobalt often serve as cofactors in enzymatic reactions.

(5) ; a source of vitamins & vitamin like compounds required in small amounts and that is typically used as a coenzyme .

(6) ; a source of water that serve as a solvent to carry nutrient and waste products away from the cell and other metabolic functions & growth.

Physical requirements ;

The successful cultivation of bacteria requires a combination of the proper nutrients & the proper physical environments ;

(1) ; Temperature ;
each species of bacteria grow at temperatures within certain range , on this bases bacteria can be classified as ;

1) Psychrophiles ; which grow at O – 20 C

2) Mesophiles; which can grow between 20 – 45 C .

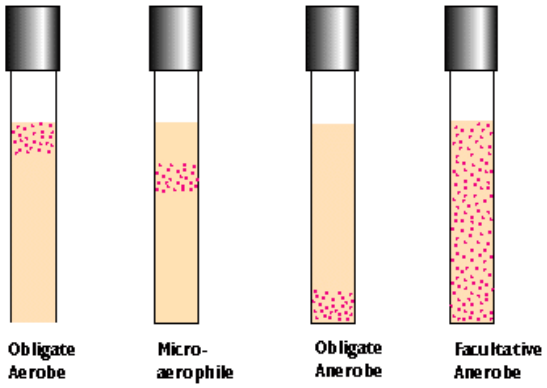
3) Thermophiles can grow between 45 -- 80 °C .

4) Extreme Thermophiles optimum temperature is above 80C.

The temperature at which the most rapid growth occurs during a short time is known as the optimum temperature.

(2) ; Oxygen ; bacteria can be divided into four groups in responses to free oxygen ;

1 ; aerobic bacteria ; oxygen requiring organisms
2 ; anaerobic bacteria ; grow in the absence of oxygen
they are killed by oxygen; they lack certain enzymes (e.g. catalase [breaks hydrogen peroxide, H_2O_2, to H_2O and O_2, peroxidase (NADH + H_2O_2 converted to



NAD and O_2), superoxide dismutase (superoxide [O_2^-] to H_2O_2) which detoxify both hydrogen peroxide and oxygen free radicals (superoxide) produced as side-products during metabolism in the presence of oxygen.
3 ; facultatively anaerobic bacteria; grow under either aerobic or anaerobic conditions (grow better with O_2) Ordinarily an aerobe but can grow in absence of O_2.
4 ; microaerophilic bacteria; grow best in small amounts of atmospheric oxygen (2-10% O_2 normal atmospheric O_2 is 20%)

(3); Acidity or alkalinity (pH) ; For most bacteria, the optimum pH to grow best is at a neutral or slightly alkaline (pH ; 7.2 – 7.6), however, some bacteria can grow in more acidic conditions & termed acidophilic (below pH 5.5) , while others are alkaline tolerant & known as basophilic (above pH 8) .

(4); Light and other radiations; Darkness provides a favorable condition for growth & viability, ultraviolet rays are bactericidal & direct sunlight or even diffuse daylight through window glass shortens the survival of microorganisms, also bacteria are killed by ionizing radiation.

(5) ; Moister & desiccation ; Four – the fifth weight of the bacterial cell consists of water & moister is necessary for growth, drying is injurious to the cell & bacterial species differ widely in their ability to survive when dried.

(6) ; Osmotic pressure ; . As a result of the presence of a semi-permeable C.M. bacteria are subjected to osmotic phenomena so interior osmotic pressure must be balanced against exterior pressure and each microorganism displays an optimum salt concentration for growth, bacteria require a high concentration of NaCl known as halophiles, while the halo-

tolerant bacteria are those that grow in high NaCl concentration but grow best at lower levels, exposure of bacteria to solutions of high salt concentration (e.g. 2 to 25 per cent sodium chloride) may cause plasmolysis i.e. shrinkage of the protoplast and retraction from the cell wall due to water withdrawal while transfer from concentrated to a weak solutions or distilled water may cause plasmoptysis , the correct osmotic pressure in culture medium is essential for the survival of the cells.

Lec. Five ; Antimicrobial agents & sterilization methods

Prepared by ; Prof. Dr. Hayder altee

The term control as used in microbiology refers to the reduction in number & / or activity of the total microbial flora , the main reasons for controlling microorganisms are ;

(1) to prevent transmission of disease & infection
(2) to prevent contamination by or growth of undesirable microorganisms
(3) to prevent deterioration تدهور & spoilage تلف of materials by microorganisms
(4) to eradicate microorganisms from a host that is infected

Any chemical substances inhibiting the growth or causing the death of microorganisms is known as " antimicrobial agents " at a concentration suitable for practical application , if the substance causes a cessation توقف of growth which is reversed when the chemical is removed , it is called a" static agent " & if the substance kills the microorganisms it is called " cidal agents ". (bactericidal, fungicidal, viricidal, etc.) , this is often depends on the concentration of the drug , a static agent may become cidal if the concentration increased

There are several factors which affect the antimicrobial action such as ;
1; the concentration of the antimicrobial agent .
2; the number of microbes .
3; microbial characteristics (spore & biofilms forming ability)
4; environment (presence of organic materials e.g., serum, blood , pH and temperature)
5; Time of exposure .

microorganisms can be removed , inhibited or killed by physical or chemical agents & a variety of techniques & agents are available & act in many different ways & each has its own limits of practical application, in microbial control several specific terms are used to describe the agents or the process ;

(a) ; **disinfectants** ; this is a term applied to chemicals used to kill infectious agents , they are usually used in the treatment of inanimate جماد objects , such as surfaces , water ….. & are not meant to come into direct contact with human , because their potential toxicity to human may not be important , the main criteria used in the choice of a disinfectant are its ability to kill a wide range of microorganisms rapidly , inexpensive غير مكلف , non-toxic and non-corrosive . One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly. Phenol is the standard disinfectant, and the corresponding rating system is called the "phenol coefficient. (Phenol co-efficient indicates Efficiency of a disinfectant) " The disinfectant to be tested is compared with phenol on a standard microbe (usually Salmonella typhi or Staphylococcus aureus). Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective have a coefficient < 1.

(b) **Antiseptic** (mild disinfectant) ; this term refers to relatively non toxic & non irritant antimicrobial agents that may be applied topically to living tissue (skin or body surface) either to kill or to inhibit the growth of pathogens to reduce the possibility of infection. Some antiseptics are germicides, capable of destroying microbes (bacteriocidal), while others are bacteriostatic and prevent their growth.

(c) **Chemotherapeutic agents** ; this term describes the chemicals that are used to kill or inhibit the growth of microorganisms already established in the tissue of the host , these agents

need to act at a concentration that can be tolerated by the host & must have selective toxicity . so an ideal chemotherapeutic agents must characterized by ;

(1) ; selectively toxic to microbe but non toxic or less toxic to the host .
(2) ; readily delivered to the site of infection in effective concentrations to come in contact with the pathogen .
(3) ; leave the host natural defense mechanisms unaltered .
(4) ; does not disrupt the hosts health and do not produce undesirable side effects .
(5) ; does not lead to the development of antimicrobial resistance .
(6) ; microbicidal and broad spectrum rather than microbistatic .
(7) ; relatively soluble and functions even when highly diluted in body fluids.
(8) ; Nonallergenic
(9) ; Stability (should be degraded and excreted by the body slowly)
(10) : Resistance by microorganisms not easily acquired
(11) ; Long shelf life.مدة الصلاحية
(12) ; Reasonable cost

the most widely chemotherapeutic agents used are antibiotics which function in four major modes ;

a- 1; inhibition of cell wall synthesis ; (examples ; bacitracin , cephalosporines , cycloserine , penicillins , vancomycin) ; Peptidoglycan is the component of the cell walls of bacteria responsible of their strength , injury to the cell wall or inhibition of its function may lead to the lyses of the cell . antibiotics affecting the synthesis of the Peptidoglycan would have no effect on L – form cells because these cells lack cell wall.

2 ; inhibition of protein syntheses ; (examples ; streptomycin ,chloramphinicol , tetracycline , neomycin , erythromycin , puromycin , getamycin , kanamynin ,….) ; bacteria have 70 S ribosomes while mammalian cells have 80 S ribosomes , the subunits of each type of ribosomes are different & this explain why antimicrobial drugs can inhibit protein synthesis in bacterial ribosomes without having a major effect on mammals.

3; inhibition of nucleic acid synthesis ; (examples ; nalidixic acid , novobiocine , sulfonamides , trimethoprin , rifampin ,…) these antibiotics combine with & alter the functioning of nucleic acid although in general they are too toxic for therapeutic use.

4 ; alteration of C.M. permeability ; (examples ; amphotericine B , colistin , nystatin , polymyxins ,…) the C.M. serve as a selective permeable barrier , if the function of it is disrupted the Cytoplasmic contents will escape from the cell & the cell damage or death occur .

Viruses remain resistant to antibiotics because of their intracellular parasitism & it relay on the host for their metabolism & any attempt to interfere with them will result in inhibition & death of the cell.

Sterilization & Disinfection

Sterilization is a term referring to any process that removes or kills all forms of microbial life from an article including viruses, bacteria, fungi & spores, both pathogenic & non pathogenic organisms, sterility is an absolute state. An article should never be described as being relatively sterile or semi sterile, it is either sterile or not. There are four main methods used for sterilization;

1 ; Heat
2 ; filtration
3 ; Radiation
4 ; Chemical disinfection

Disinfection (removing pathogens)

Disinfection means the freeing of an article from some or all of its pathogenic microorganisms to reduce the number to a level at which they pose يشير الىno danger of disease, Disinfectants are applied to non-living objects . Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores, so it is less effective than sterilization , the term is relative & disinfection may be described as being partially or highly effective according to the proportion of the pathogenic organisms killed or removed . washing ,cleaning & ventilation تهوية may remove the majority of the harmful microorganisms from an article or room , heat may be used to disinfect eating utensils اواني& clothing , chemical disinfectant may be used to wipe contaminated floors & furniture .

Sterilization by heat

Moist heat is much more effective than dry heat , moist heat kills microorganisms by coagulating enzymes & structural proteins , while dry heat kill microorganisms by distractive oxidation .

1- Thermal death point (TDP); lowest temperature at which all cells in a culture are killed in 10 min.
2- Thermal death time (TDT) ; time during which all cells in a culture are killed .
3- Decimal reduction time (DRT) ; minutes to kill 90% of a population at a given temperature.

Sterilization by moist heat

Moist heat may be employed ;

(1) at temperatures below 100 C , in the pasteurization of milk , it may be used either 63 - 66 C for 30 minutes or 72 C for 20 seconds (the flash method) .

(2) at a temperature of 100 C in boiling water or free steam for 5 to 10 minutes is sufficient to kill all non spore forming organisms .

(3) at temperatures above 100 C as in the autoclaves (saturated steam under pressure) the first two procedures may be used for disinfection but only the third ensures sterilization .

Sterilization by dry heat

(1) Red heat ; materials held in the flame of Bunsen burner until they are seen to be red .
(2) Flaming ; direct exposure for few seconds in a gas or spirit flame.
(3) Hot air oven ; This is the main means of sterilization by dry heat it is usually used at 160 C for one hour .
(4) Infra red radiation ; heating at or above 200 C by infra red radiation can be employed for surgical instruments .
(5) incineration حرق; is used for the destruction of carcasses , infected laboratory animals and other infected materials to be disposed off .
(6) desiccation تجفيف ; by the use of any heat source even sun light.

Factors influencing sterilization by heat;

(1) The temperature & time ; the relationship is inversely related , shorter times sufficing at higher temperatures & heating must be hot enough for long enough , that's mean the recommended minimal times are the times for which the microbes themselves should be held at the given temperature & do not include heating up time .

(2) The number of microorganisms and spores ; the time for complete sterilization increases in relation to the number initially present because it affect & in practice it is usually to minimize the number of contaminating bacteria by cleaning procedures before applying heat for sterilization .

(3) The species , strain & spore forming ability ; this greatly affect the susceptibility to heat either as the thermal death point or thermal death time .

4-The nature of the material in which the organisms heated ; a high content of organic substances protect

spores & cells against the lethal action of heat , proteins , gelatin , sugars , starch , nucleic acids , fats & oils all act in this way .

Sterilization by Radiation

Radiation may be used in two forms, ionizing and nonionizing. Ionizing radiation, in the form of gamma rays or electron beams, is of short wavelength and high energy. This method is used for the sterilization of disposable supplies, such as syringes, catheters, and gloves. these radiation are lethal to all cells because they induce damage in D.N.A. by various mechanisms including the production of free radicals, Nonionizing radiation in the form of ultraviolet rays is of long wavelength and low energy. Because of its poor penetrability, the usefulness of nonionizing radiation is limited; it can be used to disinfect surfaces and some transparent objects. the effectiveness of ultra violet light (260 nm) as a sterilizing agent increases with decrease in wavelength , these radiation induce thymine – thymine dimmers in D.N.A.

Sterilization by filtration

Filtration methods may be used with both liquid and air , It is possible to render fluids free from bacteria by passing them through special filters with a pore size of less than 0.45 micron , this method is useful in sterilization of air and heat-sensitive solutions, such as serum, parenteral solutions, vaccines, and antibiotics , however these solutions will not be mycoplasma or virus free so these must not regarded as safe for clinical use & should not referred to as sterile.

Chemical disinfectants

chemical agents are used mainly as disinfectants. Some chemical agents, however, may be used to sterilize. These are known as *chemosterilizers*. Chemical agents exert their killing effect by the following mechanisms:

1. Reaction with components of the Cytoplasmic membrane
2. Denaturation of cellular proteins

3. Reaction with the thiol (–SH) groups of enzymes
4. Damage of RNA and DNA

The major groups of chemical disinfectants are ;

1 ; Phenol and phenolic compounds;

are very effective disinfectant, a 5% aqueous solution of phenol kill vegetative cells but spores and viruses are more resistant, its activity reduced at alkaline pH and by organic material ,

low temperature and the presence of soap . phenolic compounds produce a variety of effects on the cell such as ; disruption of cells , precipitation of proteins , inactivation of enzymes and leakage of amino acids from the cell.

2 ; Alcohols ;

ethyl alcohol in concentration between 50 – 90 % is effective against vegetative cells but not against spores , and above 60% concentration is effective against viruses , the mode of action of alcohol may be due to protein denaturation , dissolving lipids complexes in the cell membranes and

damaging it . and also act as dehydrating agents.

3 ; Halogens

a; (iodine) ;

it is highly effective against all kinds of bacteria , spores , viruses and fungi , but its activity affected by the amount of organic material and the extent of dehydration , iodine can be used also in the disinfection of water , iodine is an oxidizing agent and can oxidize and inactivate essential components of the cell such as protein

b ; Chlorine and chlorine compounds ;

it is widely used in the purification of municipal water and food industry , the activity of chlorine against microbes depends on the formation of hypochlorous acid formed when free chlorine is added to water.

4; Heavy metals and their compounds;

most of the heavy metals \ mercury \ silver \ copper combine with cellular proteins and inactivating them or coagulating Cytoplasmic protein and precipitating them causing damage or death of the cell.

5 ; Dyes ;
two classes of dyes have antimicrobial activity , these are ;

1 ; Triphynyl methan dyes ; it include malachite green , brilliant green and crystal violate , and as a role gram positive bacteria are more susceptible to these compounds than gram negative bacteria .

2 ; Acridine dyes ; this include acriflavine and tryptoflavine , the mode of action of dyes are uncertain , but it may interfere with oxidation processes .

6 ; Detergents;
chemically detergents are classified in to ;

1 ; anionic detergents such as soaps and sodium lauryl sulphate

2 ; cationic detergents ; such as cetyl pridinium chloride

3 ; non ionic detergents ; and these substances do not have a significant antimicrobial activity , cationic detergents are regarded as more germicidal than anionic and the quaternary ammonium compounds are in most of them cationic detergents.

7 ; Aldehydes;
two of the most effective are formaldehyde and glutaraldehyde , both are active against vegetative cells and spores, they affect the cell by reacting with its proteins and nucleic acid. Formaldehyde gas is liberated by heating formalin, the atmosphere must have a high humidity 80 -90 % & a temperature at least 18C.

8 ; gaseous agents;
Certain kinds of medical devices are made of materials that are damaged by heat, so sterilization by gaseous agents are effective and practical mean in such cases & the main agents used are;

A - Ethylene oxide; it is highly lethal to all kinds of microbes & spores and capable of rapid diffusion into dry porous materials, it is used for sterilizing articles damaged by heat , its action is by alkylation of enzymes and other proteins , it penetrate well & the usual concentration for use is 400-800 mg/L

B – propiolacton; it is carcinogenic and has low power for penetration, it is usually used in concentration 2-5 mg/L

C - Formaldehyde gas; the gas is liberated by spraying or heating formalin, the atmosphere must have a high relative humidity over 60 per cent & preferably 80 -90 % & a temperature at least 18C

Lec. six ; Pathogenicity

Pathogenicity indicates the ability of an organism to induce a disease in the host , while the establishment of a disease called pathogenesis .

Virulence: - is the degree of Pathogenicity of the organism or quantitative measure of pathogenicity . Virulence depends on the presence of certain cell structures and on bacterial exotoxins and endotoxins, , all of which are virulence factors so virulence factors can be defined as some characteristics of the microbe that increase pathogenicity .

Virulence can be measured by several factors such as;

1 - The fatality rate associated with a certain bacterial infection (LD , MLD or LD_{50} ; The dose that cause the death of 50% of the experemental animals tested under standard conditions) .

2 - The ability of bacterium to invade host tissue.

3 - or by the numbers of microorganisms necessary to cause infection in the host (ID or ID_{50} ; the dose that cause an infection of 50% of the experimental animals) , those organisms that can establish infection with a low dose are considered more virulent than those that require high numbers .

Virulence can be increased by several methods such as;

1. By passage the organisms through a host system that allow a rapid growth of it such as passage *Strept. pneumonia* in a mice.
2. Infection of the organism by special factors, such as the infection of *Corynebacterium diphtheria* by beta phage which induce the production of diphtheria toxin.

Microbial Virulence Factors

The most important factors of virulence include:-

1) Ability to Resist Phagocytosis ;

The most common mechanism for a microorganisms to evade phagocytosis is having a capsule . Many of those possessing a capsule are highly virulent , virulence in a capsulated strains becomes extremely low , another bacterial structure that protects organisms from phagocytosis is M protein of *Strep. pyogenes* and protein A of *Staph. aureus* . Some organisms evade phagocytosis by releasing potent materials in tissues that kill phagocytes. Streptococci produce hemolysins that lyse red blood cells but also induce toxic effects on white blood cells and macrophages. Pathogenic staphylococci release leukocidins called Panton-Valentine which is lethal to leukocytes

2) Surface Structures That Promote Adhesion ;

The cell surface structures that mediate attachment are called adhesins. The main adhesins in bacteria are the fimbriae (pili) and capsule .

3) Ability to Survive Intracellularly and Proliferate ;

Some pathogens are able to survive within the phagocytic cell after they have been engulfed. These organisms have developed methods to prevent being killed intracellularly. Some prevent fusion of phagosomes and lysosomes, others have a resistance to the effects of the lysosomal contents, and still others escape from the phagosome

4) Ability to Produce Extracellular Toxins and Enzymes ;

Tissue damage may occur from toxins, either exotoxins or endotoxins, or from inflammatory substances that mediated damage. Some organisms produce soluble substances, such as proteases and hyaluronidases that liquefy the hyaluronic acid of the connective tissue matrix, helping to spread bacteria in tissues, thereby promoting the dissemination of infection. most pathogenic bacteria produce toxin that are capable of damaging host tissue by different ways, bacterial toxins can be divided into two groups ;

Exotoxins ; are proteins which released into the extracellular environment produced by both gram positive and gram negative bacteria , while endotoxin produced by gram negative bacteria only , also exotoxins are heat labile and released into the medium without cell lysis and are highly potent. Most exotoxins are destroyed by heating to 100^{o}C. Some toxins can be converted to TOXOIDS which are no longer toxic, but can stimulate antibody production against the toxin.

Endotoxin ; which are also referred as lipopolysaccaride (L.P.S.) is an integral part of the cell wall of gram negative bacteria and released from cells after death and lysis , it is very heat stable , has a low toxicity in comparison to exotoxins , it is toxic in milligram quantities while most exotoxins are toxic in microgram quantities , the toxicity of LPS has been shown to reside in the lipid A portion. Both toxins may be transported by blood and cause effects at tissues remote from the original point of entry or growth .

Differences between Endotoxin and Exotoxin

	Endotoxin	Exotoxin
Chemical nature	Lipopolysaccaride	Protein
Relationship to cell wall	Part of outer membrane	Extracellular, diffusible
Denatured by boiling	No	Usually
Antigenicity	No	Yes
Pyrogenicity	Yes	Occasionally
Form toxoid	No	Yes
Enzymatic activity	No	Usually
Potency	Relatively low (less than 100ug)	Relatively high (1ug)
Specificity	Low	High

STEPS IN THE DISEASE PROCESS

the disease process pass through a series of sequential stages:

INFECTION = The pathogen establishes itself in or on the host. No symptoms are yet present and the host is unaware of the infection.

INCUBATION PERIOD = This is the period of time it takes for the pathogen to establish itself to the point where the first disease symptoms appear. This varies widely, for most bacteria it takes 2 to five days, but for some like T.B. or leprosy it may be 20 to 30 years.

INITIAL SYMPTOMS = These refer to the first symptoms that clearly demonstrate an illness. Subclinical cases (asymtomatic cases) are very common

ACUTE = This refers to the clinical symptoms, where the disease is in full flower and the patient is usually seriously or clearly ill.

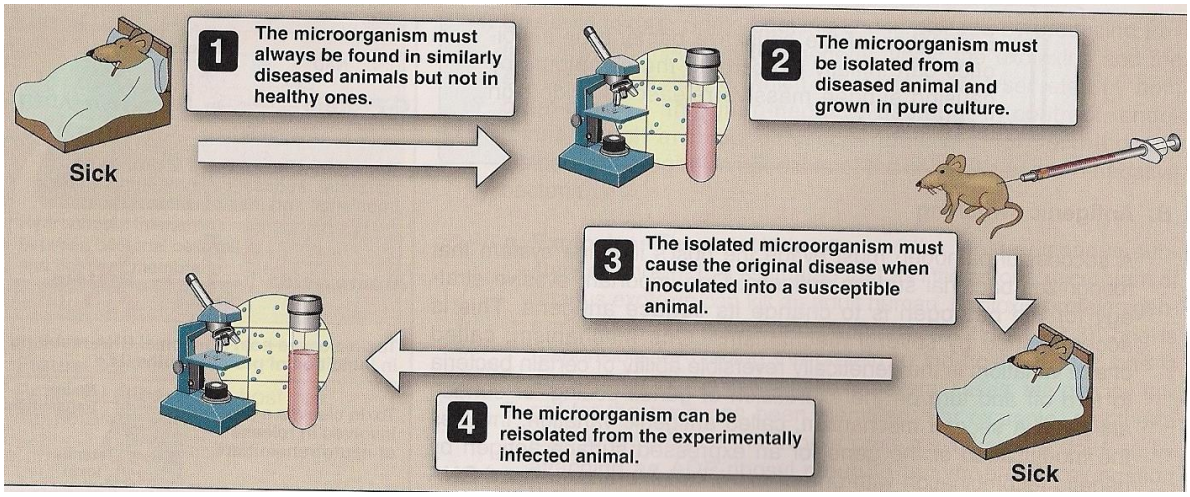
RECOVERY = Period during which the symptoms decline and the patient recovers. Recovery may take many paths.

1. In many cases the etiological agent is totally eliminated and the patient returns to full health.
2. In other cases, the patient shows a full recovery but the infectious agent is still present. Under these conditions the patient becomes a CARRIER and remains capable of spreading the virulent form of the infectious agent for some period.
3. In many cases a disease becomes CHRONIC. The victim makes a partial recovery, but they are still less well than normal .

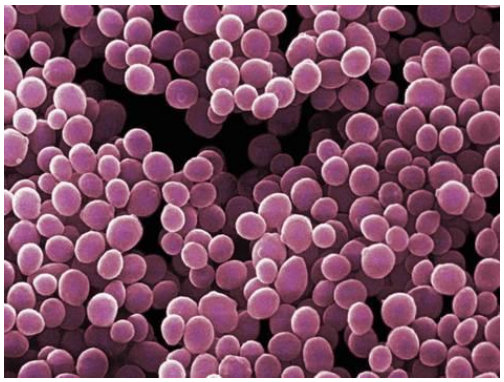
Koch's postulates

Robert Koch (1843-1910) using criteria developed by his teacher, Jacob Henle (1809-1895), established the relationship between Bacillus anthracis and anthrax; his criteria became known as Koch's Postulates and are still used to establish the link between a particular microorganism and a particular disease:

1) The microorganisms must be present in every case of the disease but absent from healthy individuals .
2) The suspected microorganisms must be isolated from the diseased individuals and grown in pure culture .
3) The same disease must result when the isolated microorganism is inoculated into a healthy host .
4) The same microorganism must be re-isolated again from the experimentally infected animals.


Sick
1 The microorganism must always be found in similarly diseased animals but not in healthy ones.
2 The microorganism must be isolated from a diseased animal and grown in pure culture.
3 The isolated microorganism must cause the original disease when inoculated into a susceptible animal.
Sick
4 The microorganism can be reisolated from the experimentally infected animal.

Luc. seven ; genus Staphylococcus



Facultative anaerobes, gram positive cocci occur in grape like clusters some single cells, pairs and short chains are seen, they are non motile , non spore forming cocci with a diameter of 1 micron and are catalase positive , major components of the normal flora of the skin and nose, the most important species are *Staph.aureus, Staph.epidermidis* and *Staph.saprophyticus*, the ability of *Staph.aureus* to form coagulase separate it from the other less virulent species.

Staph. aureus

Staph. aureus is a Gram-positive cocci that is frequently found in the human respiratory tract and on the skin. It is positive for catalase and nitrate reduction. Although *Staph. aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. Disease-associated strains often

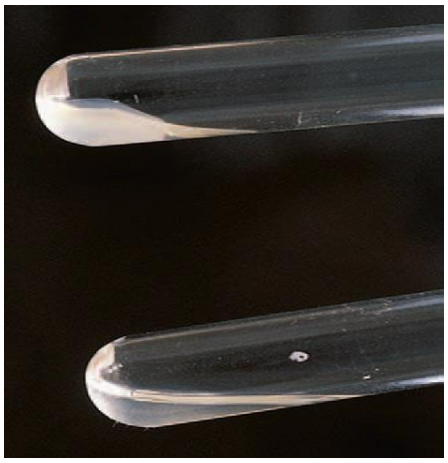
promote infections by producing potent toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic *Staph. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine, most strains are relatively heat stable and withstanding temperatures as high as 60 C for 30 min., facultative anaerobic and produce the enzyme catalase that catalyzes the following reaction $2\ H_2O_2$---------- $2\ H_2O + O_2$

morphologically it is similar to streptococci but can be differentiated from it by testing for the enzyme catalase; staphylococci possess this enzyme while streptococci do not.

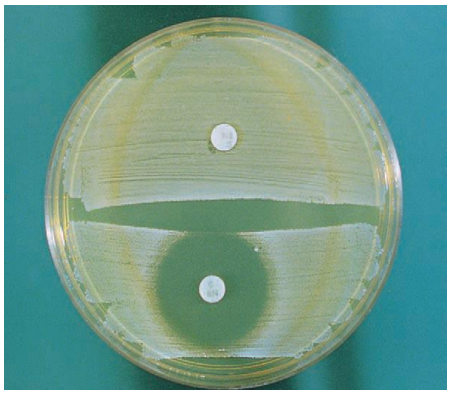
it is the commonest causes of opportunistic infections in the hospital and community, including pneumonia, osteomyelitis, septic arthritis, bacteremia, endocarditis, abscesses, boils and other skin infections. Extracellular enzymes produced by *Staph. aureus* include: coagulase, fibrinolysin, DNAse, lipases and hyaluronidase. *Staph. aureus* is responsible for many infections but it may also occur as

a commensal. The presence of it does not always indicate infection, it can survive from hours to weeks, or even months, on dry environmental surfaces, it can infect tissues when the skin or mucosal barriers have been breached.

However it is now known that not all *Staph. aureus* produce coagulase, an enzyme that converts fibrinogen into fibrin, causing blood plasma to clot. it is most important test used to distinguish *Staph.aureus* from other Staphylococci .



Staphylococci that do not produce coagulase are referred to as coagulase-negative staphylococci (CoNS). The most clinically significant species in this group are *Staph. epidermidis* and *Staph. saprophyticus.*



Staph . *epidermidis* has been known to cause various hospital acquired infections, whereas *Staph*. *saprophyticus* is associated mainly with urinary tract infections in young

women.

Isolates that are coagulase negative are further identified by testing for **novobiocin Susceptibility** *Staph.saprophyticus* is resistant to novobiocin, whereas most other coagulase-negative staphylococci are susceptible

Virulence factors;

The pathogenicity of S. aureus can be attributed to a number of virulence factors including ;

1-Enzymes

Staph. aureus produces various enzymes such as coagulase which clots plasma and coats the bacterial cell to prevent phagocytosis. Hyaluronidase (also known as spreading factor)

breaks down hyaluronic acid present in the intracellular ground substance that makes up connective tissues and helps in spreading of bacterial cells during infection. *Staph aureus* also produces DNAse (deoxyribonuclease) which breaks down the DNA, lipase to digest lipids, staphylokinase to dissolve fibrin and

aid in spread, and beta-lactamase (penicillinase) for drug resistance.

2-Toxins ;

Pathogenic Staphylococci release a number of different toxins such as;

(1) it produces four types of hemolysins which are referred as alpha, beta, gamma and delta, the alpha toxin is the only hemolysine of clinical importance it attack the membranes of erythrocytes causing lysing of them in addition it can damage platelets and macrophages and cause severe tissue damage .

(2) ; Leukocidin (Panton-Valentine leukocidin) : a none hemolytic toxin and thought to kill white blood cells by causing them to degranulate .

(3) ; enterotoxins are heat- stable exotoxins that cause a variety of symptoms including diarrhea and vomiting. Nine serologically distinct enterotoxins have been identified that fall into the following groups, A to E and G to J. Because the enterotoxins are stable at 100° C for 30 minutes,

reheating contaminated food will not prevent disease. Staphylococcal food poisoning is most commonly caused by enterotoxins A, B, and D.

(4) ; Exfoliatin; it is a heat stable toxin and responsible for the Staphylococcal scalded skin syndrome (SSSS) which occurs most commonly in infants and young children. also known as Ritter's disease.

(5) ; the Staphylococci also produce a toxin associated with toxic shock syndrome (TSS) , the toxin is now referred to as TSS-toxin1 (TSST-1) , the syndromes include fever, rash, vomiting , diarrhea and toxic shock

In addition to the exotoxins produced by *Staph. aureus* it appears to produce carbohydrate antiphagocytic capsule , it also produce ((protein A)) a cellular components in the cell wall of *Staph* . *aureus* . This substance is capable of binding to the Fc

portion of immunoglobulin IgG. and block phagocytosis.

Staph. epidermidis
Non hemolytic, white colonies (non pigmented) , coagulase negative do not ferment mannitol , Less common cause of opportunistic infections than *Staph. aureus* , but still significant and can infect prosthetic heart valves and venous catheters and may cause sub acute bacterial endocarditis.

Staph. aureus is more virulent than *Staph. epidermidis* for several reasons
1; it is more resistant to phagocytosis than *Staph. epidermidis*
2; it can survive in phagocytic cells as much as 5% of population , while *Staph. epidermidis* can not
3; only *Staph. aureus* produce alpha toxin
4*; Staph. aureus* can produces enterotoxin causing food poisoning

Laboratory diagnosis

A specimen is sent to the laboratory for identification . A Gram stain is first performed to guide the way, which should show typical Gram-positive bacteria, cocci, in clusters , however *Staph. aureus* can not be differentiated from other gram positive cocci on morphologic grounds, Second, the specimen is cultured on blood agar because it promotes pigment formation & permit direct detection of hemolyses , *Staph. aureus* usually produce a golden-yellow pigment , and also cultured on mannitol salt agar, which is a selective medium with 7–9% NaCl that allows *Staph. aureus* to grow, producing yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH. Furthermore,

catalase . coagulase , DNAse and lipase tests are done. Bacteriophage testing or serotyping may be utilized

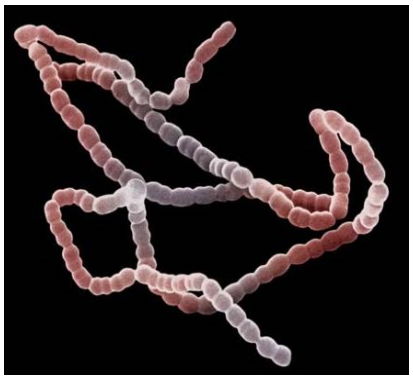
	Coagulase production	Mannitol fermentation	Beta hemolyses	Gelatin liquefaction	proteolysin	Growth in 7,5% Nacl
Staph. aureus	+	+	+	+	+++	+
Staph. Epidermidis	__	__	__	+ / _	+	_
Staph.citreus	__	__	__	__	__	_

Antibiotic therapy

Staphylococci (including both coagulase positive & coagulase negative) can produce penicillinase that degrade penicillin and methicillin are often ineffective, thus other penicillinase-resistant β-lactam antibiotic for

example, oxacillin or flucloxacillin , Combination therapy with gentamicin may be used to treat serious infections , Vancomycin is currently the drug of choice but now resistance to vancomycin is spreading now.

Lec. eight; The Streptococci



Streptococci are facultative anaerobic, Gram-positive organisms that often occur as chains or pairs and are Catalase – negative. Streptococci are subdivided serologically into groups depending on a specific carbohydrate antigen on their cell wall {Lancefield classification(Rebecca Lancefield 1895 – 1981} and the various groups are designated A through O .The most important groups of streptococci are A, B and D., infectious disease (particularly pharyngitis) is caused by group A. *Streptococcus pneumoniae* (a major cause of human pneumonia) and *Streptococcus mutans* and others called

viridans streptococci (among the causes of dental caries).

The Streptococcal cell wall contains proteins antigens (M,R,T) but the M-protein is the most important one and it exist in about 80 different forms and classification of Streptococci depending on this protein is known as (Griffiths classification), the M-protein is the sole protective antigen and needed for virulence, it is strongly antiphagocytic, toxic to platelets, determine adhesion to the epithelial cells surface, and strains lacking M-protein are either of reduced virulence or a virulent, production of secretory IgA will cause coating of M-protein and thus preventing adherence.

Also Streptococci may be classified on the bases of their hemolytic reaction on blood agar; there are three types of hemolysis reactions (alpha, beta, and gamma).

Alpha refers to partial hemolysis with a green coloration seen around the colonies; beta refers to complete clearing and gamma means there are no lyses.

Virulence Factors & Pathogenesis

Virulence factors of group A streptococci include

(1) M protein causes the streptococcal cell resist phagocytosis and lipoteichoic acid plays a role in adherence to mucosal cells.

(2) a hyaluronic acid capsule that inhibits phagocytosis;

(3) other extracellular products, such as pyrogenic (erythrogenic) toxin, which causes the rash of scarlet fever

(4) extracellular products, including hemolysins, toxins, and enzymes such as

streptokinase, streptodornase (DNase B) .

(5) Other products produced by *S. pyogenes* are streptolysins. streptolysin O (The *O* refers to this hemolysin being oxygen labile) . streptolysin S (S is oxygen stable) , all of these products play a role in virulence

Group A streptococcus (*S. pyogenes*)

This organism is beta hemolytic bacteria and bacitracin sensitive (minimum 10 mm inhibition zone) , other beta hemolytic streptococci usually are resistant so this test is a presumptive test to identify

beta hemolytic group A streptococci from other beta hemolytic strept.

Of other groups , it causes suppurative, but non-invasive pharyngitis and less frequently the skin infection, impetigo. Group A streptococcal infections also known as group A *Streptococcus* (GAS) affect all ages with peak incidence at 5-15 years of age and is the

causative agent of a wide range of infections such as ;

1- Rheumatic fever ; is an inflammatory disease affecting primarily the heart and joints.
2- Acute glomerulonephritis. This is an immune complex disease of the kidney.
3- Scarlet fever. The characteristic rash is caused by erythrogenic (pyrogenic)

toxins which are phage encoded

4- Bacteremia and toxic-shock Production of pyrogenic toxins (A, B and C) are involved in the pathogenesis. This disease can progress very quickly (a few days) and is life-threatening.

Laboratory diagnosis

1. Direct detection - the antigen is extracted from a throat swab. The antigen extract will then bind with antibody specific to the group A streptococcal carbohydrate.
2. Lancefield grouping of isolated beta hemolytic colonies
3. Colonies are beta hemolytic and their growth is inhibited by bacitracin
4. Patient serum shows antibodies to streptolysin O or other streptococcal antigens. Beta hemolysis is caused by

two hemolysins O and S; the former is inactive in the presence of oxygen & is only cause beta hemolysis under anaerobic conditions.

5. the capsule of the Streptococci can be fixed and become visible microscopically in a test known as quelling test which is useful in microbial identification

Group B streptococcus (*S. agalactiae*)

S. agalactiae causes pneumonia and meningitis in neonates after transmission from the normal flora of the mother during delivery. It may affect the elderly with occasional systemic bacteremia. They can also colonize the intestines and the female reproductive tract

The organism can be identified on the basis

of beta hemolysis, hydrolysis of hippurate (positive) , bacitracin susceptibility (negative = resistant) and positive CAMP reaction ((Christie, Atkins, and Munch-Petersen)) .

Group B streptococci produce a factor that increases beta hemolysis of a *Staph. aureus* indicator strain and give positive reaction with an arrowhead zone

Group D streptococcus

Group D streptococci are divided into those that will grow in 6.5% saline (enterococci) and those that will not (non-enterococci). Enterococci much more commonly cause human disease than non-enterococci. Enterococci are often resistant to penicillin.

In 1984, many organisms formerly considered *Streptococcus* were moved into

the genera *Enterococcus* and *Lactococcus*. the most commonly isolated is *E. (S.) faecalis* as the name implies enterococci are found in the gut flora and infection often follows from fecal contamination. A significant cause of urinary tract infections and also opportunistic infections . Colonies are usually alpha or gamma hemolytic.

Viridans streptococci

These are a diverse group of species commonly found orally (including *S. mutans*) and cause endocarditis after release into the bloodstream from tooth extraction . They are also involved in dental caries. They are alpha hemolytic and negative for other tests described above. They are non-groupable .

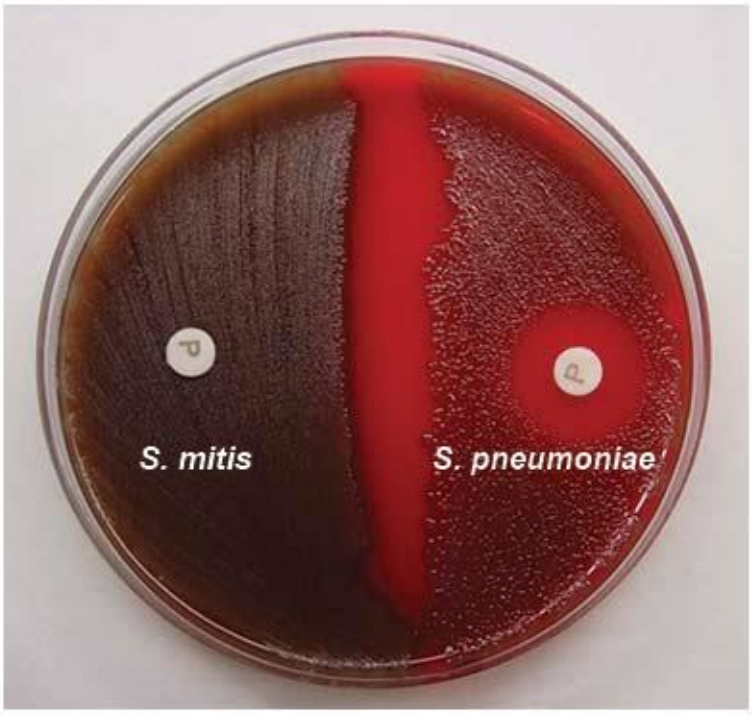
Alpha-hemolytic *Streptococcus pneumonia*

S. pneumoniae (sometimes called pneumococcus), is a leading cause of bacterial pneumonia and occasional etiology of otitis media, sinusitis, meningitis, and peritonitis. most strains prefer an atmosphere of 5-10 % CO_2 for primary culture, Pneumococcus shows a well defined capsule .

colonies on blood agar are small, flat with a raised rim & around the colonies there is a greenish discoloration (alpha hemolysis)

and are confused sometimes with other alpha hemolytic Streptococci such as *Strep. viridans*, the pneumococci is distinguishable from the latter organisms by the following

Pneumococci	viridans streptococci
1. soluble in bile	non- bile soluble
2. sensitive to optochin	resistant to it
3. ferment inulin	dose not
4. capsulated	Uncapsulated
5. draughtsman colonies	not so (small convex colonies)



Left Side

S. mitis

Resistant to optochin

Right Side

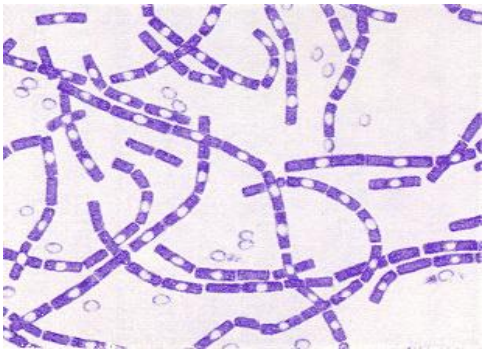
S. pneumoniae

Susceptible to optochin

it is sensitive to a wide range of antibiotics including penicillins, tetracyclines & sulphonamides.

Lec. nine; genus Bacillus

The genus bacillus includes large aerobic gram positive rods occurring in chains, most members of this genus are saprophytic organisms prevalent in soil,



The endospores are ellipsoidal shaped and located in the center. At least 48 species are known but only *B. anthracis* and *B. cereus* cause disease in humans.

B. anthracis is responsible for the disease anthrax. This is a disease primarily of animals but humans can acquire via handling, inhaling or ingesting contaminated animal products.

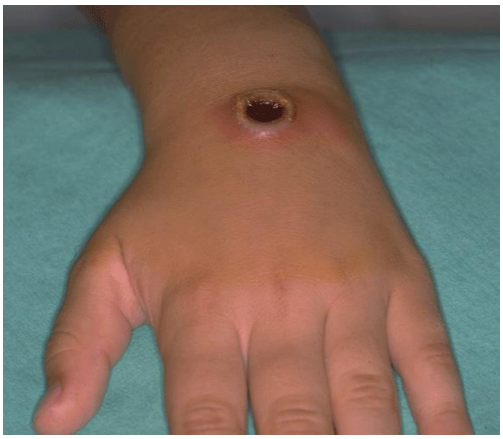
B. cereus is predominantly responsible for food poisoning in humans. *B. cereus* food poisoning results from the ingestion of preformed enterotoxins, producing predominantly vomiting and diarrhea. The vomiting form occurs within 1-6 hours is most often associated with ingestion of a heat stable toxin from contaminated rice, while the diarrheal form occurs within 8-12 hours is most often associated with ingestion of a heat

labile toxin from contaminated meat or vegetables.

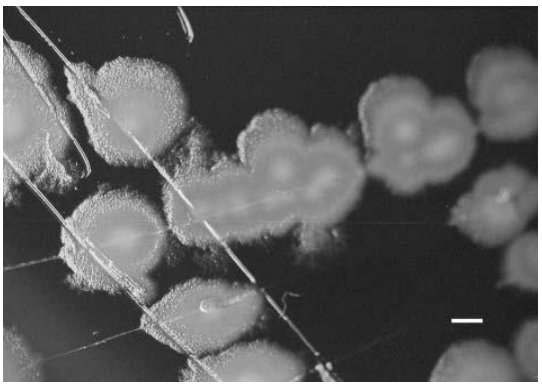
B.anthracis cause anthrax when spores usually enter through injured skin (cutaneous anthrax the most common form 95%) , after 1-7 days it causes a localized, inflammatory, black, necrotic lesion , Spread of bacteria causes regional lymph tenderness which may be followed by a toxic septicemia and death.

(gastrointestinal anthrax) it is rarely but also fatal (causes death to 25%) , results from ingestion of spores. with contaminated food produces systemic symptoms which can lead to death.

the highly fatal form of anthrax caused through inhalation of spores into the lung (pulmonary anthrax , wool sorters' disease) and characterized by sudden, massive chest edema followed by cardiovascular shock.which leads to death.



Robert Koch in 1877 grow the organism in artificial culture using pure culture techniques *Bacillus anthracis* is very large, Gram-positive, spore forming rod, 1 - 1.2µm in width x 3 - 5µm in length, non motile, found singly or in short chains in smears of infected tissues,



colonies have a rough, uneven surface, the cells in culture are non capsulated but in the host body they usually have a capsule and unlike most other bacterial capsules, it contains a glutamic acid polypeptide, the Bacilli that does not produce a capsule is not virulent and does not induce anthrax in the test animals, the capsule gene is on a plasmid. The bacterium can be cultivated in ordinary medium under aerobic or anaerobic conditions.

The optimal temperature for growth is 35C but when grown at 42 -43C the organism becomes attenuated or a virulent, the loss of virulence is due to the loss of capsule.

Pathogenicity of *Bacillus anthracis*

The pathogenicity of *Bacillus anthracis* is due to two major determinants of virulence:

1) the capsule
2) the production of anthrax toxin.

The capsule itself is nontoxic, but functions to protect the organism against complement and the bactericidal components of serum and phagocytes.

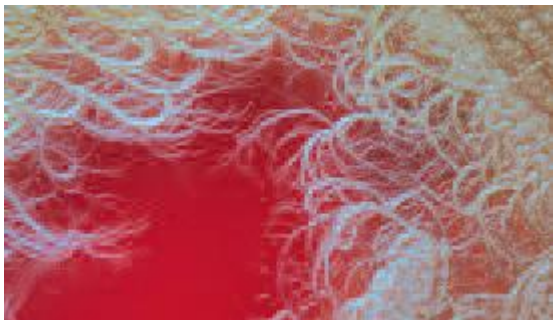
Anthrax Toxin

Anthrax toxin is made up of three proteins; protective antigen (PA) so named because of its use in producing protective anthrax vaccines, edema factor (EF) responsible for the severe edema usually seen in *B. anthracis* infections, and lethal factor (LF) is responsible for tissue necrosis,

PA mediates cell entry of edema factor and lethal factor, it binds to specific cell receptors, and following proteolytic activation it forms a membrane channel that mediates entry of the two factors into the cell. EF combine with PA and form a toxin known as edema toxin, LF plus PA form lethal toxin which is a major virulence factor and cause of death in infected animals. Each component of the toxin is a thermo labile protein. The three factors exhibits no significant biological activity. However, combinations of two or three of the toxin components yield the effect of the toxin. The anthrax vaccine is a preparation of the protective antigen recovered from a virulent, none capsulated strain of *Bacillus anthracis* that produces PA during active growth.

Diagnostic laboratory tests

Several nonselective and selective media for the detection and isolation of *Bacillus anthracis* have been described , Specimens to be examined are fluid or pus from a local lesion, blood and sputum. Stained smears often show chains of large gram positive rods.



Anthrax bacilli when grown on blood agar produce non hemolytic gray to white colonies with a rough texture and comma shaped outgrowths (Medusa head) may project from the colony.

In semisolid medium, anthrax bacilli are always nonmotile, while related nonpathogenic organisms (eg; *B.cereus*) exhibit motility.

demonstration of capsule requires growth on bicarbonate containing medium in 5 – 7 % carbon dioxide.

Lysis by a specific anthrax gamma bacteriophage may be helpful in identifying the organism.

An enzyme – linked immunoassay (ELISA) may be used to measure antibodies against edema and lethal toxins,

a positive result is a fourfold change or a single titer of greater than 1:32. The following table provides the differential

characteristics that are used to distinguish *Bacillus anthracis* from most strains of *B. cereus* and *B. thuringiensis* which is an insect pathogen.

Characteristic	*B. anthracis*	*B. cereus* and *B. thuringiensis*
growth requirement for thiamin	+	-
hemolysis on sheep blood agar	-	+
glutamyl-polypeptide capsule	+	-
lysis by gamma phage	+	-
Motility	-	+

Treatment of Anthrax

Antibiotics should be given to unvaccinated individuals exposed to inhalation anthrax. Penicillin, erythromycin, tetracycline and ciprofloxacin are effective if administered

early. Antibiotic treatment is also known to lessen the severity of disease in individuals who acquire anthrax through the skin. Inhalation anthrax was formerly thought to be nearly 100% fatal despite antibiotic treatment, particularly if treatment is started after symptoms appear. Vaccines composed of killed bacilli are not effective, acquired immunity is due to antibodies against the capsule and the toxin, high risk peoples should be vaccinated with the protective antigen portion of the toxin .

Lec. ten; *Corynebacterium diphtheriae*

Diphtheria is one of the most important diseases of bacterial etiology. The causative organism is toxin-producing *Corynebacterium diphtheriae*, which infects the tissues of the pharynx .

Corynebacteria are small and pleomorphic, the genus includes many species of aerobic and facultative anaerobic gram positive rods, there are more than 60 species in the genus, and at least 40 are thought to be clinically significant.

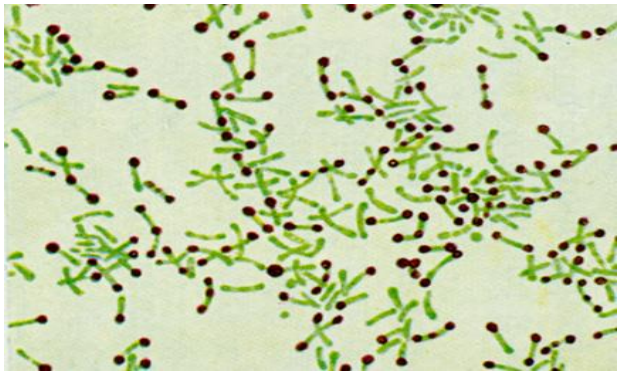
the cells tend to have clubbed ends and often remain attached after division, forming chine's letter arrangements, spores are not formed , growth is generally best under aerobic conditions on media enriched with blood but many strains will grow an aerobically, colonies on blood agar are typically small (1 to 2 mm) and most are nonhemolytic,

catalase is produced and many strains form acid through carbohydrate fermentation

Corynebacterium diphtheriae are Gram-positive, catalase positive, aerobic, non motile, rod-shaped bacteria. They have a club-shaped or V-shaped arrangements and forms resembling Chinese letters, grows best under strict aerobic conditions, colonize the upper R.T.(pharynx & nose) and less commonly the skin.

C. diphtheriae is a pathogenic bacterium that causes diphtheria. It is also known as the Klebs-Löffler bacillus, because it was discovered in 1884 by German bacteriologists Edwin Klebs and Friedrich Löffler .

it produces a powerful exotoxine that is responsible for the disease, other

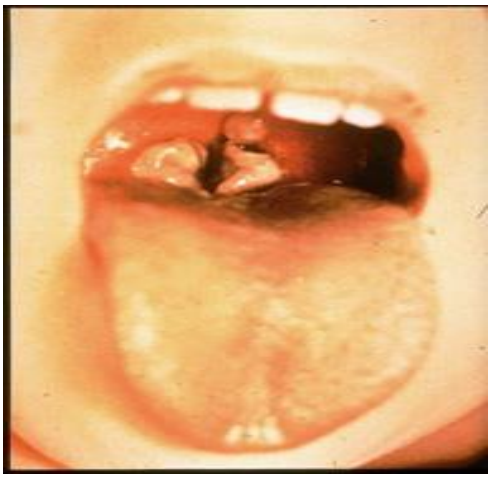


Corynebacteria are nonpathogenic commensal inhabitants of the pharynx, nasopharynx and skin,

they are collectively referred as diphtheroids. The term *diphtheroid*, meaning “diphtheria-like,” is sometimes used in reference to this Gram staining morphology.

C. diphtheriae are identified by growth on Loffler's media followed by staining by Albert's stain for metachromatic granules (polyphosphate granules), the term metachromatic refers to the color differences of the intracellular polyphosphate granules (reddish) compared to the rest of the cell (green).

Diphtheria is an upper respiratory tract illness characterized by sore throat, low fever, and an adherent membrane (called a pseudo membrane) on the tonsils, pharynx, and/or nasal cavity. Diphtheria toxin produced by *C. diphtheriae*, can cause systemic toxic effects. The gene for toxin synthesis is encoded on a bacteriophage which act as genetic determinant controlling toxin production, toxigenecity remaining as long as the cells are lysogenic when bacterium is cured from phage it loses its toxigenecity , so *C.diphyheriae* not infected with phage do not cause diphtheria (lysogenic conversion) .



organism spreads by airborne upper respiratory tract droplets or direct contact with respiratory secretions of symptomatic individuals .

Dust and clothing also contribute to transmission as the organism may survive up to 6 months in dust and vomits . once the toxin entered the cells, antitoxin is no longer effective.

Pathogenicity and virulence factors

Corynebacterium diphtheriae causes two different forms of disease in humans: respiratory and cutaneous diphtheria. In the cutaneous form of diphtheria, which is prevalent in the tropics, the toxin is also absorbed systemically, but systemic complications are less common than from upper respiratory infections. Cutaneous diphtheria consists of non healing ulcers with a dirty gray membrane.

C. diphtheriae has little invasive capacity but the major virulence determinant is diphtheria toxin

(DT) which has a potent cytotoxic effect and has both local and systemic effects,

locally its action on epithelial cells leads to necrosis and inflammation, forming a pseudo membrane composed of a coagulum of fibrin, leukocytes and cellular debris, absorption and circulation of D.T. allows binding throughout the body and inhibits protein synthesis in eukaryotic cells , myocardial cells are most affected .

Toxin consists of two factors A and B. Fragment A is responsible for the cytotoxicity, and fragment B binds to receptors on the eukaryotic cells and mediates the entry of fragment A into the cell.

Diphtheria toxin is protein in nature (M.W. 62,000 Dalton) it is extremely potent, lethal dose for humans is 130 ng/kg of body weight .

C. diphtheriae also produces **diphthin** , which is a protease that inactivates IgA.

and produce the enzyme **neuraminidase** which splits N-acetylneuraminic acid (NAN) from cell surfaces to produce pyruvate which acts as a growth stimulant.

Three strains of *C. diphtheriae* are recognized, gravis, intermedius and mitis. All strains produce the identical toxin and are capable of colonizing the throat.

The subspecies differ slightly in their colonial morphology and biochemical properties, such as the ability to metabolize certain nutrients, but all may be toxigenic (and therefore cause diphtheria) or non-toxigenic.

The differences in virulence between the three strains explained by their differing abilities to produce the toxin in rate, quantity and growth rates.

Distinguishing characters of sub species of *C.diphtheriae*

Organism	Urease test	Nitrate reduction	Esculin hydrolysis	Glycogen fermentation	Lipophilic property
C.diphtheriae subsp. Gravis	–	+	–	+	–
C.diphtheriae subsp. Intermedius	–	+	–	–	+
C.diphtheriae subsp. Mitis	--	+	--	--	--

Laboratory identification;

In order to accurately identify *C. diphtheriae*, a Gram stain is performed to show gram-positive,
highly pleomorphic organisms with no particular arrangement.

Special stains like Alberts's stain and Ponder's stain are used to demonstrate the metachromatic granules formed in the polar regions.

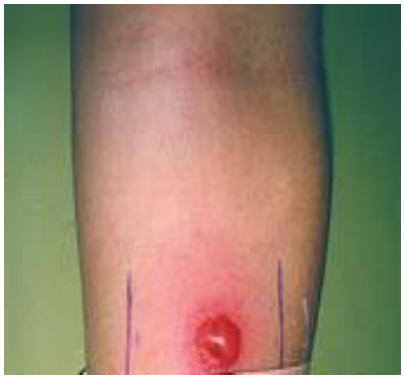
C.diphtheriae can be isolated easily from an enrichment selective medium such as Löffler's medium or Tinsdale's agar which contain potassium tellurite, an inhibitor of other respiratory flora, and on which the organism produces black colonies due to the reduction of tellurite by the bacteria

Immunity to Diphtheria

Acquired immunity to diphtheria is due to toxin-neutralizing antibody (antitoxin). Toxoid is given in

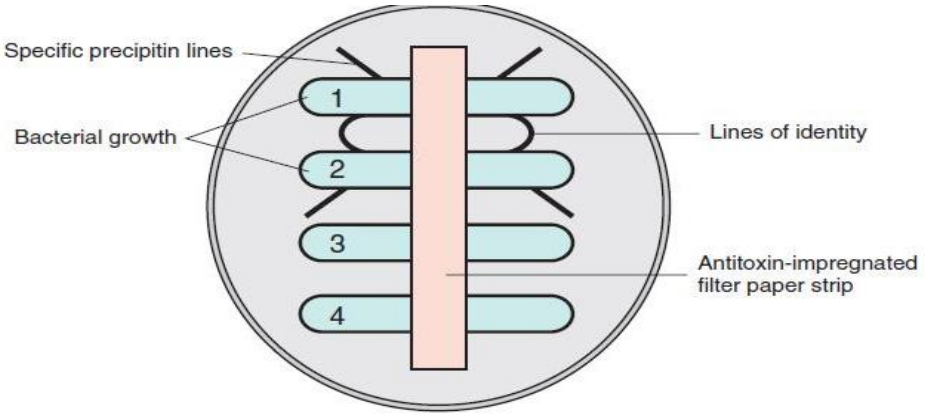
2 or 3 doses (1 month apart) for children as a primary immunization at an age of 3 - 4 months. A booster injection should be given about a year later, and it is advisable to administer several booster injections during childhood. Usually, infants are immunized with a trivalent vaccine containing diphtheria toxoid, pertussis vaccine, and tetanus toxoid (DPT vaccine).

Immunity can be monitored by Schick test. The test can be used to determine whether or not a person is susceptible to diphtheria . It involves injecting of a small amount (0.1 ml) of diluted (1/50 MLD) diphtheria toxin intradermally into one arm of the person and evaluating the injection site after 48 hours. A positive test (inflammatory reaction) indicates susceptibility (no



immunity). The absence of a reaction, A negative test indicates immunity (antibody neutralizes toxin).

Test for Toxigenicity.



Elek's test for toxogenicity is used to determine whether the organism is able to produce the diphtheria toxin or not.

a filter paper strip impregnated with diphtheria antitoxin is buried just beneath the surface of a special agar plate (of low iron content because the addition of iron inhibits toxin production very quickly) , strains to be tested and known positive and negative toxigenic strains are streaked on the agar surface in a line across the plate and at a right angle to the antitoxin paper strip, after 24 hours incubation at 37C , plates are examined for the

presence of fine precipitin lines at a 45 degree angle to the streaks, the presence of precipitin lines indicates that the strain produced toxin that react with the homologous antitoxin.

Treatment;

Treatment of diphtheria requires neutralization of toxin followed by eradication of the organism, a single dose of antitoxin inactivates any circulating toxin although it does not affect toxin already bound to a cell surface receptor,

C. diphtheriae is sensitive to several antibiotics such as penicillin, erythromycin, clindamycin, rifampicin and tetracycline.

Penicillin and erythromycin appear to be the drugs of choice .

Luc eleven ; Haemophilus and Bordetella

HAEMOPHILUS ;

Members of the genus *Haemophilus* are small, nonmotile Gram-negative bacteria. The genus contains many species but *H. influenzae* is the most common pathogen. *Haemophilus* are present in the normal flora of the human mouth and respiratory tract.

Haemophilus influenzae

The organism is a small Gram negative rod which can be grown on chocolate agar and requires hemin (factor X) and nicotinamide-adenine-dinucleotide (factor V) for growth. Growth with 5% CO_2 is enhanced.

H.influenza does not grow on normal media which requires both factors (X and V),

H. parainfluenzae which requires factor V only and *H. ducreyi* which requires factor X only.

H. influenzae will only grow on blood agar around colonies of *Staphlyococcus aureus (satellite phenomenon),*

H. influenzae strains are divided on the basis of capsular polysaccharides (A - F) or the absence of a capsule (nontypeable).

Antigenic structure

Capsulated *H.influenzae* contains capsular polysaccharides of one of 6 types (A-F); these polysaccharides resemble those of pneumococci and sometimes give serologic cross reactions with pneumococcal types.

The somatic antigen of H.influenzae consists of at least 2 proteins; the P substance constitutes much of the bacterial body, whereas the M substance is a labile surface antigen. Capsulated *H.influenzae* can be typed by a test is analogous to the "Quelling test "for pneumococci.

Pathogenesis:

The most common severe types of *Haemophilus influenzae* disease are:

Pneumonia (lung infection)

Bacteremia (bloodstream infection)

Meningitis ; This is the most serious infection caused by capsulated Haemophilus influenza type b.

H. influenzae produces no exotoxin.

Diagnostic lab.Tests ;

Specimens consist of nasopharyngeal swabs, pus, blood and spinal fluid for smears and cultures;

1- Direct identification; when organisms are present in large numbers in specimens, they may be identified by immunofluorescence or may be mixed directly with specific antisera (type B) and a capsule swelling test performed.

2- Culture ; The organism grows well in culture on the appropriate media (isovitalex enriched chocolate agar) until typical colonies can be identified with the capsule swelling test (in 36 – 48 h),

3- it also can be differentiated from related gram negative bacilli by observing the requirements for X and V factors, hemolysis on blood agar and by immunologic means. Blood cultures may be delayed since commercially prepared blood culture broth does not contain X and V factors.

Characteristics and growth requirements of some hemophilic organisms.

Organism	Hemolysis	Requires		Capsule
		X	V	
H.influenzae	--	+	+	+
H.parainfluenzae	--	--	+	+
H.haemolyticus	+	+	+	--
H.suis	--	+	+	+
H.haemoglobinophilus	--	+	--	--
B. pertussis	+	--	--	+

other *H.* species;

H. ducreyi is the causative agent of chancroid (soft chancre), a sexually transmitted disease. The chancroid is an ulcer on the genitalia with marked swelling and tenderness. The regional lymph nodes are enlarged and painful. Incubation is generally 3 to 14 days after exposure.

H. ducreyi is a fastidious organism and laboratory diagnosis is made by isolation on chocolate agar with 1% isovitalex and vancomycin. The organism can be isolated from the ulcerated chancroid exudate and a stained smear shows Gram-negative short rods occur in strands. Serologically chancroid can be diagnosed by using killed *H. ducreyi* cells serving as a skin test antigen (Ducrey's skin test), the test become positive 1-2 weeks after infection and may remain positive for years, chancroid infection give no permanent immunity.

H.aegyptius (kock-Weeks Bacillus)

This species is very similar to *H.influenzae* and causes conjunctivitis.

Treatment and prevention:

The mortality rate of untreated *H.influenzae* meningitis may be up to 90%, many strains of *H.influenzae* type B *are* susceptible to ampicillin; also most strains are still susceptible to chloramphenicol. Vaccines are available.

BORDETELLA

Bordetella organisms are small, gram-negative coccobacilli which are strict aerobes. The three species of this genus vary in motility and certain biochemical characteristics. The most important human pathogen in this genus is *B. pertussis* which causes whooping cough. The disease is spread via the respiratory route and the organism is non-invasive. Two other important species of *Bordetella* are *B.parapertussis* cause a mild pharyngitis and *B.bronchiseptica* is usually an animal pathogen (i. e. cough in dogs). It is rarely a cause of human disease, but can cause broncho-pulmonary symptoms in severely immunosuppressed individuals.

Bordetella pertussis

It is extremely small; slow growing, strictly aerobic, Gram negative, with toluidine blue stain bipolar metachromatic granules can be demonstrated, capsulated, non-motile coccobacilli (short rod). Compared to other Bordetella species, *B. pertussis* does not grow on common laboratory media. It forms acid but not gas from glucose and lactose and does not require X and V factors, It requires complex enriched media, commonly employed is Bordet-Gengou medium (potato-blood- glycerol agar).

B. pertussis can be distinguished from *B. parapertussis* in that *B. pertussis* is oxidase positive but urease negative, while *B. parapertussis* is oxidase negative and urease positive. *B. bronchiseptica* is positive for both enzymes.

<u>Clinical findings;</u>

After of an incubation period of about 2 weeks, the “catarrhal stage “develops, with mild coughing and sneezing. During this stage large numbers of organisms are sprayed in droplets and the patient is highly infectious but not very ill. During the “paroxysmal stage “the cough develops to its characteristic “whoop“ upon inhalation, the W.B.Cs is high (16000 – 30000 C/ml) with an absolute lymphocytosis, most of the patients with whooping cough are less than a year old, although older children may also get the disease.

Antigenic structure;

B. pertussis possesses many antigens; most external are an agglutinogen and a hemagglutinin. The cell wall contains a heat stable toxin, the protective antigen, and a histamine-sensitizing factor. Upon disruption of the cell, the protoplasm contains a heat labile toxin and several other antigens, phase 1 variants contain larger amounts of the protective antigens than other variant phases, it also contains peptides that promote marked lymphocytosis in the host.

Diagnosis;

Specimens consist of nasopharyngeal swabs or cough droplets expelled onto a cough plate held in front of the mouth of the patient during a paroxysm النوبة.

1- Culture: the medium of choice is Bordet-Gengou agar containing 0.5 unit penicillin G for each ml. of medium; this tends to inhibit other respiratory flora but permits growth of *B. pertussis* in 2-5 days at 35C in a moist environment. The organism grows as small transparent hemolytic colonies on blood agar. The slow growth rate makes direct immunofluorescent staining of smear made from a nasopharyngeal swab may give rapid positive results.
2- Serology; serologic tests on patients are of little diagnostic help because a rise in agglutinating antibodies does not occur until the third week of illness.

Immunity;

Recovery from whooping cough or adequate vaccination is followed by immunity; second infections may occur but are mild.

Prevention and treatment:

Erythromycin or ampicillin during the catarrhal stage promotes the elimination of the organisms; treatment after the onset of the paroxysmal phase rarely alters the clinical course. Pertussis vaccine is combined with diphtheria and tetanus toxoid in the DTP vaccine is available.

Lec. twelve ; genus Mycobacterium

The *Mycobacteria* are rod shaped bacteria belong to the family *Mycobacteriaceae* and are part of the CMN group (*Corynebacteria, Mycobacteria and Nocardia*). As a group, they produce characteristic long chain fatty acids termed mycolic acids.

Mycobacteria are non-motile and Catalase positive . The most important species in this genus for human are *M. tuberculosis & M. leprae*

Mycobacterium tuberculosis First discovered in 1882 by Robert Koch, is the etiologic agent of tuberculosis (TB) in humans and the lung is most commonly affected but lesions may also occur in kidneys, bones, lymph nodes and meninges,

It is a large non motile rod-shaped bacterium, 2 - 4 micrometers in length and 0.2 - 0.5 um in width, obligate aerobe and has a slow generation time, 15 - 20 hours.

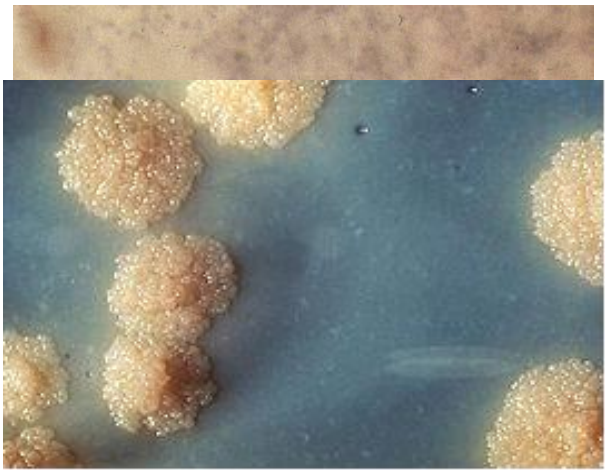
Do not stain easily, and cannot be classified as either gram positive or gram negative , but once stained it resist decolorization by acid or alcohol and are therefore called acid-fast bacilli (AFB), this property partially due to their high content of certain lipids especially mycolic acid.

The cell wall of *M.tuberculosis* is a major determinant of virulence, it contains complex lipids. Over 60% of the mycobacterial cell wall is lipid. The lipid fraction consists of three major components, mycolic acids, cord factor, and wax-D.

The high concentrations of lipids in the cell wall of *M. tuberculosis* have been associated with these properties of the bacterium:

- Impermeability to stains and dyes
- Resistance to many antibiotics
- Resistance to killing by acidic and alkaline compounds
- Resistance to osmotic Lysis via complement deposition

• Resistance to lethal oxidations and survival inside of macrophages



One acid-fast staining method for *M.tuberculosis* is the Ziehl-Neelsen stain. When this method is used, the smear is fixed, stained with carbol-fuchsin (a pink dye), and decolorized with acid-alcohol. The smear is counterstained with methylene-blue. Acid-fast bacilli appear pink.

The finding of tubercle bacilli in pathological materials signifies that the patient has an active infection and identification must be confirmed by culture but TB does not grow on ordinary medium.

Two media are used to grow MTB Middle brook's medium which is an agar based medium and Lowenstein-Jensen medium which is an egg based medium.

MTB colonies are yellow, dry & wrinkled تجاعيد when grown on either medium.

Both types of media contain inhibitors to prevent contaminants from growing.

Virulence Factors

M. tuberculosis does not possess the classic bacterial virulence factors such as toxins, capsules and fimbriae, they multiply slowly in the body & they have no ability to resist capture by phagocytes ,

their pathogenicity is due to their ability to resist destruction by lysosomal enzymes.

when being inside the phagocytes & they proceed to multiply & when the macrophage become laden with numerous bacilli, it dies & disintegrate & the liberated bacilli repeat the cycle again ,

Mycolic Acids are thought to be a significant determinant of virulence. Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme, and oxygen radicals in the phagocytic granule. They also protect extracellular mycobacteria from complement deposition in serum.

Cord factor is toxic to mammalian cells and is also an inhibitor of PMN migration.

the termination of the infection appears to depend on the development of cell mediated immunity & the production of activated macrophages which have the ability to kill ingested bacilli.

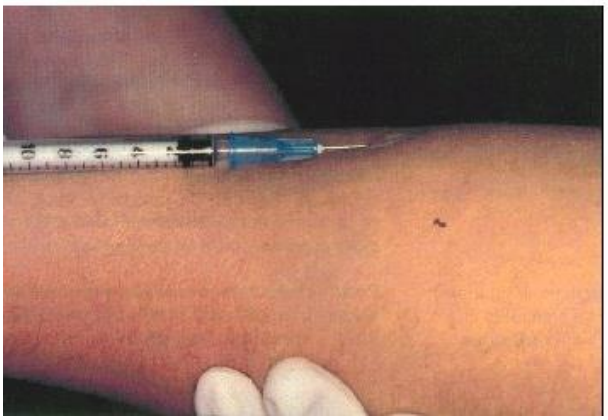
Diagnosis ;

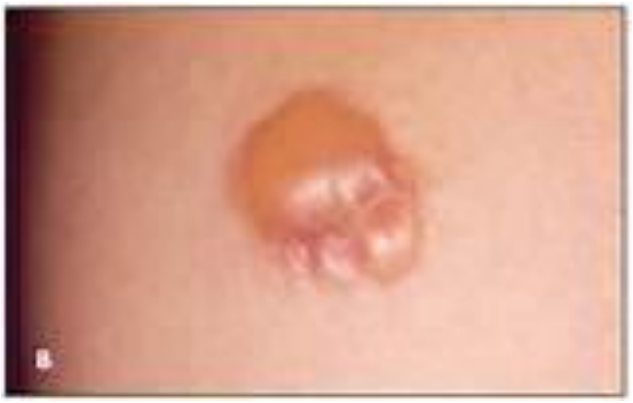
The diagnosis of tuberculosis requires detection of acid-fast bacilli in sputum via the Ziehl-Neelsen method .The presence of acid-fast bacteria in sputum is a rapid test for tuberculosis.

The organisms must be then cultured from

sputum. *M. tuberculosis* will grow very slowly producing distinct non-pigmented colonies (2-6 weeks) and can be differentiated from most other mycobacteria by the production of niacin.

There are two kinds of tests that are used to determine if a person has been infected with TB bacteria: the tuberculin skin test and TB blood tests.





Skin testing for delayed hypersensitivity is performed by tuberculin skin test (also called the Mantoux tuberculin skin test) by injecting a small amount of PPD (purified protein derivative) as the test antigen (called tuberculin) in a 0.1 ml volume intradermal in the forearm. The test is read within 48-72 hours to

detect a reaction on the arm.

The test is considered positive if the diameter of the resulting lesion is 10 mm or greater. The lesion is characterized by erythema (redness) and swelling and indurations (raised and hard).

A positive test does not mean that the patient has active tuberculosis; it indicates exposure or infection to *M. tuberculosis* at some time in the past.

90% of people that have a lesion of 10 mm or greater are currently infected with MTB. 100% of people that have a lesion of 15 mm or greater are currently infected with MTB . the reader should not measure erythema (redness) , the diameter of the indurated area should be measured across the forearm (perpendicular to the long axis)

False positive tests usually manifest themselves as lesser reactions. These lesser reactions could indicate prior exposure or infection with other mycobacteria or vaccination with BCG.

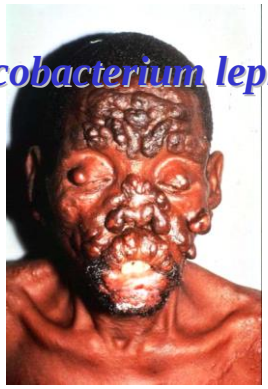
Tuberculosis Treatment

Tuberculosis is usually treated with four different antimicrobial agents the course of drug therapy usually lasts from 6-9 months. The most commonly used drugs are rifampin, ionized , pyrazinamide and ethambutol or streptomycin.

Prevention

A vaccine against MTB is available. It is called BCG (Bacillus of Calmette and Guerin, named after the two Frenchmen that developed it). BCG consists of a live attenuated strain derived from *Mycobacterium bovis*. The vaccine is not 100% effective. Studies suggest a 60-80% effective rate in children.

Mycobacterium leprae



Other human pathogens belonging to the *Mycobacterium* genus include *Mycobacterium leprae,* the causative agent of leprosy or Hansen's disease (discovered in 1873 by G.A. Hansen). a chronic disease often leading to disfigurement تشوه. The organism infect the skin because of its growth at low temperature causes skin sores, nerve damage, and muscle weakness that gets worse over time.

the organism dose not grow in culture media, but grows well in the armadillo

which has a low body temperature . Leprosy is thought to be transmitted via droplets from the nose and mouth during close prolonged contact with affected individuals .

M.leprae is indistinguishable from *M.tuberculosis* by staining except 5% H_2SO_4 substitute 20% in Z.N. stain.

Leprosy may appear in one of two forms;

1) lepromatous leprosy; this disease begins as hypo pigmentation of the skin , nerve cells are infected too , nodular lesions arise that contain large number of bacilli, nasal secretions and oozing نار sores shed large numbers of microbe and cause spread and more infectious , this

type of infection is associated with a weak immune response .

2) Tuberculoid leprosy; in this type of infection, Regions of skin lost sensation surrounded by nodules , Lose pigmentation , Causes strong cell mediated response and activated macrophages keep microbe under control , the bacilli are scanty in the lesion and there is a strong tendency toward spontaneous healing as a result of good cell mediated immunity.

M. leprae cannot be cultivated on laboratory media. Laboratory diagnosis is based only on direct microscopic examination of acid fast smears of

material from the lesions or biopsy material that show acid-fast bacilli with the Ziehl-Neelsen stain.

Treatment with antibiotics (initially with dapson and now multidrug) is effective and the overall disease incidence worldwide is down.

Lec. Thirteen; Genus Clostridium

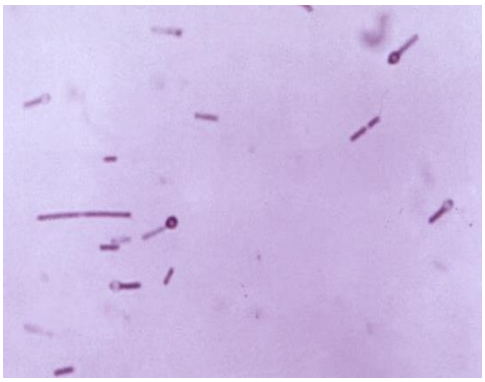
This genus consist of anaerobic gram positive bacilli which form spores that in the most cases distend their bodies, such organisms widely distributed in nature as soil saprophytes and as intestinal commensals of mammals, they include the causative organisms of some very serious human diseases such as botulism, tetanus and gas gangrene.

Clostridia are able to ferment a wide variety of organic compounds. A variety of foul smelling compounds are formed during the fermentation of amino acids and fatty acids. The clostridia also produce a wide variety of extracellular enzymes to degrade large biological molecules (e.g. proteins, lipids, collagen, cellulose, etc.) these enzymes play a role in invasion and pathology.

Clostridium tetani

This organisms is the causative agent of tetanus in human and animals, it is generally seen as straight slender rod shaped , motile with peritrichous flagella, spore forming, spores are terminal , spherical two to four times the diameter of the bacilli producing the drum stick appearance , *Cl.tetani* produces two Exotoxins:-

1) Tetanolysine.
2) Tetanospasmin.



tetanolysine is oxygen labile and does not play a significant role in pathogenesis, while tetanospasmin is an extremely powerful poison , tetanospasmin is a neurotoxin and travels from the site of infection to the central nervous system along the motor nerves while the organism is non-invasive and thus remains in the local wound ,

the effect of the toxin is to block the release of inhibitory neurotransmitters (glycine and gamma butyric acid) Glycine normally prevents contraction of muscles; therefore, the generalized muscular spasms characteristic of tetanus may occur. Cardiac failure can lead to death in approximately 55-65% of affected persons. the toxin is produced by growing cells , germination of the spores and development of vegetative cells that produce toxin are aided by

1) necrotic tissue 2) calcium salts

3) pyogenic infections, all of which aid establishment of low oxidation – reduction potential.

The incubation period for tetanus may range from 10 to 14 days , Infection usually occurs when spores germinate and produce tetanus toxin. The first symptoms of tetanus are trismus (locked jaw), stiffness of the neck,

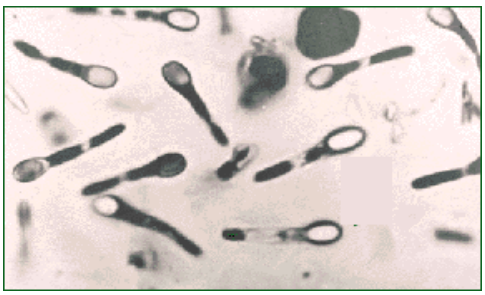
difficulty in swallowing and rigidity of abdominal muscles, clinical tetanus dose not induce immunity because the disease is produced by amounts of neurotoxin that are too small for immunization.

Diagnosis

The diagnosis of tetanus is usually based on clinical findings, however bacteriological studies can confirm the diagnosis, direct culture of unheated material on blood agar incubated anaerobically is often the best method of detection. However material from the wound or from a mixed sporing subculture may be heated at various temperatures and for various times to exclude non sporing bacteria, the heated specimens are then seeded onto solid media and incubated an aerobically.

Treatment and Immunity

Tetanus is an emergency situation and requires hospitalization. The patient is immediately treated with antitoxin . Drugs can control muscle spasms. The wound requires aggressive washing and treatment with antibiotics. toxoid given to women before or during the first 6 months of pregnancy prevents tetanus neonatorum, Whenever a previously-immunized individual sustains a potentially dangerous wound, a booster of toxoid should be taken.



Cl. Perfringens

It is gram positive bacilli with oval sub terminal spore giving it tennis – racket form, non motile,

colonies on blood agar are surrounded by a zone of beta hemolysis.

Cl. perfringens is divided into five types (in some papers to six A to F), A through E on the basis of its ability to produce various toxins.

Type	Alpha	Beta	Epsilon	Iota
A	+	--	--	--
B	+	+	+	--
C	+	+	--	--
D	+	--	+	--
E	+	--	--	+

Only types A,C and D are pathogenic for human , type A is the causative agents of gas gangrene and food poisoning,

Clostridium perfringens is associated with two types of food poisoning: type A, a relatively mild and self-limited gastrointestinal illness, and type C, a more serious but rarely seen disease type C cause necrotizing enteritis .

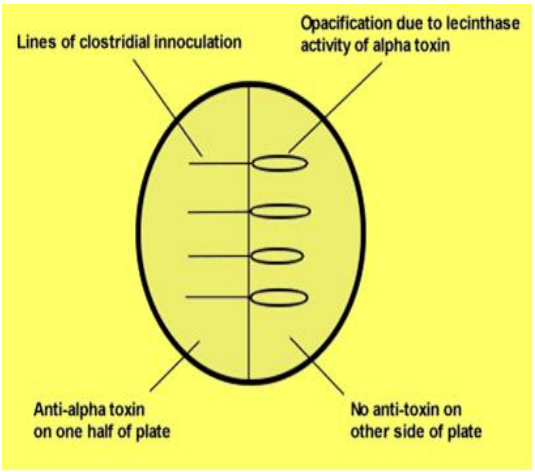
The principle toxin of *Cl.perfringens* is the alpha toxin known as phospholipase C (lecithinase) Most cases of Clostridial gas gangrene occur following dirty wounds or bad surgical procedures, several Clostridial species are able to cause a gas gangrene such as ; *Cl. perfringenes*, *Cl. novyi*, *Cl. Septicum*, *Cl. bifermentans*, *Cl. histolyticum and Cl. Sordelli.* Once pathogenic Clostridia are established in damaged tissue , they rapidly invade the surrounding healthy tissues by elaborating toxins, many of these toxins are enzymes such as ; neuraminidase, protease, DNase, hyaluronidase, lecithenase, collagenase, the affected tissues undergo necrosis and autolysis.

Certain widely distributed strains which differ slightly from ordinary type A strain in being able to survive prolonged boiling, whereas ordinary strains are killed in about 5 minutes are responsible for many outbreaks of food poisoning, the ingested vegetative cells reach the intestine and undergo sporulation , the heat labile enterotoxin is released during sporulation, usual onset of symptoms is 8 – 12 hours following ingestion of contaminated food with large number of cells (more than 10^8 cell) , the disease is rather mild and symptoms include abdominal pain and acute diarrhea lasting 12 to 24 h. fever and vomiting are uncommon, the disease is self limiting and usually over within 24 hrs.

Laboratory diagnosis;

Specimens should be taken from the deeper areas of the wound for microscopical examination and culture , also the cells can be identified by Nagler

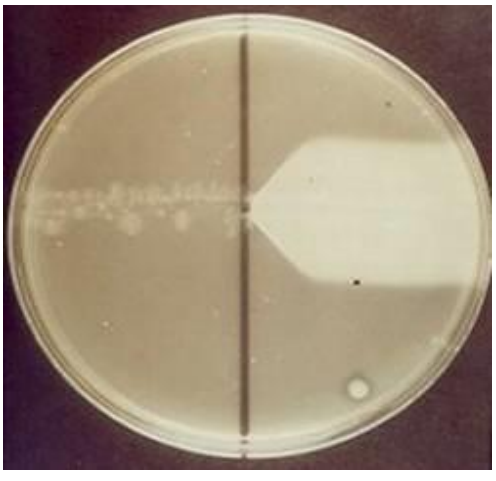
reaction a test for the identification of alpha toxin of *Cl.perfringens* , the addition of antitoxin to cultures on egg yolk agar prevents visible opacity, due to lecithinase action which is normally observed around colonies.



Nagler reaction test

Treatment;

Adequate surgical attention to the wound is of the utmost importance, necrotic and devitalized tissue is carefully removed and antibiotic therapy is started immediately with very high doses, penicillin, metronidazole and an aminoglycoside may be given in combination.



Cl. Botulinum:-

It is anaerobic gram positive bacilli, motile, with an oval sub terminal spore, spores of some strains are able to withstand moist heat at 100C for several hours, and they are destroyed by moist heat at 120C within 5 minutes.

eight types of *Cl. Botulinum* (A,B,C1,C2,D,E,F,G) have been differentiated on the basis of their toxins production,

types A ,B & E are associated with human botulism , but types C & D are encoded by bacteriophage and also caused disease in human.

Cl. botulinum produce a powerful exotoxins that is the most potent exotoxin known in nature (10 ng can kill a normal adult). it is destroyed at 80C for 30 to 40 minutes ,

Food-borne botulism is not an infection but an intoxication. Botulism results from eating uncooked foods in which contaminating spores germinate and the multiplying vegetative cells elaborate neurotoxin which absorbed from the stomach and the upper small bowel.

spores are relatively heat resistant and may survive the sterilizing process of improper canning procedures. The anaerobic environment produced by the canning process may further encourage the outgrowth of spores. The organisms grow best in neutral or "low acid" vegetables .

Clinical symptoms begin 18-36 hours after toxin ingestion with weakness, dizziness and dryness of the mouth. Nausea and vomiting may occur. Neurologic features soon develop, including blurred vision, inability to swallow, difficulty in

speech, descending weakness of skeletal muscles and respiratory paralysis.

the toxin block release of acetylcholine at synapses and neuromuscular junctions, flaccid paralysis results. symptoms usually begins 12 to 36 h. after ingestion of contaminated food .

Treatment:-

The priorities are

1) To remove unabsorbed toxin from the stomach and intestinal tract.
2) To neutralize unfixed toxin by giving polyvalent antitoxin (A, B & E).
3) To give relevant intensive care and support. Recovery is very gradual occurring over weeks to months, there is no need for using antibiotics, since in the usual adult forms of botulism the organism does not multiply in the patient's body.

Lec. fourteen the enteric bacteria

The enteric bacteria are gram negative , non sporulating, facultativelly anaerobic rods that are present in the intestinal tract of humans and many animals, the majority of enteric bacteria do not produce disease in the intestinal tract but it may act as an opportunistic pathogens i.e.; typically non-pathogenic microorganisms that act as a pathogen & cause disease in patients whose defense mechanisms have been weakened , or if they do not reside in the intestinal tract their natural habitat .

most of these bacteria belong to the family enterobacteriaceae which includes 176 species among 44 different genera ,

although similar bacteria classified in other families are also considered to be enteric bacteria because of their intestinal habitat, enteric bacteria are differentiated on the basis of cultural, biochemical and antigenic characteristics, there are three major classes of antigens associated with these bacteria

1) The O – antigen or cell wall antigens they are lipopolysaccharide in composition.
2) The H- antigens or flagellar antigens, they are proteins.
3) The K- antigens or capsular antigens, they are polysaccharides.

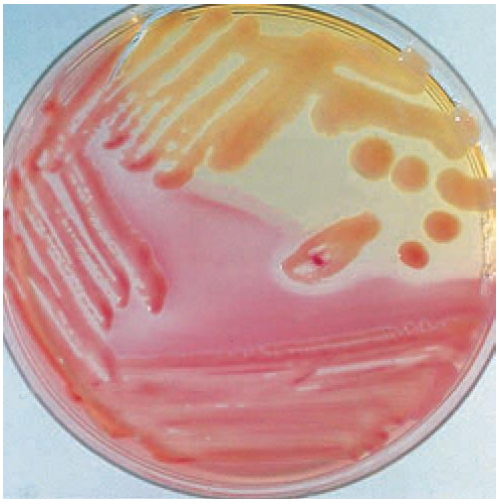
infections produced by enteric bacteria includes, UTI, pneumonia,

wound infections, bacteremia, meningitis , abscesses and endocarditis.

The substances responsible for endotoxicity of enteric bacteria is lipid-A portion of the cell wall . In addition to the LPS endotoxin common to all gram negative bacteria some enterobacteriaceae also produce exotoxins which act on host cells by damaging membranes, inhibiting protein synthesis or altering metabolic pathways, the end results of these actions may be cell death . They also produce hemolysins. The activity of hemolysins is not limited to red cells since the alpha-hemolysins of *E. coli* also lyses lymphocytes, and a pore-forming cytotoxin that causes leakage of cytoplasmic contents and

cell death. the beta-hemolysins inhibit phagocytosis.

One of the most famous members of the family is *Escherichia coli*, a bacterium which has been studied extensively in laboratories all over the world.



Escherichia coli ; is a major cause of urinary tract infections and bacteremia, since the intestinal tract is it's only natural habitat , its presence in environmental samples is considered a proof of fecal contamination, the identification of *E.coli* is based on colony morphology on differential and selective media, biochemical and serological reactions.

it ferment carbohydrates including lactose with the production of acid and gas, They are often isolated on agar containing lactose and a pH indicator such as MacConkey (MAC) agar, Hektoen enteric (HE) agar and xylose-lysine-desoxycholate (XLD) agar,. Colonies which ferment lactose will produce acid to cause a color shift in the indicator. *E. coli* is a lactose fermenter, while *Shigella, Salmonella* and *Yersinia* are non-fermenters.

E.coli strains with K-antigens 1,2,3,5,12 &13 are found with high frequency in extra intestinal infections, other coliform organisms including the genera klebsiella, enterobacter, Serratia ,citrobacter and *E.coli* are referred to as coliform

bacteria, because they share similar morphological and biochemical characteristics, but unlike *E.coli* they are found in soil, water, food and intestinal tract, most of them ferment lactose, sometimes they cause infection in burns, U.T., R.T. and bacteremia.

Pathogenesis of *E. coli*

Pathogenic strains of *E. coli* are responsible for three types of infections in humans:

1) urinary tract infections (UTI),

2) neonatal meningitis, and

3) intestinal diseases (gastroenteritis)

Urinary Tract Infections

Uropathogenic *E. coli* (UPEC)

Causes 90% of the urinary tract infections. They produce factors that allow them to attach to the urinary epithelial mucosa . The ability of *E. coli* to cause UTIs are :

1) the production of pili, both P fimbriae and Type 1 fimbriae (common pili) which allow cells to adhere to epithelial cells

2) Cytolysins ; can kill immune cells and inhibit the phagocytosis and chemotaxis of certain white blood cells.

3) Aerobactin allows the bacterial cell to chelate iron , free iron is generally unavailable within the host for use by bacteria.

Neonatal Meningitis

E. coli strains invade the blood stream of infants from the nasopharynx or GI tract and are carried to the meninges.

The K-1 antigen is considered the major determinant of virulence. It inhibits phagocytosis, complement, and host's immunological responses .

Intestinal Diseases Caused by *E. coli*

Five classes of *E. coli* that cause diarrheal diseases are now recognized:

1. Enterotoxigenic *E. coli* (ETEC)

ETEC is an important cause of diarrhea in infants and travelers, sometimes referred to as traveler's diarrhea , ETEC strains may release one or both

of two toxins into the small intestine. They produce a heat-labile toxin (LT), which is similar in action to cholera toxin from. (LT) stimulate the secretion of chloride out of the cell and the blockage of NaCl absorption, the net effect is the accumulation of water and electrolytes into the bowel lumen causing diarrhea without fever. ETEC may also produce a heat stable toxin (ST) that binds to glycoprotein receptors on the cell causes an LT like net secretion of fluids and electrolytes into the bowel.

2. Enteroinvasive *E. coli* (EIEC)

EIEC differs greatly from EPEC and ETEC strains. Enteroinvasive strains produce dysentery with direct penetration, invasion, and

destruction of the intestinal mucosa and colon . The clinical syndrome is identical to *Shigella* dysentery and includes a dysentery-like diarrhea with fever. EIEC do not produce LT or ST toxin. Unlike typical *E. coli*, EIEC are non-motile, and do not ferment lactose. infections seem to occur in adults and children alike.

3. Enteropathogenic *E. coli* (EPEC)

EPEC induce watery sometimes bloody diarrhea. They cause infantile diarrhea , do not produce ST or LT toxins , not as invasive as *Shigella,* and

unlike ETEC, they cause an inflammatory response. The illness is characterized by low-grade fever, vomiting and diarrhea.

4. Enterohemorrhagic *E. coli* (EHEC)

they are the primary cause of hemorrhagic colitis (HC) or bloody diarrhea, EHEC are characterized by the production of verotoxin or Shiga toxins . The serotype O157:H7 has been associated with hemorrhagic diarrhea . EHEC are "moderately invasive". *E. coli* O157:H7 produces two cytotoxins: verotoxin I and verotoxin II. Verotoxin I is identical to the Shiga toxin produced by *Shigella dysenteriae* type I. Verotoxin II is biologically similar to (but

immunologically different from) verotoxin l.

5. Enteroaggregative *E. coli* (EAEC)

These strains are associated with persistent diarrhea in young children , cause non-bloody diarrhea without invading or causing inflammation. the organisms produce a hemolysin and a heat-labile enterotoxin.

Isolation and identification of *Enterobacteriaceae*

The coliforms are easily cultivated and identified by routine laboratory procedures. As a group, the

Enterobacteriaceae are oxidase negative, capable of reducing nitrates to nitrites, ferment glucose, ferment lactose and motile (except *Klebsiella*). Many conventional and rapid identification systems are available. All *Enterobacteriaceae* are identified biochemically. Important serotypes can be differentiated by their O H and K (capsular) antigens.

Chemotherapeutic: Moderate or broad spectrum antibiotics are generally useful. Susceptibility tests should be performed when appropriate

Lec. fifteen; Other enteric bacteria

Genus Klebsiella ; genus Klebsiella have no specific growth requirements and grow well on standard laboratory media, most strains can survive with citrate and glucose as their sole carbon sources and ammonia as their sole nitrogen source.

The most distinctive bacteriological feature of the genus Klebsiella are the absence of motility and the presence of polysaccharide capsule.

This gives the colonies a mucoid character and form the basis of a serotyping system, over 80 capsular types have been defined , human infections of the respiratory tract are caused by capsular types 1 and 2 , those of the urinary tract, by types 8,9,10 and 24. several types of pili are also present on the surface and probably aid in the adherence to respiratory and urinary epithelium.

K. pneumoniae is a gram negative , non motile, lactose fermenting , facultative anaerobic, rod-shaped bacterium , can cause destructive changes to human lungs resulting in bloody sputum., some strains of *K. pneumoniae* produce a heat stable enterotoxin that induces hyper secretion of fluids and electrolytes into the small intestine and cause diarrhea,. In recent years, klebsiellae have recognized as important nosocomial pathogens in hospital patients .

Isolation

Klebsiellas grow readily on ordinary media used to isolate Enterobacteriace e.g., nutrient agar, tryptic soy agar, bromocresol purple lactose agar, blood agar, as well as more differential plating media such as MacConkey agar, eosin-methylene blue agar (EMB), and bromo-thymol blue agar . Klebsiella pneumonia colonies are lactose positive with a mucoid aspect and sometimes

stickiness ملتصقة, depending on the strain and the composition of the medium

Diagnosis

Because gram negative rods of the coliform group all resemble each other, only the presence of large capsules of Klebsiella is diagnostic, specimens are plated both on blood agar and on differential media that contain special dyes and carbohydrates, this permits the rapid recognition of lactose fermenting and non lactose fermenting colonies , organisms isolated on differential media are further identified by biochemical and serological tests.

Difference between *Escherichia coli* and *Klebsiella*

	E. coli	*Klebsiella*
1	Non-capsulated	Capsulated
2	Motile	Non-motile
3	Indole and MR positive	Indole and MR negative
4	Citrate and VP negative	Citrate and VP positive
5	Urease negative	Urease positive
6	Colonies not mucoid	Colonies mucoid
7	Slender and long	Short and thick

Treatment

antibiotics used to treat susceptible isolates include ampicillin, piperacillin, ticarcillin, ceftazidime, cefepime, levofloxacin, norfloxacin, gatifloxacin, moxifloxacin, meropenem, and ertapenem.

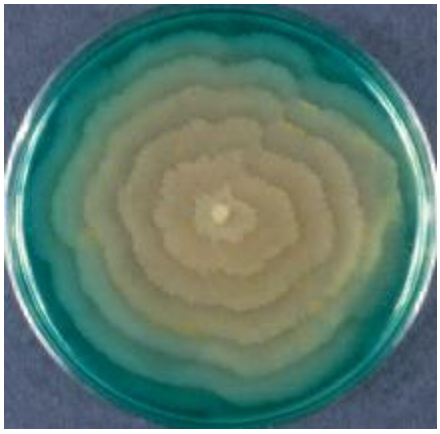
Genus Proteus ;

The genus *Proteus* consists of four species: *P. mirabilis, P. vulgaris, P. penneri,* and *P. myxofaciens*. *P. mirabilis* and *P. vulgaris* are widely recognized as human pathogens. Proteus are motile, Gram-negative rods ,
they are characterized by their ability to deaminate phenylalanine and hydrolyze urea , species are free living in water , soil , sewage as saprophytes . They are opportunistic pathogens responsible for many human infections most are associated with prolonged hospitalization and isolated from urine, wounds, ear and bacteremic infections.

Most strains of *P. mirabilis* are sensitive to ampicillin and cephalosporin. *P. mirabilis*, once attached to the urinary tract, infects the kidney more commonly than *E. coli.*

P .vulgaris commonly occur in the normal flora of the intestinal tract and isolated less often in the laboratory and usually targets immunosuppressed individuals *P. vulgaris* is not sensitive to ampicillin and cephalosporin.

Proteus species do not ferment lactose and are not considered to be coliform organisms, they hydrolyze urea by the enzyme urease into CO_2 and ammonia which cause an increase in the alkalinity of urine and decrease in solubility of calcium and magnesium phosphates this leads to the formation of stones , also ammonia may directly damage urinary tract epithelium.

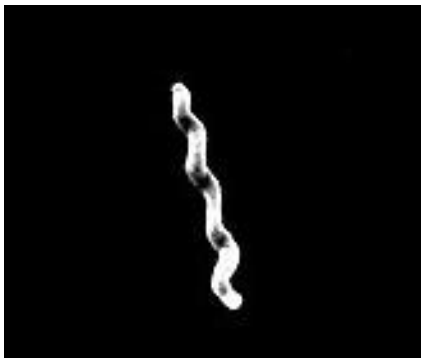


Pr.mirabilis and *Pr.vulgaris* can swarm on agar surface in a thin film resulting in a waves of growth and this character makes them readily recognized in the laboratory. Motile strains of Proteus contain H antigen in addition to the O antigen. Certain strains labeled OX share specific polysaccharides with some rickettsiae. The OX strain are agglutinated by sera of patients with rickettsial diseases (Weil-Felix test), Proteus produces infections in human only when it leaves its normal habitat in the intestinal tract. it is frequently found in chronic urinary tract infections and produces bacteremia, pneumonia and focal lesions , there are great variations among strains of Proteus in antibiotic sensitivity, gentamicin and amikacin are the most active drugs, *Pr.mirabilis* is often inhibited by penicillin G and ampicillin .

Identification

Proteus species do not ferment lactose , It is oxidase-negative but catalase , nitrate and urease positive. On the species level, indole is considered reliable, as it is positive for *P. vulgaris*, but negative for P. mirabilis. Species can be motile, and have characteristic "swarming" patterns. . Flagellated (swarming, motile) variants were therefore designated H forms, non- flagellated (non swarming, non motile) variants designated as O forms Serological classification depends on O and H antigens, known as Kauffman–White classification

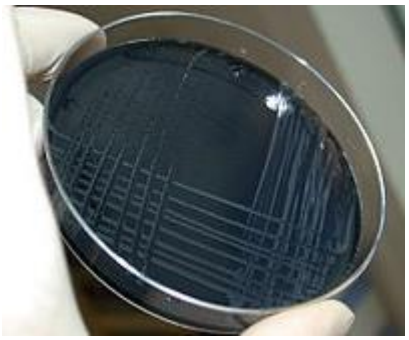
Genus Campylobacter



campylobacter are motile, curved, helical-shaped, oxidase-positive, gram negative rods similar in morphology to vibrios, the cells have polar flagella and are often attached at their ends giving pairs of (S) appearance, more than a dozen Campylobacter species have been associated with human disease, of these *C.jejuni* is the most common potential causes of human gastroenteritis and diarrhea,

Campylobacter jejuni is a species of bacteria commonly infects the intestinal tract of several animal (including chickens, cattle and sheep) and transmitted to man in milk or meat products. it grows well on Blood-free , charcoal-based selective medium agar (CSM) for isolation under microaerophilic conditions (requires oxygen at reduced tension 5 %) and grows best at 42 C , growth usually requires 2 to 4 days, sometimes as much as a week,

in contrast to vibrios it does not break down carbohydrates to get energy but uses amino acids and metabolic intermediates .



C.jejuni infection typically begins 1 – 7 days after ingestion with fever and lower abdominal pain that may be severe enough to mimic acute appendicitis, these are followed within hours by dysenteric stool. The organism is invasive but less than Shigella., the illness resolves spontaneously after a few days to one week.

Diagnosis

The diagnosis is confirmed by isolation of the organism from stool, A selective blood agar medium (Skirrow's medium) can be used , greater selectivity can be gained with an infusion of a cocktail of antibiotics: vancomycin, polymixin-B, trimethoprim and actidione (Preston's agar) that inhibit the normal facultative flora of the bowel. Plates

must be incubated in microaerophilic atmosphere at 42°C, the normal avian body temperature, rather than at 37°C.

They usually grow in scanty amounts on the plate.

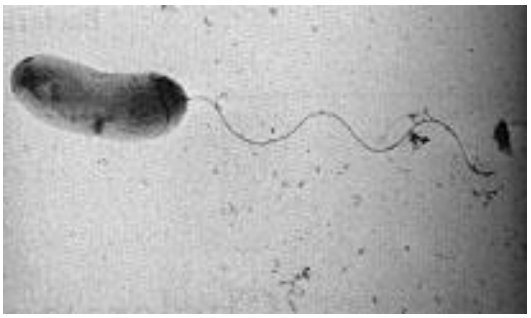
Treatment;

The disease is usually self-limiting. Severe or prolonged cases (more than 1 week) may require ciprofloxacin, erythromycin, azithromycin or norfloxacin. Fluid and electrolyte replacement may be required for serious cases.

Lec. sixteen; intestinal pathogens

The organisms that produce enteric diseases in human gain entrance to the body by the oral route and multiply in the intestinal tract, some of these pathogens produce enteric diseases by excreting exotoxins (called enterotoxins because of the site of activity) while others are invasive, penetrating intestinal epithelial cells and passing into intestinal tissue and in some cases into other areas of the body, a few enteric pathogens are both toxigenic and invasive

Toxigenic enteric pathogens (*V.cholerae*) ;



They are thin, curved, comma shaped aerobic or facultative anaerobic Gram-negative, actively motile by means of a single polar flagellum. They are non-lactose fermenting, Growth occurs in alkaline pH (7.5 to 9.6) between 22 and 40°C (optimum 37°C). It grows well in ordinary media. , produce indole and are oxidase positive. In most ways vibrios are related to enteric bacteria, Vibrios are distinguished from enterics by being oxidase-positive and motile by means of polar flagella.

cholera as a disease occur after ingestion of adequate number of cholera vibrios usually in water or food , they multiply in the intestine and do not penetrate the intestinal epithelium and produce an enterotoxin which cause watery diarrhea and the consequent dehydration may be rapidly fatal, a healthy person may become hypotensive within an hour of the onset of symptoms and may die within 2-3 hours if no treatment is provided. More commonly, the disease progresses from the first liquid stool to shock in 4-12 hours, with death following in 18 hours to several days.

There are five species within the genus Vibrio, of which *V.cholerae* and *V.parahaemolyticus* are the major pathogens, both produce diarrhea, but in ways that are entirely different. *V. parahaemolyticus* is an invasive organism affecting primarily the colon usually transmitted by ingestion of raw seafood; *V. cholerae* is noninvasive, affecting the small intestine through secretion of an enterotoxin. however, other vibrios, known as non-agglutinable (NAG) vibrios or non-cholera vibrios are capable of producing diarrhea in humans (cholera-like disease) with limited outbreaks . Morphologically and biochemically it resembles Vibrio cholera but It is non-agglutinable with O1 antiserum of Vibrio cholera.

V.cholerae is divided into six serogroups O;1 through O;6 and four biotypes, two of the biotypes classical and El-Tor belonging to the serogroup O;1 are responsible for epidemic disease , serogroup O;1 is further divided into three serotypes known as Ogawa, Inaba and Hikojima in relation to their content of A,B,C antigens.

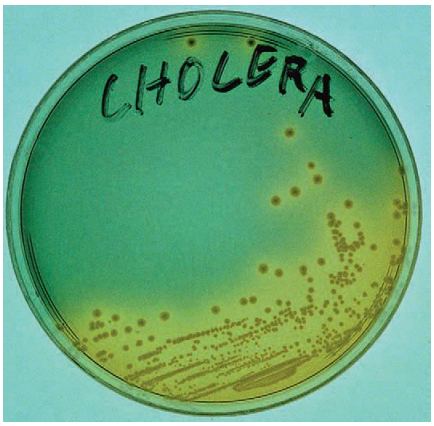
Serotype	O Antigens
Ogawa	A, B
Inaba	A, C
Hikojima	A, B, C

Diagnosis;

a bacteriologic diagnosis is done by isolation of *V.cholareae* from stool, the organism grows on common media such as blood agar and Mac Conkey agar, Most vibrios have relatively simple growth requirements and will grow in synthetic media with glucose as a sole source of carbon and energy , most species require 2-3% NaCl or a sea water base for optimal growth, but its isolation is enhanced by the use of selective medium Thiosulfate Citrate Bile salt Sucrose (T.C.B.S.) as yellow colonies, once isolated, the organism is readily identified by biochemical and serological test with specific O;1 antisera.

Immunity to Cholera

After natural infection by *V. cholerae*, circulating antibodies can



be detected against several cholera antigens including the toxin, somatic (O) antigens, and flagellar (H) antigens. These antibodies are also raised by parenteral injection of antigens as vaccine components. Antibodies directed against *Vibrio* O antigens are considered "vibriocidal" antibodies because they will lyse *V. cholerae* cells in the presence of complement and serum components. Antibodies reach a peak 8-10 days after the onset of clinical illness, and then decrease, returning to the baseline 2 - 7 months later . Immunization of killed *V.cholerae* is of little value, prevention depends on good general hygiene.

Treatment;

The most important immediate treatment of cholera is replacement of lost body fluids and electrolytes, antibiotics such as tetracycline and chloramphenicol effectively eliminate *V.cholerae* in most patients. Vaccination is partially effective.

Genus Brucella (invasive intestinal pathogens)

Brucellosis is an important zoonotic disease (principally is a disease of the genitourinary tract of domestic animals) it is caused by bacteria of the genus Brucella, Brucella are fastidious aerobic nonmotile gram negative bacilli, four species of Brucella cause human disease, *B.abortus (cattle) B.melitensis (sheep & goats), B.suis (pigs), and B.canis (dogs),* all species produce catalase, oxidase and urease but do not ferment carbohydrates, they are differentiated by carbon dioxide requirements, H_2S production and susceptibility to dyes (thionin and basic fuchsin) .

Differentiation of Brucella species

Organism	Growth in presence of		H_2S production	CO_2 requirements
	Thionine 1;25000	Basic fuchsin 1;50000		
B.abortus	--	+	++	+
B.melitensis	--	+	--	--
B.suis	+	--	+	--
B.canis	+	--	--	--

they are pathogenic of mammals and tend to localize in the pregnant uterus and mammary glands of these animals, the most common rout of infection in human is the gastrointestinal tract after ingestion of infected milk , milk products or meat, Brucella organisms do not produce exotoxins but they do elaborate endotoxin, the principle features of the disease are fatigue, sweating, malaise, headache, joints pain and pains in muscles, the organism is widely distributed throughout the body including blood , virulent strains of Brucella are capable of intracellular multiplication in cells of the reticuloendothelial system. A definitive diagnosis of brucellosis is dependent on isolation of the organism from blood, feces, urine, bone marrow and lymph nodes, isolation of Brucella from clinical specimens usually needs prolonged incubation(2 – 4 weeks although most are positive in 2 – 5 days) on blood agar or enriched media and requires 5-10 % CO_2 , also the second tool for diagnosis is the serological tests.

Immunity; although antibodies are formed in the course of brucellosis, there is little evidence they are protective .

Treatment; the most commonly used drugs are the tetracyclines (sometimes with Streptomycin) or cotrimoxazole, if these fail a course of gentamycin may be beneficial.

Genus Shigella (toxigenic and invasive)

Organisms are nonmotile, gram negative rod-shaped bacteria, do not ferment lactose, H_2S negative, there are four species within this genus, *S.dysenteriae, S.boydii, S.flexneri and S.sonnei*, all of the species are pathogenic to human and cause dysentery, infection occur as a result of consuming food or water contaminated with the organisms, ingestion of (10 -200 cell) can cause infection and on this basis they are considered to be the most effective of the enteric pathogens in causing human disease, *S.dysenteriae* type 1 produces an exotoxins that cause peripheral paralysis and death when injected into mice or rabbits and is therefore considered to be a neurotoxin, it is also highly toxic for humans and induces fluid secretion in the intestinal lumen and thus has enterotoxin activity too, Shigella penetrates mucosal epithelial cells of the colon and kills them resulting in superficial ulceration, bleeding and an inflammatory response, the infected individual develops fever, severe abdominal cramps and has frequent stools.

Diagnosis

Several media have been used such as eosin methylene blue (EMB) agar, MacConkey agar, ENDO agar, Hektoen enteric (HE) agar) and Salmonella-Shigella (SS) agar , isolates are identified with further biochemical tests and slide O antisera. Shigella is non motile and can be differentiated from Salmonella on the basis of a motility test or a flagellar stain.

Transmission

Shigella is transmitted from an infected person to another usually by a fecal-oral route. *Shigella* are present in the diarrheal stools of infected persons while they are ill and for a week or two afterwards. Most *Shigella* infections are the result of the bacterium passing from stools or soiled fingers of one person to the mouth of another person.

Immunity

Once someone has had shigellosis, they are not likely to get infected with that specific type again for at least several years. However, they can still get infected with other types of *Shigella*

Treatment

The antibiotics commonly used are ampicillin, trimethoprim/sulfamethoxazole, nalidixic acid and ciprofloxacin.

Lec. seventeen ; genus Salmonella

(Invasive enteric pathogens capable of systemic spread)

genus *Salmonella* is a member of the family *Enterobacteriaceae*. *Salmonella* is a Gram-negative facultative anaerobic rod-shaped bacterium, live in the intestinal tracts of warm and cold blooded animals. non-lactose fermenters, motile with peritrichous Flagella, most species produce hydrogen sulfide which can readily detected by growing them on media containing ferrous sulfate such as TSI. In humans, *Salmonella* are the cause of two diseases called Salmonelloses (enteric fever) resulting from bacterial invasion of the bloodstream caused by *S.typhi , S.paratyphi* A, B, and C, and food poisoning caused by other members of Salmonella, resulting from a food borne infection or intoxication.

Salmonella typhi

This species is entirely parasitic and man is it's only natural host, epidemics are usually spread via water supplies , food or when the sanitary conditions are poor and the source is always a human patient or carrier thus one person e.g.; food handlers can cause a lot of spread, the organisms entered the body through the mouth and invades the intestinal epithelium and in the early stages of the enteric fever bacteria localized chiefly in the Payer's patches of the small intestine, then the organisms penetrates within the first week and pass into the blood stream where it is disseminated in macrophages and typical feature of a systemic bacterial infection are noted, finally loading in the gall bladder , organisms are shed in the intestine for some weeks, at this time gastroenteritis (including diarrhea) is noted again, in the second week the nature of the infection is more obvious with the appearance of Rose-spot skin eruption , diarrhea and ulceration of Payer's patches may lead to

intestinal perforation which are common causes of death in untreated cases.

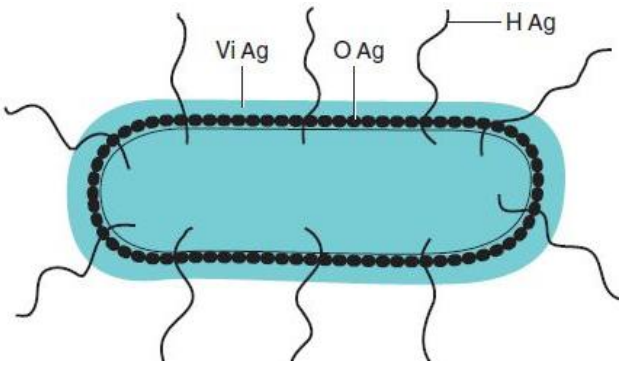
Salmonella usually produce gastro-enteritis only when ingested in large doses , An oral dose of at least 10^5 *Salmonella typhi* cells are needed to cause typhoid , whereas at least 10^9 *S. typhimurium* cells (oral dose) are needed to cause symptoms of a toxic infection, the symptoms are headache, fever, abdominal pain and diarrhea, they usually come on 12 to 36 hr. after eating contaminated food, vomiting may occur but is not commonly, recovery may take a week or two.

Salmonella excretion by human patients may continue long after clinical cure. A symptomatic carriers are potentially dangerous when unnoticed. About 5% of patients clinically cured from typhoid remain carriers for months or even years. Antibiotics are usually ineffective on *Salmonella* carriage (even if salmonellae are susceptible to them) because the site of carriage may not allow penetration by the antibiotic.

Antigenic Structure

The genus *Salmonella* has three kinds of antigens .

1) Somatic (O) or cell wall antigens are heat stable (resistant to prolonged heating at 100 C) , also resistant to alcohol and dilute acids. Approximately 60 somatic O antigenic groups exist; however, 98% of *Salmonella* isolates belong to serogroups A through G. it is present in two forms major O antigen and it is regarded as a group specific and minor O



antigen which is sub group specific

2) The " Vi " antigens, Vi (from the term ***vi****rulence*) (referred to as "K" capsular antigens in other *Enterobacteriaceae*) , a heat- labile antigen is surface polysaccharide capsular antigen found in *Salmonella Typhi* and a few strains of *Salmonella Choleraesuis*. The capsular antigen plays a significant role in preventing phagocytosis of the organism. , Salmonella species having "Vi" antigens tend to be more virulent than those lacking them .
3) Flagellar (H) Antigens
   are heat-labile proteins and inactivated by heating over 60C and also by alcohol and acids. Flagellar antigens occur in two forms called phase 1(specific phase) and phase 2 (non specific phase) and the organisms tend to change from one phase to the other , this is called phase variation The Kauffmann-White classification of Salmonellae is based on agglutination with O antigens and phase 1 and phase 2 H antigens and classified this genus over 2000 serotype.

<u>Virulence Factors</u>

Factors responsible for the virulence of salmonellae includes;

1) The role of fimbriae in adherence , It is apparent that fimbriated strains appear more virulent than nonfimbriated strains.
2) their ability to invade intestinal mucosa.
3) Enterotoxin produced by certain *Salmonella* strains that cause gastroenteritis

<u>Isolation and Identification of *Salmonella* ;</u>

During the first week of typhoid , Salmonella can usually isolated from blood , but positive blood cultures are less common as the disease progresses, fecal culture which may be

positive at any stage is more often , so in the second and third weeks Salmonella may also found in urine .

The most commonly used media selective for *Salmonella* are SS agar, bismuth sulfite agar, Hektoen enteric (HE) medium, brilliant green agar and Xylose-lisine-deoxycholate (XLD) agar. All these media contain both selective and differential ingredients .

Treatment ;

Chloramphinicol, amoxicillin , cotrimoxazole are effective drugs. . Three types of typhoid vaccines are currently available for use , No typhoid vaccine is 100% effective and is not a substitute for being careful about what you eat or drink.

The paratyphoid bacilli ;

S.paratyphi A,B,C which also cause enteric fever but usually a milder form than that due to *S.typhi* .

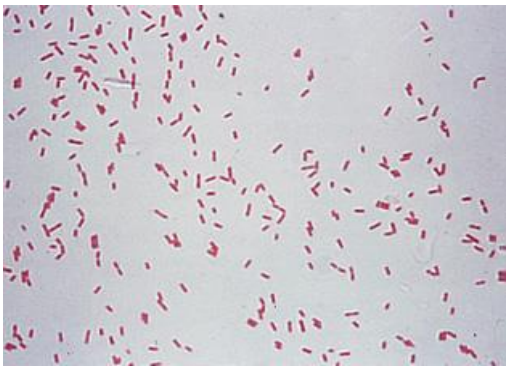
Other Salmonella;

Most of the other Salmonella types are animal pathogens which occasionally attack human

Lec. eighteen ; *Pseudomonas aeruginosa*

Genus Pseudomonas

They are mostly saprophytes free-living bacterium, commonly found in water, and soil . They are involved as secondary invaders causing suppurative and inflammatory lesions. There are a large number of Pseudomonas species (greater than 140 species) , the most important of which is *P.aeruginosa* , Pseudomonas species are most frequently seen as contaminants but has become recognized as opportunistic pathogen . It causes a variety of infections, particularly in patients with severe burns , cancer and AIDS patients . The case fatality rate in these patients is near 50 percent.



Characteristics;

- *Pseudomonas aeruginosa* is a Gram-negative rod , motile by means of a single polar flagellum. , non fermentative aerobes that can utilize acetate for carbon and ammonium sulphate for nitrogen.

• has very simple nutritional requirements, it does not require enriched media for growth . It is often observed growing in distilled water , which is evidence of its minimal nutritional needs.

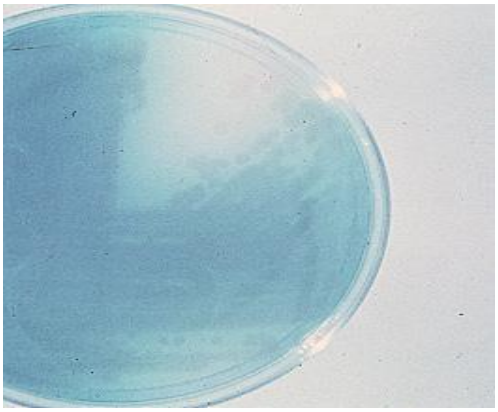
• Its optimum temperature for growth is 37 degrees, and it can survive and multiply over a wide temperature rang (20- 42 C).

• It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.

colonies has a characteristic intense fruity odor, hemolysis is usually produced on blood agar, the positive oxidase reaction of *P.aeruginosa* differentiates it from the enterobacteriaceae,

P. aeruginosa strains produce two types of soluble pigments, the fluorescent pigment pyoverdin (fluorescin, a yellow pigment is produced by *P.aeruginosa* and other free living less pathogenic Pseudomonas species) and the blue pigment pyocyanin .

Some strain may be non-pigmented. its production of blue, yellow or rust colored pigments differentiates it from most other gram negative bacteria, the blue pigment, pyocyanin, is produced only by *P.aeruginosa*, pyocyanin and fluorescin combined produce a bright green color that diffuses throughout the medium.



Pseudomonas aeruginosa is notorious for its resistance to antibiotics and is a dangerous pathogen , its resistance to antibiotics is due to

1) the permeability barrier afforded by its Gram-negative outer membrane.
2) its tendency to colonize surfaces as a biofilm making the cells impervious to antibiotics.
3) Since its natural habitat is the soil, living in association with the bacilli, actinomycetes and molds has developed resistance to a variety of their naturally-occurring antibiotics.
4) Moreover, *Pseudomonas* maintains antibiotic resistance plasmids.

Diagnosis;

Diagnosis of *P. aeruginosa* infection depends upon isolation and laboratory identification of the bacterium. It grows well on most laboratory media and commonly isolated on blood agar or eosin-methyline blue agar. It is identified on the basis of its inability to ferment lactose, a positive oxidase reaction, its fruity odor, pyocyanin production and its ability to grow at 42°C.

Pathogenesis **;**

It is commonly encountered in secondary infection of wound, burns and chronic ulcers of skin Localized infections can lead to generalized, and occasionally fatal bacteremia , it also responsible for a number of nosocomial infections including urinary tract infections following catheterization, pneumonia resulting from contaminated respirators, also eye and ear infections.

Most *Pseudomonas* infections are both invasive and toxinogenic.

Toxinogenesis;

In addition to the LPS endotoxin characteristic of other Gram-negative bacteria , *Pseudomonas* produces many exoenzymes including hemolysins, leukocidins and proteases. Also it produce Exotoxins of two types A and B. Exotoxin A is the most toxic product produced by *Pseudomonas*. It is a polypeptide and inhibits protein synthesis in the cell .

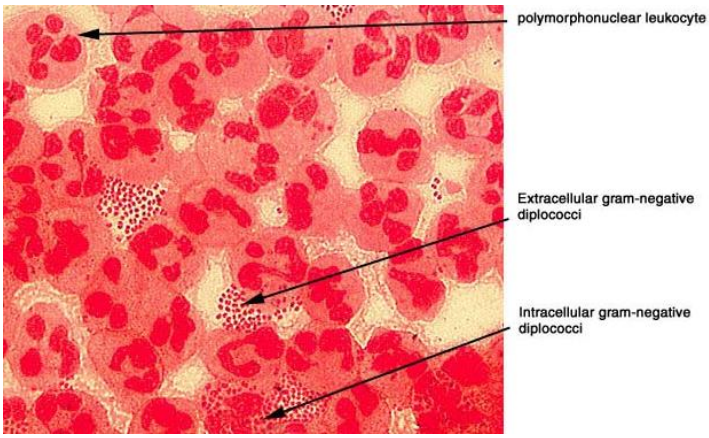
Pseudomonas has an antiphagocytic slime layer. Exoenzyme S is produced by bacteria growing in burned tissue and may be detected in the blood before the bacteria are. It has led to the suggestion that exoenzyme S may act to impair the function of

phagocytic cells in the bloodstream and internal organs as a preparation for invasion by *P.aeruginosa.*

Treatments ;

Pseudomonas aeruginosa is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and fluoroquinolins, Clinical infection may not be treated with one antibiotic. The combination of gentamicin and carbenicillin is frequently used to treat severe *Pseudomonas* infections.

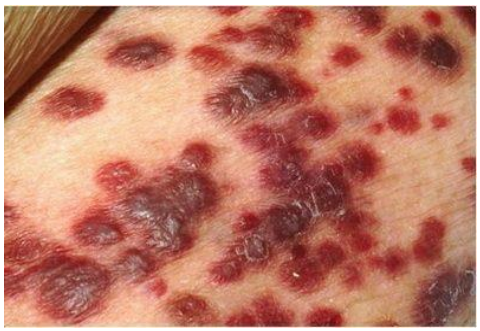
Lec. nineteen ; The Pathogenic Neisseria



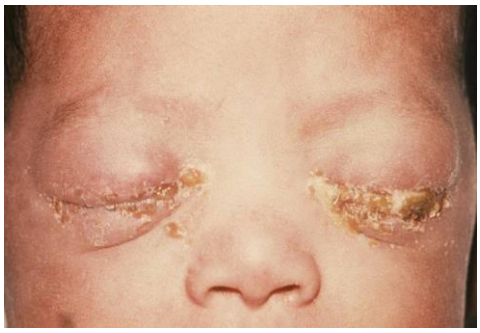
The family *Neisseriaceae* consists of Gram-negative aerobic bacteria , oxidase & catalase positive, non motile. The genus *Neisseria* contains 12 species that can be isolated from humans two of them are important pathogens , *N. gonorrhoeae* causes gonorrhea and *N. meningitides* which cause meningitis , in stained films the cocci are usually arranged in pairs of kidney shaped many of which are inside pus cells.

N. gonorrhoeae (Also referred as gonococcus) is a relatively fragile organism, susceptible to temperature changes, drying, uv light, and other environmental conditions. Strains of *N. gonorrhoeae* are variable in their cultural requirements so that media containing hemoglobin, NAD, yeast extract and other supplements are needed for isolation and growth . Cultures are grown at 35-36 C in an atmosphere of 3-10% added CO_2.

Neisseria gonorrhoeae infections are acquired by sexual contact and usually affect the mucous membranes of the urethra in males and the endocervix in females, although the infection may disseminate to a variety of tissues. The majority of infected women are asymptomatic while greater than 90% of infected men are symptomatic . gonococci infecting the genitalia may invade blood stream and be carried to various parts of the body to give rise to a variety of syndromes collectively called disseminated gonococcal infection (DGI) , the two most common (DGI) are acute septic arthritis and dermatitis.



In addition to venereal transmission, *N. gonorrhoeae* may be transmitted by nonsexual contact, neonatal ophthalmia is the infection of the neonate eyes with this bacteria during the passage through the birth canal of infected mother.



Virulence Factors

The pathogenic *Neisseria* have several characteristics that contribute to their virulence, including the following:

- Receptors for human transferrin
- Capsule; serve as protective device of the organism
- Pili (fimbriae) ; *N. gonorrhoeae* is divided into five morphologically distinct colony types. Types T1 through T5 are based on the presence or absence of pili, they are important in

1) attachment of the organism to host tissues
2) inhibit phagocytosis of the organism by neutrophils
3) aid in the exchange of genetic material between cells.

- cell outer membrane proteins I, II, and III,

• Endotoxin ; is a major virulence factor that mediates damage to body tissues and cause the inflammatory response.
• Immunoglobulin A (IgA) protease that inactivates the IgA by cleaving the heavy chain ,
• it also produce cytotoxic factor that damage ciliated epithelial cells.

Diagnosis;

The finding of Oxidase-positive, gram negative diplococci within WBCs (PMNs) in a purulent urethral exudates strongly suggests the diagnosis of gonorrhea in men, however a similar finding in exudates from women is of limited value, to confirm the results of gram stain *N. gonorrhoeae* must be isolated & identified, isolation may be done on blood agar, chocolate agar, New York City (NYC) medium or Thayer- martin medium when the specimens are from sites that harbor a normal flora because it contains antibiotics (vancomycin, colistin, nystatin & trimethoprim) to inhibit the growth of organisms other than *N. gonorrhoeae.* plates should be incubated at 35° C in a 3% to 5% CO2 atmosphere by use of a candle jar .

Treatment

Penicillin is the drug that preferred in the treatment , unless allergy to it or resistant strains , alternative therapeutic agents include cefoxitin, tetracycline, and spectinomycin.

The recommended treatment for uncomplicated infections is a third-generation cephalosporin. Sex partners should be treated too.

Neisseria meningitides (Also referred as meningococcus) ;

It is identical in its staining and morphological characteristics to *Neisseria gonorrhoeae .* it is the causative agent of meningitis. infections occur most commonly in children between the ages of 6 months and 2 years, meningococci are transmitted from person to person by airborne droplets of the nasopharyngeal secretions, it can be carried in the upper R.T. of healthy people asymptomatically and serve as reservoir in transmitting the disease to susceptible hosts. The disease occur as sporadic cases or in epidemics in closely packed communities.

Neisseria meningitidis is usually cultivated in a peptone-blood base medium in a moist chamber containing 5-10% CO_2 at 37 degrees. In stained films made from a purulent C.S.F. the organism can be rapidly recognized and identified by its characteristic shape, arrangement and its intracellular situation.

Neisseria species can be differentiated by a variety of tests, the inability of *N. gonorrhoeae & N. meningitides* to grow at 22 C & their fastidious nutrient requirements distinguish these two species from most of the other non pathogenic species, also *N.meningitidis* can be distinguished from *N. gonorrhoeae* by maltose fermentation which is fermented by the first & not by the second. The genus Neisseria also include non pathogenic species (*N. flavesens, N. mucosa, N.subflava, N.sica*) that are part of the normal flora and may confused with the meningococci & gonococci, but these species may cause disease (eg; endocarditis and meningitis).

Fermentation reactions of some Neisseriae species

Organisms	Dextrose	Acid production from			Reduction of	
		maltose	sucrose	Lactose	NO_3	NO_2
N.meningitidis	+	+	--	--	--	Variable
N.gonorrhoeae	+	--	--	--	--	--
N. sicca	+	+	+	–	–	+
N.flavescens	–	–	–	–	–	+
N.catarrhalis	–	–	–	–	+	+

Virulence Factors

With few exceptions, *N. meningitidis* exhibits the same cellular structural characteristics as *N. gonorrhoeae*: the virulence factors pili, polysaccharide capsule, the cellular membrane proteins and endotoxin. Many virulent meningococcal strains produce IgA1 protease, an enzyme that aids invasiveness. encapsulated strains are most often associated with epidemics.

Treatment

The drug of choice for treatment of confirmed *N. meningitidis* meningitis is penicillin, but rifampin or a sulfonamide is recommended as prophylaxis for close contacts.