

BACTERIAL GENETICS

Dr. Bincy Joseph

Assistant Professor

PGIVER, Jaipur.

BACTERIAL GENETICS

- The methods of gene transfer in bacteria includes
- Bacterial conjugation
- Transformation
- Transduction



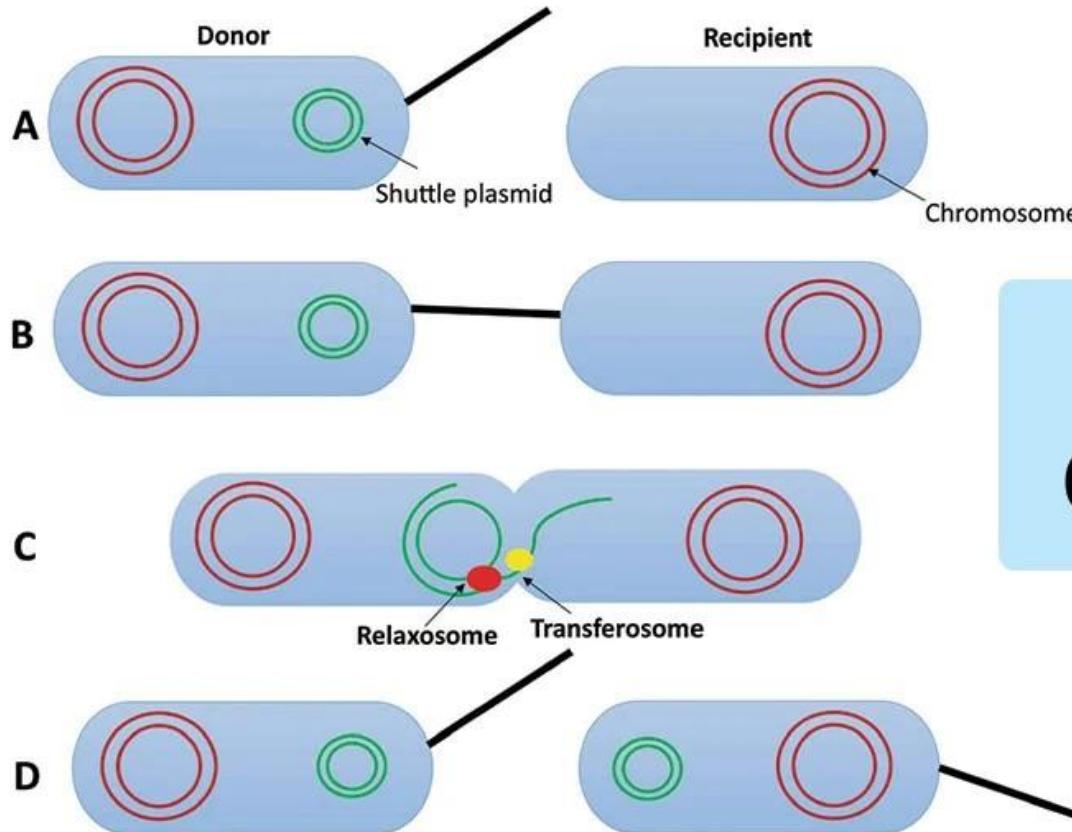
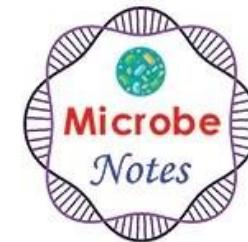
BACTERIAL CONJUGATION

- Bacterial conjugation was first demonstrated by **Joshua Lederberg and Edward Tatum** in 1946
- It is the transfer of genetic material from one bacterial cell to another by direct cell to cell contact through sex pili



DIFFERENT TYPES OF CONJUGATION

- F+ x F- conjugation
- Here the transfer of F plasmid from donor cell / F+ cell to recipient / F – cells take place
- The F+ strain contains an extra chromosomal F factor carrying the genes for sex pilus formation and plasmid transfer.
- The sex pilus is used to establish contact between F+ and F- cells.
- Once the contact is made the pilus retracts bringing the cells into close physical contact
- The F factor then replicates and one strand is moved to the recipient cell through the lumen of sex pilus
- After the entry of plasmid into the recipient cell, the entering strand is copied to produce double stranded DNA
- Now the F- recipient become F+



Bacterial Conjugation

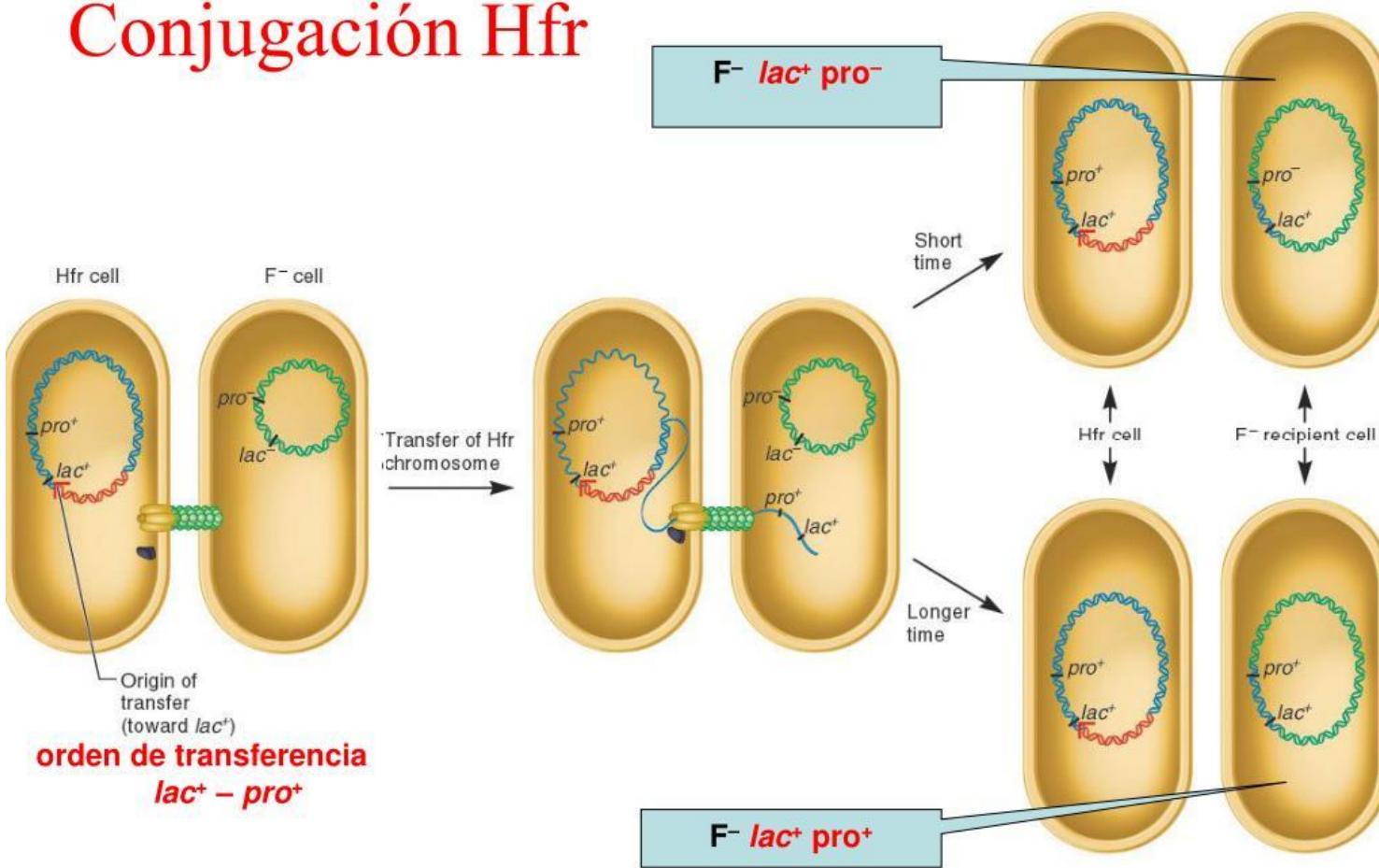
The  Biology Notes



HFR CONJUGATION

- It is called high frequency conjugation because of high frequency of recombinants produced by this mating.
- In Hfr strains the F factor is integrated into the bacterial chromosome rather than free in the cytoplasm
- So these plasmids also known as episomes
- After integration also these plasmids can produce pili carry out replication and transfer of genetic material to F- recipient.
- But here rather than transferring itself , the F factor directs the transfer of host chromosome
- Here only part of F factor is transferred to the recipient cell, so the F- recipient cells remain F- itself

Conjugación Hfr



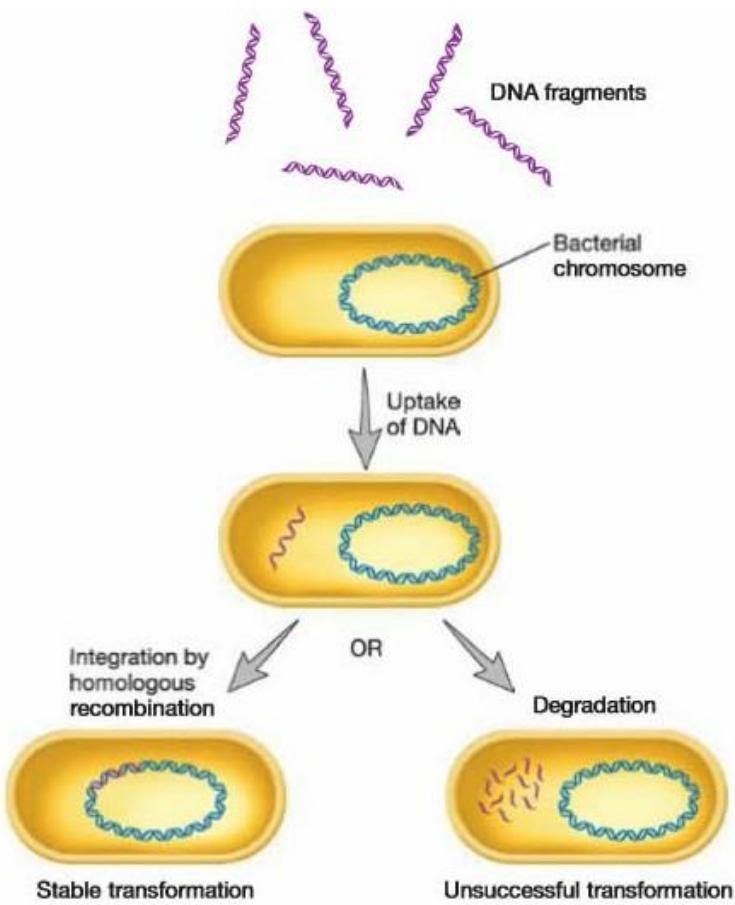
F' CONJUGATION

- The F factor is an episome when leaves the bacterial chromosome,, pick up a portion of bacterial chromosome along with it.
- Since it contains bacterial DNA also it is known as F' plasmid. Then it conjugate with F- recipient
- F'x F- conjugation is similar to F+ x F- mating and the recipient become F' +ve
- Conjugation occur mainly in E. coli and other Gram- negative bacteria.

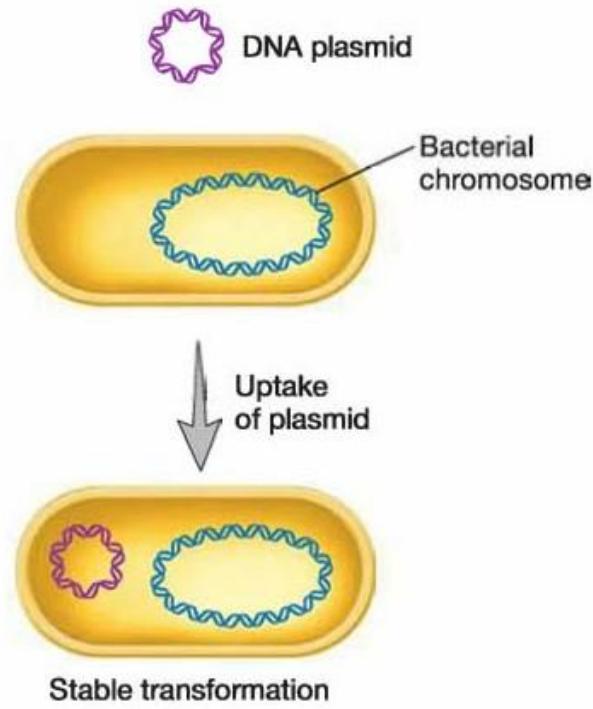


DNA TRANSFORMATION

- The transformation is the uptake of Naked DNA molecule/ fragment from the medium and incorporation of these molecule into the recipient chromosome in a heritable form
- Transformation was discovered by Frederich Griffith in 1928
- Natural transformation was discovered in **Streptococcus, Bacillus and Pseudomonas**
- A cell that is able to take up DNA and get transformed are called **competent cells**
- The mechanism of transformation has been intensively studied in **Streptococcus pneumoniae**



(a) Transformation with DNA fragments



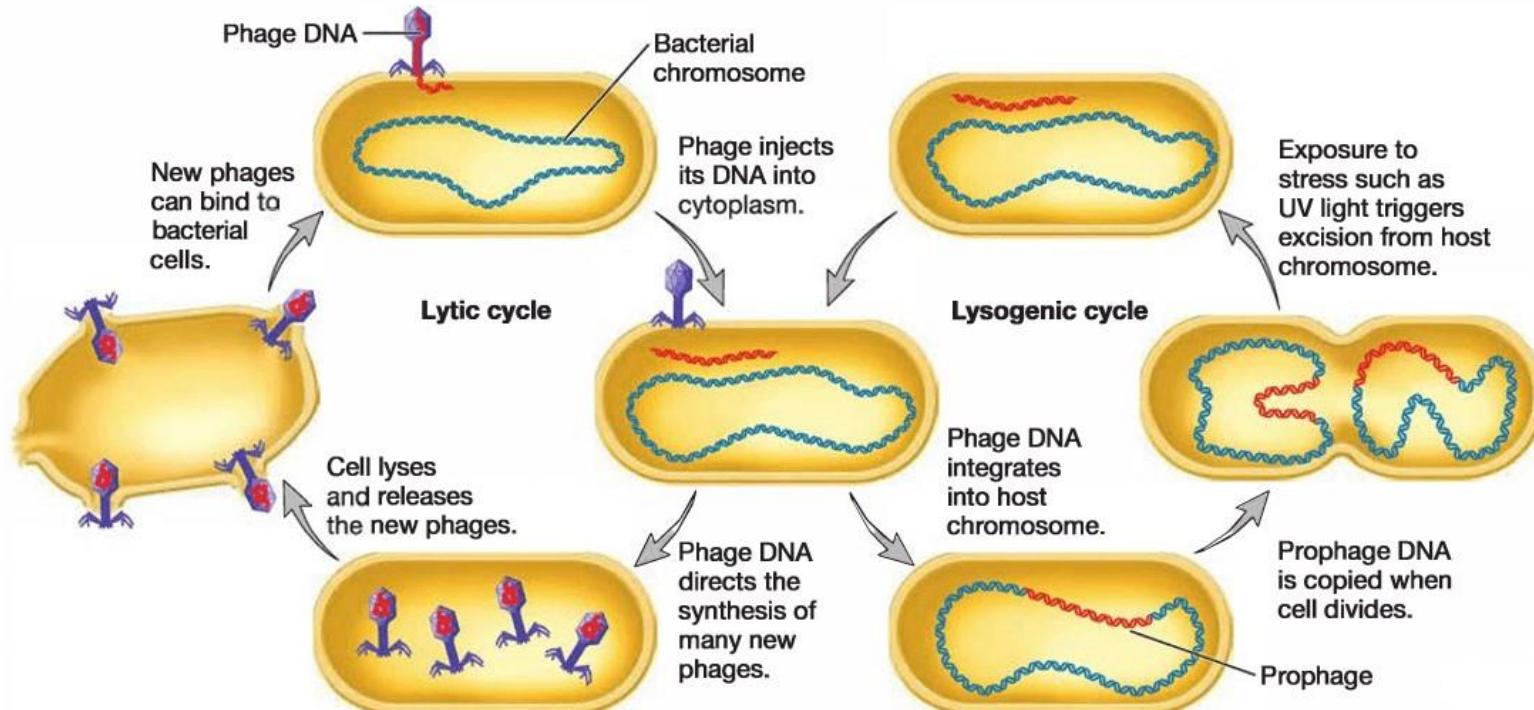
(b) Transformation with a plasmid

- The competent cells bind with double stranded DNA fragments, if the fragment is moderately large.
- Then the DNA is cleaved by endonucleases to double stranded fragments of 5 to 15 kilo bass
- It is an energy dependant procedure and one strand is hydrolyzed by exonuclease and the other strand associates with small protein and move through the plasma membrane
- Then the single stranded fragment get integrated to the bacterial genome
- We can artificially makes the cells competent by electrical shock and treatment with Calcium chloride

TRANSDUCTION

- Transduction is the bacteriophage mediated transfer of genes from one bacteria to other .
- It is discovered by Joshua Lederberg and Zinder in 1951 in *Salmonella enterica* serovar Typhimurium
- Viruses that infect bacteria are called bacteriophages
- Some phage replicate in their bacterial host immediately after entry
- After the number of replicated phages reaches a certain number , they cause the host cell to lyse and infect new host cells
- These phages are called virulent phages and the process is called lytic cycle





- Other bacteriophage after entry into the bacterium, instead of replicating insert their genome into the bacterial chromosome
- Phage DNA inserted into the bacterial genome is called prophage
- The host bacterium is not harmed by this
- Here the phage genome also replicate along with the bacterial genome
- These bacteriophages are called **temperate phages** and the relationship between the virus and host is called **lysogeny**
- The bacteria that have been lysogenised are called **lysogens**

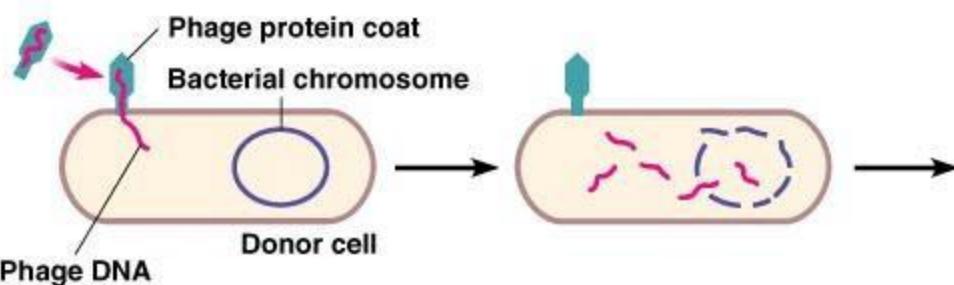
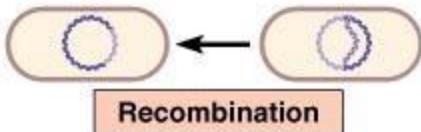
- These temperate phages can be induced to switch to a lytic cycle of growth under certain condition such as UV irradiation
- Here the prophage excise from the bacterial genome and enter into the lytic cycle



TWO TYPES OF TRANSDUCTION

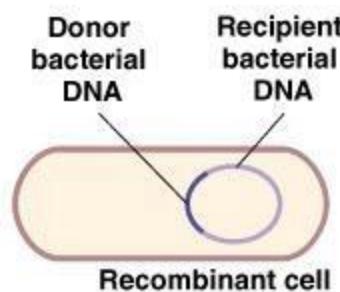
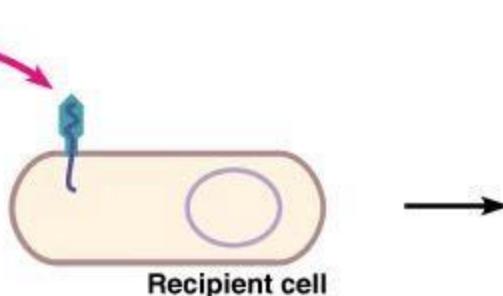
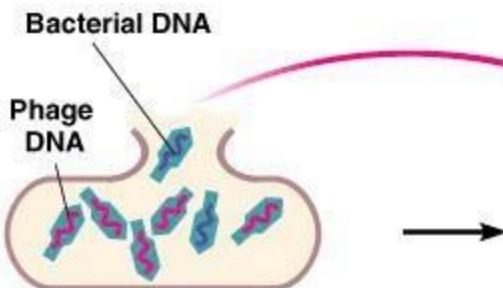
- **Generalized transduction**
- Generalized transduction occur during the lytic cycle of virulent phage and some temperate phages can transfer any part of the bacterial genome
- Here during the assembly stage when viral chromosomes are packed into the protein capsids, random fragments of partially degraded bacterial chromosome also may be packed by mistake
- Because the capsid can contain only a limited quantity of DNA, the viral DNA is left behind





- 1** A phage infects the donor bacterial cell.

- 2** Phage DNA and proteins are made, and the bacterial chromosome is broken down into pieces.



- 3** Occasionally during phage assembly, pieces of bacterial DNA are packaged in a phage capsid. Then the donor cell lyses and releases phage particles containing bacterial DNA.

- 4** A phage carrying bacterial DNA infects a new host cell, the recipient cell.

- 5** Recombination can occur, producing a recombinant cell with a genotype different from both the donor and recipient cells.

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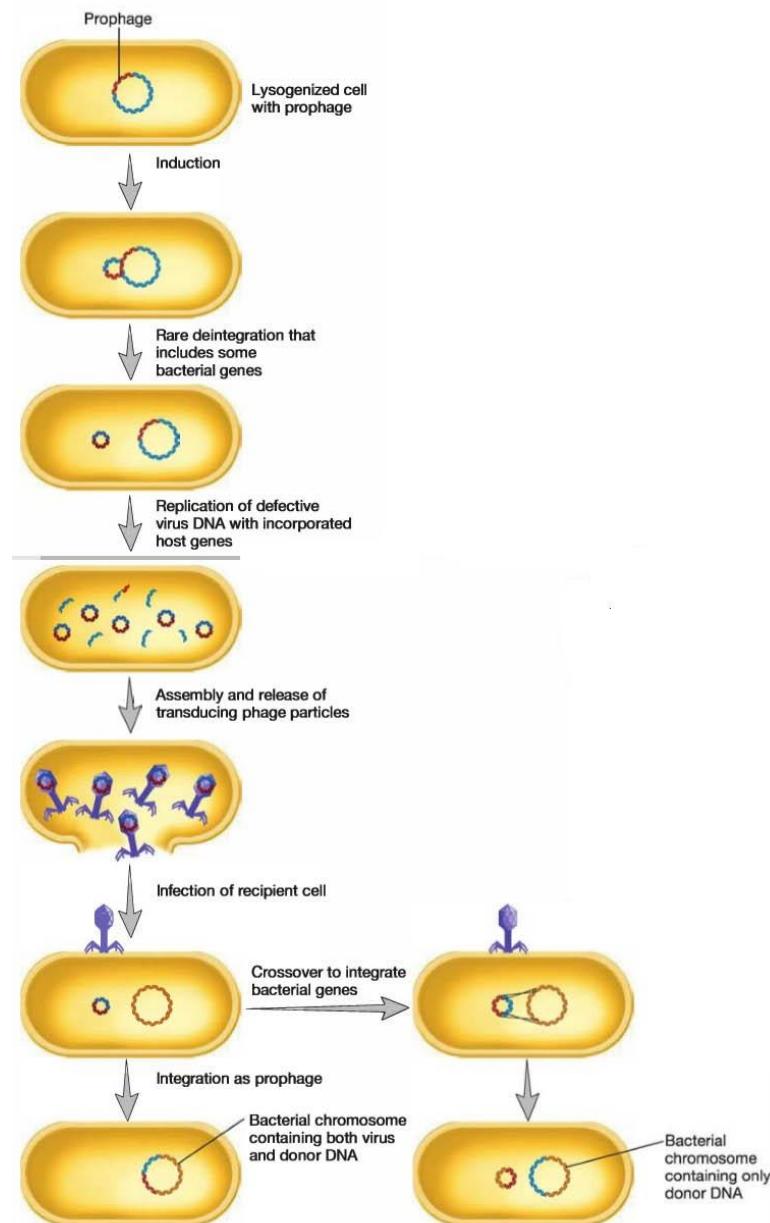
- The resulting viral particle often injects DNA into other bacterial cell, but can not initiate a lytic cycle
- As in transformation, one DNA has been injected, it must be incorporated into the recipient cells chromosome for stable transduction



SPECIALISED TRANSDUCTION

- In specialized transduction the transducing particle carries only specific portion of the bacterial genome
- It occurs in the lysogenic life cycle of temperate phage
- Here when a prophage is induced to leave the bacterial genome, it will carry some portion of bacterial gene next to the integration site
- The transducing phage will inject bacterial genes into another bacterium and get incorporated into the recipients genome

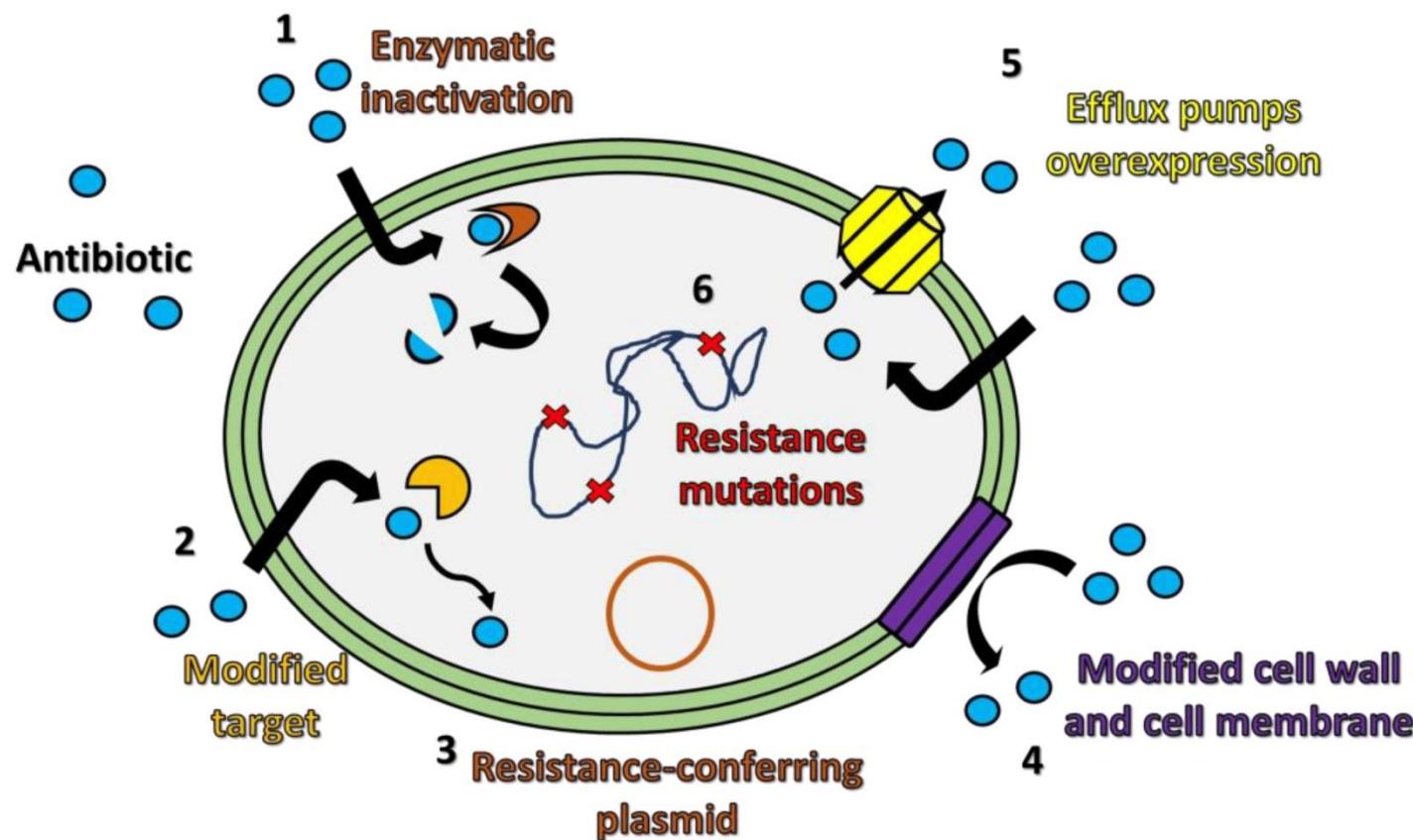




ANTIBACTERIAL RESISTANCE

- Resistance to antibiotics occur as a result of drug inactivation, drug target modification or decreased intracellular accumulation associated with reduced membrane permeability or increased drug efflux
- In broad terms resistance can be
 - **Innate (intrinsic)**
 - **Acquired (extrinsic)**
- Innate resistance is chromosomally encoded and is due to cell wall complexity, efflux mechanism or enzyme inactivation of antibiotic
- Acquired resistance can arise from a mutation of gene/ transfer of genetic material encoding resistance gene *via* conjugation, transformation and transduction





GENUS LISTERIA AND ERYSEPELOTHRIX



॥પણેણ નિદ્યં સર્વલોકાપકારકમ्॥

**Dr. Bincy Joseph
Assistant Professor
Veterinary Microbiology**

LISTERIA

- Kingdom Bacteria
- Division Firmicutes
- Class Bacilli
- Order Bacillales
- Family *Listeriaceae*
- Genus *Listeria*



HISTORY:

- *L. monocytogenes* first described by Murray (1926) who named it as Bacterium monocytogenes because of characteristic monocytosis infection in laboratory animals (rabbits).
- It was renamed *Listerella hepatolytica* by Pirie (1927) and the present name given by him in 1940.
- The *Listeria monocytogenes* was first isolated by Gill (1929) from sheep.



INTRODUCTION

- Gram positive rods/ coccobacillary up to 0.4-0.5 µm in length, old cultures stain gram negative
- From rapidly growing cultures or tissues the cells can appear coccal
- Motile by few (1-5) peritrichous flagella-end - over - end tumbling motility, Flagella are produced at room temperatures, but not at 37°C
- Beta hemolytic - CAMP Test positive with *Staphylococcus aureus* (*L. monocytogens* and *L. seeligerii*) or *Rhodococcus equi* (*L. ivanovii*).
- Psychrophilic- Grows well at refrigerated temperature - Basis of a cold- enrichment technique.
- Facultative intracellular bacterium of the reticulo endothelial system that causes listeriosis (circling disease, silage disease)

- Small, Gram-positive rods
- Grow on non-enriched media
- Catalase Positive, Oxidase-negative, Facultative anaerobes
- Non-acid fast, Non-spore forming
- Tolerates wide temperature and pH ranges
- Small haemolytic colonies on blood agar
- Tumbling motility at 25°C (non motile at 37°C)
- Aesculin hydrolysed
- Environmental saprophytes
- Outbreaks of listeriosis often related to silage feeding



- The genus is composed of six species, three of which are pathogenic.
- *Listeria monocytogenes*, the most important of these pathogens, has been implicated world-wide in diseases of many animal species and humans.
- It was first isolated from laboratory rabbits with septicaemia and monocytoisis (Murray et al., 1926).
- The organism can grow over a wide temperature range from 4°C to 45°C and can tolerate pH values between 5.5 and 9.6.
- The other two pathogens, *L. ivanovii* and *L. innocua*, are less frequently implicated in diseases of animals.



HABITAT AND ECOLOGY:

- *Listeria monocytogenes*, is wide spread in the environment throughout the world.
- Found in soil, dust, mud, vegetation, silage, sewage and most of the animals that have been tested.
- Grow well under a wide temperature from 4 - 44°C, relatively resistant to high salt concentrations (10% NaCl) and can grow from pH 5-9.
- Over growth of the bacterium in improperly prepared silage (poorly fermented silage with pH>5) / vegetation is the source of infection to animals.
- Found in the faeces of both clinically ill and sub clinically infected ruminants & humans (Healthy carriers).



CULTURAL CHARACTERISTICS:

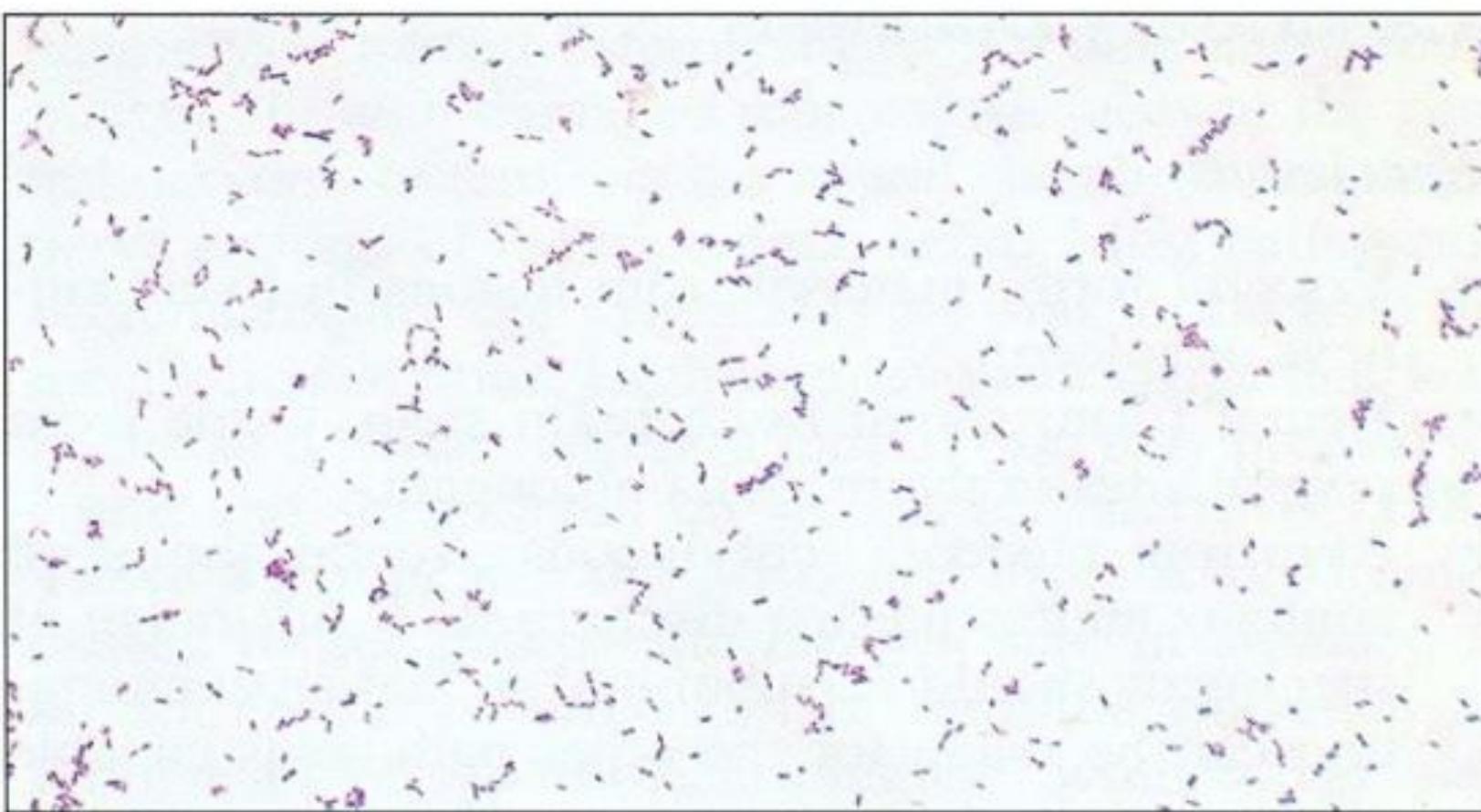
- Can grow on ordinary medium.
- Blood agar plates -produce small **bluish white** colonies with or without hemolysis.
- Commercial selective and indicator media include – **PALCAM agar (polymixin-acriflavin-lithium chloride - ceftazidime-esculin-mannitol agar)** to isolate from foods.
- On brain heart infusion agar-blue-green sheen when light is reflected obliquely at 45°C.
- Grow between 4 and 45°C (heat tolerant).
- Grows well at refrigerated temperature.



BIO-CHEMICAL PROPERTIES:

- Catalase positive
- motility in tube media at 25°C
- H₂S negative, Indole negative.
- Bile Esculin Agar (*BEA*) positive
- Glucose, trehalose, and salicin positive
- Growth at 4°C
- Nitrate and urease negative
- Resistant to drying and can survive up to two years in dry soil and feces.
- **Anton Test:** Broth culture in eye sac of rabbit or guinea pig produces conjunctivitis





203 *L. monocytogenes* in a stained smear from a culture with both Gram-positive rods and cocci present.
(Gram stain, $\times 1000$)

VIRULENCE FACTOR

- **Internalins (InlA)** – are surface associated proteins that act to facilitate the uptake of the bacterium into epithelial cells
- **Listeriolysin O (LLO)** - is a cholesterol-binding haemolysin. It is a pore forming toxin that facilitates the escape of the organism from the endosome to the cytosol. Inhibits phagolysosome formation.
- **Phospholipase C** - lyse the phagosome membrane and escapes the organism into cytosol. It is phosphatidyl inositol independent.
- **ActA** – a surface protein (transmembrane protein) that facilitates the rearrangement of actin to propel the organism through the cell and into an adjacent cell



DIFFERENTIATION OF LISTERIA SPECIES

- The pattern of haemolysis on sheep blood agar, CAMP tests and acid production from a short range of sugars are useful differentiating laboratory methods for Listeria species.
- Sixteen serotypes, based on cell wall and flagellar antigens, are recognized.
- Phage typing is reproducible and discriminating but its diagnostic applications are limited.
- A chemiluminescent DNA probe assay is available for rapid and specific identification of *L. Monocytogenes* from colonies on primary isolation plates.
- DNA fingerprinting methods are currently used in reference laboratories.



<i>Listeria</i> species	Haemolysis on sheep blood agar	CAMP test		Acid production from sugars		
		<i>S. aureus</i>	<i>R. equi</i>	D-mannitol	L-rhamnose	D-xylose
<i>L. monocytogenes</i>	+	+	-	-	+	-
<i>L. ivanovii</i>	++	-	+	-	-	+
<i>L. innocua</i>	-	-	-	-	V	-
<i>L. seeligeri</i>	+	+	-	-	-	+
<i>L. welshimeri</i>	-	-	-	-	V	+
<i>L. grayi</i>	-	-	-	+	V	-

v variable reactions

Clinical manifestations of infections of Listeria spp.

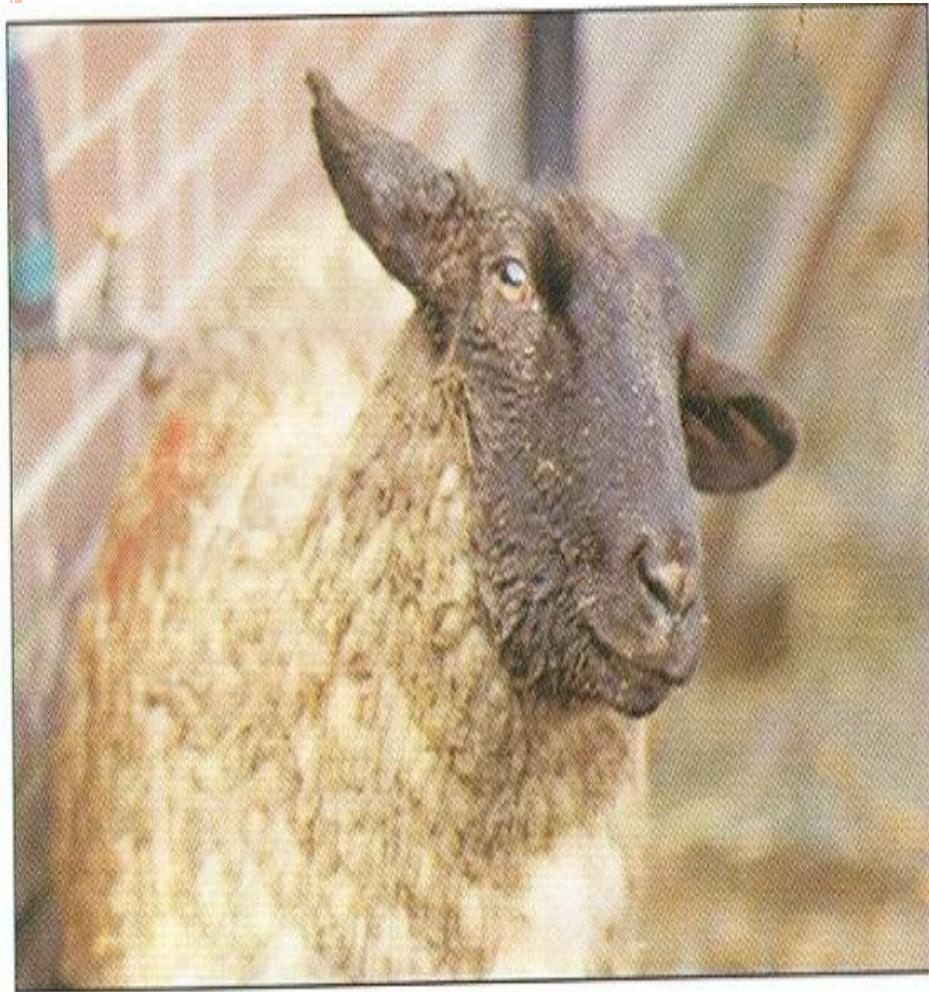
Species	Hosts	Forms of disease
<i>Listeria monocytogenes</i>	Sheep, cattle, goats	Encephalitis (neural form) Abortion Septicaemia Endophthalmitis (ocular form)
	Cattle	Mastitis (rare)
	Dogs, cats, horses	Abortion, encephalitis (rare)
	Pig	Abortion, septicaemia, encephalitis
	Birds	Septicaemia
<i>L. ivanovii</i>	Sheep, cattle	Abortion
<i>L. innocua</i>	Sheep	Meningoencephalitis (rare)

- Encephalitis (infection of the central nervous system).
 - "**Circling disease**" -- caused by brain involvement
 - Unilateral facial paralysis results in drooling of saliva and drooping of the eyelid and ear
- Abortion (infection of the uterus or the fetus).
 - Generally during the **last two months of gestation** because of production practices
 - Occurs in winter
 - Associated with consumption of spoiled silage
 - Animals that abort are resistant to re-infection

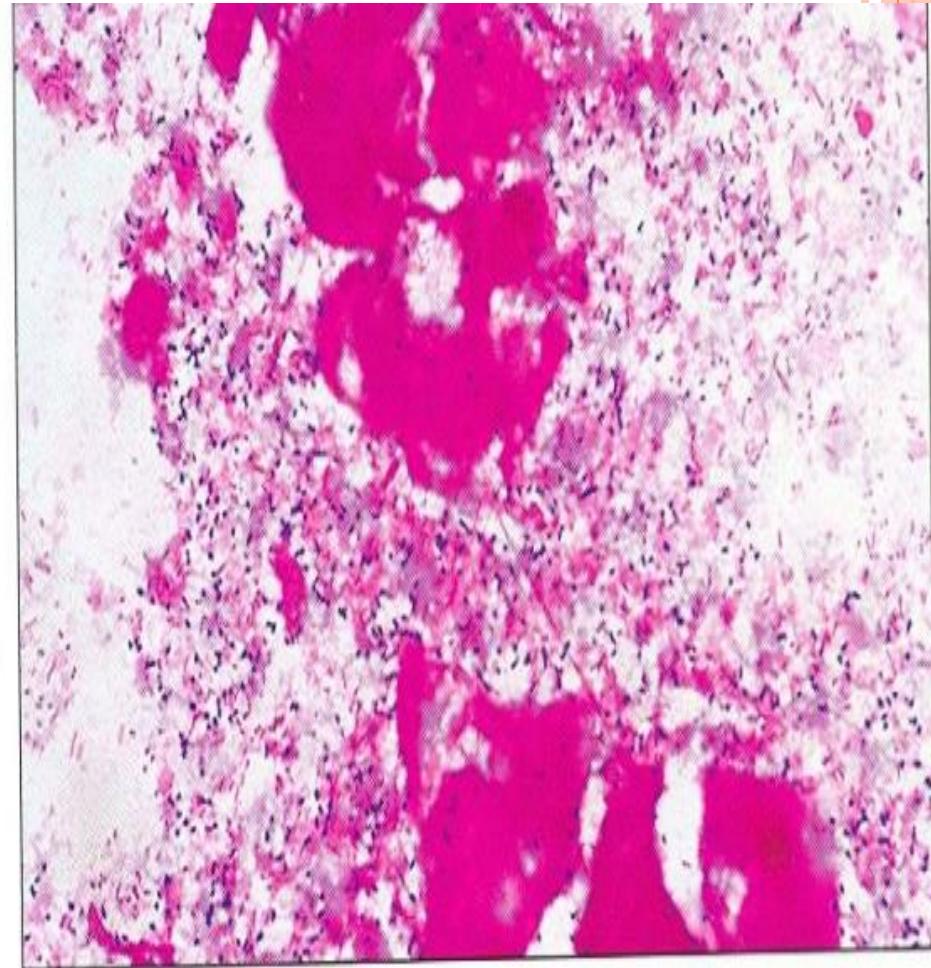


- Septicemia (infection of the blood).
 - Primarily occurs in young animals
 - Can be exhibited by sudden death
 - Kerato-conjunctivitis
- Mastitis (infection of the mammary gland).
 - Bacteria can be shed in milk
 - Shedding can persist for more than 3 years





198 *Listeria monocytogenes*: neural form of listeriosis in a silage-fed sheep showing unilateral facial paralysis.



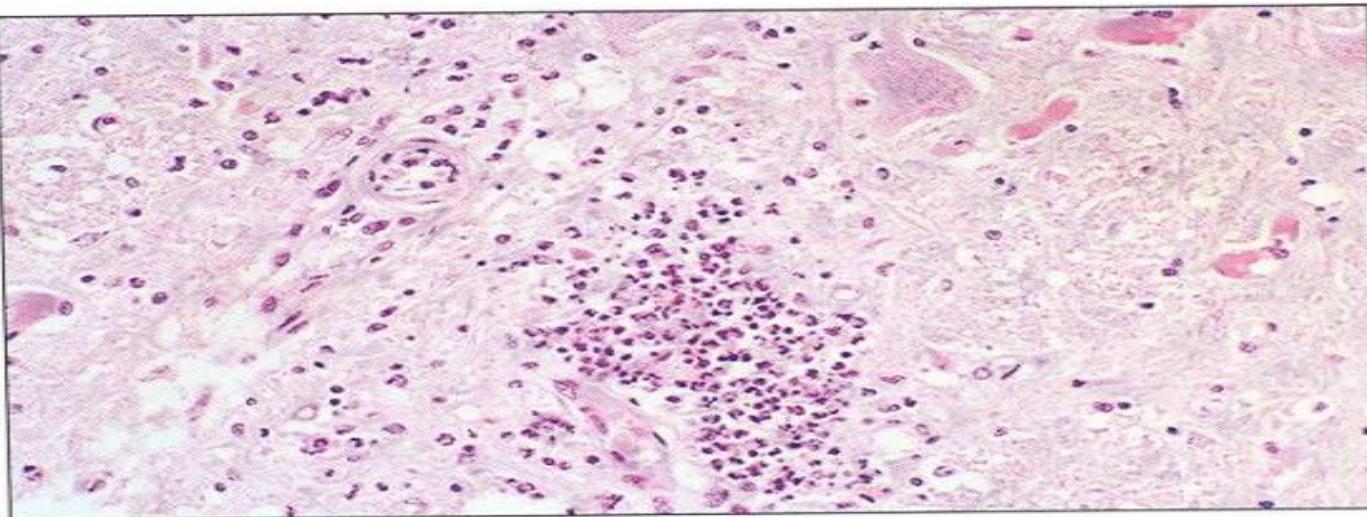
199 *L. monocytogenes* in a Gram-stained smear of material from a placenta (bovine abortion). ($\times 1000$)

DIAGNOSIS

- Characteristic neurological signs or abortion in association with silage feeding may suggest listeriosis.
- Cerebrospinal fluid (CSF) and tissue from the medulla and pons of animals with neurological signs should be sampled.
- Fresh tissue is required for isolation of organisms and fixed tissue for histopathological examination.
- Specimens from cases of abortion should include cotyledons, foetal abomasal contents and uterine discharges.
- Smears from cotyledons or from liver lesions may reveal Gram-positive coccobacillary bacteria.

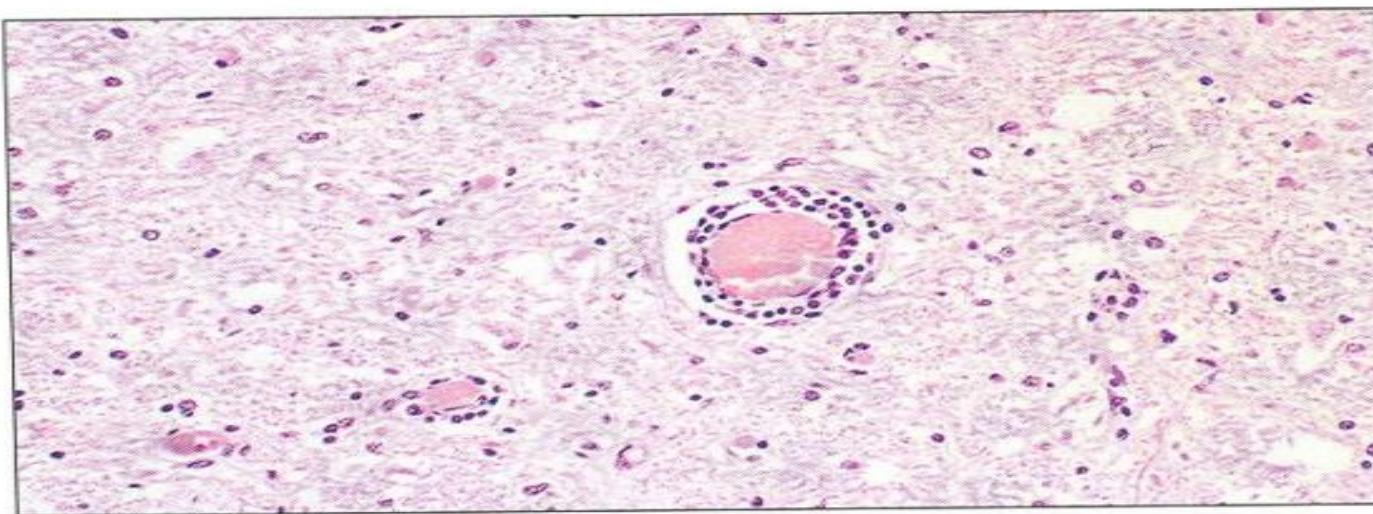
- Histological examination of brain tissue reveals microabscesses and heavy perivascular mononuclear cuffing in the medulla and elsewhere in the brain stem.
- White cell numbers exceeding $1.2 \times 10^7 /L$ and a protein concentration of greater than 0.4g/L in CSF are found in neural listeriosis.
- Inoculation in developing chicken embryos causes development of focal necrotic lesions on the chorio allantoic membrane (CAM).

200



200 A microabscess in the medulla of a sheep with listeriosis. (H&E stain, $\times 400$)

201



201 Perivascular cuffing in an ovine medulla indicative of the neural form of listeriosis. (H&E stain, $\times 400$)

ISOLATION METHODS

- Specimens from cases of abortion and septicaemia can be inoculated directly onto blood, selective blood (PALCAM) and MacConkey agars. The plates are incubated aerobically at 37°C for 24 to 48 hours.

Cold-enrichment

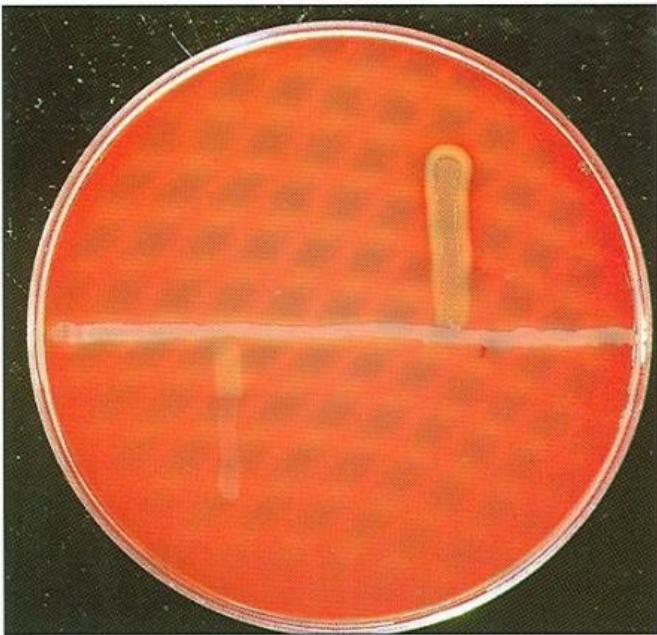
- Small pieces of spinal cord and medulla are homogenized and a 10% suspension is made in a nutrient broth.
- Broth suspension is placed in the refrigerator at 4°C and sub cultured into blood agar once weekly for up to 12 weeks.



Identification criteria for *L. monocytogenes* isolates:

- Colonies are small, smooth and flat with a blue-green colour when illuminated obliquely.
- Individual colonies are usually surrounded by a narrow zone of complete haemolysis.
- CAMP test is positive with *Staphylococcus aureus* but not with *Rhododococcus equi*
- Aesculin is hydrolysed.
- Isolates incubated in broth at 25°C for 2 to 4 hours exhibit a characteristic tumbling motility.
- Most isolates of animal origin are virulent, a characteristic which can be confirmed by animal inoculation (Anton test).

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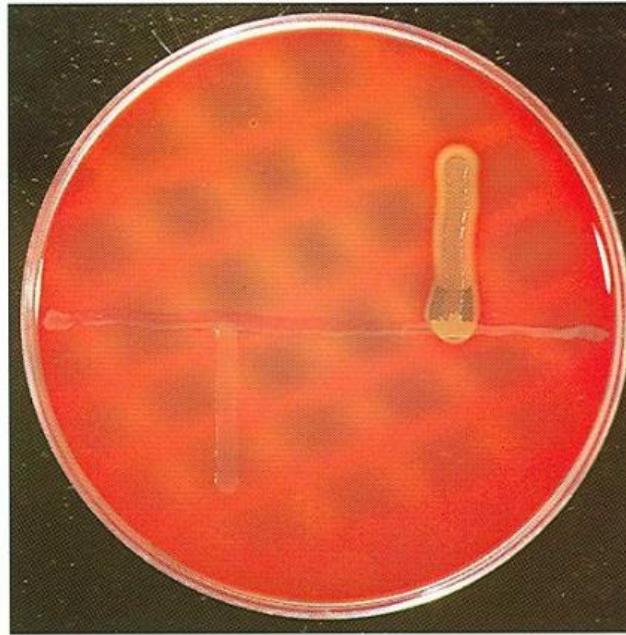


205 CAMP test with *Staphylococcus aureus* (horizontal) showing enhancement of the effect of the staphylococcal beta-haemolysin by *L. monocytogenes* (left) but not by *L. ivanovii* (right).

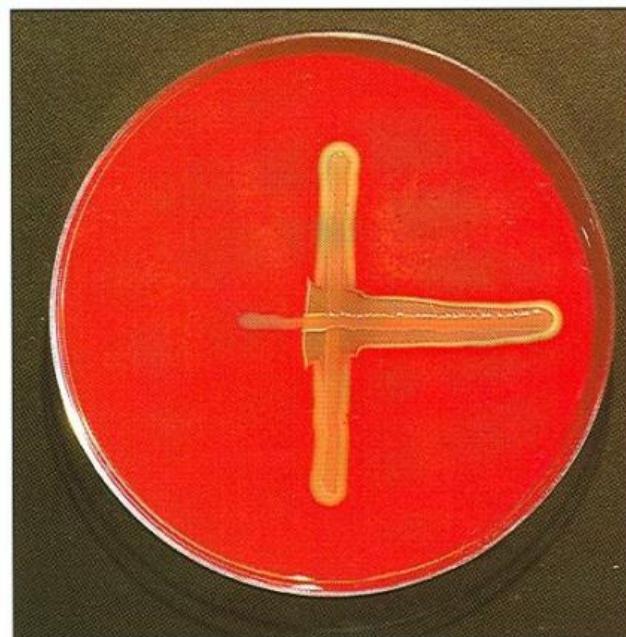
206 CAMP test with *Rhodococcus equi* (horizontal): no reaction by *L. monocytogenes* (left) and enhancement of haemolysis by *L. ivanovii* (right).

207 *Rhodococcus equi* streaked across (left to right) a vertical streak of *L. ivanovii* giving an enhanced haemolytic effect.

206



207



ANTON'S TEST:

- Inoculation of live bacterial suspension into the conjunctiva of a rabbit or guinea pig only
- *L. monocytogenes* causes a purulent kerato-conjunctivitis within 24-36 hrs of inoculation.

ERYSIP ELOTHRIX



CLASSIFICATION

- Kingdom Bacteria
- Phylum Firmicutes
- Class Erysipelotrichi
- Order Erysipelotrichales
- Family Erysipelotrichidae
- Genus *Erysipellothrix*



INTRODUCTION

- *Erysipelothrix rhusiopathiae* (previously named *Erysipelothrix insidiosa*) is, a non-motile, non-spore forming, Gram- positive, facultative anaerobe.
- It is catalase-negative, oxidase-negative, resistant to high salt concentrations (8.5% NaCl) and grows in the temperature range 5°C to 42°C and in the pH range of 6.7 to 9.2.
- Gram-positive, small rods (smooth form) or filaments (rough form) Growth on non-enriched media Small colonies, Exhibit alpha haemolysis or non haemolytic.
- Causes **swine erysipelas** and **turkey erysipelas**



USUAL HABITAT

- It is claimed that up to 50% of healthy pigs harbour *E. rhusiopathiae* in tonsillar tissues.
- Carrier pigs excrete the organism in faeces and in oro-nasal secretions.
- The bacterium has also been isolated from sheep, cattle, horses, dogs, cats, poultry.



COLONIAL MORPHOLOGY AND HAEMOLYTIC ACTIVITY

- Non-haemolytic, pin-point colonies (0.05 mm) appear after incubation for 24 hours and, after 48 hours, a narrow zone of greenish, incomplete haemolysis develops around the colonies.
- Smooth colonies are up to 1.5 mm in diameter, convex and circular with even edges while rough colonies are slightly larger, flat and opaque with irregular edges.
- A '**bottle-brush**' type of growth is characteristic of rough isolates when they are stab- inoculated into **nutrient gelatin** and incubated at room temperature for up to 5 days.



BIOCHEMICAL REACTIONS

- Catalase negative, Oxidase negative
- Coagulase-positive, few pathogens produce this enzyme apart from some staphylococci.
- H₂S production is detected by a thin, black central line in triple sugar iron (TSI) agar when this medium is stab-inoculated at 37°C for 24 hours.



VIRULENCE FACTORS

- A **capsule** which protects against phagocytosis, ability to adhere to endothelial cells and the production of **neuraminidase**, an enzyme which enhance cell penetration.
- In septicaemic form, vascular damage is characterized by swelling of endothelial cells, adherence of monocytes to vascular walls and widespread micro-thrombus formation.
- Localization of the bacteria in **joint synovia** and on **heart valves** via haematogenous spread leads to chronic lesions at these sites.
- Long term articular damage may result from an immune response to persistent bacterial antigens. Viable organisms are rarely isolated from chronically affected joints.

Box 14.1 Clinical manifestations of *Erysipelothrix rhusiopathiae* infection in domestic animals.

- Pigs (swine erysipelas)
 - septicaemia
 - ‘diamond skin’ lesions
 - chronic arthritis
 - chronic valvular endocarditis
 - abortion
- Sheep
 - polyarthritis in lambs
 - post-dipping lameness
 - pneumonia
 - valvular endocarditis
- Turkeys (turkey erysipelas)
 - septicaemia
 - arthritis
 - valvular endocarditis

SWINE ERYSIPelas

- Sub-clinically-infected carrier pigs are the main reservoir of infection.
- Pigs with acute disease excrete large numbers of organisms in faeces.
- Infection is usually acquired through ingestion of contaminated food or water and less commonly through minor skin abrasions.
- The frequency of disease outbreaks in free-range pigs may be reduced by keeping them on concrete.
- Pigs under 3 months of age are normally protected by maternally-derived antibodies while animals over 3 years of age usually have acquired a protective active immunity through exposure to strains of low virulence.
- Factors which may predispose to disease development include changes in diet, extreme ambient temperatures and fatigue.

CLINICAL MANIFESTATIONS:

- **Pigs (swine erysipelas)** – It occurs in 4 forms. There are two acute and two chronic forms. Chronic arthritis has the most significant negative impact on productivity.
- **Septicaemic form** – It occurs after incubation period of 2-3 days. During an outbreak, some pigs will be found dead and others are febrile, depressed and walk with a stiff, stiled gait or remain recumbent. Mortality may be high and pregnant sow with septicaemic form may abort.

- **Diamond skin form** – Systemic signs are less severe and mortality rates are much lower than septicaemic form. Pigs are febrile and cutaneous lesions are seen as small, light pink or purple, raised areas to more extensive and characteristic diamond-shaped erythematous plaques. Some of these lesions resolve within one week; others become necrotic and may slough.
- **Chronic arthritis** – It is commonly seen in older pigs. There is stiffness, lameness or reluctance to bear weight on affected limbs. Joint lesions, which may be initially mild, can lead to erosion of articular cartilage with eventual fibrosis and ankylosis
- **Chronic valvular endocarditis** – It is the least common form. In this form, wart like thrombotic masses are present, usually on the mitral valves.



- **Sheep** - polyarthrjtis in lambs - post-dipping lameness - pneumonia - valvular endocarditis
- **Turkeys (turkey erysipelas)** - septicaemia - arthritis -valvular endocarditis

DIAGNOSIS:

- Diamond-shaped skin lesions are pathognomonic.
- Specimens for laboratory examination include blood for haemoculture and postmortem specimens of liver, spleen, heart valves or synovial tissues. Organisms are rarely recovered from skin lesions or chronically affected joints.
- Microscopic examination of specimens from acutely affected animals may reveal slender Gram-positive rods. Filamentous forms may be demonstrable in smears from chronic valvular lesions.
- Blood and MacConkey agar plates, inoculated with specimen material are incubated aerobically at 37°C for 24-48 hours. Selective media, containing either sodium azide (0.1%) or crystal violet (0.001%), may be used for contaminated samples.

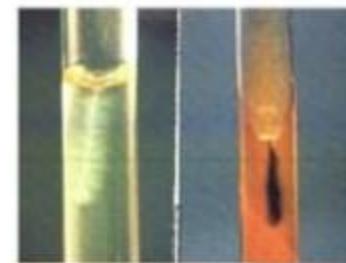
IDENTIFICATION CRITERIA FOR ISOLATES

- Colonial morphology after incubation for 48 hours.
- Absence of growth on MacConkey agar.
- Appearance in Gram-stained smears from colonies
- Negative catalase test
- Coagulase production
- H₂S production in TSI agar slants
- Biochemical test profile.
- Serological tests are not applicable for diagnosis.



Erysipelothrix rhusiopathiae: Laboratory Diagnosis

- Alpha hemolytic on SBA
- Non-motile
- Catalase negative
- Hydrogen sulfide production on TSIA
- VP negative
- Gelatin stab culture yields a test tube brush-like pattern at 22°C



FURTHER READINGS

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease



HISTORY OF MICROBIOLOGY

Dr. Bincy Joseph

Assistant professor

PGIVER, Jaipur

MICROBIOLOGY



- The development of microbiology as a scientific discipline has depended on the availability of the microscope and the ability to isolate and grow pure cultures of microorganisms
- The development of these techniques in large part grew out of studies
- disproving the Theory of Spontaneous Generation
- establishing that microorganisms can cause disease

Microbiology

Microbiology

Definition

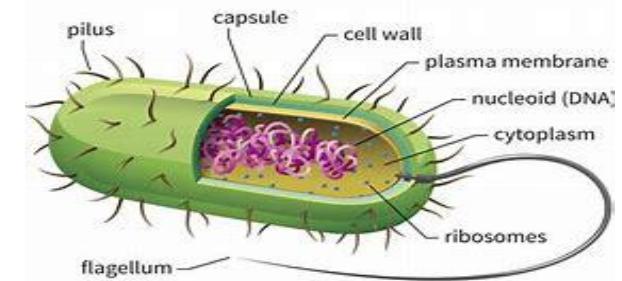


- Microbiology often has been defined as the study of organisms and agents too small to be seen clearly by the unaided eye—that is, the study of microorganisms

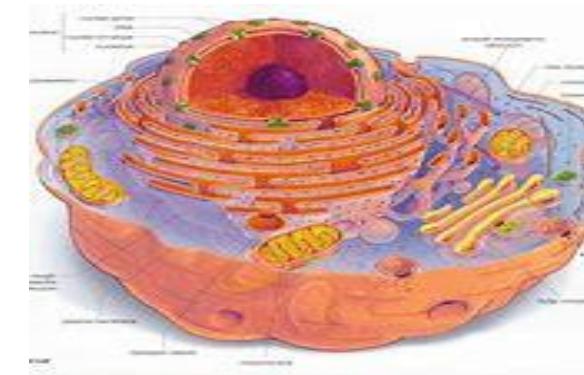
Thiomargarita and Epulopiscium are the two bacteria which can be seen by naked eye



- Prokaryotic cells [Greek pro, before, and karyon, nut or kernel; organisms with a primordial nucleus] have a much simpler morphology than eukaryotic cells
- Lack a true membrane-delimited nucleus

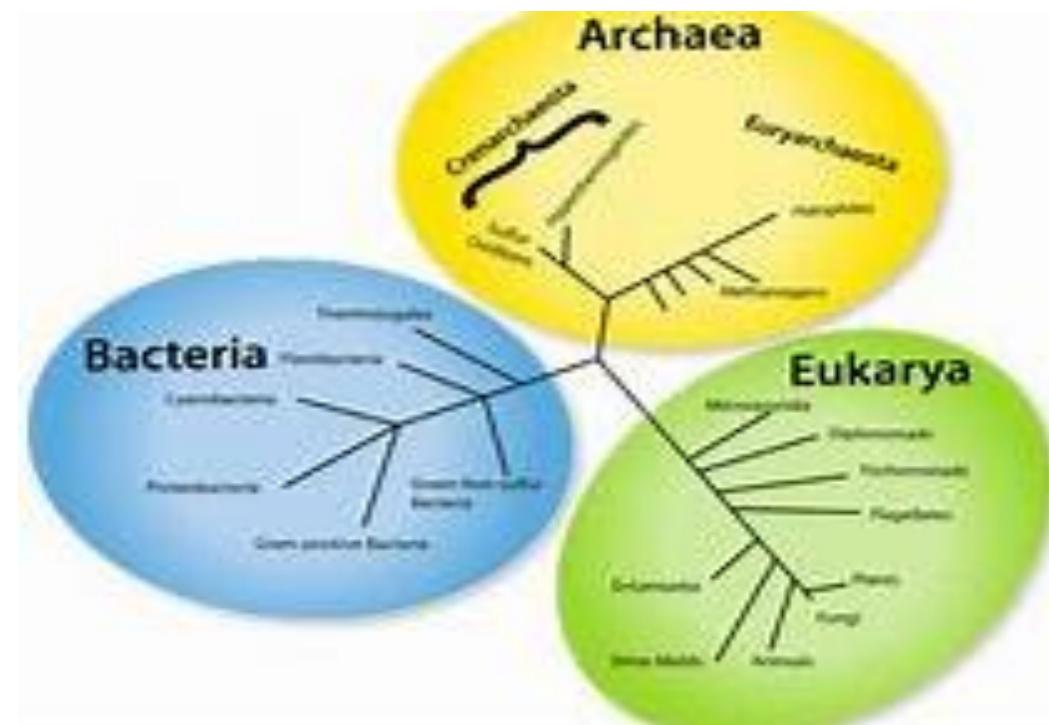


- In contrast, eucaryotic cells [Greek, eu, true, and karyon, nut or kernel] have a membrane-enclosed nucleus
- They are more complex morphologically and are usually larger than prokaryotes



THREE DOMAINS OF LIFE

- Carl Woese in 1970
- Based on the difference in Ribosomal RNA (rRNA) sequence



3 domains of life

Bacteria

- Bacteria are prokaryotes
- usually single-celled organisms.
- Most have cell walls that contain the structural molecule peptidoglycan
- They are abundant in soil, water, and air and are also major inhabitants of our skin, mouth, and intestines.
- Some bacteria live in environments that have extreme temperatures,

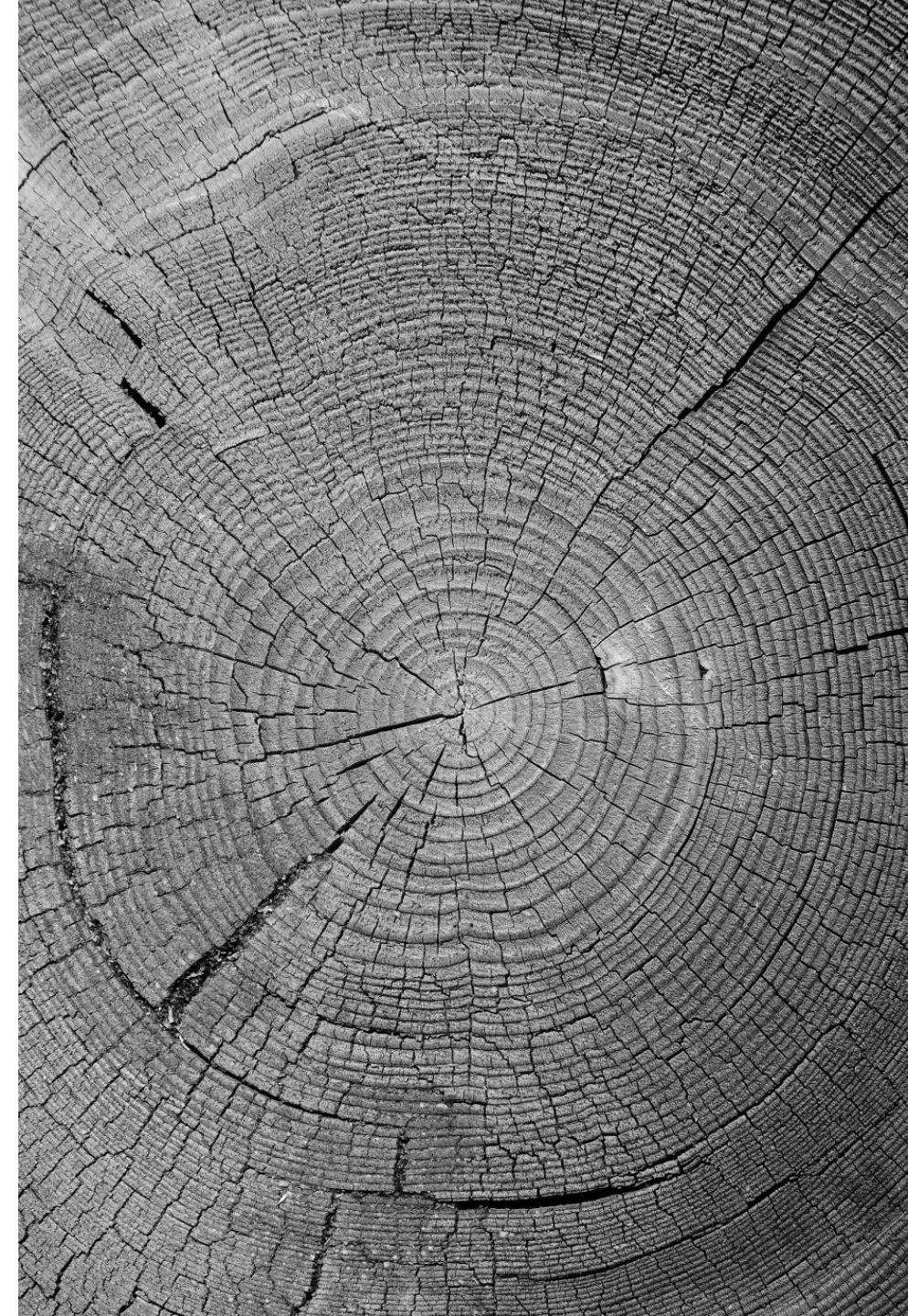
Archae

- Archaea are prokaryotes
- most notably their unique ribosomal RNA sequences.
- They also lack peptidoglycan in their cell walls
- Have unique membrane lipids
- Some have unusual metabolic characteristics, such as the methanogens, which generate methane gas. Many archaea are found in extreme environments. Pathogenic archaea have not yet been identified

Eukarya

- Domain Eukarya includes microorganisms classified as protists or Fungi.
- Protists are generally larger than prokaryotes and include unicellular algae, protozoa, slime molds, and water molds.
- Algae are photosynthetic protists that together with the cyanobacteria produce about 75% of the planet's oxygen

History of microbiology



Theories of spontaneous generations

- Spontaneous generation—that living organisms could develop from non living matter.
- Even Aristotle (384–322 B.C.) thought some of the simpler invertebrates could arise by spontaneous generation.
- This view finally was challenged by the Italian physician Francesco Redi (1626–1697), who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously
- English priest John Needham (1713–1781) reported the results of his experiments on spontaneous generation.
- Lazzaro Spallanzani (1729–1799) improved on Needham's experimental design by first sealing glass flasks that contained water and seeds. If the sealed flasks were placed in boiling water for 3/4 of an hour, no growth took place as long as the flasks remained sealed

Theories of spontaneous generation

- Theodore Schwann (1810–1882) allowed air to enter a flask containing a sterile nutrient solution after the air had passed through a red-hot tube. The flask remained sterile.
- Georg Friedrich Schroder and Theodor von Dusch allowed air to enter a flask of heat-sterilized medium after it had passed through sterile cotton wool.
- No growth occurred in the medium even though the air had not been heated.

Swan neck flask experiments- Louis Pasteur

- Pasteur , first filtered air through cotton and found that objects resembling plant spores had been trapped.
- If a piece of the cotton was placed in sterile medium after air had been filtered through it, microbial growth occurred. Next he placed nutrient solutions in flasks, heated their necks in a flame, and drew them out into a variety of curves, while keeping the ends of the necks open to the atmosphere
- The English physicist John Tyndall (1820–1893) dealt a final blow to spontaneous generation in 1877
- **Ferdinand Cohn** (1828–1898) discovered the existence of heat-resistant bacterial endospores

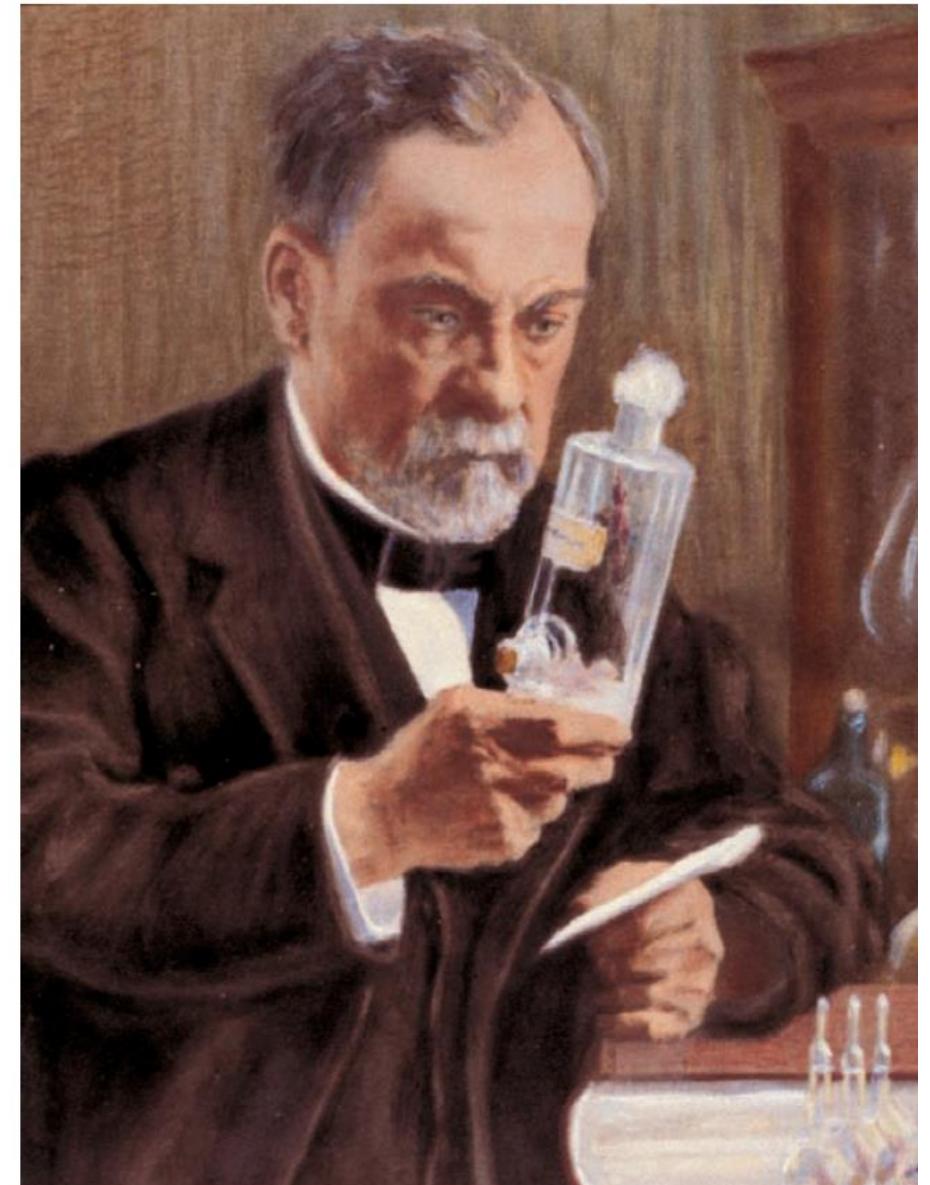


Figure 1.4 Louis Pasteur. Pasteur (1822–1895) working in his laboratory.

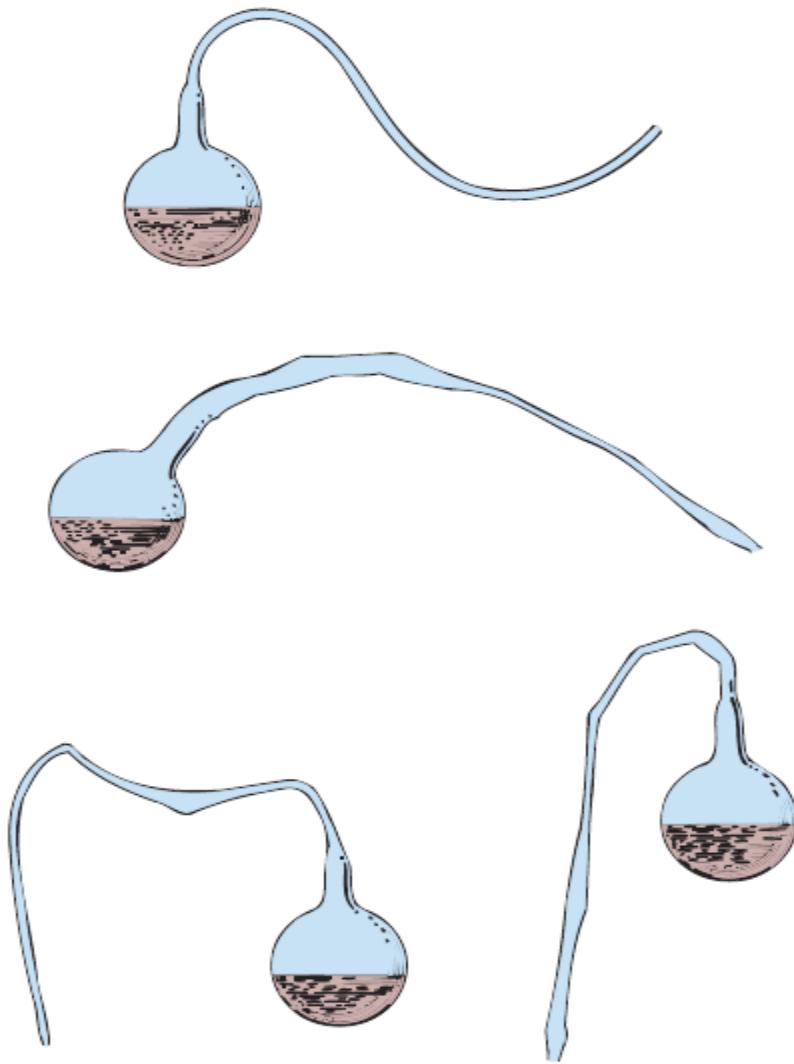
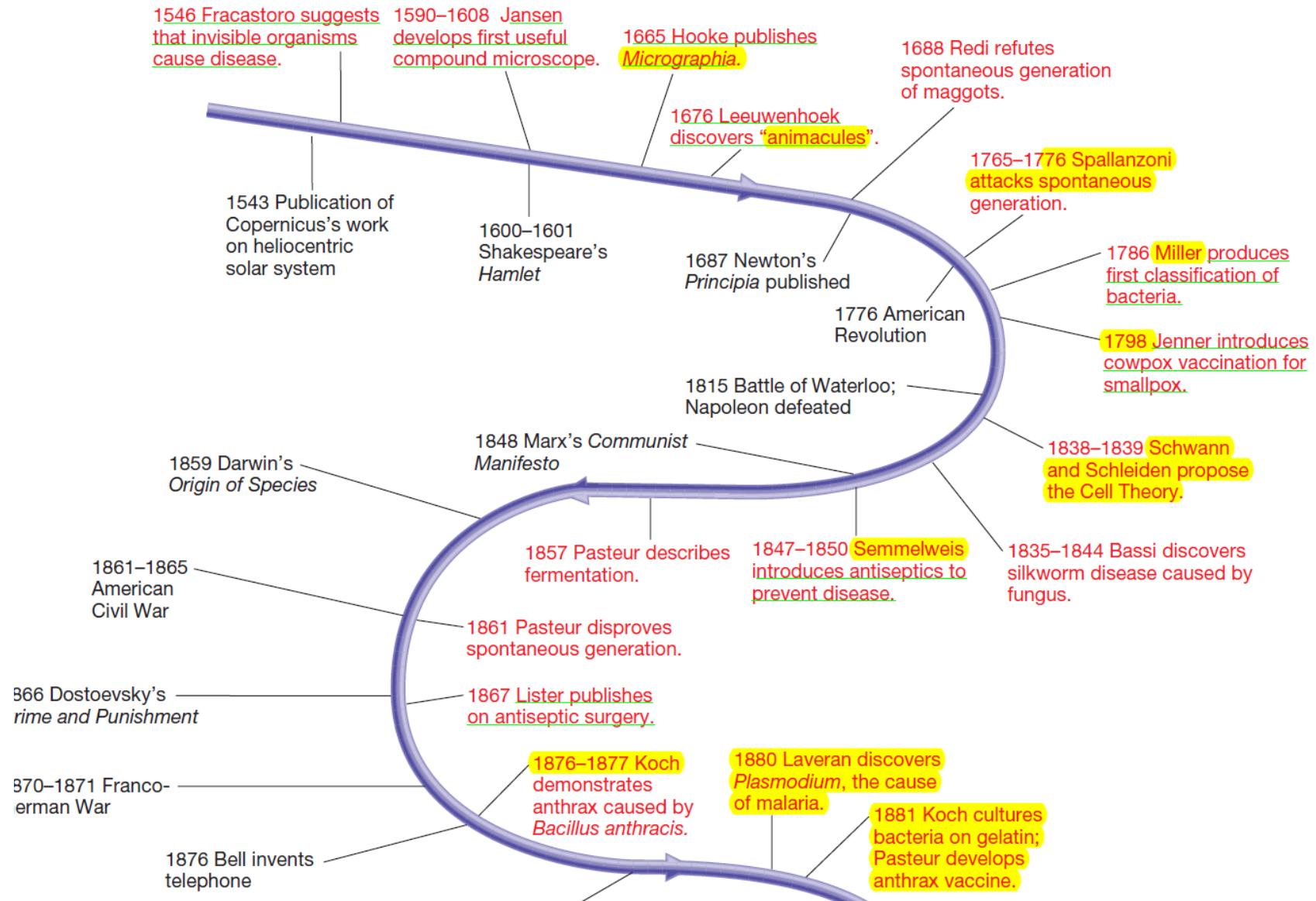


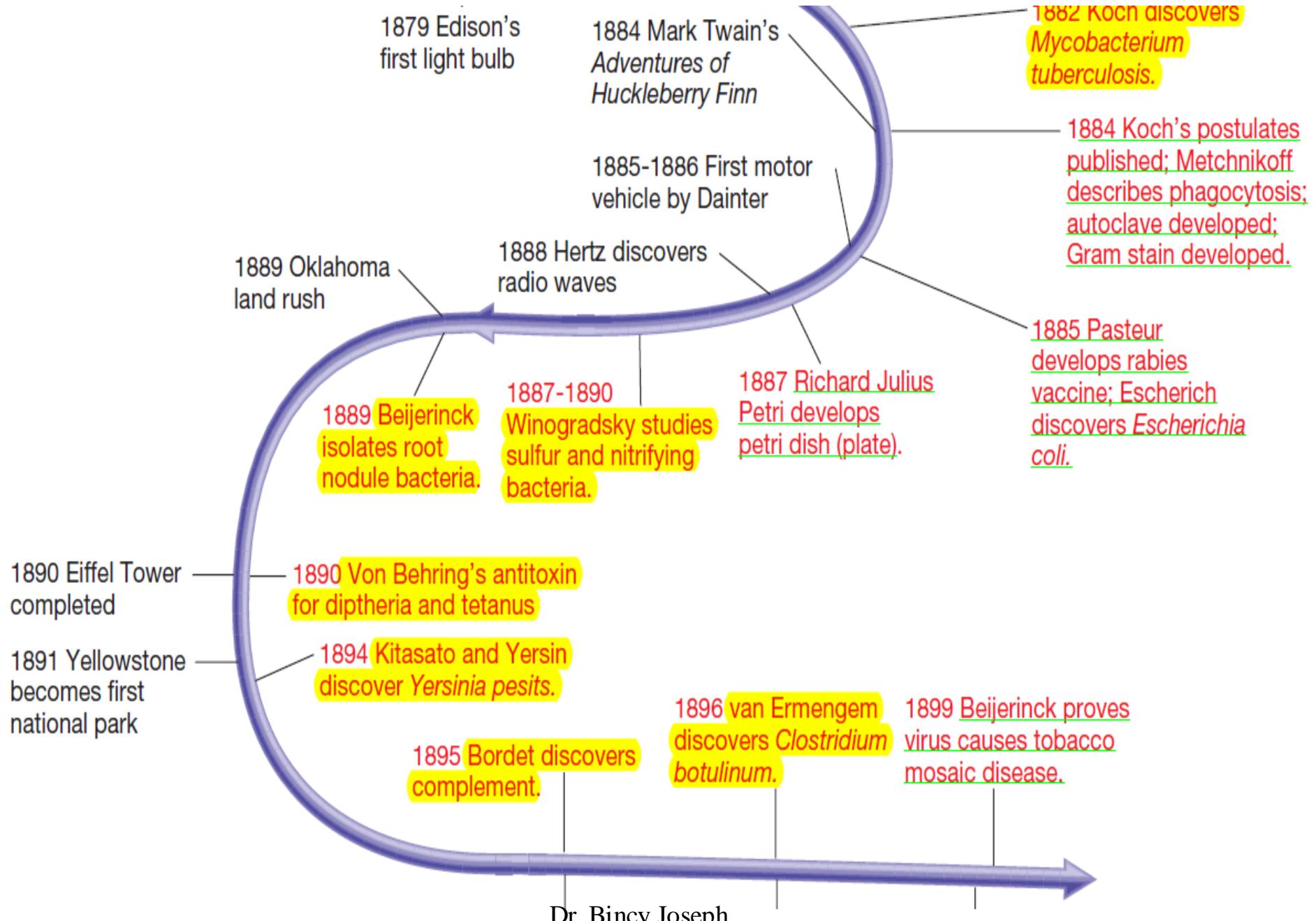
Figure 1.5 The Spontaneous Generation Experiment.
Pasteur's swan neck flasks used in his experiments on the spontaneous generation of microorganisms. *Source: Annales Dr. Bincy Joseph*

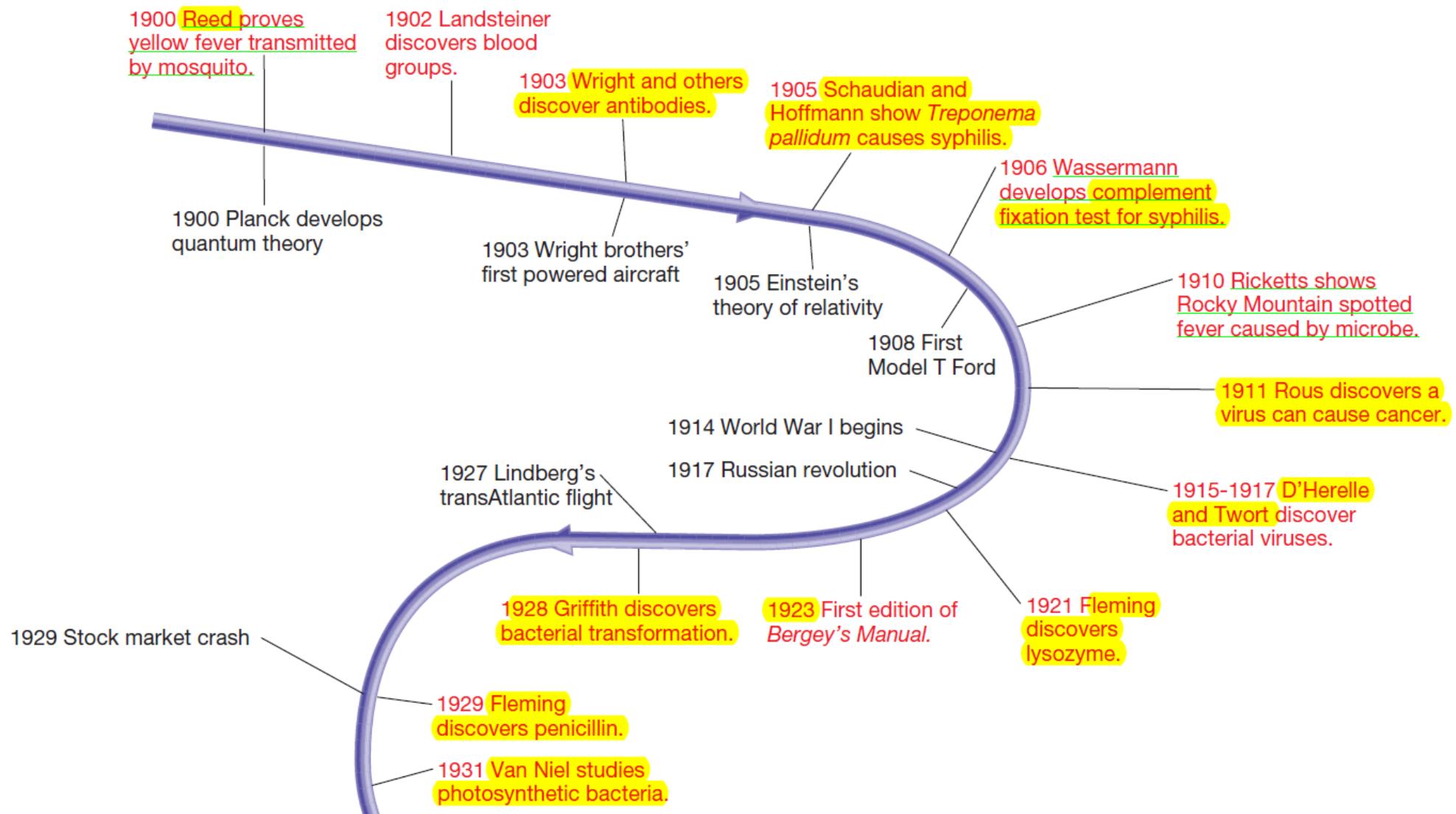


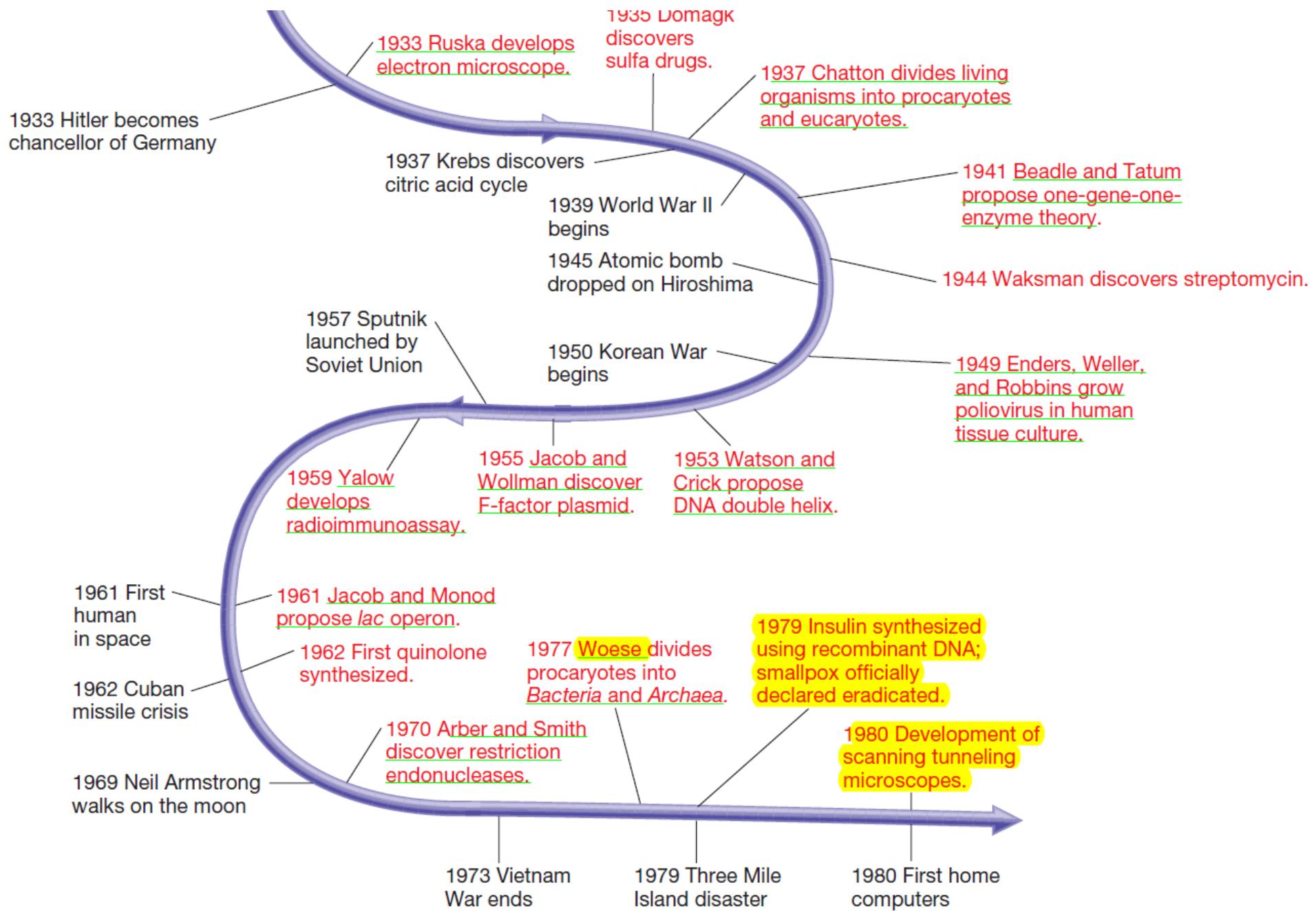
The golden age of microbiology

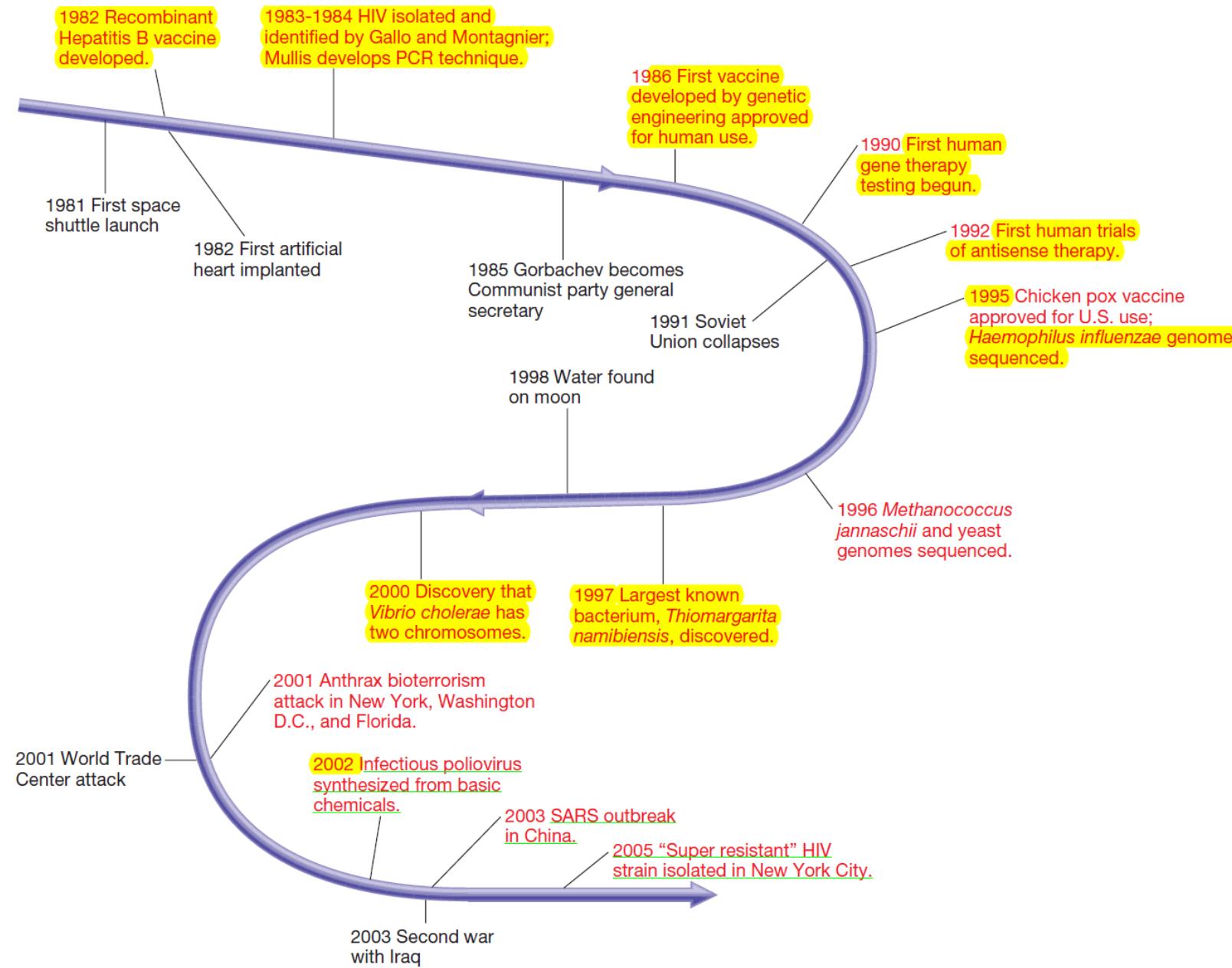
1857-1914











Germ theory of disease

Microorganism causes disease

Agostino Bassi (1773–1856) first showed a microorganism could cause disease when he demonstrated in 1835 that a silkworm disease was due to a fungal infection.

In 1845, M. J. Berkeley proved that the great Potato Blight of Ireland was caused by a water mold

in 1853, Heinrich de Bary showed that smut and rust fungi caused cereal crop diseases.



- Joseph Lister (1827–1912) developed a system of antiseptic surgery heat sterilized, and phenol was used on surgical dressings and at times sprayed over the surgical area



Robert Koch

First demonstrated the role of bacteria causing disease

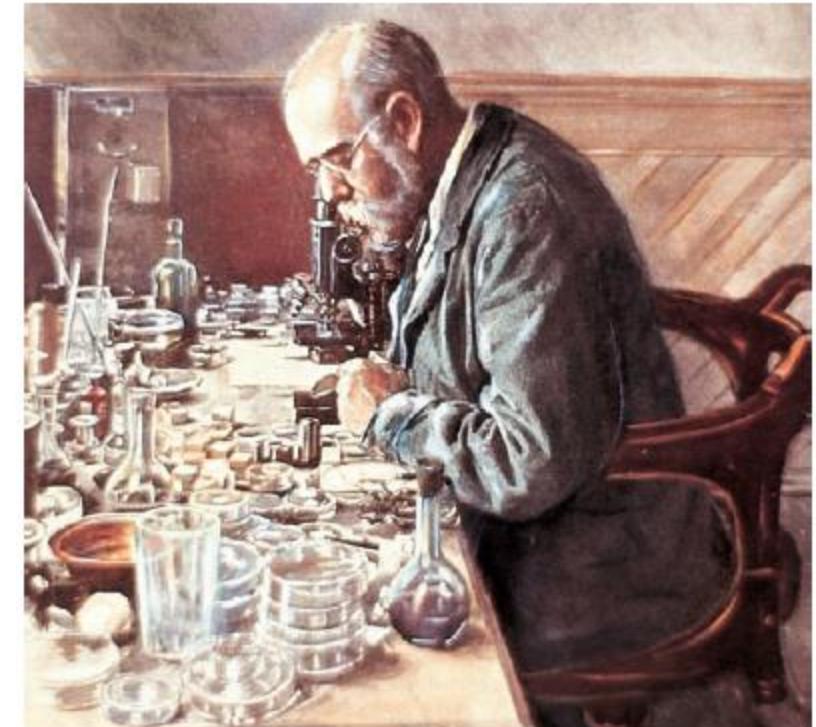
He isolated the organism *Bacillus anthracis*, the causative agent of anthrax

Mycobacterium tuberculosis also discovered

Pure culture techniques introduced

He cultured organism on sterile cut surfaces of boiled potatoes

He first tried the use of gelatin as a solidifying agent for the medium, it is not an ideal solidifying agent as it can be digested by many bacteria and also melts at 28°C



KOCH POSTULATES

1. Microorganism must be present in every case of disease but absent from healthy organisms
2. The suspected microorganism must be isolated and grown in pure culture
3. The same disease must result when the isolated microorganism is inoculated into healthy host
4. The same microorganism must be isolated again from the diseased host

Other discoveries

Charles Chamberland constructed bacterial porcelain filter

Dimitri Ivanovsky and Marinus Beijerinck- Tobacco mosaic virus

Louis Pasteur

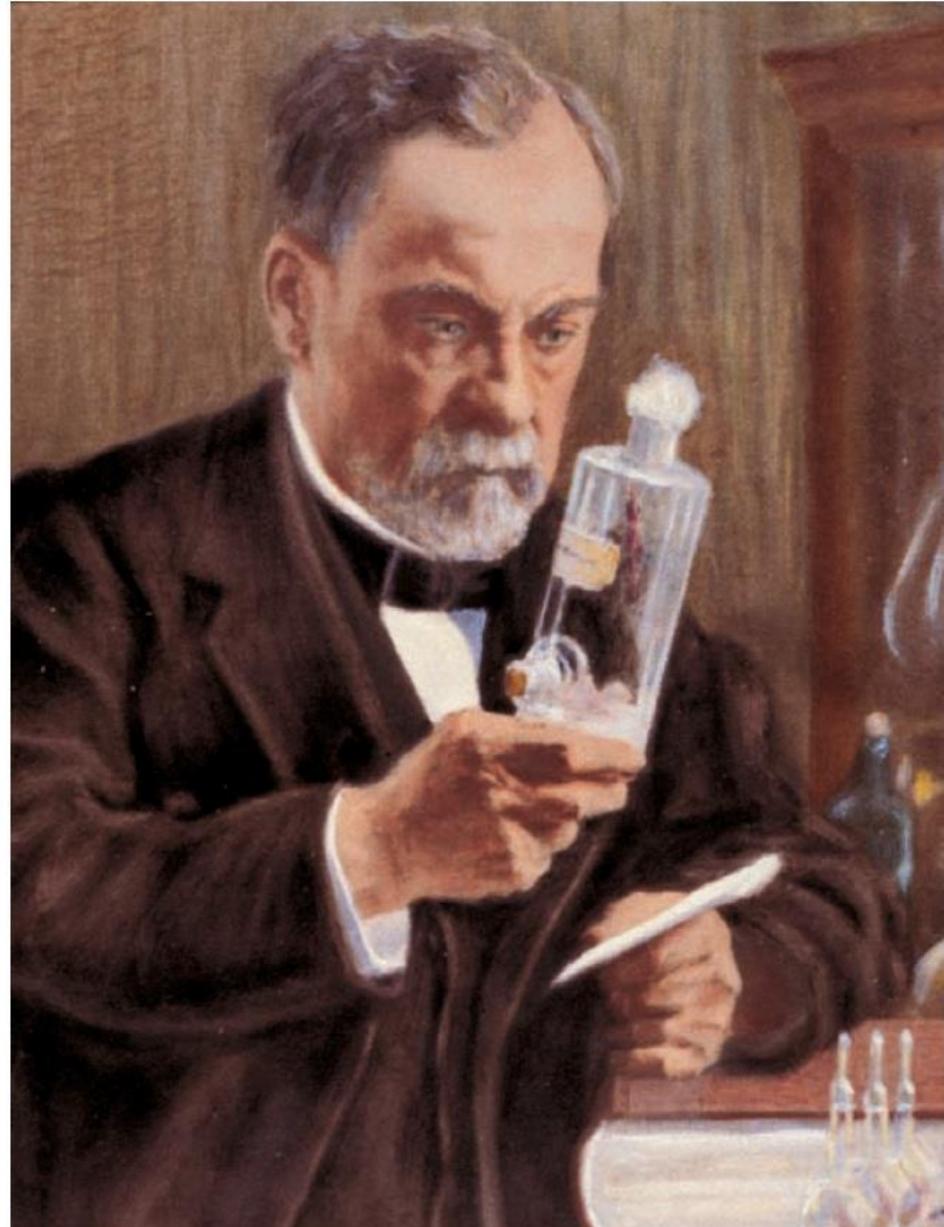
Pasteur and Rox discovered that incubating the culture for long intervals between transfers will attenuate the bacteria, that is they lost their ability to cause disease

He called this attenuated culture as vaccine

Louis Pasteur developed the term vaccine to give respect to Edward Jenner , who used cow pox lesions to protect from small pox

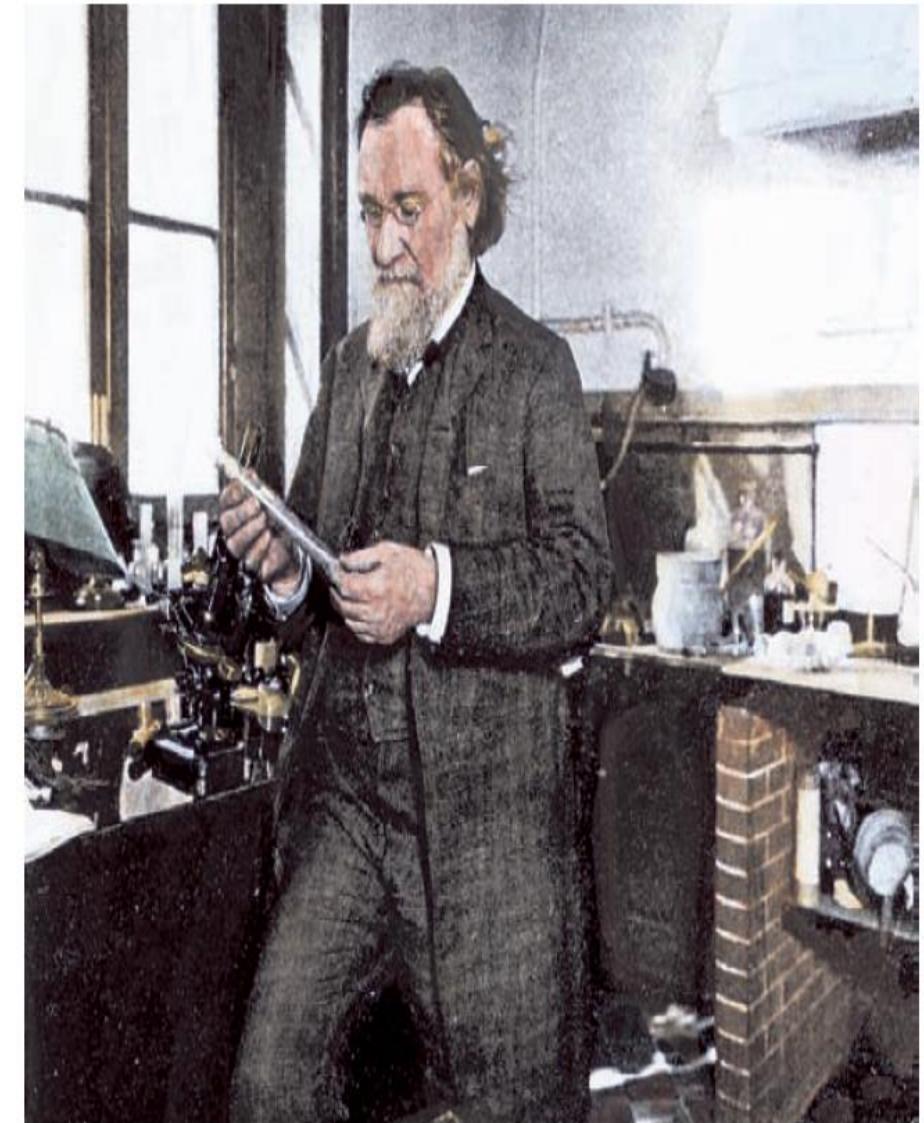
Pasteur and Chamberland developed an attenuated anthrax vaccine in two ways: by treating the with Potassium bichromate and by incubating bacteria at 42°C to 43°C

Pasteur prepared Rabies vaccine by inoculating into Heterologous host Rabbit in which virulent rabies virus and then , once the animal dies, its brain and spinal cord dried and used as vaccine

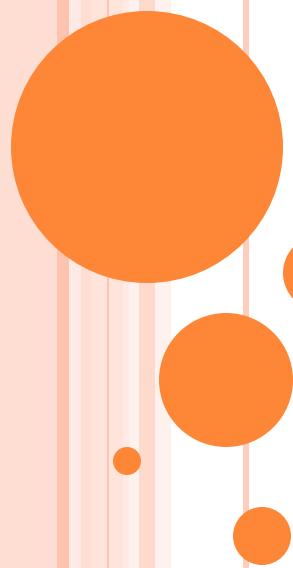


Emil von Behring and Shibasaburo Kitasato, developed anti toxin

Elie Metchnikoff discovered phagocytes and phagocytosis



Thank you



MICROSCOPY

Dr. Bincy Joseph
Assistant Professor
PGIVER, Jaipur

MICROSCOPY

- Microbiology usually is concerned with organisms so small that they cannot be seen distinctly with the unaided eye
- Because of the nature of this discipline, the microscope is of crucial importance
- Thus it is important to understand how the microscope works and the way in which specimens are prepared for examination.



LENSES AND BENDING OF LIGHT

- When a ray of light passes from one medium to another, **refraction occurs—that is, the ray is bent at the interface**
- The **refractive index is a measure of how greatly a substance** slows the velocity of light
- The direction and magnitude of bending is determined by the refractive indices of the two media forming the interface
- When light passes from air into glass, a medium with a greater refractive index, it is slowed and bent toward the normal, a line perpendicular to the surface



LENSES AND BENDING OF LIGHT

- As light leaves glass and returns to air, a medium with a lower refractive index, it accelerates and is bent away from the normal
- Thus a prism bends light because glass has a different refractive index from air, and the light strikes its surface at an angle



LENSES AND BENDING OF LIGHT

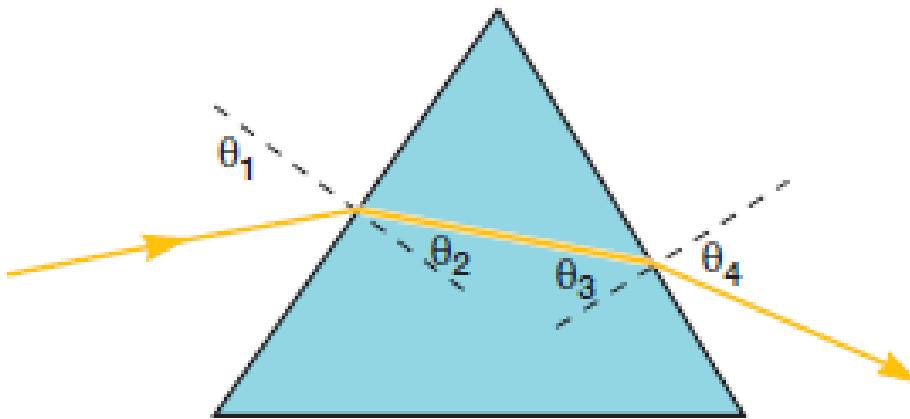


Figure 2.1 The Bending of Light by a Prism. Normals (lines perpendicular to the surface of the prism) are indicated by dashed lines. As light enters the glass, it is bent toward the first normal (angle θ_2 is less than θ_1). When light leaves the glass and returns to air, it is bent away from the second normal (θ_4 is greater than θ_3). As a result the prism bends light passing through it.

LENSES AND BENDING OF LIGHT

- Lenses act like a collection of prisms operating as a unit
- When the light source is distant so that parallel rays of light strike the lens,a convex lens will focus these rays at a specific point, the **focal Point (F)**
- **The distance between the center of the lens and the focal point is called the focal length (f)**



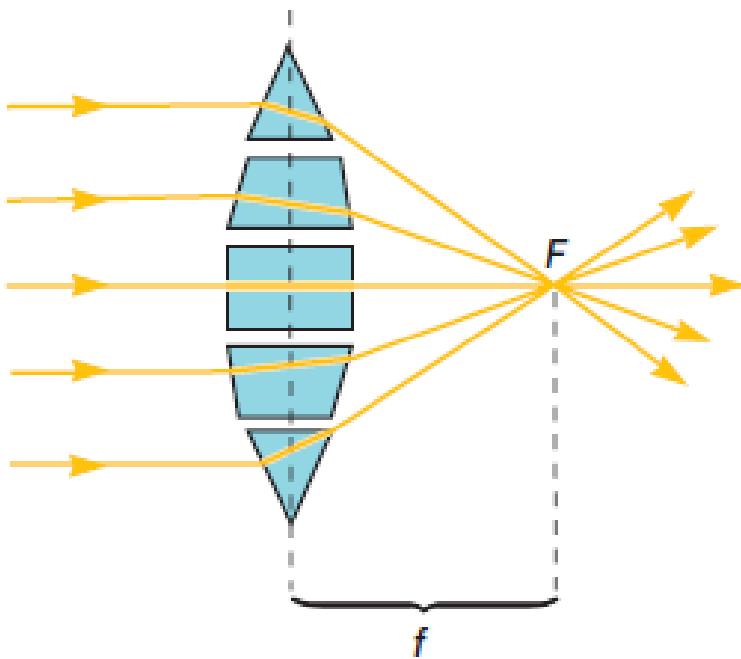


Figure 2.2 Lens Function. A lens functions somewhat like a collection of prisms. Light rays from a distant source are focused at the focal point F . The focal point lies a distance f , the focal length, from the lens center.

THE LIGHT MICROSCOPE

- Bright-field, Dark-field, Phase-contrast, and Fluorescence microscopes are most commonly used
- **Compound microscope:** the magnified image by objective lens is further magnified by eyepiece lense



BRIGHT FIELD MICROSCOPE

- The ordinary microscope is called a **bright-field microscope because** it forms a dark image against a brighter background.
- The microscope consists of a sturdy metal body or stand composed of a base and an arm to which the remaining parts are attached
- **A light source, either a mirror or an electric illuminator,** is located in the base
- Two focusing knobs, the fine and coarse adjustment knobs, are located on the arm and can move either the stage or the nosepiece to focus the image.

BRIGHT FIELD MICROSCOPE

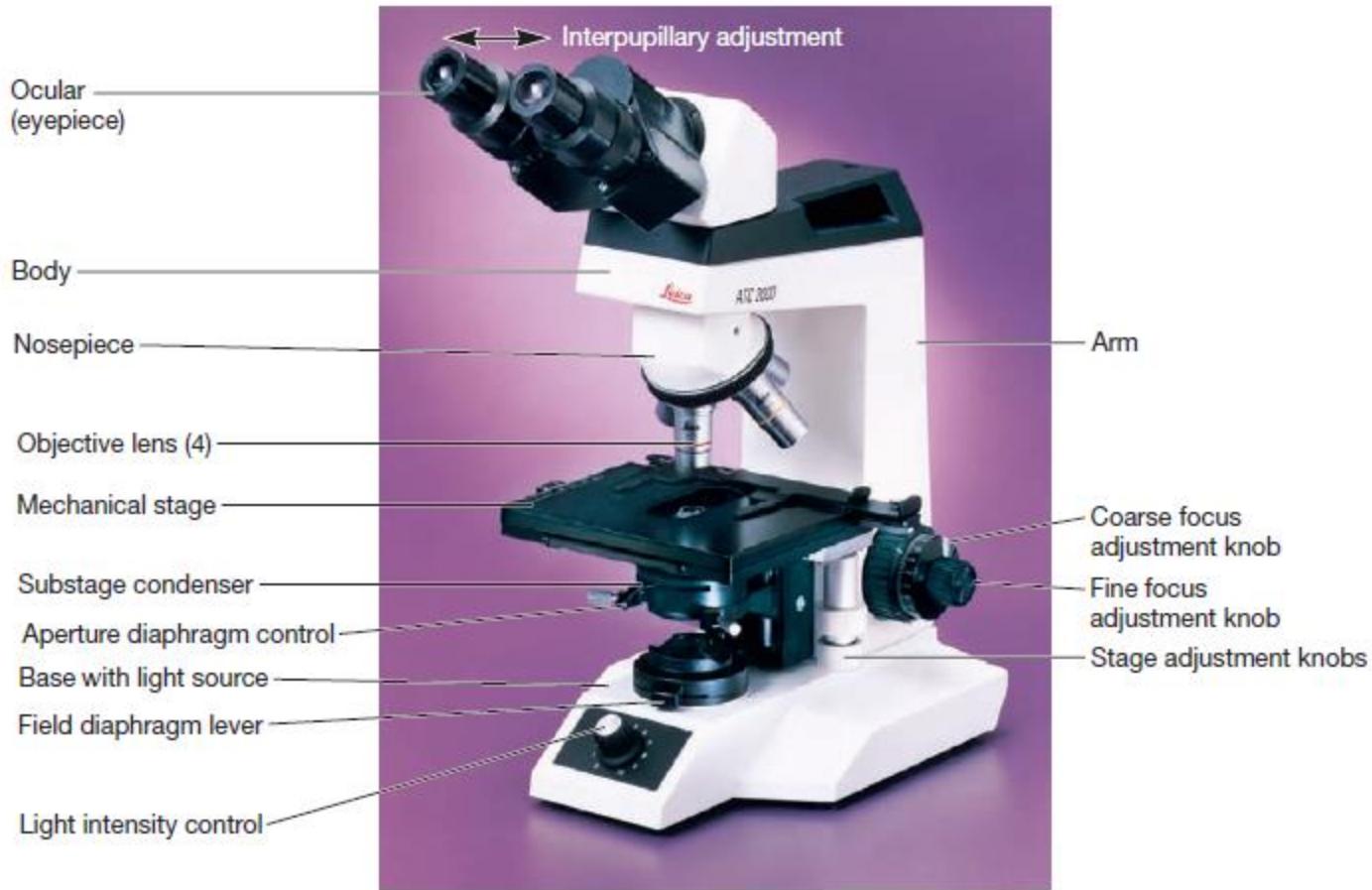
- The stage is positioned about halfway up the arm and holds microscope slides by either simple slide clips or a mechanical stage clip
- A mechanical stage allows the operator to move a slide around smoothly during viewing by use of stage control knobs
- The **substage condenser is mounted within or beneath the stage** and focuses a cone of light on the slide
- Its position often is fixed in simpler microscopes but can be adjusted vertically in more advanced models.

BRIGHT FIELD MICROSCOPE

- The curved upper part of the arm holds the body assembly, to which a nosepiece and one or more **eyepieces or ocular lenses** are attached.
- More advanced microscopes have eyepieces for both eyes and are called binocular microscopes
- The nosepiece holds three to five **objective lenses of** differing magnifying power and can be rotated to position any objective beneath the body assembly
- Ideally a microscope should be **parfocal**—that is, the image should remain in focus when **objectives** are changed.

BRIGHT FIELD MICROSCOPE

- The image one sees when viewing a specimen with a compound microscope is created by the objective and ocular lenses working together
- Light from the illuminated specimen is focused by the objective lens, creating an enlarged image within the microscope
- The ocular lens further magnifies this primary image
- The total magnification is calculated by multiplying the objective and eyepiece magnifications together.
- For example, if a 45 objective is used with a 10x eyepiece, the overall magnification of the specimen will be 450.



MICROSCOPIC RESOLUTION

- **Resolution** is the ability of a lens to separate or distinguish between small objects that are close together
- The Abbé equation states that the minimal distance (d) *between two* objects that reveals them as separate entities depends on the
 - wavelength of light (λ) used to illuminate the specimen
 - **numerical aperture of the lens ($n \sin \theta$), which is the ability** of the lens to gather light.

$$\frac{d}{\text{---}} = \frac{0.5\lambda}{n \sin \theta}$$



MICROSCOPIC RESOLUTION

- As d becomes smaller, the resolution increases, and finer detail can be discerned in a specimen
- d becomes smaller as the wavelength of light used decreases and as the numerical aperture (NA) increases.
- Thus the greatest resolution is obtained using a lens with the largest possible NA and light of the shortest wavelength, light at the blue end of the visible spectrum



MICROSCOPIC RESOLUTION

- It is defined by two components: n is the refractive index of the medium in which the lens works (e.g., air) and θ is 1/2 the angle of the cone of light entering an objective
- If the cone of light has a very wide angle and spreads out rapidly after passing through a specimen, closely packed objects appear widely separated and are resolved
- The angle of the cone of light that can enter a lens depends on the refractive index (n) of the medium in which the lens works, as well as upon the objective itself
- The refractive index for air is 1.00 and $\sin \theta$ cannot be greater than 1 (the maximum is 90° and $\sin 90^\circ$ is 1.00)



NUMERICAL APERTURE

- The only practical way to raise the numerical aperture above 1.00, and therefore achieve higher resolution, is to increase the refractive index with immersion oil, a colorless liquid with the same refractive index as glass
- **If air is replaced** with immersion oil, many light rays that did not enter the objective due to reflection and refraction at the surfaces of the objective lens and slide will now do so
- **An increase in numerical** aperture and resolution results



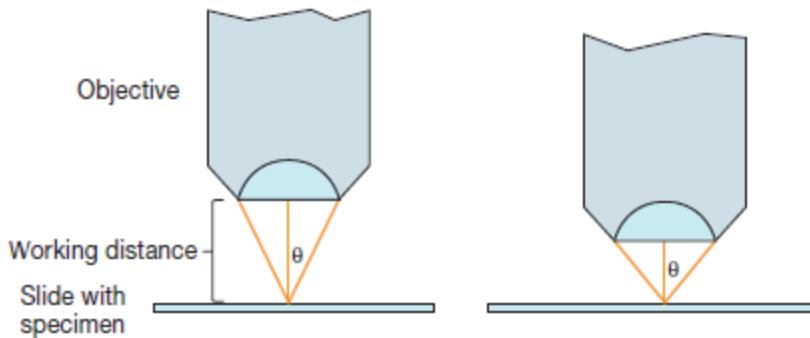


Figure 2.5 Numerical Aperture in Microscopy. The angular aperture θ is $1/2$ the angle of the cone of light that enters a lens from a specimen, and the numerical aperture is $n \sin \theta$. In the right-hand illustration the lens has larger angular and numerical apertures; its resolution is greater and its working distance smaller.

NUMERICAL APERTURE

- Numerical aperture is related to another characteristic of an objective lens, the working distance.
- The **working distance of an** objective is the distance between the front surface of the lens and the surface of the cover glass (if one is used) or the specimen when it is in sharp focus
- Objectives with large numerical apertures and great resolving power have short working distances



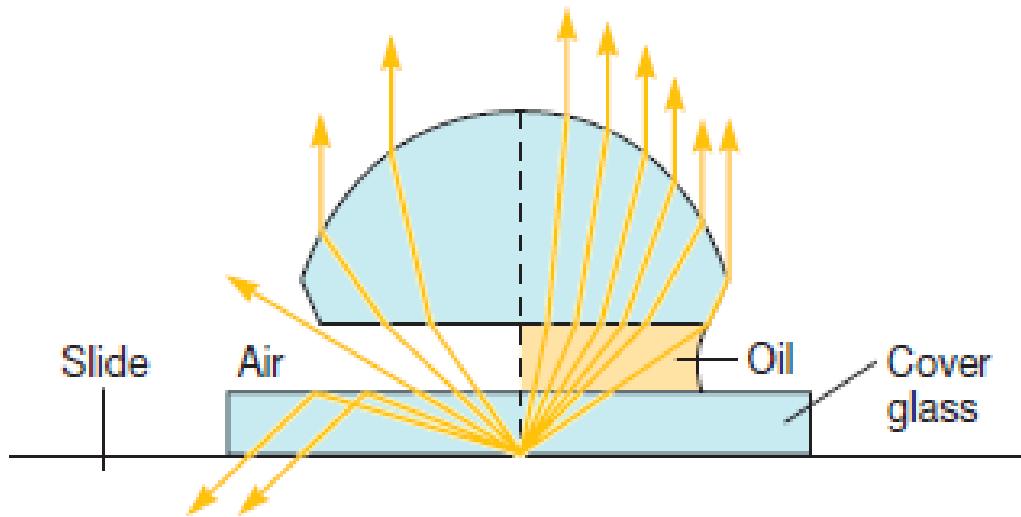


Figure 2.6 The Oil Immersion Objective. An oil immersion objective operating in air and with immersion oil.

- The maximum theoretical resolving power of a microscope with an oil immersion objective (numerical aperture of 1.25) and blue-green light is approximately 0.2 m.

$$d = \frac{(0.5)(530 \text{ nm})}{1.25} = 212 \text{ nm or } 0.2 \mu\text{m}$$

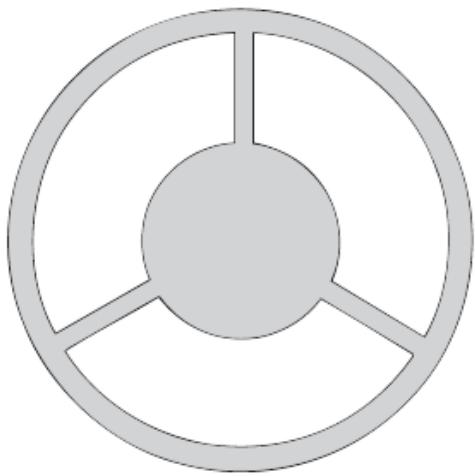


Table 2.2 The Properties of Microscope Objectives

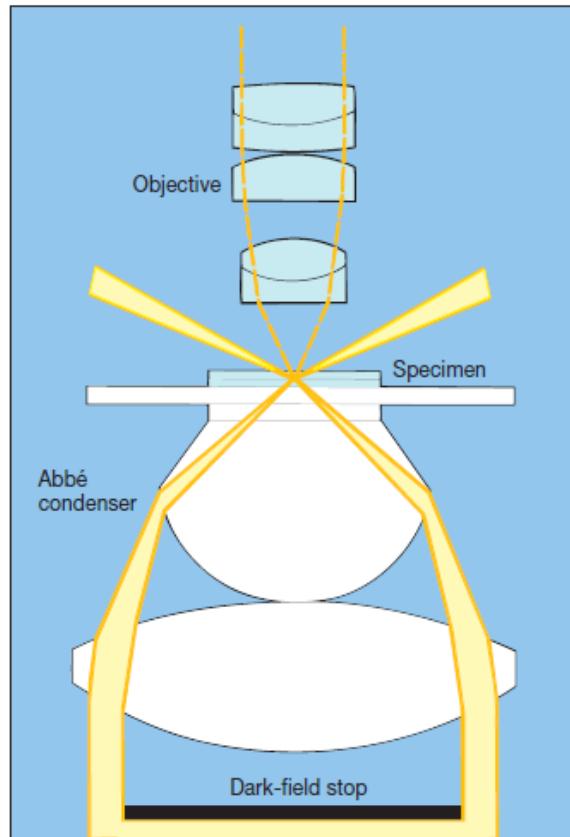
Property	Objective			
	Scanning	Low Power	High Power	Oil Immersion
Magnification	4×	10×	40–45×	90–100×
Numerical aperture	0.10	0.25	0.55–0.65	1.25–1.4
Approximate focal length (f)	40 mm	16 mm	4 mm	1.8–2.0 mm
Working distance	17–20 mm	4–8 mm	0.5–0.7 mm	0.1 mm
Approximate resolving power with light of 450 nm (blue light)	2.3 μm	0.9 μm	0.35 μm	0.18 μm

THE DARK FIELD MICROSCOPE

- To observe living,unstained cells and organisms
- A hollow cone of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective
- Only light that has been reflected or refracted by the specimen forms an image
- **The field** surrounding a specimen appears black, while the object itself is brightly illuminated
- ***The dark-field microscope*** can reveal considerable internal structure in larger eucaryotic microorganisms
- *It also is used to identify certain bacteria*
- like the thin and distinctively shaped *Treponema pallidum* the causative agent of *syphilis*, *leptospira* and other *spirochaetes*



(a)

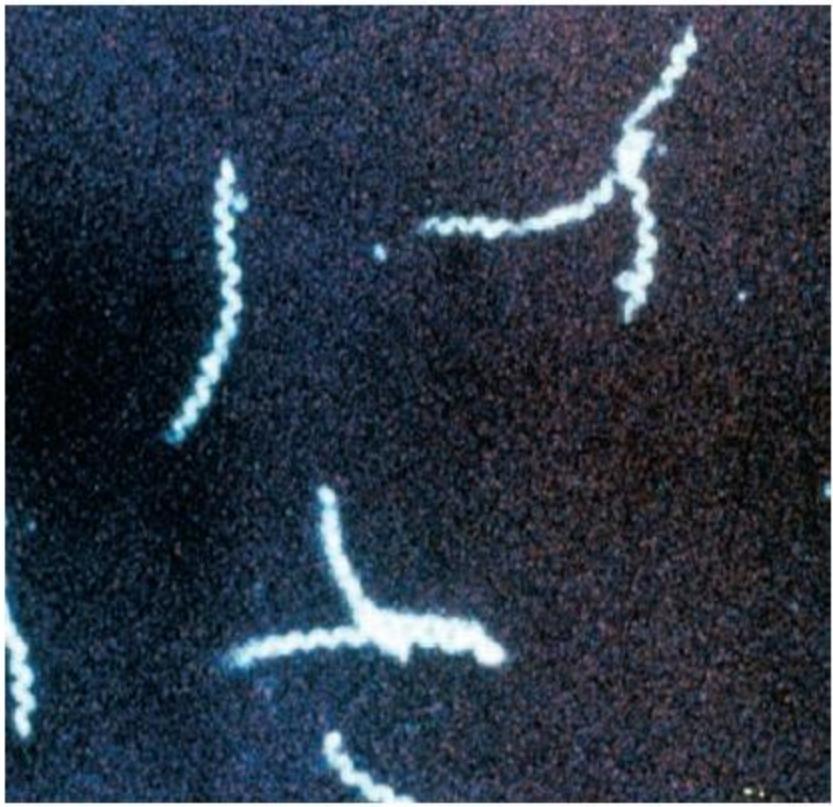


(b)

Figure 2.7 Dark-Field Microscopy. The simplest way to convert a microscope to dark-field microscopy is to place (a) a dark-field stop underneath (b) the condenser lens system. The condenser then produces a hollow cone of light so that the only light entering the objective comes from the specimen.



12/29/2023 Dr. Bincy Joseph



PHASE CONTRAST MICROSCOPE

- Unpigmented living cells are not clearly visible in the brightfield microscope because there is little difference in contrast between the cells and water
- A **phase-contrast microscope converts slight differences** in refractive index and cell density into easily detected variations in light intensity and is an excellent way to observe living cells
-

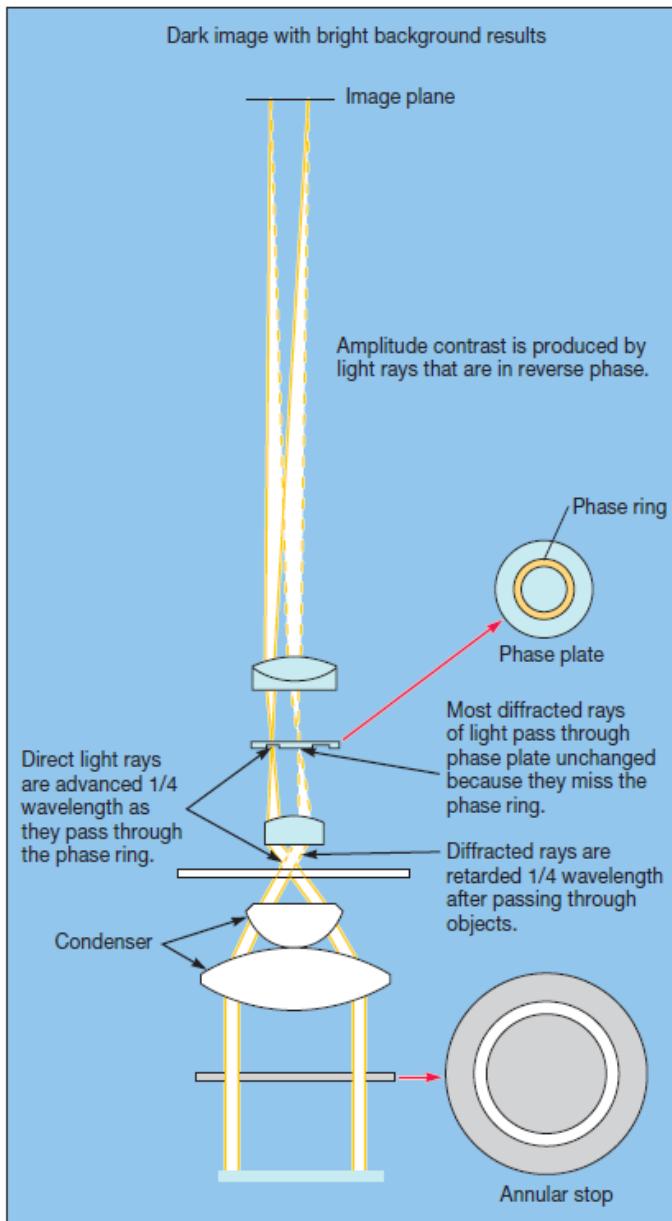


Figure 2.9 Phase-Contrast Microscopy. The optics of a dark-phase-contrast microscope.

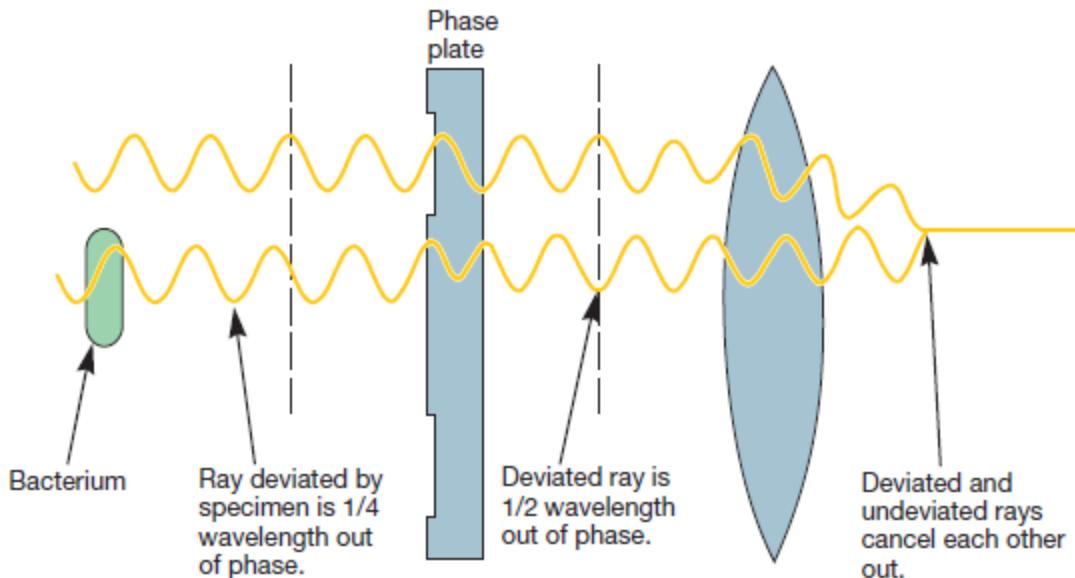


Figure 2.10 The Production of Contrast in Phase Microscopy. The behavior of deviated and undeviated or undiffracted light rays in the dark-phase-contrast microscope. Because the light rays tend to cancel each other out, the image of the specimen will be dark against a brighter background.

PHASE CONTRAST MICROSCOPE

- The condenser of a phase-contrast microscope has an annular stop, an opaque disk with a thin transparent ring, which produces a hollow cone of light
- **As this cone passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about $\frac{1}{4}$ wavelength**
- The deviated light is focused to form an image of the object
- Undeviated light rays strike a phase ring in the phase plate,a special optical disk located in the objective, while the deviated rays miss the ring and pass through the rest of the plate.

PHASE CONTRAST MICROSCOPY

- If the phase ring is constructed in such a way that the undeviated light passing through it is advanced by $1/4$ wavelength, the deviated and undeviated waves will be about $1/2$ wavelength out of phase and will cancel each other when they come together to form an image
- The background, formed by undeviated light, is bright, while the unstained object appears dark and well-defined
- This type of microscopy is called dark-phase-contrast microscopy

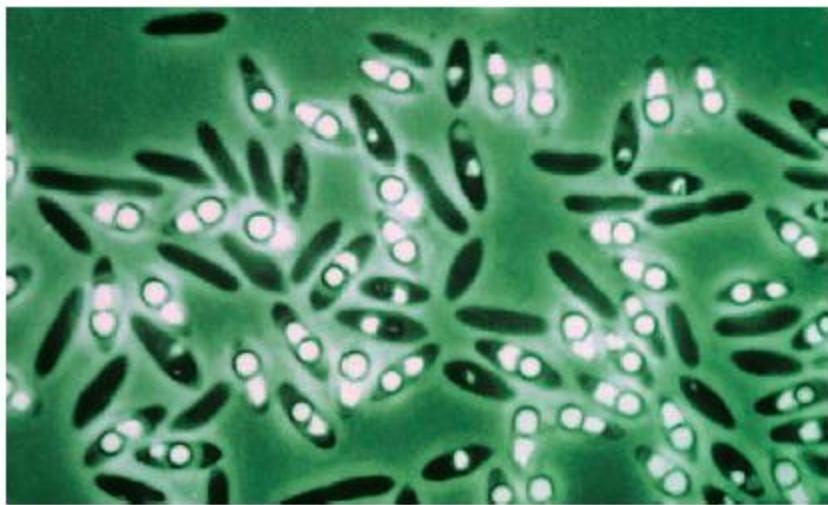


PHASE CONTRAST MICROSCOPY

- Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells, and detecting bacterial components such as endospores and inclusion bodies



(c) *Pseudomonas*: phase-contrast microscopy

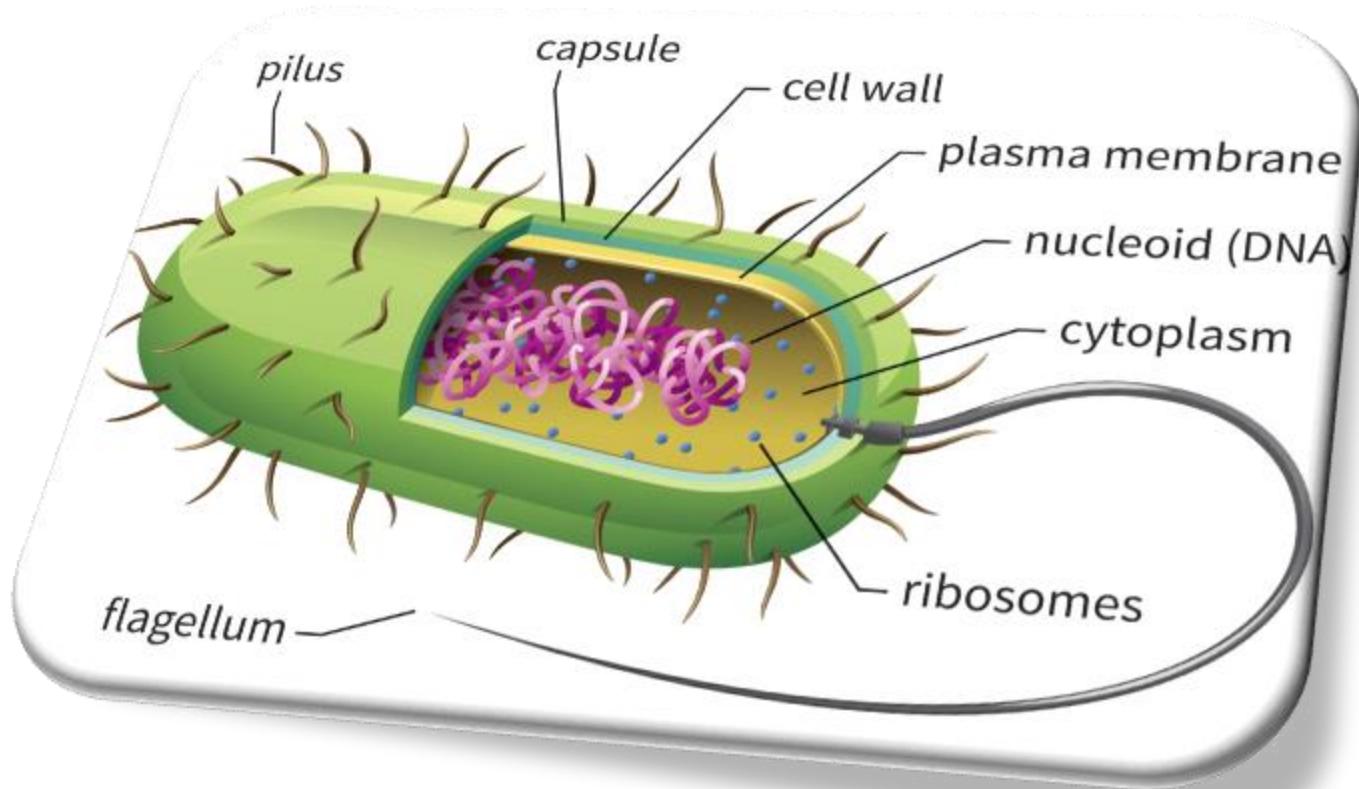


(d) *Desulfotomaculum*: phase-contrast microscopy

12/29/2023 Dr. Bincy Joseph

THANK YOU





PROKARYOTIC CELL STRUCTURE

Dr. Bincy Joseph
Assistant Professor
PGIVER, Jaipur

BACTERIAL CELL SHAPE ARRANGEMENT AND MORPHOLOGY

- **Cocci** (*s. coccus*) are roughly spherical cells.
- **Diplococci** (*s., diplococcus*) arise when cocci divide and remain together to form pairs
- Long chains of cocci (**Streptococci**) result when cells adhere after repeated divisions in one plane
 - Eg. Streptococcus, Enterococcus, and Lactococcus*
- *Staphylococcus* divides in random planes to generate irregular grapelike clumps
- Divisions in two or three planes can produce symmetrical clusters of cocci.
- In **Micrococcus** cocci divide in two planes to form square groups of four cells called tetrads
- In the genus **Sarcina**, cocci divide in three planes producing cubical packets of eight cells.

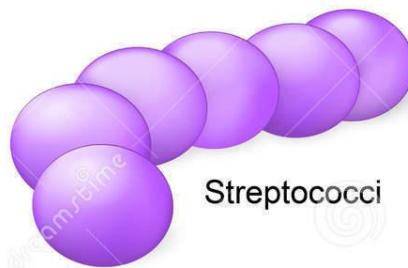


DIFFERENT TYPES OF COCCI

COCCI



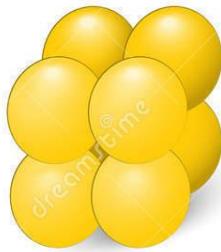
Coccus



Streptococci



Diplococci



Sarcina



Tetrad



Staphylococci



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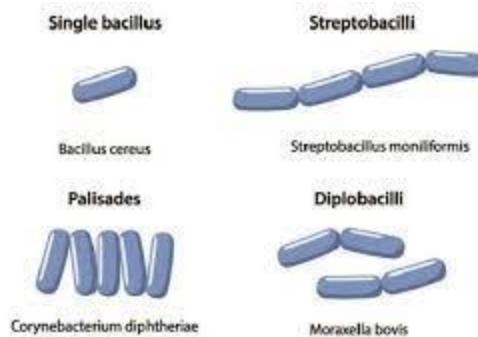


ID

CC



- Rod shaped bacteria are called a bacillus (pl., bacilli).
- *Bacillus megaterium* is a typical example of a bacterium with a rod shape
- Coccobacilli being so short and wide that they resemble cocci.
- Rods can occur singly, in pairs or in chains
- Vibrio: comma shaped bacteria



Coccobacillus



Bacillus



Diplobacilli



Streptobacilli



Types of Bacilli Bacteria

Flagellate Rods



**Endospore-Forming
bacilli**



Piliocades



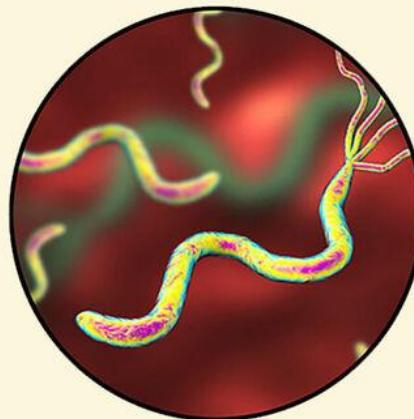
CONTD..

- Spiral-shaped prokaryotes can be either classified as **spirilla**, which usually have tufts of flagella at one or both ends of the cell or *spirochetes*.
- *Spirochetes are more flexible and* have a unique, internal flagellar arrangement.
- Actinomycetes typically form long filaments called hyphae that may branch to produce a network called a **mycelium**
- some prokaryotes are variable in shape and lack a single, characteristic form
- *These are called **pleomorphic*** even though they may, like *Corynebacterium*,

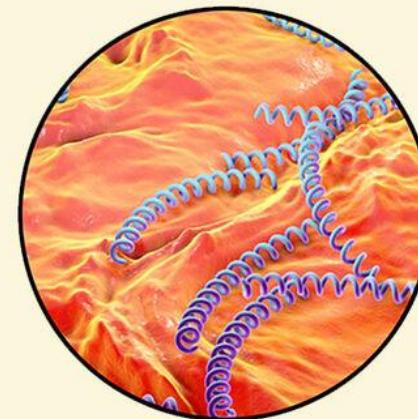
SPIRAL-SHAPED BACTERIA



Vibrio
(*Vibrio cholerae*)



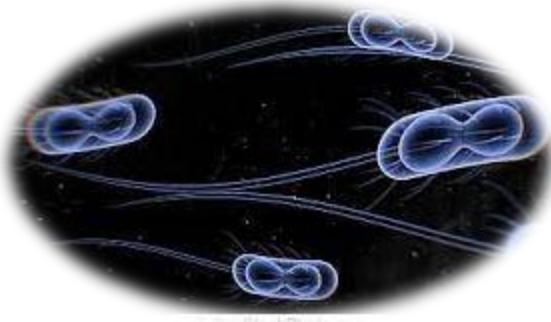
Spirilla
(*Helicobacter pylori*)



Spirochaetes
(*Treponema pallidum*)



- The smallest bacteria is Nanobacteria of about 0.05-0.2um in size
- The largest bacteria : Thiomargarita namibiensis



Nanobacteria

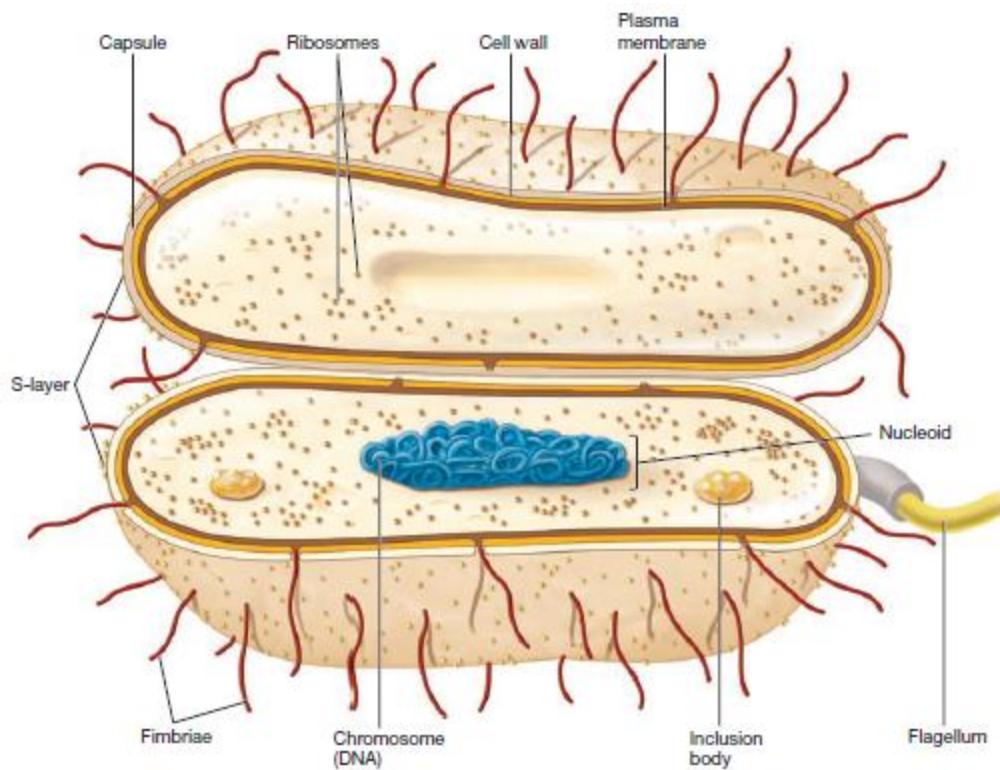


DID YOU KNOW?

The largest known bacterium, the marine **THIOMARGARITA NAMIBIENSIS**, can be visible to the naked eye and sometimes attains 0.75 mm (750 µm).



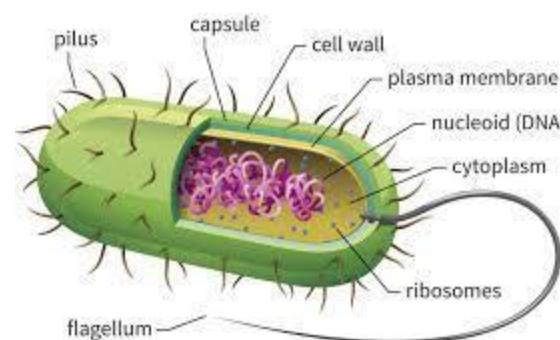
PROKARYOTIC CELL STRUCTURE



3.4 Morphology of a Prokaryotic Cell.

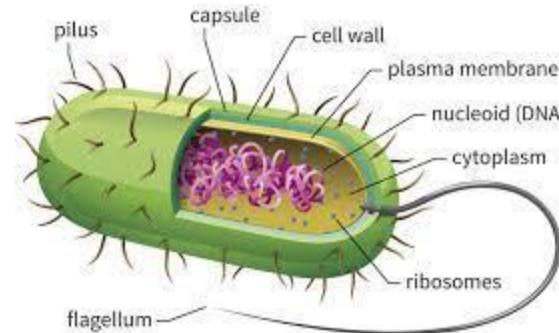
PROKARYOTIC CELL STRUCTURE

- Prokaryotic cells almost always are bounded by a chemically complex cell wall.
- Interior to this wall lies the plasma membrane.
- This membrane can be invaginated to form simple internal membranous structures of bacteria
- The prokaryotic cell does not contain internal membrane-bound organelles,



PROKARYOTIC CELL STRUCTURE

- The genetic material is localized in a discrete region, the nucleoid, and usually is not separated from the surrounding cytoplasm by membranes.
- Ribosomes and larger masses called inclusion bodies are scattered about the cytoplasmic matrix.
- Many prokaryotes use flagella for locomotion.
- In addition, many are surrounded by a capsule or slime layer external to the cell wall



FUNCTIONS OF PROKARYOTIC CELL STRUCTURE

Table 3.1

Functions of Prokaryotic Structures

Plasma membrane	Selectively permeable barrier, mechanical boundary of cell, nutrient and waste transport, location of many metabolic processes (respiration, photosynthesis), detection of environmental cues for chemotaxis
Gas vacuole	Buoyancy for floating in aquatic environments
Ribosomes	Protein synthesis
Inclusion bodies	Storage of carbon, phosphate, and other substances
Nucleoid	Localization of genetic material (DNA)
Periplasmic space	Contains hydrolytic enzymes and binding proteins for nutrient processing and uptake
Cell wall	Gives prokaryotes shape and protection from osmotic stress
Capsules and slime layers	Resistance to phagocytosis, adherence to surfaces
Fimbriae and pili	Attachment to surfaces, bacterial mating
Flagella	Movement
Endospore	Survival under harsh environmental conditions



PLASMA MEMBRANE

- The **plasma membrane encompasses the cytoplasm of both** procaryotic and eucaryotic cells.
- It is the chief point of contact with the cell's environment
- The plasma membrane also serves as a **selectively permeable barrier**:
- It allows particular ions and molecules to pass, either into or out of the cell, while preventing the movement of others.
- Thus the membrane prevents the loss of essential components through leakage while allowing the movement of other molecules.



- The prokaryotic plasma membrane also is the location of a variety of crucial metabolic processes: **respiration, photosynthesis, and the synthesis of lipids and cell wall constituents**
- The membrane contains special receptor molecules that help prokaryotes detect and respond to chemicals in their surroundings.



FLUID MOSAIC MODEL OF PLASMA MEMBRANE

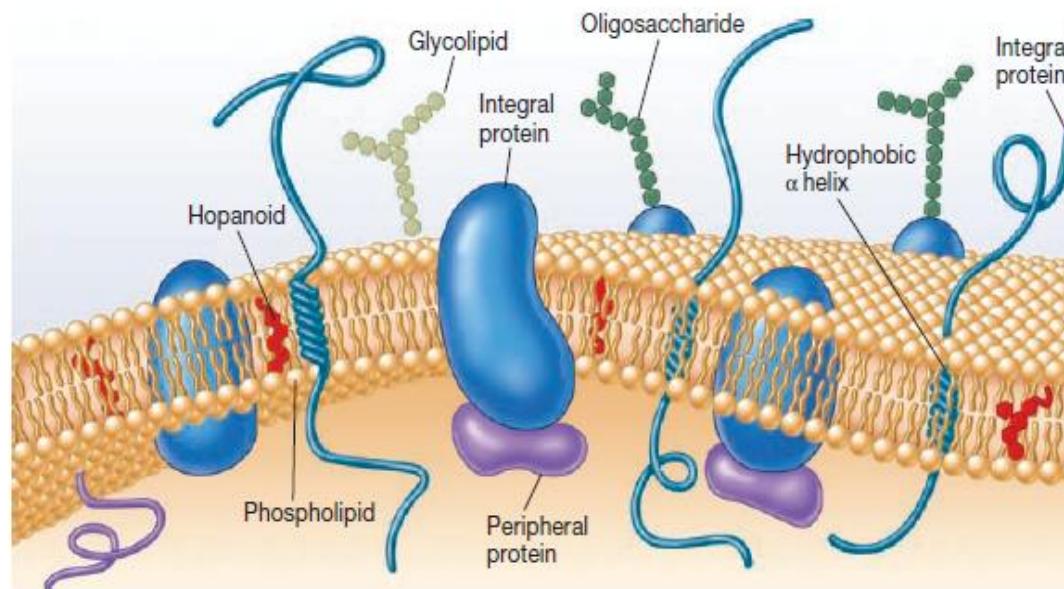


Figure 3.5 Bacterial Plasma Membrane Structure. This diagram of the fluid mosaic model of bacterial membrane structure shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains. Other membrane lipids such as hopanoids (red) may be present. For the sake of clarity, phospholipids are shown in proportionately much larger size than in real membranes.

- Bacterial membranes differ from eukaryotes in lacking sterols like cholesterol
- Many bacterial membranes have sterol like molecules called **Hopanoids**



BACTERIAL CELL WALL

- The cell wall is the layer, usually fairly rigid, that lies just outside the plasma membrane
- It is one of the most important prokaryotic structures for several reasons:
 - It helps determine the shape of the cell
 - It helps protect the cell from osmotic lysis
 - It can protect the cell from toxic substances
 - In pathogens, it can contribute to pathogenicity.
 - The prokaryotic cell wall also is the site of action of several antibiotics.



BACTERIAL CELL WALL STRUCTURE

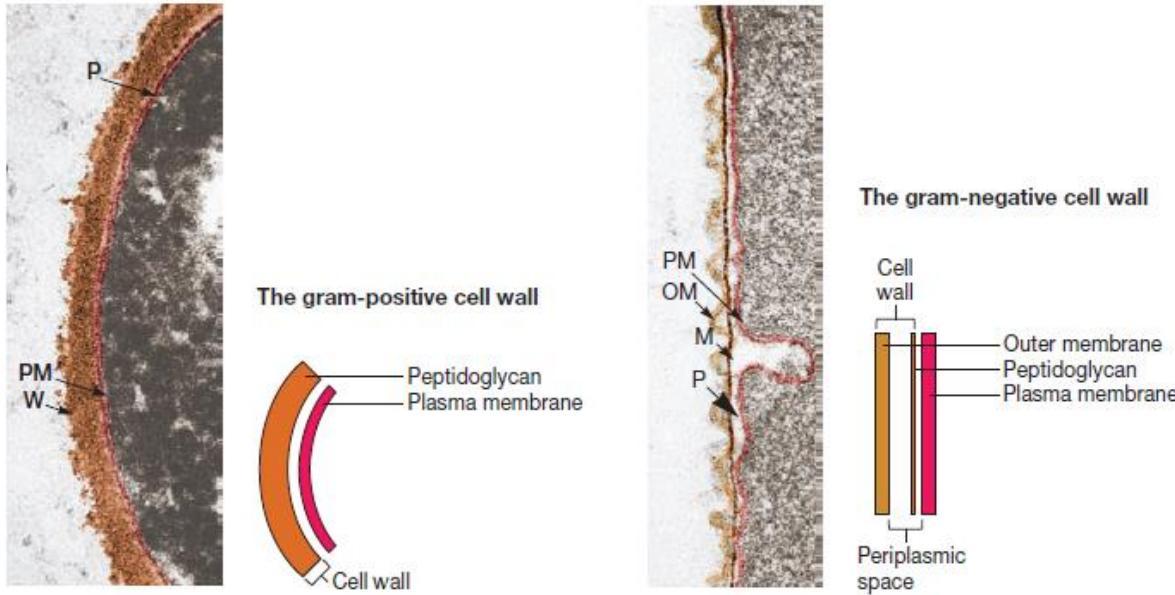


Figure 3.17 Gram-Positive and Gram-Negative Cell Walls. The gram-positive envelope is from *Bacillus licheniformis* (left), and the gram-negative micrograph is of *Aquaspirillum serpens* (right). M; peptidoglycan or murein layer; OM, outer membrane; PM, plasma membrane; P, periplasmic space; W, gram-positive peptidoglycan wall.

BACTERIAL CELL WALL

- After Christian Gram developed Gram staining 1884 bacteria are classified into two groups
- Gram-positive bacteria stained purple, whereas gram-negative bacteria were colored pink or red by the technique
- The gram-positive cell wall consists of a single 20 to 80 nm thick homogeneous layer of **peptidoglycan (murein) lying** outside the plasma membrane
- Gram-negative cell wall is quite complex as it has a 2 to 7 nm peptidoglycan layer covered by a 7 to 8 nm thick **outer membrane**.



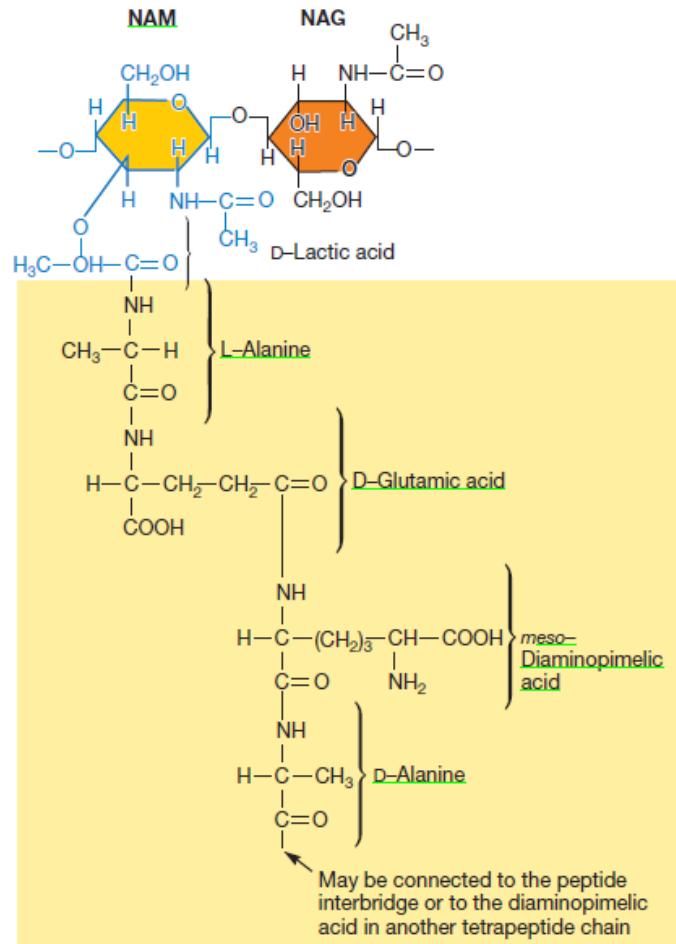
- Because of the thicker peptidoglycan layer, the walls of Grampositive cells are more resistant to osmotic pressure than those of gram-negative bacteria
- All the structures from the plasma membrane outward the **cell envelope**.
- Therefore this includes the plasma membrane, cell wall, and structures like capsules



PEPTIDOGLYCAN STRUCTURE

- Peptidoglycan, or murein, is an enormous meshlike polymer composed of many identical subunits.
- The polymer contains two sugar derivatives, *N-acetylglucosamine* and *N-acetylmuramic acid* (the lactyl ether of *N-acetylglucosamine*), and several different amino acids.
- Three of these amino acids are not found in proteins: D-glutamic acid, D-alanine, and *mesodiaminopimelic acid*.
- The presence of D-amino acids protects against degradation by most peptidases, which recognize only the L-isomers of amino acid residues.

PEPTIDOGLYCAN SUBUNIT

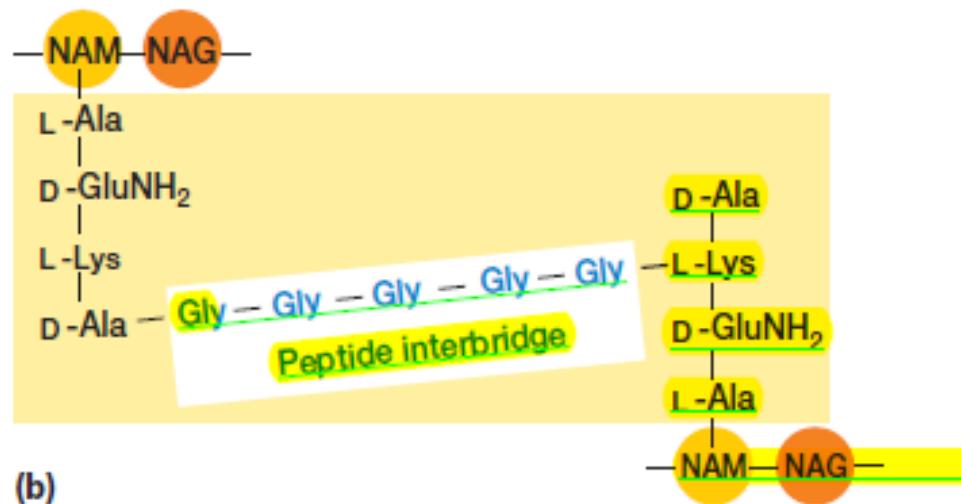
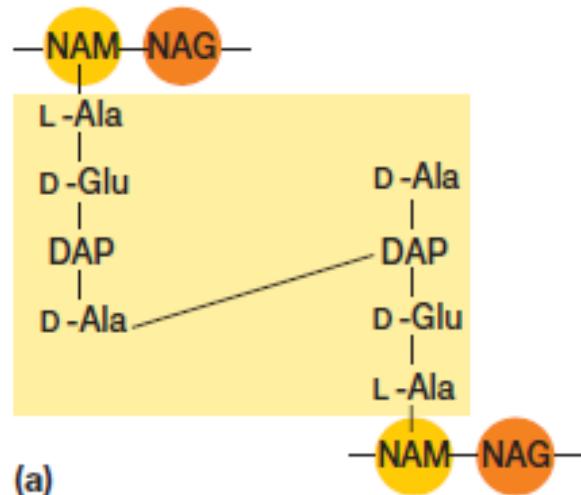


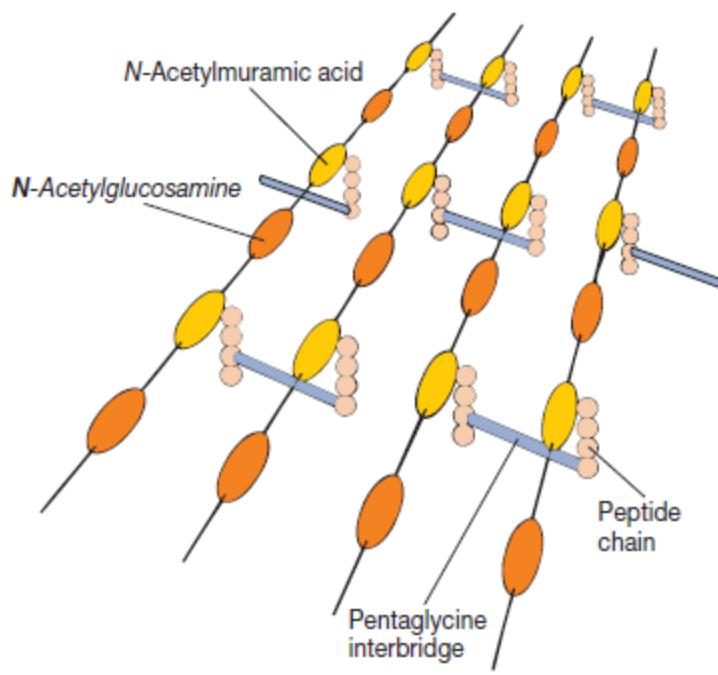
- Peptidoglycan is a polymer composed of alternating *N-acetylglucosamine* and *Nacetylmuramic acid* residues
- A peptide chain of four alternating D- and L-amino acids is connected to the carboxyl group of *Nacetylmuramic acid*
- Many bacteria replace *meso-diaminopimelic acid* with another diaminoacid, usually L-lysine



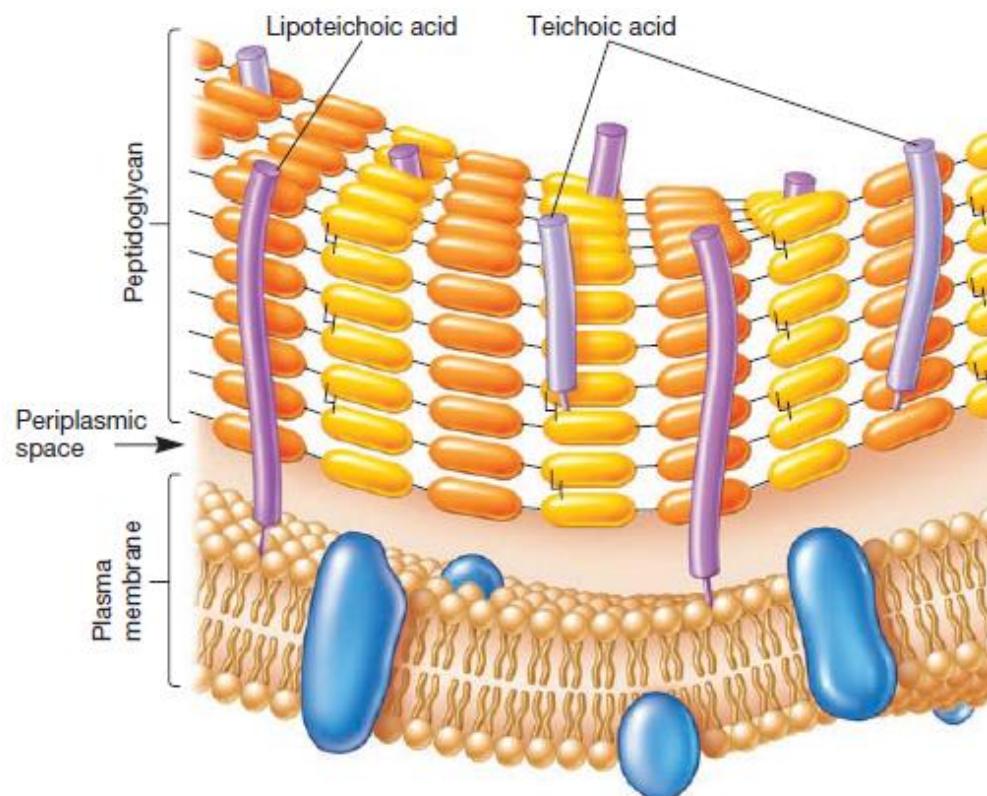
- In order to make a strong, meshlike polymer, chains of peptidoglycan subunits must be joined by cross-links between the peptides.
- Often the carboxyl group of the terminal D-alanine is connected directly to the amino group of diaminopimelic acid, but a **peptide interbridge may be used instead (penta glycine bridge)**
- Most gram-negative cell wall peptidoglycan lacks the peptide interbridge.







GRAM POSITIVE CELL WALL

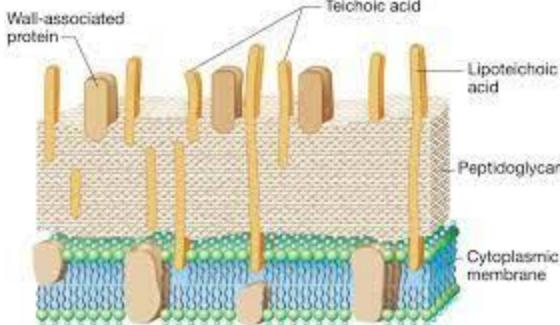


.23 The Gram-Positive Envelope.

- Gram-positive bacteria normally have cell walls that are thick and composed primarily of peptidoglycan
- Peptidoglycan in grampositive bacteria often contains a peptide interbridge
- **In addition, gram-positive cell walls usually contain** large amounts of **teichoic acids, polymers of glycerol or ribitol joined by phosphate groups**
- **Amino** acids such as D-alanine or sugars like glucose are attached to the glycerol and ribitol groups.



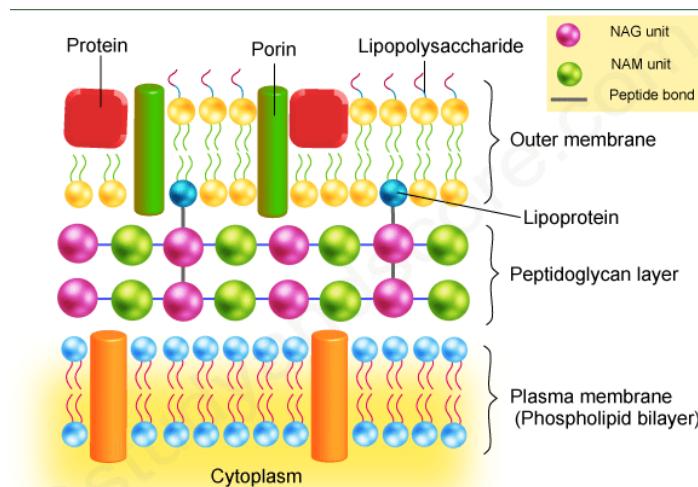
- The teichoic acids are covalently connected to either the peptidoglycan itself or to plasma membrane lipid , then they are called lipoteichoic acids.
- Teichoic acids appear to extend to the surface of the peptidoglycan, give the gram-positive cell wall its negative charge
- Teichoic acids are not present in gram-negative bacteria.



- The periplasmic space of gram-positive bacteria, lies between the plasma membrane and the cell wall and is smaller than that of gram-negative bacteria.
- An enzyme called **sortase** catalyzes the attachment of surface proteins to the gram-positive peptidoglycan

GRAM NEGATIVE CELL WALL

- Gram-negative cell walls are much more complex than gram-positive walls
- The thin peptidoglycan layer next to the plasma membrane and bounded on either side by the periplasmic space



CELL WALL STRUCTURE OF GRAM NEGATIVE BACTERIA

- Peptidoglycan layer may constitute not more than 5 to 10% of the wall weight
- The periplasmic space of gram-negative bacteria ranges in size from 1 nm to as great as 71 nm.
- It may constitute about 20 to 40% of the total cell volume, and it is usually 30 to 70 nm wide



- The outer membrane lies outside the thin peptidoglycan layer **and is linked to the cell in two ways.**
- The first is by Braun's lipoprotein, the most abundant protein in the outer membrane.
- This small lipoprotein is covalently joined to the underlying peptidoglycan, and is embedded in the outer membrane by its hydrophobic end
- The second linking mechanism involves the many adhesion sites joining the outer membrane and the plasma membrane.
- In *E. coli*, 20 to 100 nm areas of contact between the two membranes can be seen.

GRAM NEGATIVE CELL ENVELOPE

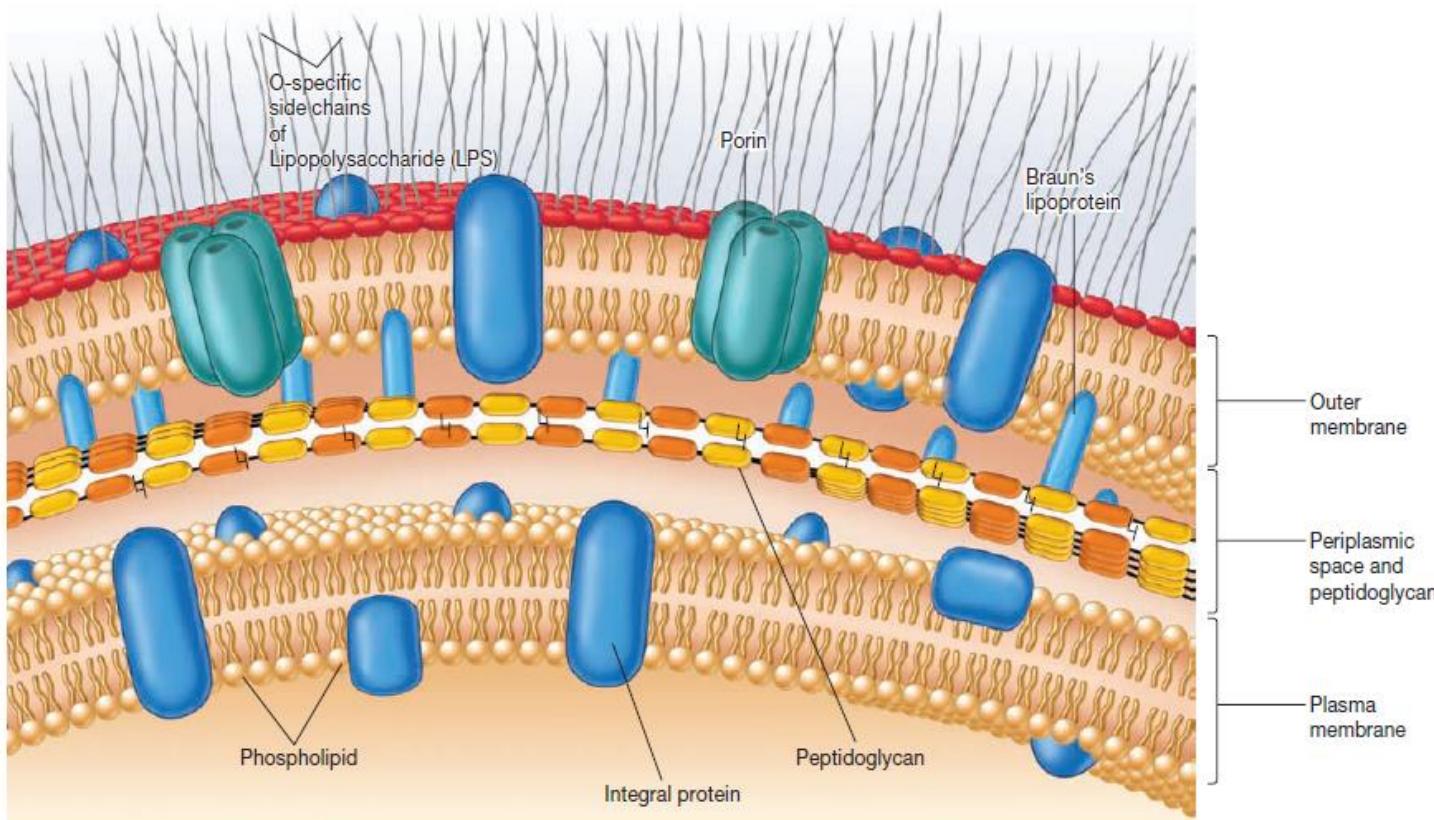
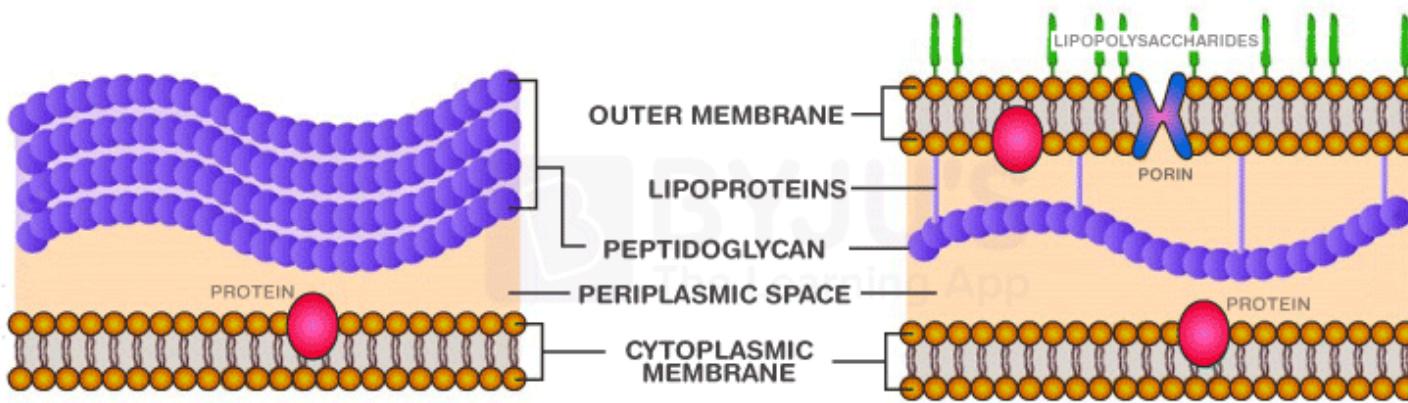


Figure 3.25 The Gram-Negative Envelope.

GRAM POSITIVE VS. NEGATIVE CELL WALL

BYJU'S
The Learning App



- Possibly the most unusual constituents of the outer membrane are its **lipopolysaccharides (LPSs)**.
- These large, complex molecules contain both lipid and carbohydrate consist of three parts: (1) lipid A, (2) the core polysaccharide, and (3) the O side chain.

FUNCTIONS OF LPS

- LPS contributes to the negative charge on the bacterial surface.
- Lipid A also helps stabilize outer membrane structure.
- LPS may contribute to bacterial attachment to surfaces and biofilm formation.
- A major function of LPS is that it aids in creating a permeability barrier., restrict the entry of bile salts, antibiotics, and other toxic substances that might kill or injure the bacterium.



- LPS also plays a role in protecting pathogenic gram-negative bacteria from host defenses
- The O side chain of LPS is also called the O antigen because it elicits an immune response.
- Importantly, the lipid A portion of LPS often is toxic; as a result, the LPS can act as an endotoxin
- If the bacterium enters the bloodstream, LPS endotoxin can cause a form of septic shock
- The outer membrane also have transport proteins called **Porin** channels



MECHANISM OF GRAM STAINING

Gram Stain

Principle of staining technique:

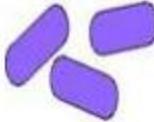
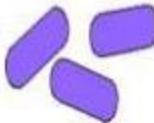
Primary stain:- Crystal Violet

Mordant(fixes the dye):- Iodine

Decolorizing agent:-Alcohol/Acetone

Counter stain;- Safranin

Gram Positive



Gram Negative



MECHANISM OF GRAM STAINING

- The difference between gram-positive and gram-negative bacteria is due to the physical nature of their cell walls.
- The peptidoglycan act as a permeability barrier preventing loss of crystal violet.
- During the procedure the bacteria are first stained with crystal violet and next treated with iodine to promote dye retention
- When gram-positive bacteria are then treated with ethanol, the alcohol shrink the pores of the thick peptidoglycan.



- Thus the dye-iodine complex is retained during this short decolorization step and the bacteria remain purple
 - In contrast, recall that gram negative peptidoglycan is very thin, not as highly cross-linked, and has larger pores
 - Alcohol treatment also may extract enough lipid from the gram-negative outer membrane to increase its porosity further
- Also the Gram positive cells have more acidic cytoplasm which have more affinity to the basic dye crystal violet

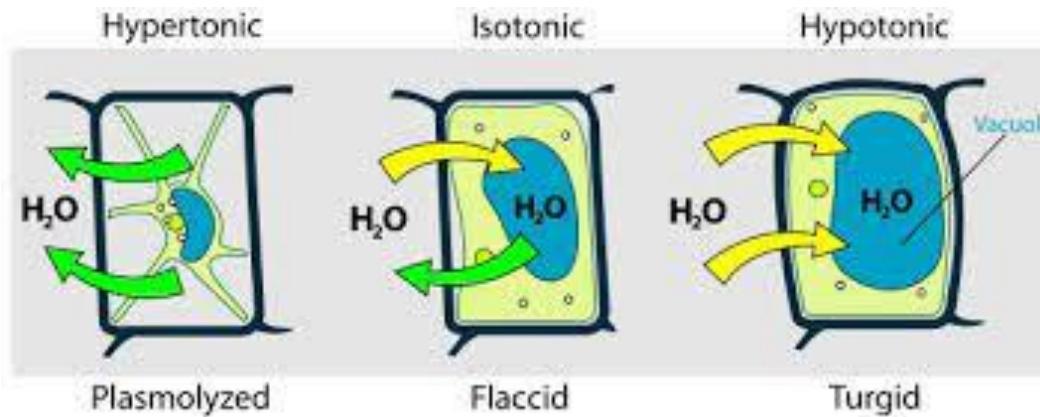


- For these reasons, alcohol more readily removes the purple crystal violet-iodine complex from gram-negative bacteria.
- Thus gram-negative bacteria are then easily stained red or pink by the counterstain safranin



CELL WALL AND OSMOTIC PROTECTION

- When cells are in hypotonic solutions water moves into the cell, causing it to swell and the cell would burst—a process called **lysis**.
- In hypertonic solutions, water flows out and the cytoplasm shrivels up—a process called **plasmolysis**.



SPHROPLAST AND PROTOPLAST

- The cell wall peptidoglycan layer can be removed by treatment with either lysozyme or penicillin
- The enzyme **lysozyme attacks peptidoglycan by hydrolyzing the bond that connects *N-acetylmuramic acid with N-acetylglucosamine***
- **Penicillin** inhibits peptidoglycan synthesis.
- In gram positive bacteria treatment with lysozyme or penicillin results in the complete loss of the cell wall, and the cell becomes a protoplast



- When gram-negative bacteria are exposed to lysozyme or penicillin, the peptidoglycan layer is lost, but the outer membrane remains. These cells are called **spheroplasts**.
- Because they lack a complete cell wall, both protoplasts and spheroplasts are osmotically sensitive and can grow only in isotonic solutions



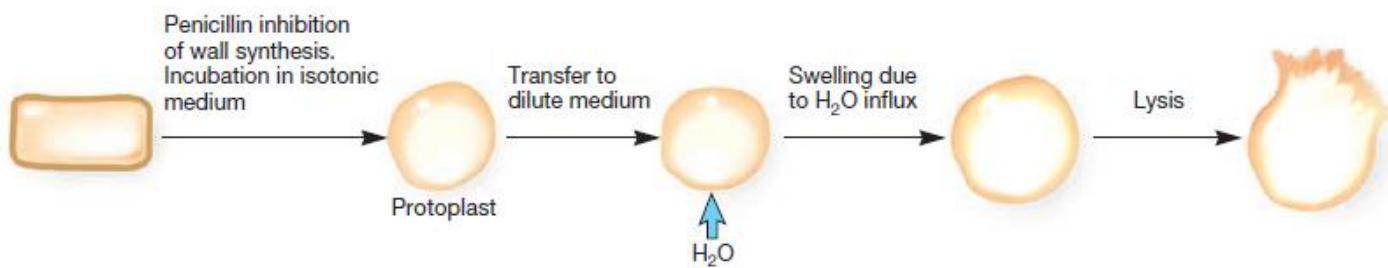


Figure 3.29 Protoplast Formation and Lysis. Protoplast formation induced by incubation with penicillin in an isotonic medium. Transfer to dilute medium will result in lysis.

Bacterial cell structure

Cell wall – Peptidoglycan

- Isotonic media:

- Gram-positive bacteria → Lysozyme → **Protoplasts**
- Gram-negative bacteria → EDTA-lysozyme → **Spheroplasts**
- If protoplasts/spheroplasts are able to grow and divide, they are called **L-forms**.

CYTOPLASMIC MATRIX

- About 70% of bacterial mass is water
- The plasma membrane and everything within is called the **protoplast**



PROKARYOTIC CYTOSKELETON

- Homologs of all three eucaryotic cytoskeletal elements (microfilaments, intermediate filaments, and microtubules) have been identified in bacteria, and one has been identified in archaea
- **The cytoskeletal filaments of** prokaryotes are structurally similar to their eucaryotic counterparts and carry out similar functions
- They participate in cell division, localize proteins to certain sites in the cell, and determine cell shape



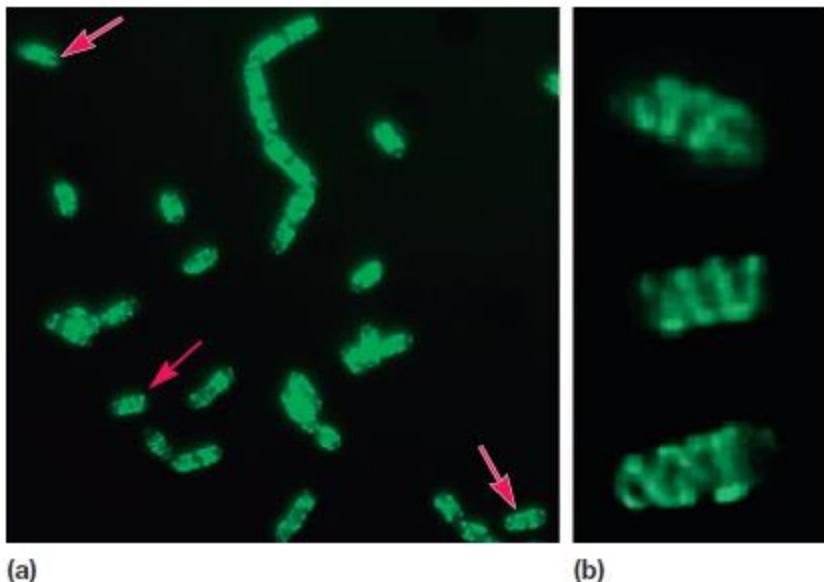


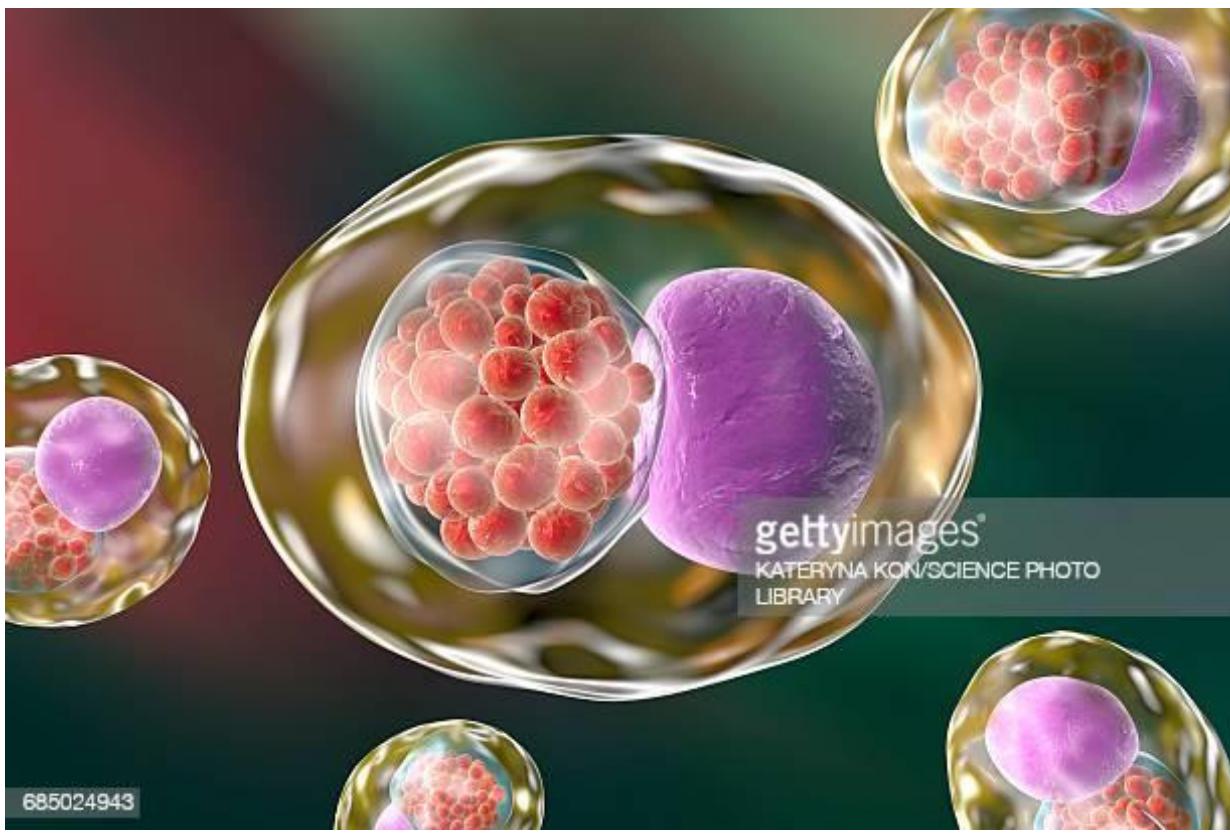
Table 3.2 | **Prokaryotic Cytoskeletal Proteins**

Prokaryotic Protein (Eucaryotic Counterpart)	Function	Comments
FtsZ (tubulin)	Cell division	Widely observed in <i>Bacteria</i> and <i>Archaea</i>
MreB (actin)	Cell shape	Observed in many rod-shaped bacteria; in <i>Bacillus subtilis</i> is called Mbl
Crescentin (intermediate filament proteins)	Cell shape	Discovered in <i>Caulobacter crescentus</i>

INCLUSION BODIES

- **Inclusion bodies, granules of organic or inorganic material that** often are clearly visible in a light microscope, are present in the cytoplasmic matrix.
- These bodies usually are used for storage (e.g., carbon compounds, inorganic substances, and energy), and also reduce osmotic pressure by tying up molecules in particulate form.
- Some inclusion bodies lies free in the cytoplasm
Eg: polyphosphate granules, cyanophycin granules, and some glycogen granules





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- Inclusion bodies enclosed by a shell about 2.0 to 4.0 nm thick are called enclosed inclusion bodies
- Examples of enclosed inclusion bodies are poly--hydroxybutyrate granules, some glycogen and sulfur granules, carboxysomes, and gas vacuoles.
- Many inclusion bodies are used for storage; their quantity will vary with the nutritional status of the cell.
- For example, polyphosphate granules will be depleted in freshwater habitats that are phosphate limited

- Organic inclusion bodies usually contain either glycogen or poly—hydroxyalkanoates
- Cyanobacteria, a group of photosynthetic bacteria, have two distinctive organic inclusion bodies.
Cyanophycin granules and Carboxysomes
- **Carboxysomes** are present in many cyanobacteria and other CO₂-fixing bacteria.
- They contain the enzyme ribulose-1, 5-bisphosphate carboxylase, called Rubisco.
- Rubisco is the critical enzyme for CO₂ fixation.,



- A most remarkable organic inclusion body is the **gas vacuole**, a structure that provides buoyancy to some aquatic prokaryotes

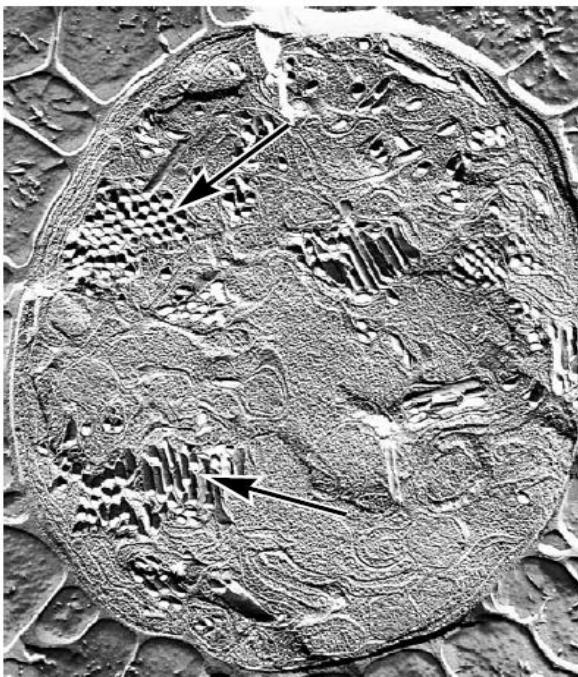


Figure 3.14 Gas Vesicles and Vacuoles. A freeze-fracture



- Two major types of inorganic inclusion bodies are seen in prokaryotes:
- polyphosphate granules and sulfur granules.
- Many bacteria store phosphate as **polyphosphate granules or volutin Granules**
- Thus volutin granules function as storage reservoirs for phosphate, an important component of cell constituents such as nucleic acids.



- These granules are sometimes called **metachromatic granules because they show the metachromatic effect**; that is, they appear red when stained with the blue dyes methylene blue or toluidine blue.
- Sulfur granules are used by some prokaryotes to store sulfur temporarily



- Inorganic inclusion bodies can be used for purposes other than storage.
- An excellent example is the **magnetosome**, which is used by some bacteria to orient in the Earth's magnetic field.
- Many of these inclusion bodies contain iron in the form of magnetite
- Eg: *Aquaspirillum magnetotactum*



RIBOSOME

- Ribosomes are very complex structures made of both protein and ribonucleic acid (RNA)
- They are the site of protein synthesis
- Prokaryotic ribosomes are called 70S ribosomes (as opposed to 80S in eukaryotes) and are
- constructed of a 50S and a 30S subunit
- **The S in 70S stands for Svedberg unit**
- **This is the unit of the** sedimentation coefficient, a measure of the sedimentation velocity in a centrifuge



PROKARYOTIC RIBOSOME

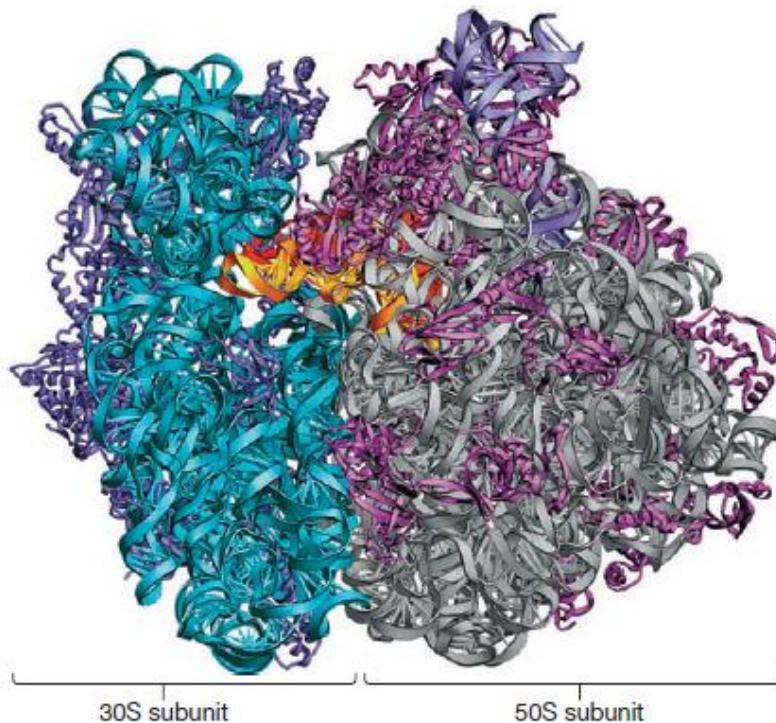


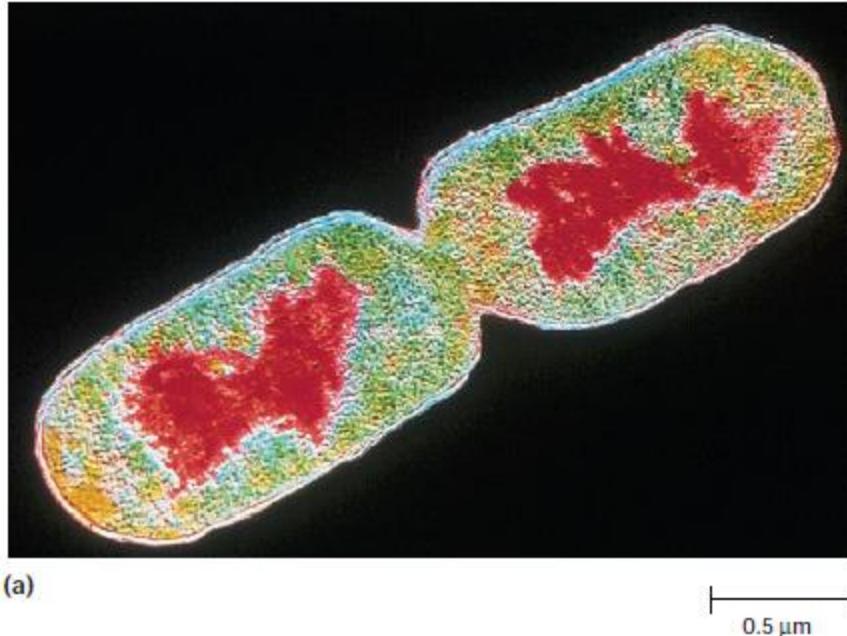
Figure 3.15 Prokaryotic Ribosome. The two subunits of a bacterial ribosome are shown. The 50S subunit includes 23S rRNA (gray) and 5S rRNA (light blue), while 16S rRNA (cyan) is found in the 30S subunit. A molecule of tRNA (gold) is shown in the A site. To generate this ribbon diagram, crystals of purified bacterial ribosomes were grown, exposed to X rays, and the resulting diffraction pattern analyzed.

THE NUCLEOID

- Prokaryotes lack a membrane-delimited nucleus
- The prokaryotic chromosome is located in an irregularly shaped region called the **nucleoid or the nuclear body or chromatin** body or nuclear region
- **Usually prokaryotes contain** a single circle of double-stranded **deoxyribonucleic acid (DNA)**,
- but some have a linear DNA chromosome (*Borrelia burgdorferi*) and some, such as *Vibrio cholerae* and *Borrelia burgdorferi* (*the causative agents of cholera and Lyme disease, respectively*), have more than one chromosome.



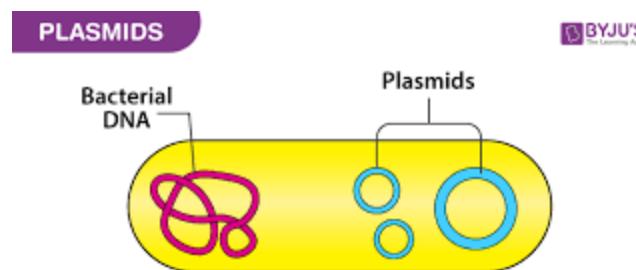
THE NUCLEOD



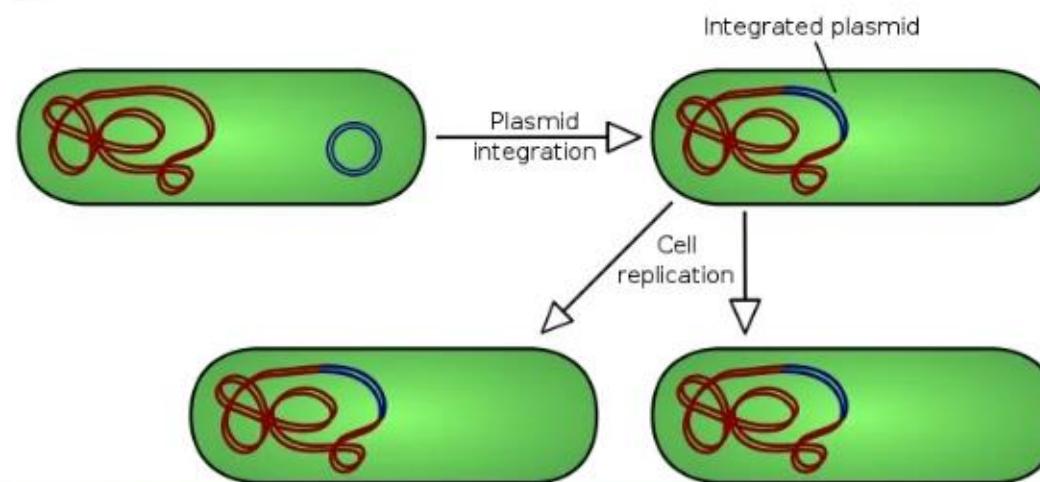
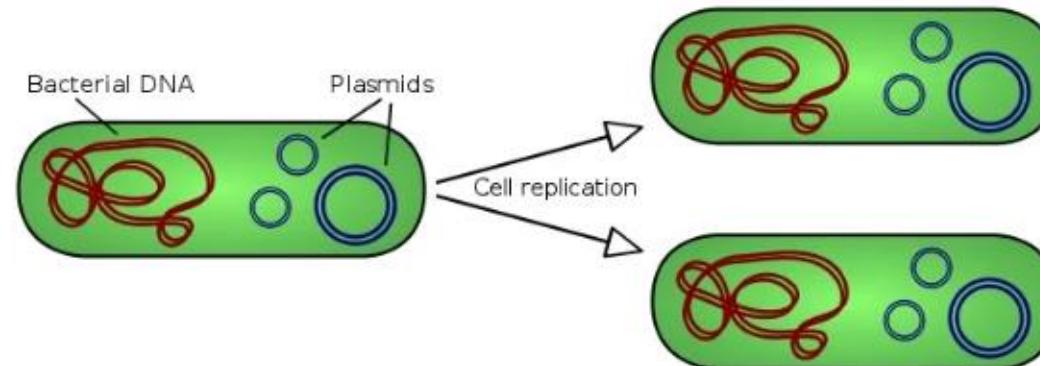
A color-enhanced transmission electron micrograph of a thin section of a dividing *E. coli* cell. *The red areas are* the nucleoids present in the two daughter cells

PLASMIDS

- Extrachromosomal DNA molecules called plasmids
- **Plasmids are small, double-stranded DNA molecules that can exist independently of the chromosome**
- Both circular and linear plasmids have been documented, but most known plasmids are circular
- *B. burgdorferi*, carries 12 linear and 9 circular plasmids.

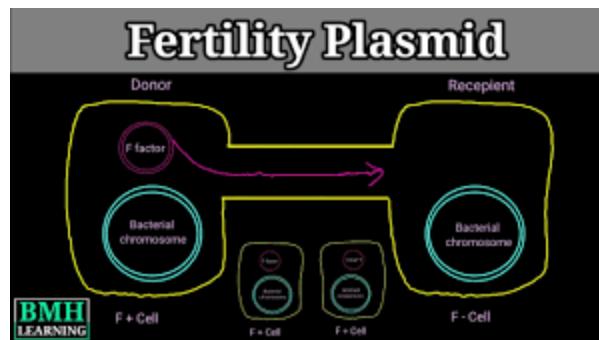


- Plasmids are able to replicate autonomously
- Single-copy plasmids produce only one copy per host cell
- Multicopy plasmids may be present at concentrations of 40 or more per cell
- Some plasmids are able to integrate into the chromosome and are thus replicated with the chromosome. Such plasmids are called **episomes**
- The loss of a plasmid is called **curing**
- Curing can be induced using acridine mutagens, UV and ionizing radiation, thymine starvation, antibiotics, and growth above optimal temperatures.



CLASSIFICATION OF PLASMID

- **Conjugative plasmids**
- **They have genes for the construction** of hairlike structures called pili and can transfer copies of themselves to other bacteria during conjugation
- Perhaps the best studied conjugative plasmid is the **F factor (fertility factor or F plasmid)**



- **Resistance plasmids or R plasmids** which confer antibiotic resistance on the cells that contain them
- Bacteriocin encoding plamids:**Bacteriocins are bacterial proteins** that destroy other bacteria. They usually act only against closely related strains
- **Col plasmids contain genes for the synthesis of bacteriocins** known as colicins, which are directed against *E. coli*
- Cloacins kill *Enterobacter species*



- **Virulence plasmids encode factors that make their hosts more pathogenic**
- For example, enterotoxigenic strains of *E. coli* cause traveler's diarrhea because they contain a plasmid that codes for an enterotoxin
- **Metabolic plasmids carry genes for enzymes that degrade substances such as aromatic compounds (toluene), pesticides (2,4-dichlorophenoxyacetic acid), and sugars (lactose)**



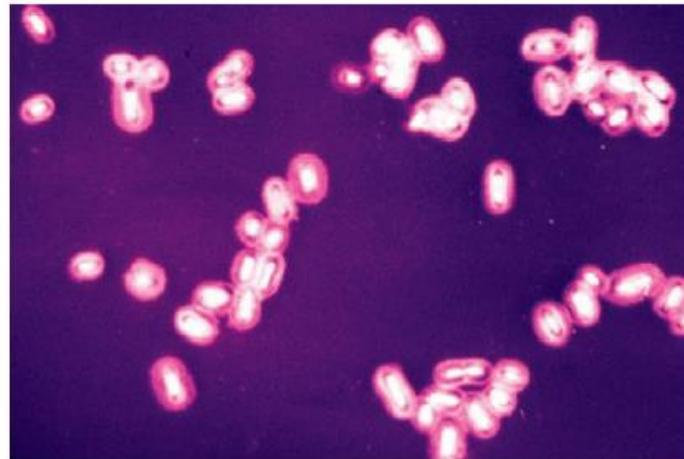
COMPONENTS EXTERNAL TO CELL WALL

Capsules, Slime Layers, and S-Layers

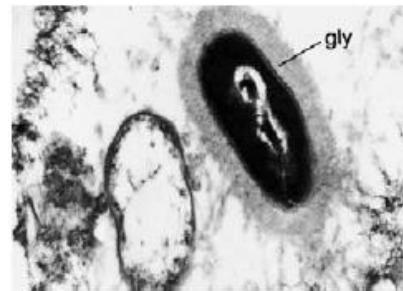
- When the layer is well organized and not easily washed off, it is called a **capsule**
- *It is called a slime layer* when it is a zone of diffuse, unorganized material that is removed easily.
- When the layer consists of a network of polysaccharides extending from the surface of the cell, it is referred to as the **glycocalyx**
- *The term Glycocalyx encompass both capsules and slime layers because they usually are composed of polysaccharides.*



- The capsule of *Bacillus* is made of polypeptide :
Poly D glutamic acid



(a) *K. pneumoniae*



(b) *Bacteroides*

- Capsule help pathogenic bacteria resist phagocytosis by host phagocytes.
- Capsules contain a great deal of water and can protect against dessiccation
- They exclude viruses and most hydrophobic toxic materials such as detergents
- The glycocalyx also aids in attachment to solid surfaces, including tissue surfaces in plant and animal hosts
- **Gliding bacteria often** produce slime, which in some cases, has been shown to facilitate motility.

S LAYER

- Many prokaryotes have a regularly structured layer called an **S layer** on their surface
- The S-layer has a pattern something like floor tiles and is composed of protein or glycoprotein
- In gram-negative bacteria the S-layer adheres directly to the outer membrane
- it is associated with the peptidoglycan surface in gram positive bacteria
- It may protect the cell against ion and pH fluctuations, osmotic stress, enzymes, or the predacious bacterium *Bdellovibrio*.



- *The S-layer also helps maintain the shape and envelope rigidity of some cells*
- It can promote cell adhesion to surfaces
- Finally, the S-layer seems to protect some bacterial pathogens against host defenses, thus contributing to their virulence



PILI AND FIMBRIAE

- Short, fine, hair like appendages that are thinner than flagella
- Helps in adhesion
- Sex pili play important role in conjugation
- Made of protein subunits called pilin

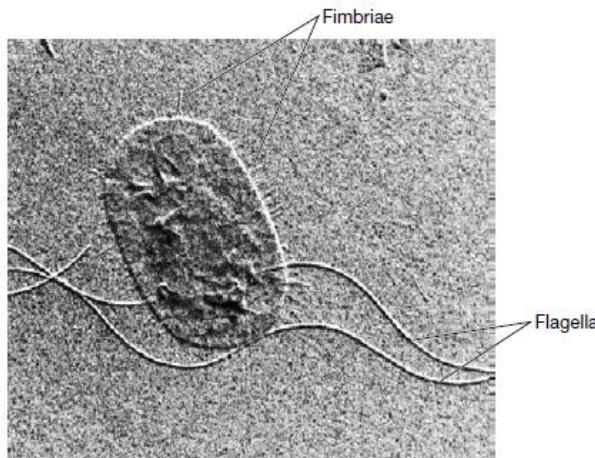
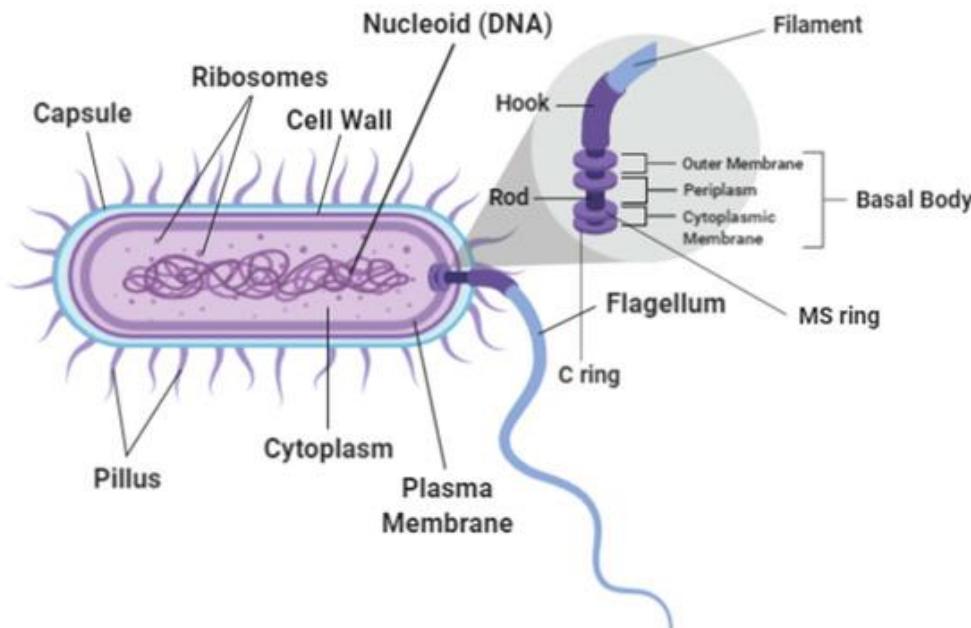


Figure 3.37 Flagella and Fimbriae. The long flagella and the numerous shorter fimbriae are very evident in this electron micrograph of the bacterium *Proteus vulgaris* ($\times 39,000$).

BACTERIAL FLAGELLA

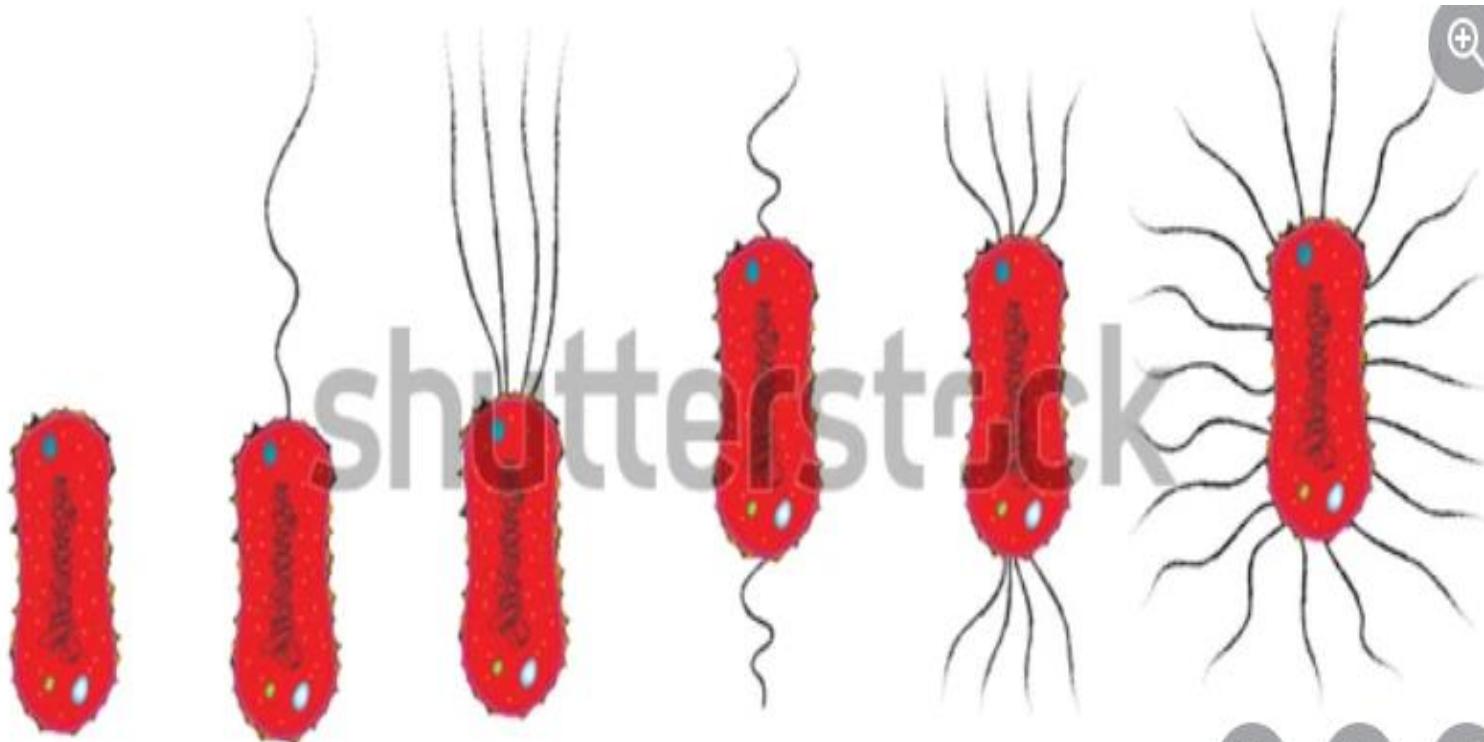
- It is the locomotory appendages of bacteria
- Bacterial flagella are slender, rigid structures, about 20 nm across and up to 15 or 20 μm long bacteria



CLASSIFICATION OF BACTERIA BASED ON FLAGELLAR ARRANGEMENT

- ❖ **Monotrichous bacteria** (*trichous means hair*) **have one** flagellum; if it is located at an end, it is said to be a **polar flagellum**
- **Amphitrichous bacteria** (*amphi means on* both sides) have a single flagellum at each pole.
- **lophotrichous bacteria** (*lopho means tuft*) **have a cluster of flagella** at one or both ends
- *Flagella are spread fairly evenly over the whole surface of peritrichous* (*peri means around*) bacteria





Atrichous

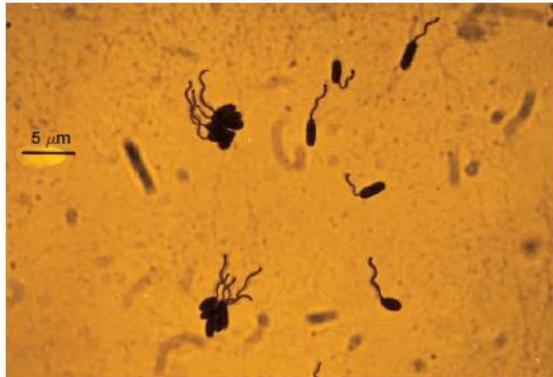
Monotrichous

Lophotrichous

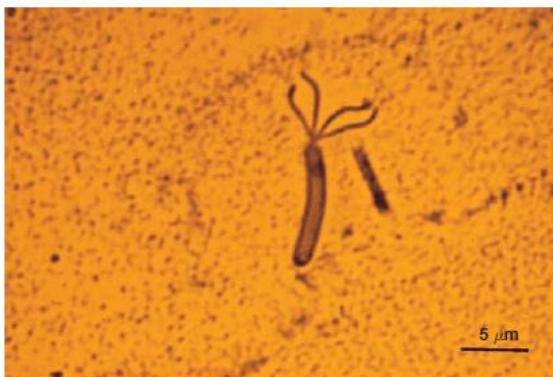
Amphitrichous

Amphilophotrichous

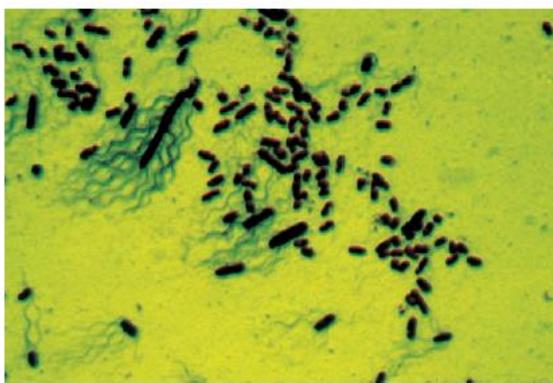
Peritrichous T



(a) *Pseudomonas*—monotrichous polar flagellation

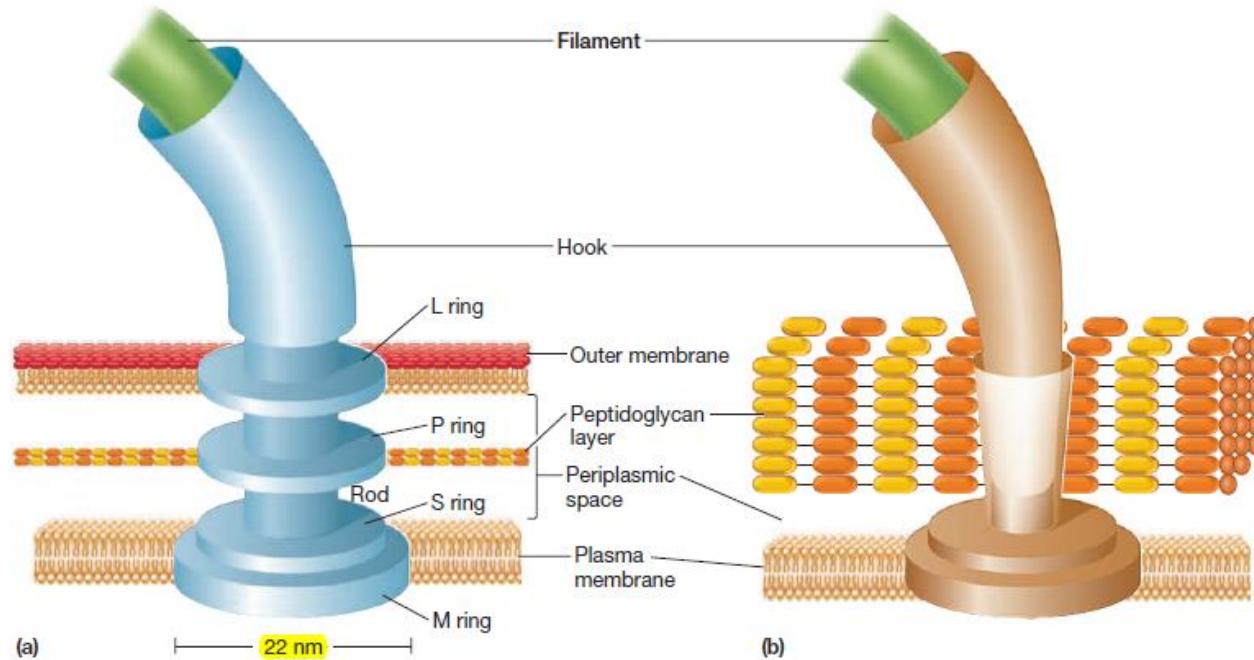


(b) *Spirillum*—lophotrichous flagellation



(c) *P. vulgaris*—peritrichous flagellation

FLAGELLAR STRUCTURE



Gram Negative Bacteria

Gram Positive Bacteria

FLAGELLAR STRUCTURE

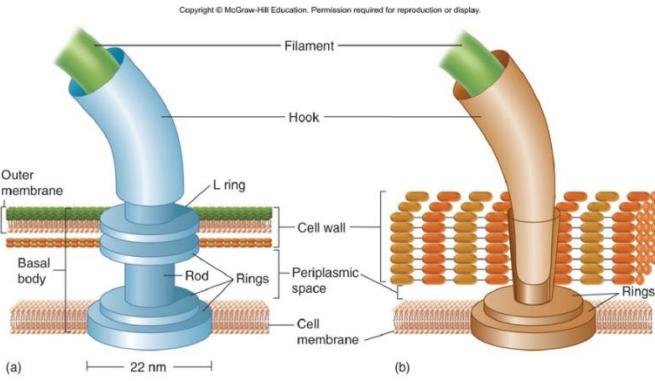
- Bacterial flagellum is composed of three parts
- The longest and most obvious portion is the **flagellar filament, which extends** from the cell surface to the tip
- A **basal body is embedded** in the cell
- A short, curved segment, the **flagellar hook, links the filament to its basal body and acts as a flexible coupling**
- The filament is a hollow, rigid cylinder constructed of subunits of the protein **flagellin and filaments ends with a capping protein.**



- The hook and basal body are quite different from the filament
- **Slightly wider than the filament, the hook is made of** different protein subunits
- The basal body is the most complex part of a flagellum
- In *E. coli* and most gram-negative bacteria, the basal body has four rings connected to a central rod
- *The outer L and P rings associate with the lipopolysaccharide and peptidoglycan layers, respectively*

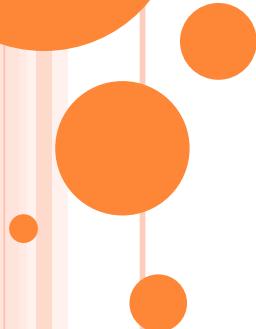
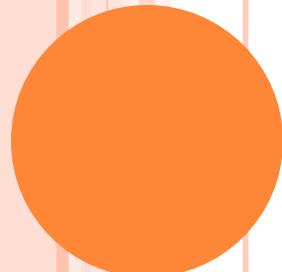
- The inner M ring contacts the plasma membrane.
- Gram-positive bacteria have only two basal body rings—an inner ring connected to the plasma membrane and an outer one probably attached to the peptidoglycan

Gram-negative (a), and Gram-positive (b)
Flagella



- Some bacteria have sheaths surrounding their flagella
- For example, *Bdellovibrio* has a membranous structure surrounding the filament.
- *Vibrio cholerae* has a lipopolysaccharide sheath





BACTERIAL ENDOSPORE

Dr. Bincy Joseph

Assistant Professor

PGIVER, Jaipur

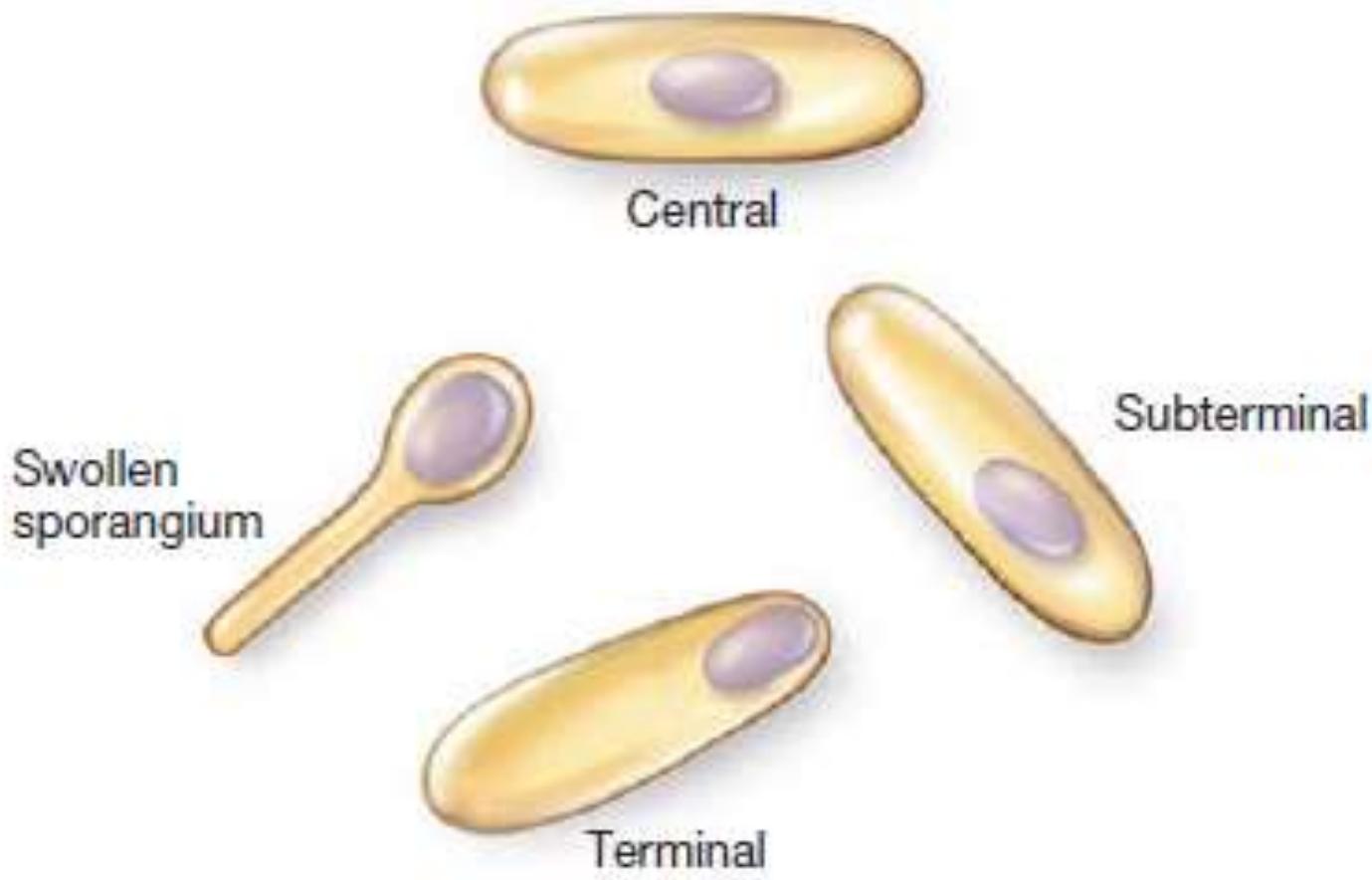
BACTERIAL ENDOSPORE

- A number of gram-positive bacteria can form a special resistant, dormant structure called an **endospore**
- **Endospores develop** within vegetative bacterial cells of several genera:
- *Bacillus and Clostridium (rods), Sporosarcina (cocci), and others.*
- *These structures* are extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants, and desiccation.
- Some endospores have remained viable for around 100,000 years.

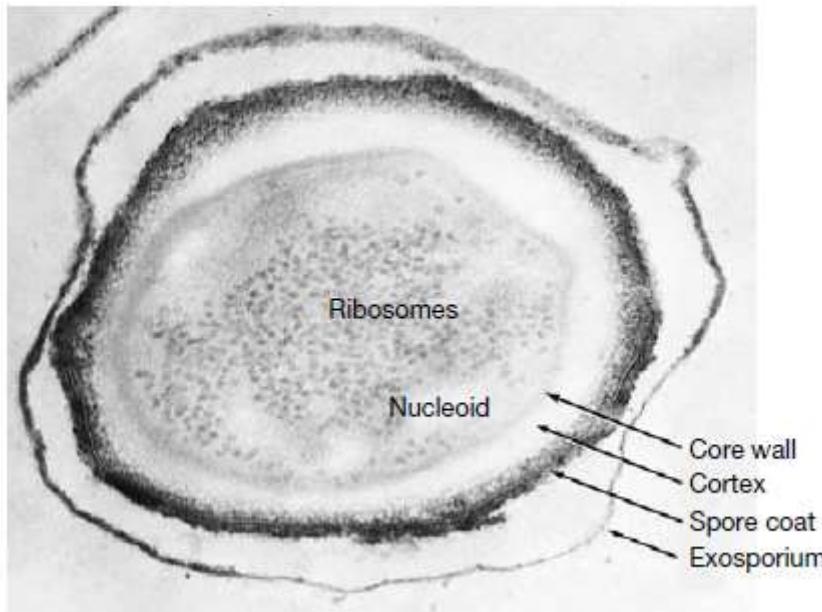


- In the environment, endospores aid in survival when moisture or nutrients are scarce
- Endospore position in the mother cell (**sporangium**) frequently differs among species, making it of considerable value in identification
- Endospores may be centrally located, close to one end (subterminal), or definitely terminal
- **Sometimes an endospore is so large that it swells the sporangium.**





STRUCTURE OF BACTERIAL SPORE



- The spore often is surrounded by a thin, delicate covering called the **exosporium**
- A **spore coat** lies beneath the exosporium, is composed of several protein layers, and may be fairly thick
- It is impermeable to many toxic molecules and is responsible for the spore's resistance to chemicals.
- The coat also is thought to contain enzymes that are involved in germination



- The **cortex**, which may occupy as much as half the spore volume, rests beneath the spore coat
- It is made of a peptidoglycan that is less cross-linked than that in vegetative cells
- The **spore cell wall** (or core wall) is inside the cortex and surrounds the protoplast or **spore core**
- The core has normal cell structures such as **ribosomes** and a nucleoid, but is metabolically inactive
- As much as 15% of the spore's dry weight consists of dipicolinic acid complexed with calcium ions **which is located in the core**



WHY SPORES ARE HIGHLY RESISTANT

- 15% of the spore's dry weight consists of dipicolinic acid complexed with calcium ions **which is located in the core play important role in heat resistance**
- Calcium does aid in resistance to wet heat, oxidizing agents, and sometimes dry heat
- Calcium-dipicolinate stabilizes the spore's nucleic acids
- In addition, specialized *small, acid-soluble DNA-binding proteins* (SASPs), are found in the endospore
- They saturate spore DNA and protect it from heat, radiation, dessication, and chemicals.
- Dehydration of the protoplast appears to be very important in heat resistance.
- The cortex may osmotically remove water from the protoplast, thereby protecting it from both heat and radiation damage.
- The spore coat also seems to protect against enzymes and chemicals such as hydrogen peroxide.
- Core also contain DNA repairing enzymes



SPOROGENESIS OR SPORULATION

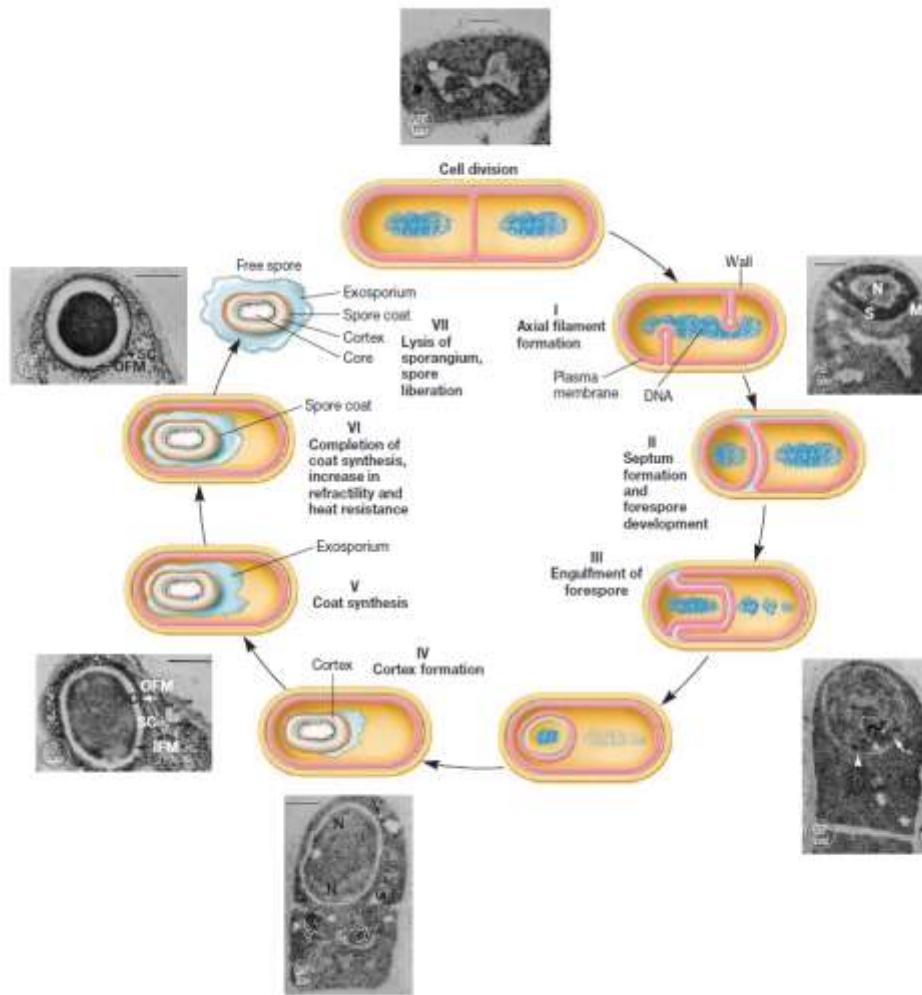
- Normally commences when growth ceases due to lack of nutrients

It is a complex process and may be divided into seven stages

- **Stage I An axial filament of nuclear material forms**
- Stage II inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum
- Stage (III) The membrane continues to grow and engulfs the immature endospore in a second membrane
- Stage IV Next, cortex is laid down in the space between the two membranes, and both calcium and dipicolinic acid are accumulated
- Stage V Protein coats then are formed around the cortex
- Stage VI maturation of the endospore occurs
- Stage VII Finally, lytic enzymes destroy the sporangium releasing the spore



SPORULATION OR SPOROGENESIS

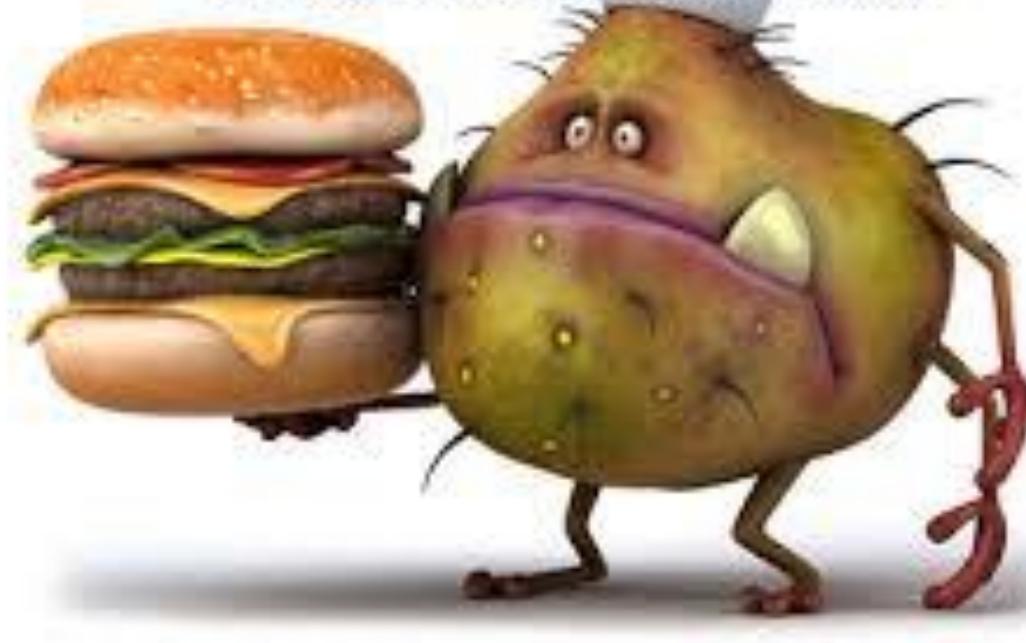


SPORE GERMINATION

- The transformation of dormant spores into active vegetative cells.
- It occurs in three stages: (1) activation, (2) germination, and (3) outgrowth
- **Often a spore will not germinate successfully, even in a nutrient-rich medium, unless it has been activated**
- **Activation** is a process that prepares spores for germination and usually results from treatments like heating.
- **Germination**, the breaking of the spore's dormant state
- This process is characterized by spore swelling, rupture or absorption of the spore coat, loss of resistance to heat and other stresses, loss of refractility, release of spore components, and increase in metabolic activity.
- Many normal metabolites or nutrients (e.g., amino acids and sugars) can trigger germination after activation.
- **outgrowth. The spore protoplast makes new components, emerges from the remains of the spore coat, and develops again into an active bacterium.**



Microbial Nutrition And Metabolism



BACTERIAL NUTRITION

Dr. Bincy Joseph
Assistant professor
PGIVER, Jaipur

- Microorganism require about 10 elements in large quantities for the synthesis of macro molecules these elements are called **macro elements**
- 95% of cell dry weight is made up of a few major elements :Carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorus, potassium, calcium, magnesium and iron
- The nutrients which are required in small amounts is called **micro nutrients/trace elements**
- Eg. Zinc, Manganese, Molybdenum



NUTRITIONAL TYPES OF MICROORGANISM

- Based on the sources of **carbon, electron and energy** micro organism are placed in different nutritional types
- A) Carbon sources
- a) **Autotrophs**: They use carbon dioxide as a sole source of carbon
- b) **Heterotrophs** : They use reduced preformed organic molecules from other organism as carbon source
- B) Energy source
- a) **Phototroph** : Light is used as source of energy



- b) **Chemotroph**: oxidation of organic / inorganic compound as a source of energy

C) Electron source

- a) **Litho trophs** : reduced inorganic molecules
- b) **Organotrophs** – organic molecules



MICROBIAL GROWTH

- Prokaryotes Reproduce by Binary fission
- Some prokaryotes reproduce by budding, fragmentation or other means



BACTERIAL GROWTH CURVE

- In growth curve, the growth of microorganism reproducing by binary fission is plotted as logarithm of number of viable cells versus the incubation time
- The growth curve has four distinct phases

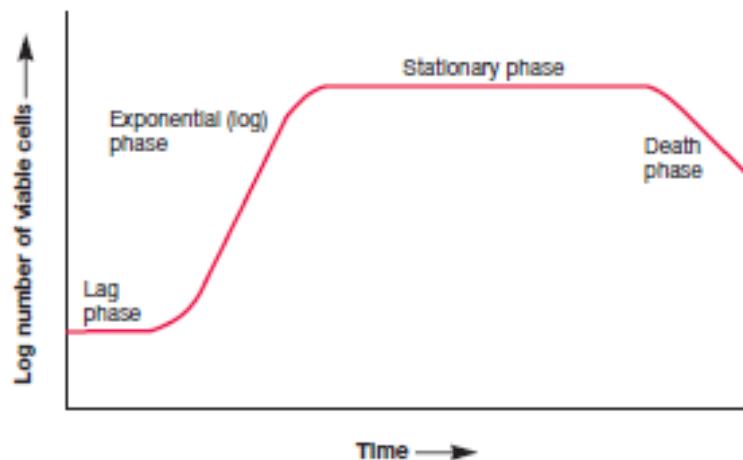


Figure 6.6 Microbial Growth Curve in a Closed System.
The four phases of the growth curve are identified on the curve and discussed in the text.

LAG PHASE

- When micro organism are introduced into fresh culture, there is no increase in cell number occurs
- So this period is called lag period
- There is no increase in the number of cells
- During this period bacteria is adapting itself to the new environment and synthesise the essential cofactors, ribosome and ATP which are required for growth



EXPONENTIAL PHASE OR LOG PHASE

- In this phase microorganism are growing and dividing exponentially
- The rate of growth is constant in exponential phase
- The population is most uniform in terms of chemical and physiological properties during this phase



STATIONARY PHASE

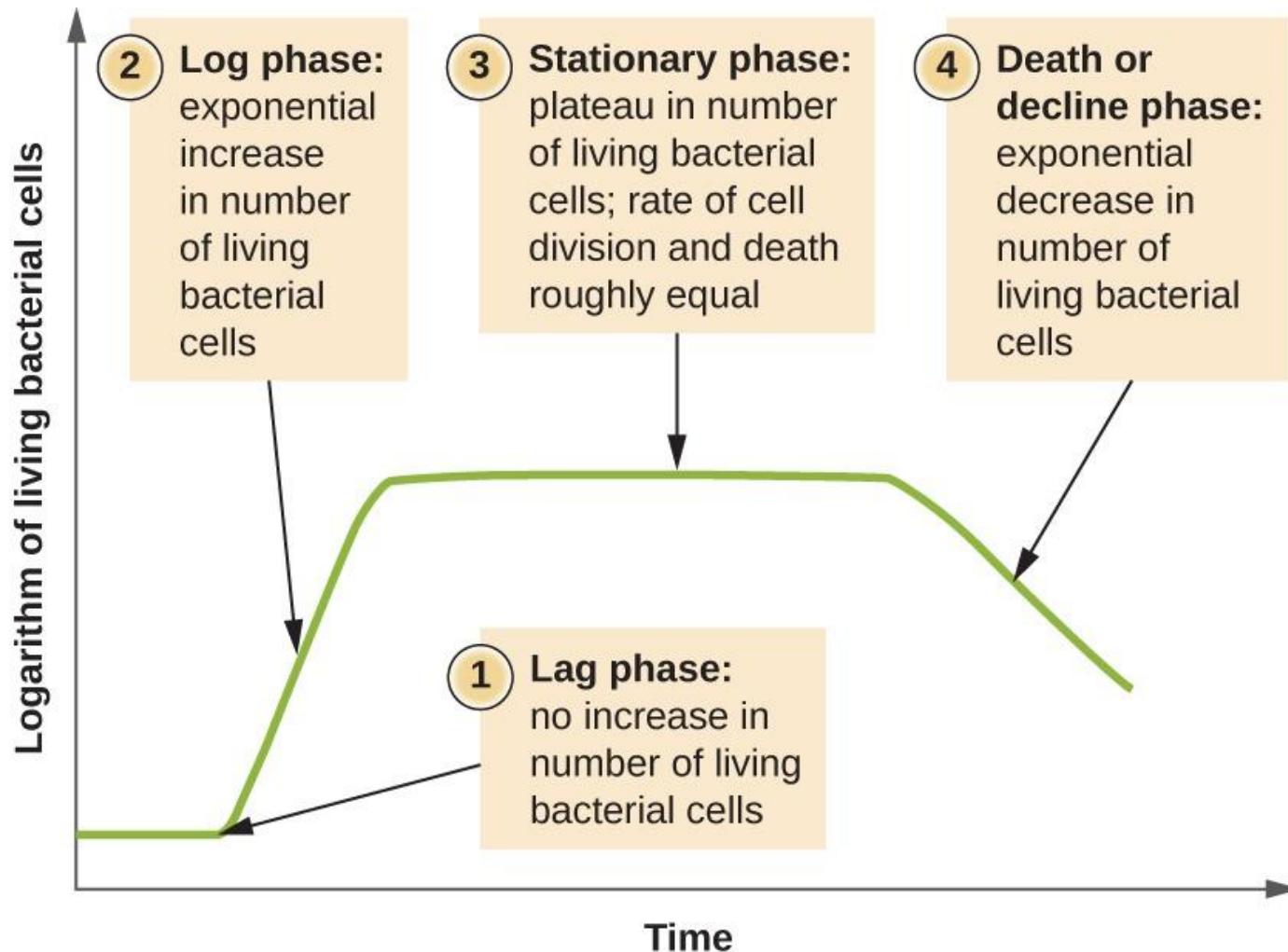
- In stationary phase total number of valuable organism remain constant
- There is a balance between cell division and cell death
- In bacteria stationary phase is achieved at a population level of around 10^9 cells per ml
- Microbial population enters into the stationary phase due to
 - Depletion of essential nutrients
 - Limited availability of oxygen for aerobic organism
 - Accumulation of toxic waste products
- Spore formulation occur in this phase



SENESCENCE AND DEATH

- Decline in viable count following stationary phase
- Nutrient depletion and accumulation of toxic waste is responsible for death phase



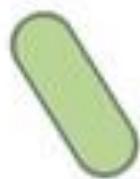


GENERATION TIME/ DOUBLING TIME

- The time required to double the number of microorganism in a population
 - Because the population is doubling in every generation
 - The increase in population always 2^n where n is the number of generations
 - So the population increase is exponential or Logarithmic
 - Let N_0 is the initial population number
 - N_t is the population at time t
-
- $N_t = N_0 \times 2^n$



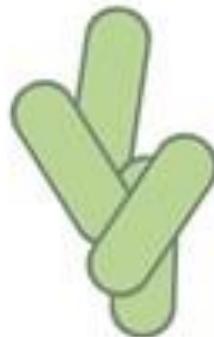
N_0



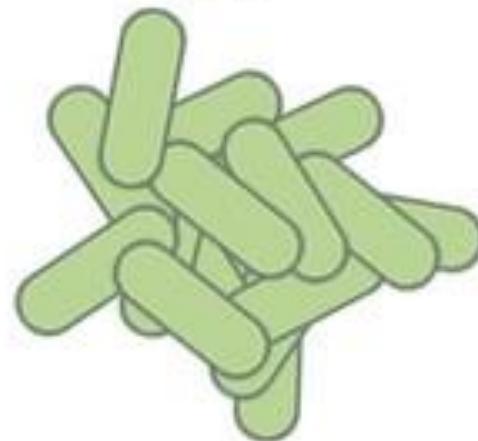
N_1



N_2



N_t



t_0



t_1



t_2



t_n



CONTINUOUS CULTURE SYSTEM

- A microbial population can be maintained in the exponential growth phase and at constant biomass concentration for long periods in continuous culture system
- Two major types of continuous culture system commonly used
 - 1) Chemostat
 - 2) Turbidostat



CHEMOSTSAT

- Sterile medium is fed into the culture vessel at the same time media containing microorganism is removed
- One essential nutrient is limiting in chemostat
- Dilution rate is constant
- Chemostat is stable and effective at lower dilutions

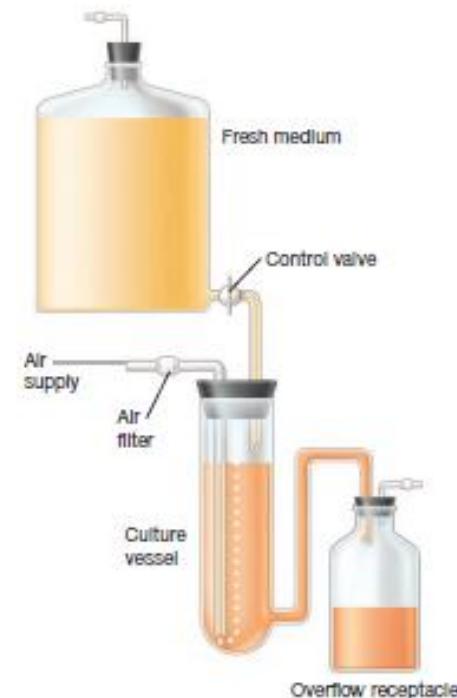


Figure 6.16 A Continuous Culture System: The Chemostat. Schematic diagram of the system. The fresh medium contains a limiting amount of an essential nutrient. Growth rate is determined by the rate of flow of medium through the culture vessel.

TURBIDOSTAT

- It has a photocell that measures the absorbance or turbidity of the culture in the growth medium
- The flow rate of media through the vessel is automatically regulated to maintain predetermined turbidity or cell density
- In turbidostat all the nutrients will be in excess
- Turbidostat operates best at high dilution rates



CLASSIFICATION OF BACTERIA BASED ON ENVIRONMENTAL FACTOR REQUIREMENT

Based on solute and water activity

- **Osmotolerant** : able to grow over a wide range of water activity or osmotic concentration

Eg: *Staphylococcus aureus*

- **Halophile**: requires high level of Sodium chloride usually above 0.2 M to grow
- Eg, *Staphylococcus aureus*
 Halobacterium



pH

- Acidophile: Growth optimum between pH 0 to 5.5
Eg: *Sulfobolus*
- Neutrophile: Growth optimum between pH 5.5 and 8.0
Eg : *Escherichia coli*
- Alkalophile: Growth optimum between pH 8.0- and 11.5



TEMPERATURE

- **Psychrophile:** Grows well at 0°C and has an optimum growth temperature of 15°C or lower
Eg: *Bacillus psychrophilus*
- **Psychrotroph:** can grow at 0-7°C has an optimum growth temperature between 20-30°C and maximum around 35°C.
Eg: *Listeria monocytogenes*
Pseudomonas flourescens
- **Mesophile :** Has optimum growth around 20-45°C
Eg: *Escherichia coli*
- **Thermophile:** Can grow at 55°C or higher. Optimum temperature of growth often between 55-65°C
- **Hyper thermophile:** has an optimum temperature between 80-113°C



PRESSURE

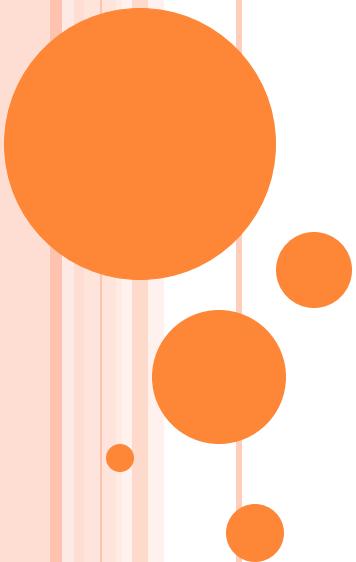
- **Barophilic:** Grow more rapid at high hydrostatic pressures



OXYGEN REQUIREMENT

- **Obligate aerobe:** Completely dependent on atmospheric O₂ for growth
 - Eg : micrococcus
 - Psuedomonas
 - Mycobacterium
- **Facultative anaerobe:** Does not require oxygen for growth, but grows better in its presence
 - Eg. *E. Coli*
- **Aerotolerant anaerobe:** grows equally well in presence or absence of O₂
 - Eg. *Streptococcus pyogenes*
- **Obligate anaerobe:** Do not tolerate O₂ and dies in its presence
 - Eg. Clostridium, Bacteriodes
- **Microaerophile:** Require O₂ levels below 2-10% for growth and is damaged by atmospheric level O₂
 - Eg. Campylobacter
 - Treponema pallidum





PATHOGENICITY AND VIRULENCE

PATHOGENICITY AND VIRULENCE

- Any organism that produce disease is called pathogen
- The ability to cause disease is called pathogenicity



- **Primary pathogen**: Any organism that cause disease in healthy host by direct interaction
- **Opportunistic pathogen**: Refers to an organism that is part of host's normal microbiota, but is able to cause disease when the host is immunocompromised or when it has gained access to other tissue sites
- **Latent stage**: there is no shedding of organism and no symptoms present in the host
- **Virulence** refers to the degree or intensity of pathogenicity

Pathogenicity vs Virulence

- Pathogenicity is the quality or state of being pathogenic, the potential ability to produce disease
 - Pathogenicity is a qualitative term, an "all-or-none" concept
-
- Virulence is the disease producing power of an organism the degree of pathogenicity within a group or species
 - Virulence is a term that quantifies pathogenicity

In two minutes



VIRULENCE FACTORS

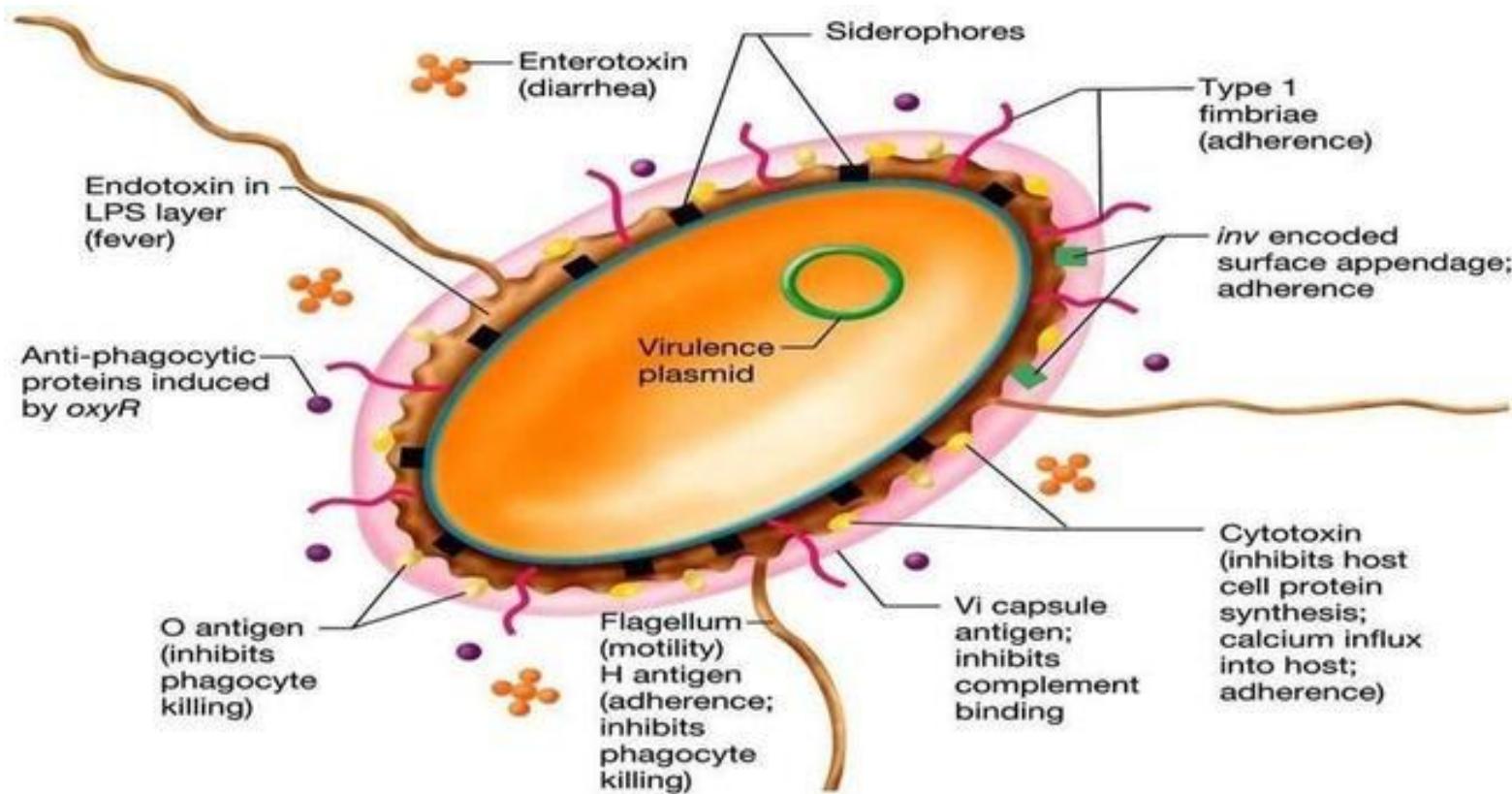
- The individual characteristic that contributes to virulence are called virulence factors
 - Eg: Capsule, Pili, and toxins
- Virulence is characterised by the characteristic of pathogen
 - a) Invasiveness
 - b) Infectivity
 - c) Pathogenic potential



- **Invasiveness** : is the ability of organism to spread to adjacent /other tissues
- **Infectivity** is the ability of organism to establish a focal point of infection
- **Pathogenic potential**: refers to the degree that the pathogen causes damage
- **Toxigenicity**: is the pathogens ability to produce toxins, chemical substance that damage the host and produce disease



VIRULENCE FACTORS OF BACTERIA



PATHOGENICITY ISLANDS

- The genes that encode major virulence factors in many bacteria are found in large segments of DNA called pathogenicity islands which carry genes responsible for virulence

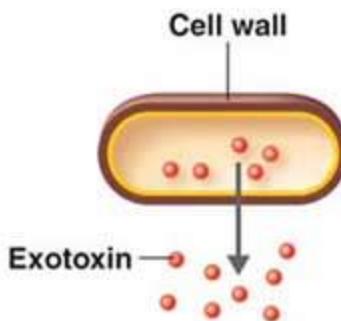
Eg: Yersinia, Salmonella

- **Intoxications:** Are disease that result from specific toxin produce by bacteria
- **Toxaemia** is a condition caused by toxins that have entered into the blood of host
- Toxins produced by bacteria is classified into two groups: **exotoxins and endotoxins**

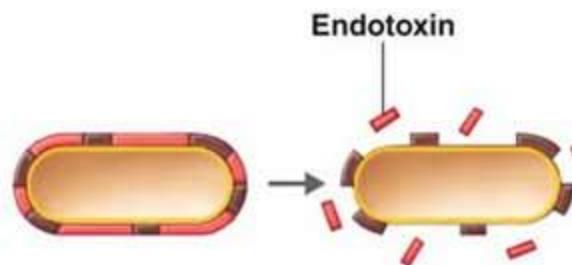


EXOTOXIN AND ENDOTOXIN

Differences Between Exotoxins and Endotoxins



(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.



(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.

EXOTOXINS

- They are soluble heat labile proteins that are released into the surroundings as the bacterial pathogen grows
- In general exotoxins are produced by Gram positive bacteria, although some Gram negative bacteria also produce exotoxins
- Exotoxins are usually encoded by plasmids and prophages
- Exotoxins are among the most lethal substances known: Botulinum toxin
- They are heat labile and can be inactivated by at 60-80°C



- Exotoxins are highly immunogenic and can stimulate production of neutralising antibodies called antitoxins
- The toxin protein can also be inactivated by formaldehyde, iodine and other chemicals to form immunogenic toxoids

Tetanus toxoid

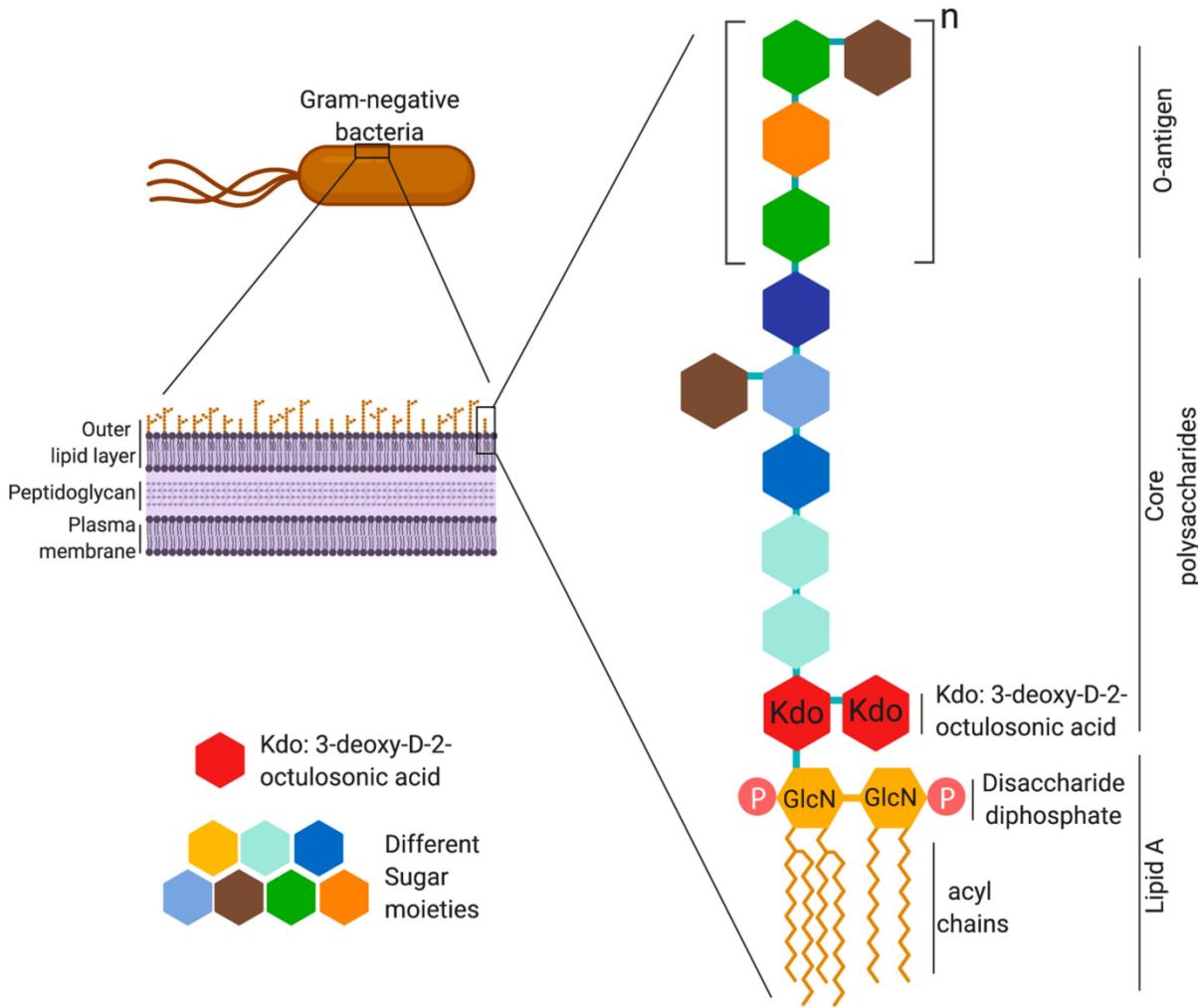
- Anthrax toxin
- Tetanus toxin
- Botulinum toxin
- Cholera toxin



ENDO TOXIN

- Endo toxin is the lipopolysacharide of Gram negative bacteria. So it is produced only by Gram negative bacteria
- It is bound to the bacterium and released only when the microorganism dies
- The toxic component of lipopolysacharide is Lipid A





GENERAL CHARACTERISTIC OF BACTERIAL ENDOTOXIN

- Heat stable
- Toxic (nanogram amounts)
- They are weakly immunogenic
- Generally similar despite the source
- Capable of producing general systemic effects like fever, shock, blood coagulation , weakness, diarrhoea, inflammation, intestinal haemorrhage, fibrinolysis
- The test used for detection of endotoxin is Limulus amoebocyte lysate test (LAL test)



DIFFERENTIATION BETWEEN ENDOTOXIN AND EXOTOXIN

Characteristic	Exotoxins	endotoxins
Chemical composition	protein	Lipopolysaccharide complex on outer membrane Lipid A portion is toxic
Disease examples	Botulism Diphtheria Tetanus	Gram negative infection
Effect on host	Highly variable between different toxins	Similar for all endotoxins
Fever	Usually do not produce fever	Produce fever by induction of IL-1 and TNF
Genetics	Frequently carried by extra chromosomal genes such as plasmids	Sythesized directly from chromosomal genes

Heat stability

CONTD..

**More heat sensitive
and inactivated at
60-80°C**

Heat stable at 250°C

Immune response

Anti toxins provide host immunity,
Highly antigenic

Weakly immunogenic and immunogenicity associated with polysaccharide

Location

Usually excreted outside the living cell

Part of outer membrane of Gram negative bacteria

Production

Produced by both Gram positive and Gram negative bacteria

Found only on Gram negative bacteria and released on bacterial death

Toxicity

Highly toxic and fatal in nanogram quantities

Less potent and less specific than exotoxin.
It causes septic shock

Toxoid production

Converted to antigenic non toxic toxoids are used for immunization

Toxoids can not be made

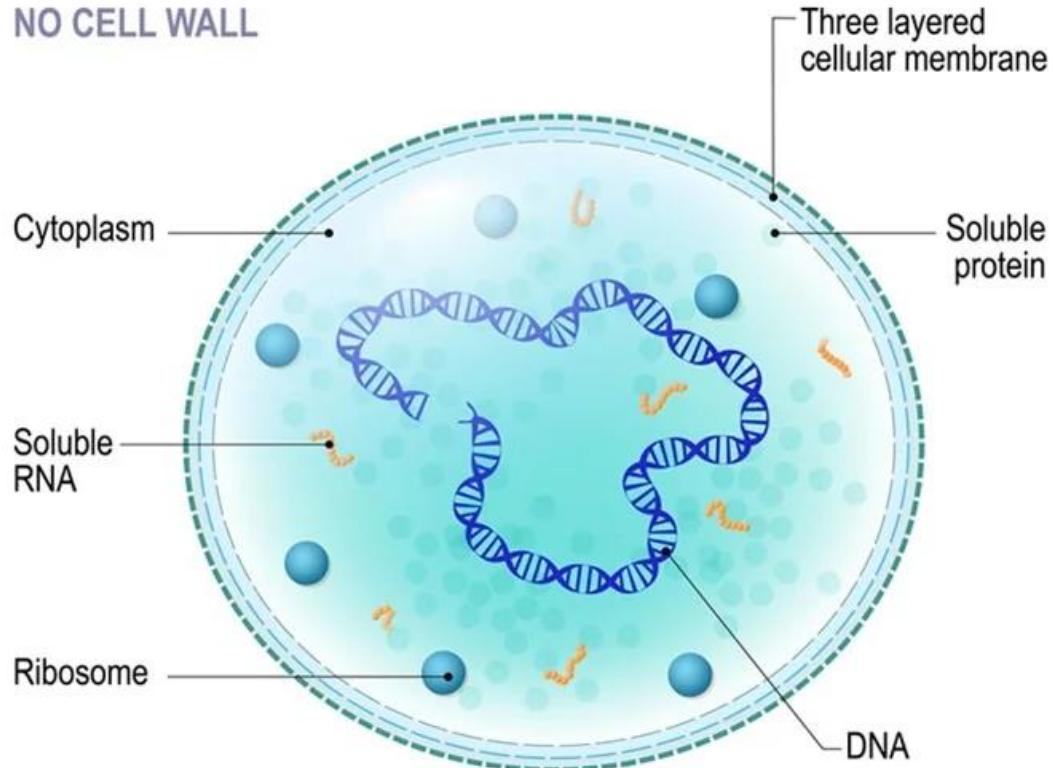


THANK YOU



Mycoplasma

NO CELL WALL

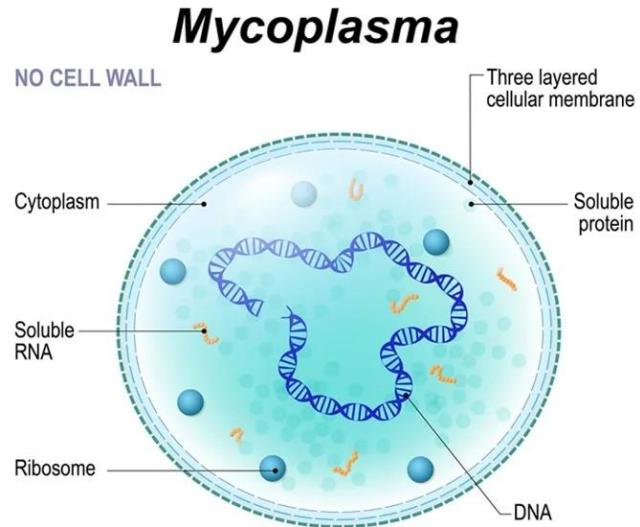


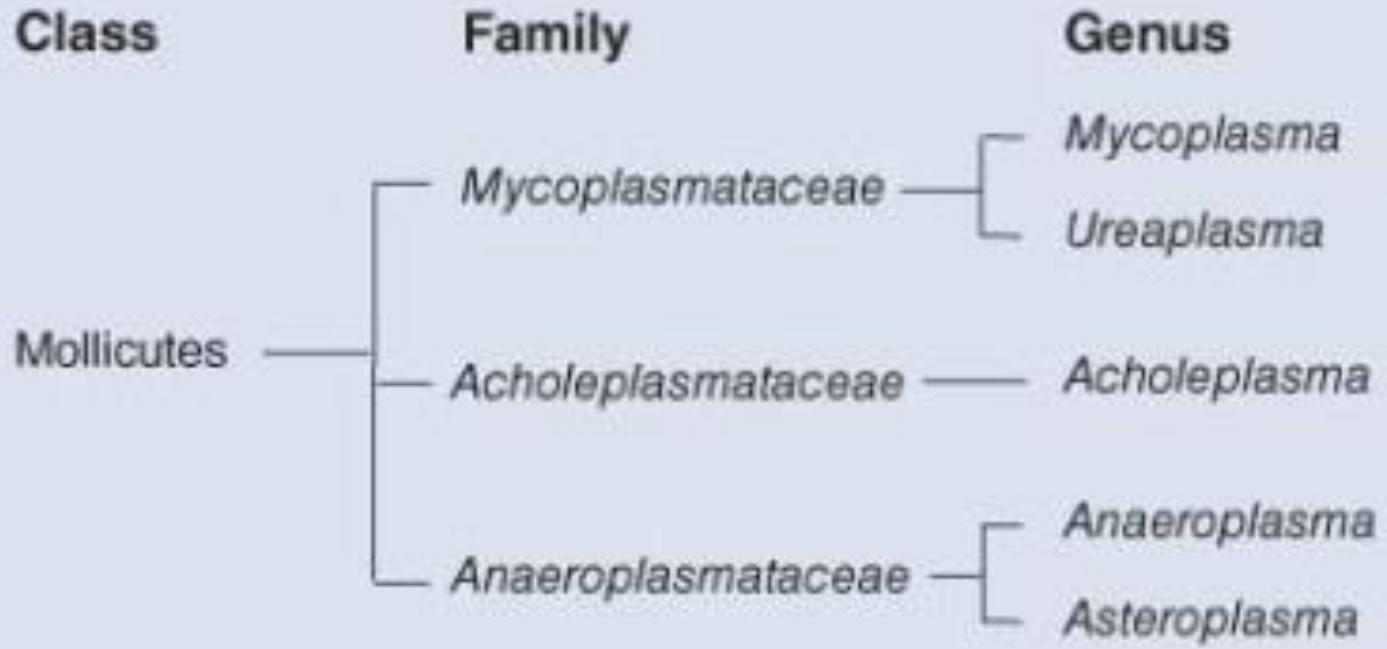
MYCOPLASMA

Dr. Bincy Joseph
Assistant professor
PGIVER, Jaipur

MYCOPLASMA

- Included in Class: Mollicutes
- This class is divided into 3 families
 - Family: Mycoplasmataceae
G. Mycoplasma
G. Ureaplasma
 - Family: Acholeplasmataceae
G: Acholeplasma
 - Family: Anaeroplasmataceae
G. Anaeroplasma
G. Asteroplasma
- The first mycoplasma identified in 1890 was *Mycoplasma mycoides* subspecies *mycoides*, the cause of contagious bovine pleuropneumonia
- Mycoplasma also known as PPLO (Pleuro pneumoniae like organisms)
- These are smallest prokaryotic cells capable of self replication and they lack cell walls





GROWTH REQUIREMENT

- *Mycoplasma* species and *Ureaplasma* species require enriched media containing animal protein, a sterol component and a source of DNA or adenine dinucleotide.
- Commercially available mycoplasma agar or broth media (often heart infusions) are supplemented with 20% horse serum and yeast extract providing amino acids and vitamins
- In addition, penicillin is used to inhibit Gram-positive bacteria, and thallous acetate is incorporated to inhibit Gram-negative bacteria and fungi
- Media are buffered at pH 7.3 to 7.8 for *Mycoplasma* species and at pH 6.0 to 6.5 for *Ureaplasma* species.
- For culturing ureaplasmas, urea is added to the medium and thallous acetate, which is toxic for these organisms, is omitted



MORPHOLOGY AND STAINING

- Morphology varies according to the species and environmental conditions and stage of growth because they lack cell wall
- The organisms are pleomorphic spherical, coccoid, coccobacillary, ring, dumb bell, long branching filamentous forms
- The organisms possess a flexible triple layered outer membrane.
- This flexibility allows them to pass through the bacterial membrane filters of pore sizes from $0.22\text{ }\mu\text{m}$ to $0.45\text{ }\mu\text{m}$
- Mycoplasmas are susceptible to desiccation, heat, detergents and disinfectants.
- However, they are resistant to antibiotics such as penicillin which interfere with the synthesis of bacterial cell walls
- Organisms divide by binary fission
- Long filamentous form breaks into round forms
- Not stained by Gram staining

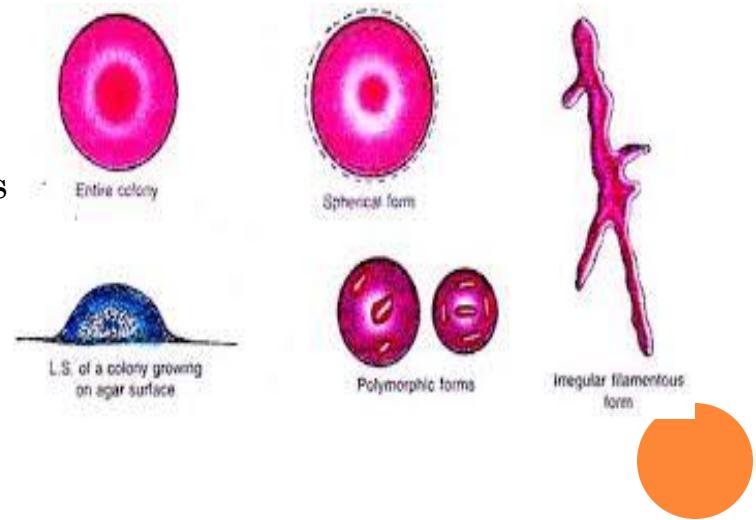
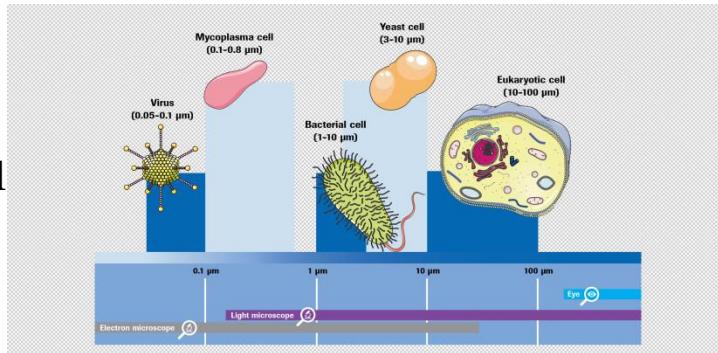
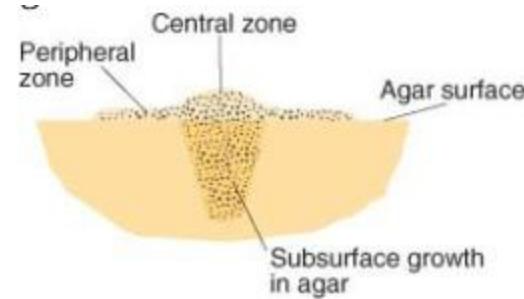
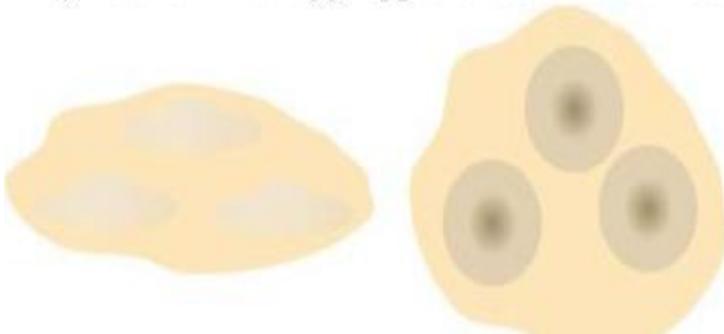
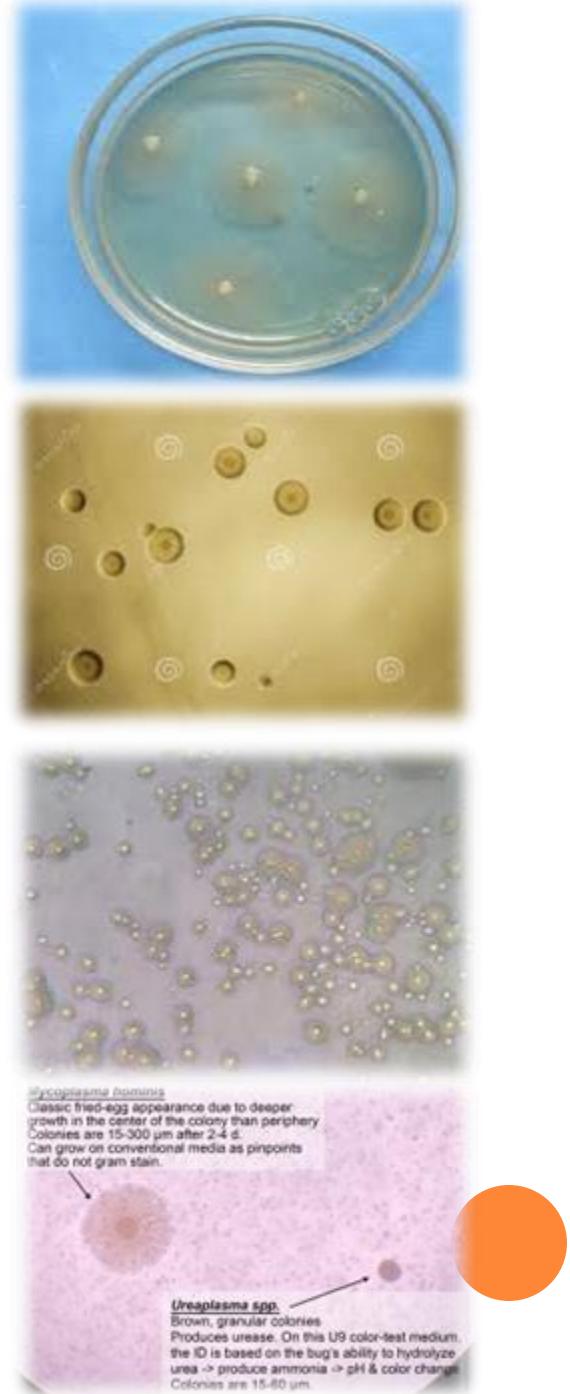


Figure 38.2 The appearance of mycoplasma microcolonies in oblique illumination (A) and in transmitted light (B). When illuminated obliquely, the microcolonies have an umbonate appearance. They have a ‘fried-egg’ appearance in transmitted light.



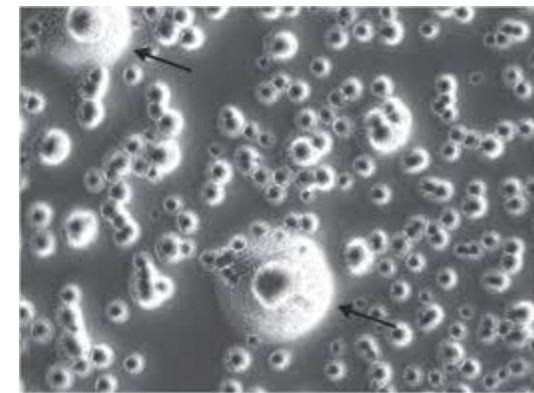
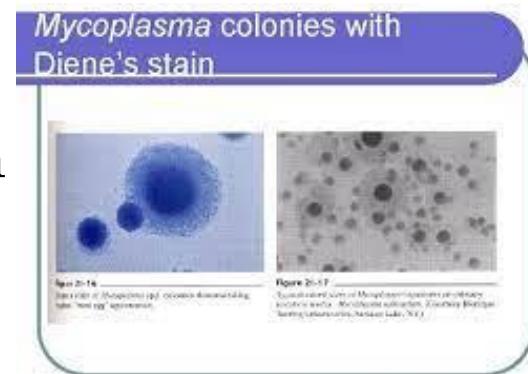
COLONY MORPHOLOGY

- Most mycoplasmas are facultative anaerobes and some grow optimally in an atmosphere of 5 to 10% CO₂.
- When examined under low power magnification colonies have 0.1-0.6 mm diameter and have a typical fried egg or bull's eye appearance.
- Some species produce colonies upto 1.5 mm diameter which can be seen without magnification
- Colonies of ureaplasma are usually 0.02-0.06 mm and they lack typical peripheral zone
- Because of tiny appearance of colony Ureaplasma are known as tiny mycoplasma or T- mycoplasma
-



DIENE'S STAINING

- Best staining method for demonstration of colonies of organism by Diene's staining method
- In this method a piece of agar containing the colony is placed on the coverslip with surface growth in contact with stain
- The centre will be stained dark blue and periphery light blue
- This staining will be retained for long time and this method is used for differentiation of Mycoplasma from L – form of bacteria
- In L form the stain will be destained within 15 minutes



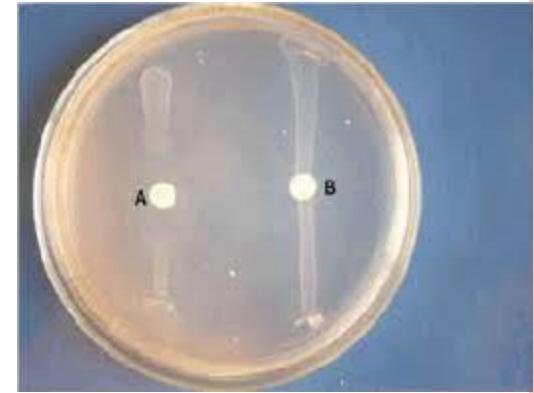
OTHER METHODS OF CULTIVATION

- Embryonated eggs through **yolk sac route**. The avian strain cause death of embryo within 2-4 days with extensive cutaneous haemorrhage and generalised oedema
- There are various cell culture employed for isolation of organism : **Hela cells and chicken heart fibroblast**



DIGITONIN SENSITIVITY TEST

- *Mycoplasma* species and *Ureaplasma* species require sterols for growth and this is reflected in their sensitivity to inhibition by digitonin
- As *Acholeplasma* species are sterol-independent, they are resistant to inhibition by digitonin.
- In the digitonin sensitivity test, a filter paper disc impregnated with digitonin is placed on medium inoculated with the isolate
- A zone of growth inhibition exceeding 5 mm around the disc indicates sensitivity to digitonin



DIFFERENTIATION BETWEEN VARIOUS GENERA

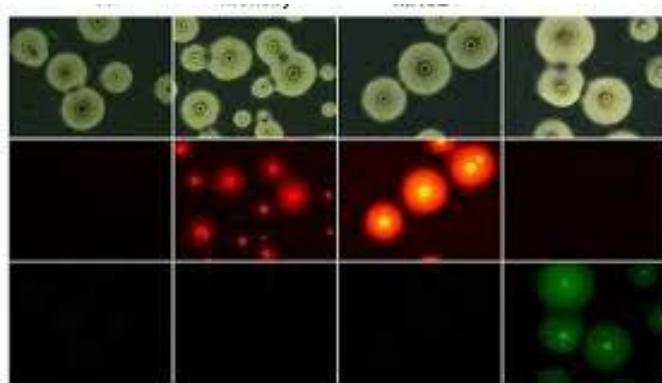
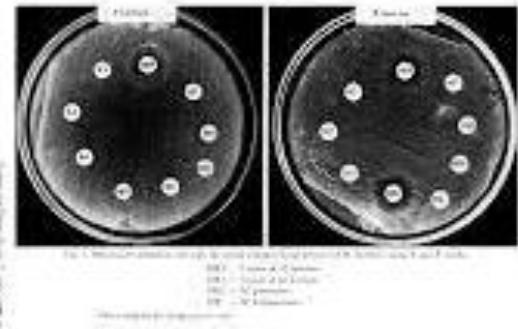
organism	Effect of digitonin	Colony size	Reqt of cholesterol	Urease production
Mycoplasma	Growth inhibition	0.1-0.6mm	Requir cholesterol	-ve
Ureaplasma	Growth inhibition	0.02-0.06 mm	Require cholesterol	+ve
Achleplasma	No Growth inhibition	Upto 1.5 mm	Don't require cholesterol	-ve

BIOCHEMICAL TESTS: USED FOR DIFFERENTIATION OF MYCOPLASMA SPECIES AFFECTING SHEEP AND GOATS

Test	<i>M. agalactiae</i>	<i>M. capricolum</i> <i>subsp.</i> <i>capricolum</i>	<i>Mycoplasma</i> <i>mycoides</i> <i>subsp.</i> <i>mycoides</i>
Glucose fermentation	-ve	+ve	+ve
Arginine hydrolysis	-ve	+ve	-ve
Phosphatase activity	+ve	+ve	-ve
Casein digestion	-ve	+ve	+ve

SPECIES IDENTIFICATION

- Certain immunological tests utilising specific antiserum are required for species identification
- **Growth inhibition test**
- Filter paper disc specific antisera are placed on agar surface and cultured with mycoplasma
- If there is inhibition of growth then +ve for that species
- **Metabolic inhibition test**
- In the presence of specific antiserum, there metabolic activity will get inhibited
- **Fluorescent antibody staining for individual colonies**



<i>Mycoplasma</i> species	Hosts	Disease conditions
<i>M. mycoides</i> subsp. <i>mycoides</i> (small colony type)	Cattle	Contagious bovine pleuropneumonia
<i>M. bovis</i>	Cattle	Mastitis, pneumonia, arthritis
<i>M. agalactiae</i>	Sheep, goats	Contagious agalactia
<i>M. capricolum</i> subsp. <i>capripneumoniae</i>	Goats	Contagious caprine pleuropneumonia
<i>M. capricolum</i> subsp. <i>capricolum</i>	Sheep, goats	Septicaemia, mastitis, polyarthritis, pneumonia
<i>M. mycoides</i> subsp. <i>capri</i> includes strains previously classified as <i>M. mycoides</i> subsp. <i>mycoides</i> (large colony type)	Goats, sheep	Septicaemia, pleuropneumonia, arthritis, mastitis
<i>M. hyopneumoniae</i>	Pigs	Enzootic pneumonia
<i>M. hyorhinis</i>	Pigs (3–10 weeks of age)	Polyserositis
<i>M. hyosynoviae</i>	Pigs (10–30 weeks of age)	Polyarthritis
<i>M. gallisepticum</i>	Chickens Turkeys	Chronic respiratory disease Infectious sinusitis
<i>M. synoviae</i>	Chickens, turkeys	Infectious synovitis
<i>M. meleagridis</i>	Turkeys	Airsacculitis, bone deformities, reduced hatchability and growth rate
<i>M. haemofelis</i>	Cats	Feline infectious anaemia

DISEASES BY MYCOPLASMA: CATTLE

- **Mycoplasma mycoides subspecies mycoides (small colony type)** is considered as most important in bovine mycolasma
- This organism causes **contagious bovine pleuropneumoniae or Brahmaputra valley disease.**
- It can be subclinical acute or fatal, characterised by rise in temperature and respiratory disturbances, cough and nasal discharge
- Animal will be reluctant to move
- In severe cases animal stand with neck extended and mouth open to facilitate breath
- Subclinically affected animal is the source of spreading and maintaining disease in a herd

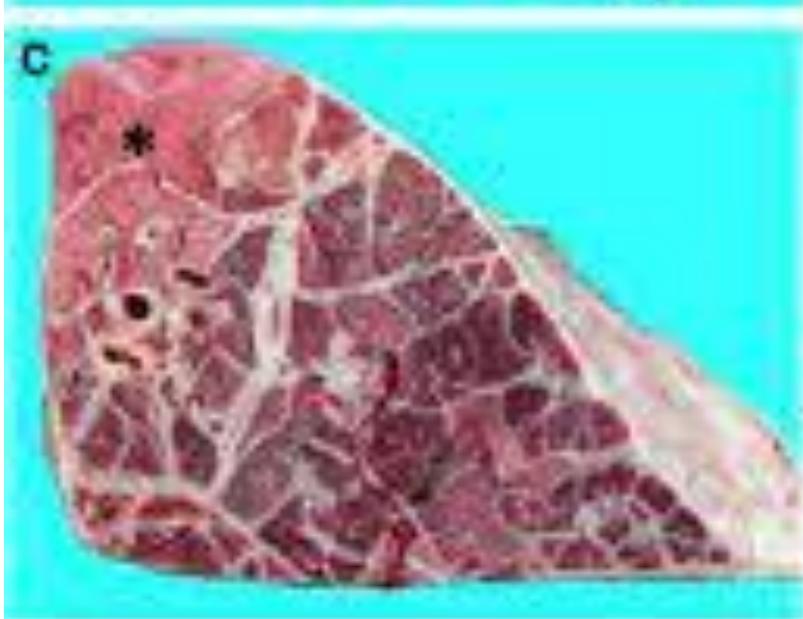


CLINICAL SIGNS AND PATHOLOGY

- Clinical signs in the acute form of CBPP include sudden onset of high fever, anorexia, depression, drop in milk yield, accelerated respiration and coughing
- Animals adopt a characteristic stance with the head and neck extended and elbows abducted
- Expiratory grunting and mucopurulent nasal discharge may be present.
- Death can occur 1 to 3 weeks after the onset of clinical signs.
- Arthritis, synovitis and endocarditis may be present in affected calves
-



- At post-mortem, the pneumonic lungs have a marbled appearance.
- Grey and red consolidated lobules alternate irregularly with pink emphysematous lobules and the interlobular septa are distended and oedematous.
- There may be abundant serofibrinous exudate in the pleural cavity
- In chronic cases,fibrous encapsulation of necrotic foci is commonly found
- These necrotic foci contain viable mycoplasmas, and breakdown of the capsules in chronically affected animals is a major factor in the persistence and spread of CBPP in endemic areas.



MYCOPLASMA BOVIS

- Most important cause of Mycoplasma mastitis in cattle
- There will be decrease in milk production and milk will become thick, intermixed with watery secretion and may progress to a purulent exudate
- *M.bovis* and *M.dispar* upper respiratory infection in calf along with other respiratory pathogen
- *M. bovigenitalium* : causes urogenital infection in cattle



DISEASES CAUSED BY MYCOPLASMA : GOAT

- *Mycoplasma mycoides* subsp. *mycoides* causes mastitis, arthritis, pneumoniae in goat and septicaemia in kids
- *Mycoplasma mycoides* subsp. *capri* causes pleuropneumoniae in goat
- *Mycoplasma capricolum* subsp. *capripneumoniae* causes **contagious caprine pleuropneumoniae (CCPP)**
- The disease is characterized by pneumonia, fibrinous pleurisy, profuse pleural exudate and a marbled appearance on the cut surface of affected lungs. Although similar in many respects to contagious bovine pleuropneumonia (CBPP), well developed necrotic areas in the lungs in chronic CCPP are rare
- *Mycoplasma agalactiae* causes contagious agalactiae in sheep and goat



DISEASES BY MYCOPLASMA : PIG

- *Mycoplasma hyorhinis* causes atropic rhinitis along with *Pasteurella multocida* and *Bordetella bronchiseptica*
- *Mycoplasma hyopneumoniae* causes enzootic pneumoniae in pig
- *Mycoplasma hyosynoviae* arthritis in pig



- *Mycoplasma ovipneumoniae*: pneumoniae in sheep
- *Mycoplasma conjunctivae*: keratoconjunctivitis
- In birds *Mycoplasma gallisepticum* cause **chronic respiratory disease (CRD)** in chicken and infectious sinusitis in turkey
- *Mycoplasma synoviae*: Infectious synovitis in chicken
- *Mycoplasma meleagridis*: air sacculitis in turkey
- *Mycoplasma pneumoniae* also known as Eaton's agent

DIAGNOSIS

- Clinical material are mucoid scrappings, tracheal exudate, pneumonic lung, mastitic milk, fluids from joints and other body fluids and swabs from lesions or suspected material to be transported in mycoplasma transport medium
- Diagnosis
- Presence of mycoplasma antigen can be demonstrated immunologically and nucleic acid detection (FAT, IPT and PCR of Nucleic acid)
- Material are inoculated on Mycoplasma medium and incubated aerobically or increased carbon dioxide in humid atmosphere at 37°C for 2 weeks
- Fluid sample can be inoculated into PPLO agar/ broth
- Colonies are identified by fried egg appearance, size, digitonin sensitivity and biochemical test



- FAT on microcolonies and growth and metabolic inhibition using specific antiserum
- Serological test
- CFT, ELISA, Rapid plate agglutintion test for poultry and CCPP
- HI for avian mycoplasmosis



TREATMENT AND CONTROL

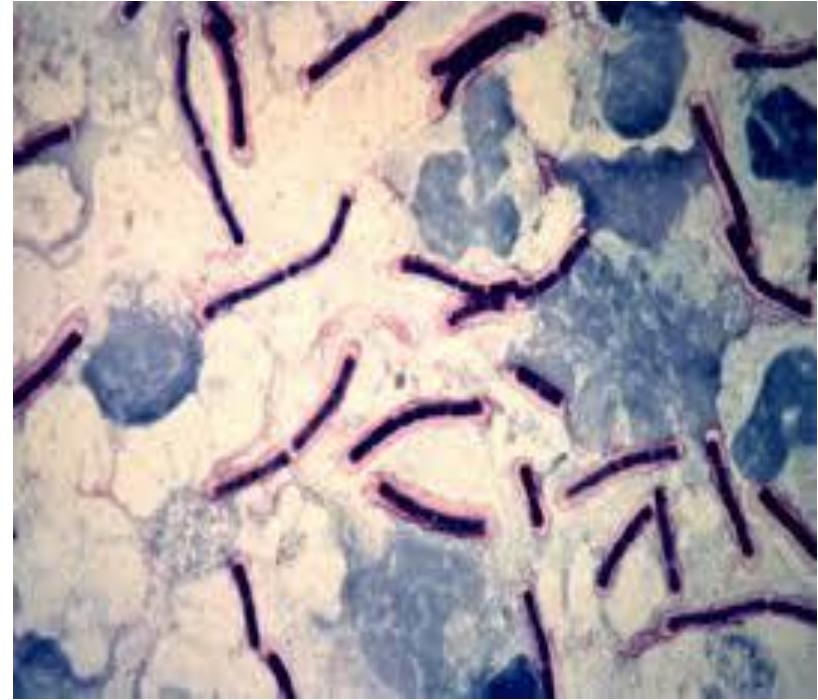
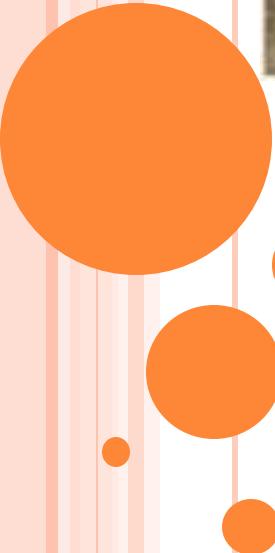
- Most of this drugs are unsatisfactory in chronic condition
- In countries where disease is exotic, slaughter of affected and incontact animals
- In endemic areas control is based on prohibitory movement of suspected animals
- Mandatory quarantine and elimination of carrier animal by serological test can be detected
- Autogenic vaccine from affected tissues also practised
- Attenuated vaccine are there and annual vaccination practised



AVIAN MYCOPLASMA (MYCOPLASMA GALLISEPTICUM)

- Antimicrobial medication with tetracycline or tylosin
- Establishment of mycoplasma free flock
- The eggs used for hatching should be dipped in tylosin
- Modified live vaccine available



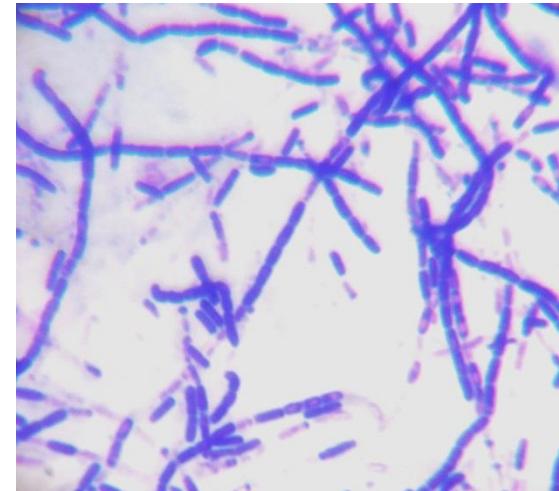


BACILLUS

Dr. Bincy Joseph
Assistant professor
PGIVER, Jaipur

BACILLUS

- Kingdom -Prokaryotes
- Division -Firmicutes
- Class -Firmibacteria
- Family -Bacillaceae
- Genus -Bacillus
- Species - *B. anthracis*



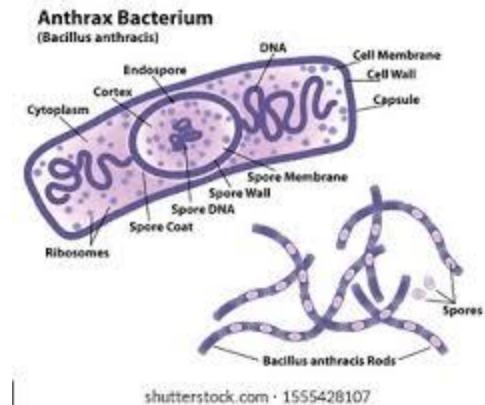
HISTORY:

- Koch (1863) – first bacillus to be isolated in pure culture : *Bacillus anthracis*
- Pasteur (1881) – used for the preparation of attenuated vaccine.
- Wade (1980) – *B. anthracis* has received much consideration as a potential agent for use in biological warfare

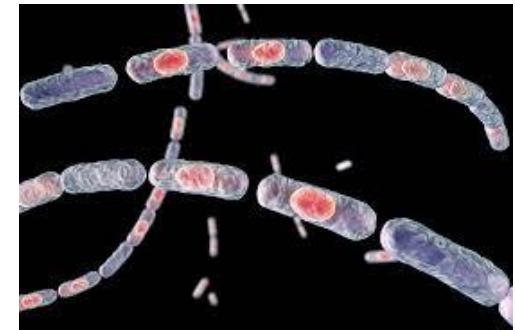


INTRODUCTION

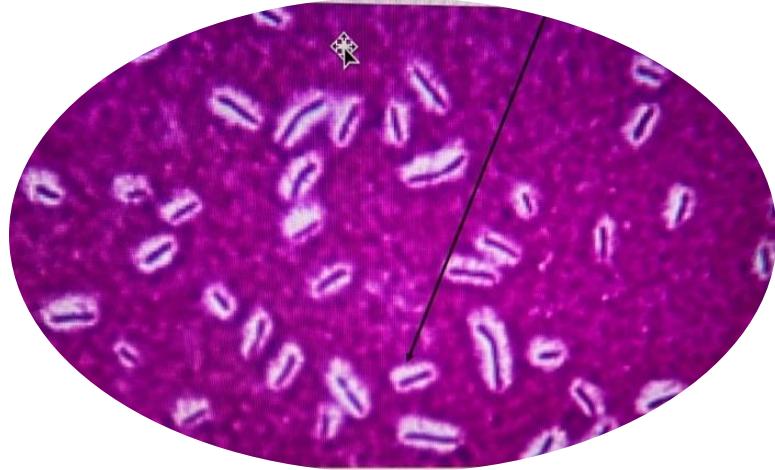
- Gram positive, Medium-to-large rods
- Arranged singly or in short chains
- Endospore forming - oval, centrally located endospores
- Aerobic (facultative anaerobic), Capsulated
- Catalase positive, Oxidase Negative
- Fermentative organisms
- Motile by peritrichous flagella except *Bacillus anthracis*.



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- Major pathogen is *B. anthracis* (*B. cereus* rarely produce disease in animals).
- Most species are saprophytes with no pathogenic potential.
- Some produce antibiotics (bacitracin, polymyxin & other polypeptides)



NATURAL HABITAT

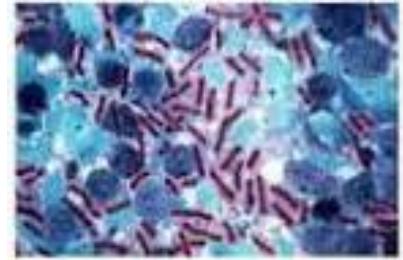
- Nearly worldwide distribution
- Ubiquitous in nature and are found in air, soil, dust, water
- *B. anthracis* natural reservoir is soil
- Anthrax zones – Soil rich in organic matter (pH< 6.0)
- Major naturally-occurring anthrax areas are tropical, subtropical
- *B. cereus* is found in dust, soil and spices
- They produce highly resistant endospores.
- In soil, endospores of *B. anthracis* can survive for more than 50 years.



MORPHOLOGICAL CHARACTERISTICS

- *B. anthracis* is rod shaped organism with truncated ends - presents a bamboo stick appearance.
- Anthracoid organisms are rod shaped with rounded ends
- Gram's stain: gram positive
- Arrangement: Forms long filaments in culture media, but filaments are never seen in tissues they are in pairs or short chains
- Spore formation: Forms oval, centrally located endospores
- Motility: Motile by peritrichous flagella except *B. anthracis*.





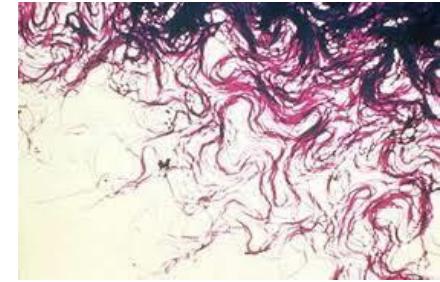
Bacillus anthracis – capsule

- Capsule is polypeptide in nature, being composed of a polymer of d-glutamic acid.
- When blood films are stained with polychrome methylene blue , an amorphous purplish material is noticed around the bacilli
- Represents the capsular material and is characteristic of the anthrax bacilli - called as **McFaydean reaction**.

Bacillus anthracis – Endospore

- Sporulation requires
 - Presence of oxygen
 - Poor nutrient conditions
- 1 spore present per cell
- Highly resistant to heat, cold, chemical disinfectants, dry periods
- Protoplast carries the material for future vegetative cell
- Cortex provides heat and radiation resistance
- Spore coat- made up of keratin, 50% of volume of bacterial spore, provides protection from chemicals & enzymes
- Sporulation occurs readily outside the body in the presence of oxygen.
- Because of lack of air or sufficient oxygen, spores are not formed in the blood and internal organs
- Sporulation takes place at an opt. temp. of 25-30° C and in atmosphere containing low partial pressure of oxygen.
- Sporulation is inhibited by anaerobic conditions and by CaCl₂.

CULTURAL CHARACTERISTICS



- Aerobic and facultative anaerobic.
- Grow well in ordinary laboratory medium and enrichment with blood or serum enhances the growth.
- On agar plates *B. anthracis* produce irregular, round, raised, dull, opaque, greyish white, 2-3mm in diameter frosted glass appearance colonies
- Under the low power microscope, the slightly serrated edge of the colony is composed of long, interlacing chains of bacilli, resembling locks of matted hair.
- Referred as medusa head or judges wig or women's curling hair type of growth.

CULTURAL CHARACTERISTICS

- Selective medium for isolation is the **PLET** medium (Knisely 1966) consisting of polymyxin, lysozyme, EDTA and thallous acetate added to heart infusion agar.
- It is useful for isolate *B. anthracis* from mixtures containing other spore-bearing bacilli
- Virulent capsulated strains form rough colonies, while avirulent attenuated strains form smooth colonies
- In gelatin stab - Inverted fir tree appearance
- On blood agar *B. anthracis* produces slight haemolysis compared with anthracoid organisms.
- *B. cereus* produce a wide zone of complete haemolysis around the colonies.
- Laboratory animals of choice for cultivation of the organisms are guinea pigs and mice

CULTURE

GELATIN STAB

- CHARACTERISTIC INVERTERED FIR TREE APPEARANCE





PLET medium (Knisely 1966) consisting of polymyxin, lysozyme, EDTA and thallous acetate added to heart infusion agar.



RESISTANCE

- Growing forms of the anthrax bacillus are only slightly resistant.
- Killed by ordinary disinfectants, by pasteurization.
- Under aerobic conditions spores are readily formed.
- If the carcass is opened, the tissues are exposed to air, and then the organisms are able to sporulate.
- Spores resist dying for long periods of time.
- Spores surviving for 60 or more years have been recorded.
- Heat fixation of smears does not kill spores
- Resist dry heat at 140°C for 2-3 hrs and boiling for 10 mts.
- Killed at 120°C for 10 min and 4% KMnO_4 treatment for 15 mts



VIRULENCE FACTORS

○ Poly D glutamic acid capsule

Plasmid mediated. Protects against phagocytosis, lytic antibodies, and complement activity.

Have two plasmids PX 01 and PX 02

- It secretes **two toxins** which are composed of three proteins component
 - The lethal toxin (PA+LF)
 - The edema toxin (PA+EF)
- a) Oedema factor (EF)
- b) Protective antigen (PA)
- c) Lethal factor (LF)
- Work in combination and have little or no toxic action as single entities
- Although protective antigen induces antibodies which confer partial immunity.
- Protective antigen acts as the binding moiety for both oedema factor and lethal factor.

- Anthrax toxin is an A/B toxin.
- Each individual anthrax toxin protein is nontoxic.
- Toxic symptoms are not observed when these proteins are injected individually into laboratory animals.
- The co-injection of PA and EF causes edema, and the co-injection of PA and LF is lethal.
- Anthrax toxin is *A / B* paradigm. The *A* component is enzymatically active, and the *B* component is the cell binding component.
- Anthrax toxin is of the form A₂B, where the two enzymes, EF and LF, are the A components and PA is the B component.



ENZYME FUNCTION OF LF AND EF

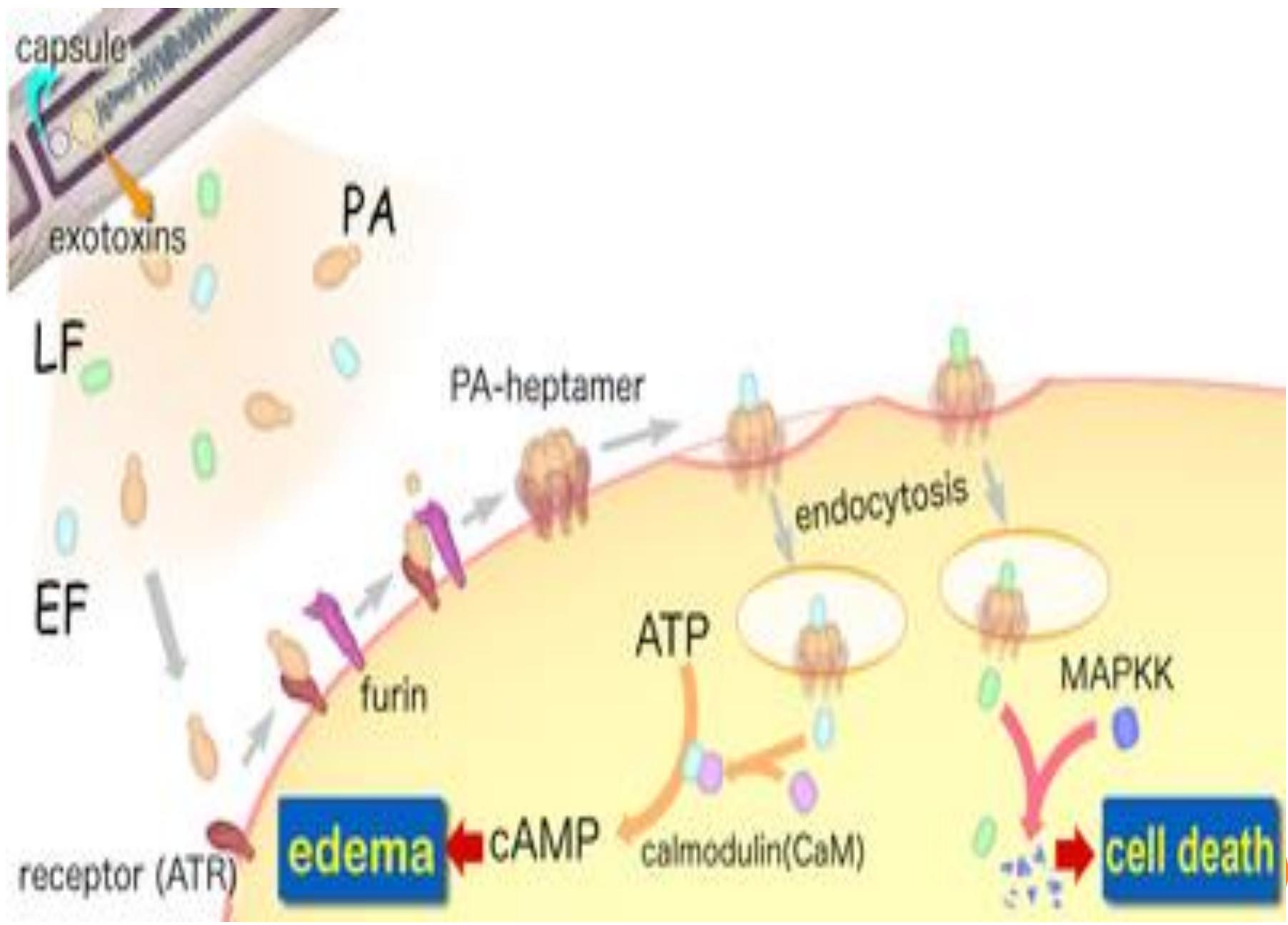
- Once in the cytosol, the EF and LF then carry out their respective damage-inducing processes
- EF acts as a Ca^{2+} and calmodulin dependent adenylate cyclase that greatly increases the level of cAMP in the cell.
- This increase in cAMP upsets water homeostasis, severely imbalances the intracellular signalling pathways, and impairs macrophage function, allowing the bacteria to further evade the immune system.



ENZYME FUNCTION OF LF AND EF

- LF also helps the bacteria evade the immune system through killing macrophages.
- Once in these cells, LF acts as a Zn²⁺-dependent endoprotease that snips off the N-terminus of mitogen-activated protein kinase kinases (MAPKK).
- This inhibits these kinases by not allowing them to efficiently bind to their substrates, which leads to altered signaling pathways and ultimately to apoptosis.





VIRULENCE FACTORS

- The resultant upset in water homeostasis causes the fluid accumulation seen in clinical disease.
- Neutrophils are the principal target of oedema factor which severely inhibits their function.
- Lethal toxin consists of lethal factor, a zinc metallo-protease and protective antigen which acts as the binding domain as for oedema factor.
- In naturally-occurring disease, local effects of the complex toxin include swelling and darkening of tissues due to oedema and necrosis.
- When septicaemia occurs, increased vascular permeability and extensive haemorrhage lead to shock and death



- Kills phagocytes & Inhibits complement.
- Capillary thrombosis occurs
- Increases capillary permeability
- Blood pressure falls
- Increase in WBC, Damages clotting mechanism
- Decreased oxygen consumption by tissues
- Suppresses CNS- myocardial hypoxia and death.
- Net effect of the toxic complex on the animal is to produce hemorrhage, edema, shock, and death.

DIFFERENTIATION OF BACILLUS SPECIES

- The ability to grow aerobically and to produce catalase distinguishes *Bacillus* species from the Clostridia which are also Gram-positive, endospore-forming rods.
- Colonial characteristics of *Bacillus* species
- *B. anthracis* colonies are up to 5 mm in diameter, flat, dry, greyish and with a 'ground glass' appearance after incubation for 48 hours.
- At low magnification, curled outgrowths from the edge of the colony impart a characteristic, 'Medusa head' appearance. Rarely, isolates are weakly haemolytic.



- *Bacillus cereus* colonies are similar to those of *B. anthracis* but are slightly larger with a greenish tinge.
- The majority of strains produce a wide zone of complete haemolysis around the colonies. Because they have some similar characteristics, *B. anthracis* and *B. cereus* require careful differentiation.
- *Bacillus licheniformis* colonies are dull, rough, wrinkled and strongly adherent to the agar.
- Characteristic hair-like outgrowths are produced from streaks of the organisms on agar media.
- Colonies become brown with age. The name of this species derives from the similarity of its colonies to lichen.
- The name *Clostridium piliforme* has been proposed for *Bacillus piliformis*, the agent of Tyzzer's disease

Table 15.1 Differentiating features of *Bacillus anthracis* and *B. cereus*.

Feature	<i>B. anthracis</i>	<i>B. cereus</i>
Motility	Non-motile	Motile
Appearance on sheep blood agar	Non-haemolytic	Haemolytic
Susceptibility to penicillin (10 unit disc)	Susceptible	Resistant
Lecithinase activity on egg yolk agar	Weak and slow	Strong and rapid
Effect of gamma phage	Lysis	Lysis rare
Pathogenicity for animals (application to scarified area at tail base of mouse)	Death in 24 to 48 hours	No effect

Difference between *B. anthracis* & Anthracoid organisms

<i>B. anthracis</i>	Anthracoid organisms
Non motile	Generally motile
Capsulated	Non capsulated
Grows in long chains	Grows in short chains
No turbidity in broth	Turbidity in broth
Inverted fir tree in gelatin	Atypical or absent
Methylene blue reduced weakly	Reduced strongly
Haemolysis weak / Absent	Strong
Liquefaction of gelatin is slow	Rapid
Lecithinase reaction is weak	Rapid
Ferments salicin slowly	Rapid
Pathogenic to G.pigs & mice	Non pathogenic
Susceptible to gamma phage	Not susceptible

Table 44. Main diseases and hosts of the *Bacillus* species.

Bacillus species	Host(s)	Disease
<i>B. anthracis</i>	Cattle and sheep	Septicaemic form of anthrax. Usually sudden death
	Pigs	Subacute anthrax with oedematous swelling in pharyngeal tissues and regional lymphadenitis or intestinal form with a higher mortality
	Horses	Oral route: septicaemia with colic and enteritis. Wound infections: localised oedema and lymphadenitis
	Carnivores (including mink)	Comparatively resistant. Disease pattern similar to that in pigs. A massive dose from eating anthrax-infected carcasses can lead to septicaemia
	Humans	Skin form: 'malignant pustule'. Pulmonary ('wool-sorters' disease') and intestinal forms are often fatal
<i>B. cereus</i>	Humans	Food poisoning
	Cattle	Rare cases of mastitis
<i>B. licheniformis</i>	Cattle and sheep	Reported as a cause of abortion
' <i>B. piliformis</i> ' (taxonomy uncertain)	Laboratory mice, foals and other animals	Tyzzer's disease. An acute fatal infection causing hepatitis, enteritis and colitis

ANTHRAX – DISEASE

- Synonyms: Malignant Pustule, Wool sorters' Disease, Hide porter's disease, Spleenic Fever
- Anthrax is an acute febrile disease of mammals caused by *B. anthracis* and characterized by an enlarged, black, soft spleen, edema and hemorrhage of subcutaneous and sub serous tissues
- *Bacillus anthracis* is pathogenic for cattle, sheep (except Algerian), mules, horses etc.
- Characterized by sudden onset and a rapidly fatal course.
- Some animals in a herd are found dead without having previously shown any evidence of a disease.



CATTLE, SHEEP AND GOAT

- **Per acute form:** may sometimes occur in herbivores; may terminate fatally in 1-2 hours, sick animals are rarely seen.
- Rapidly-developing cerebral anoxia, pulmonary edema
- **Acute form:** is fatal in less than 24 hours, the first sign is a rise in temp. to about 104-108°F.
- Excitement stage is followed by depression, respiratory distress, trembling staggers, convulsions, and death.
- Rumen stasis and a great reduction in the amount of milk produced.
- One of the most important features in fatal cases is the bloody discharge from the natural openings, particularly the anus.
- Organism found in the excretion or the blood in fairly large numbers at the time of death.

SWINE AND HORSES

- Anthrax usually assumes a localized form in swine and horses. These animals are infected only by eating heavily contaminated feed, either the raw meat of animals which have died of anthrax, or in the case of swine, infected bone or meat meal given as a feed supplement.
- Horses: course acute-to-subacute (survive ≤ 96 h)
- Symptoms: colic, edematous swellings of the throat, neck, shoulders
- Transmission: insect bite: similar to human cutaneous anthrax
- Local subcutaneous edema affects throat, ventral thorax, abdomen



HUMANS:

("Woolsorters' disease“)- fatal, cutaneous – carbuncle & intestinal meningitis.

Inhalation Anthrax (Wool sorter's Disease)

- Dust particles contaminated with spores are inhaled,
- deposit in terminal alveoli
- Spores engulfed by macrophages, transported to regional LN
- Germinate, vegetative cells produce toxin
- Extensive necrotic hemorrhage, Multiple organs
- Involved, rapid death frequently results



HUMANS:

Gastrointestinal Anthrax

- Results from ingestion of contaminated meat
- Organisms or spores penetrate oropharynx / intestinal mucosa
- Deposited in sub mucosal tissue, multiply and produce toxin
- Usually extends to regional Lymph node, systemic symptoms develop

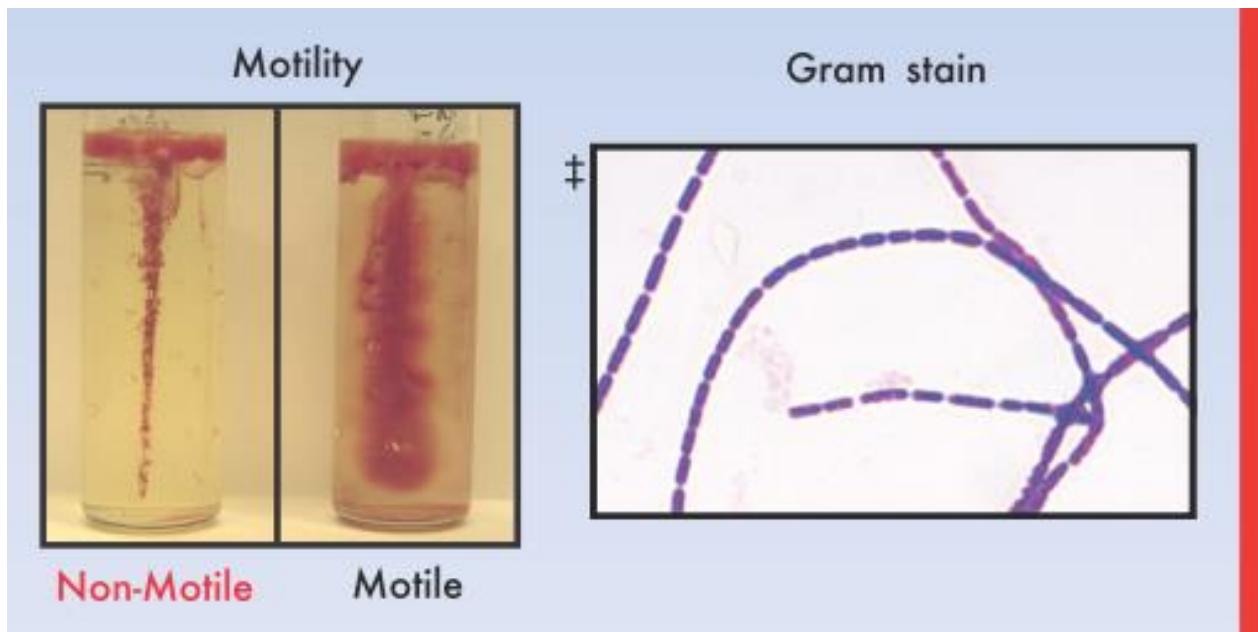
Cutaneous Anthrax

- Malignant carbuncle folliculitis



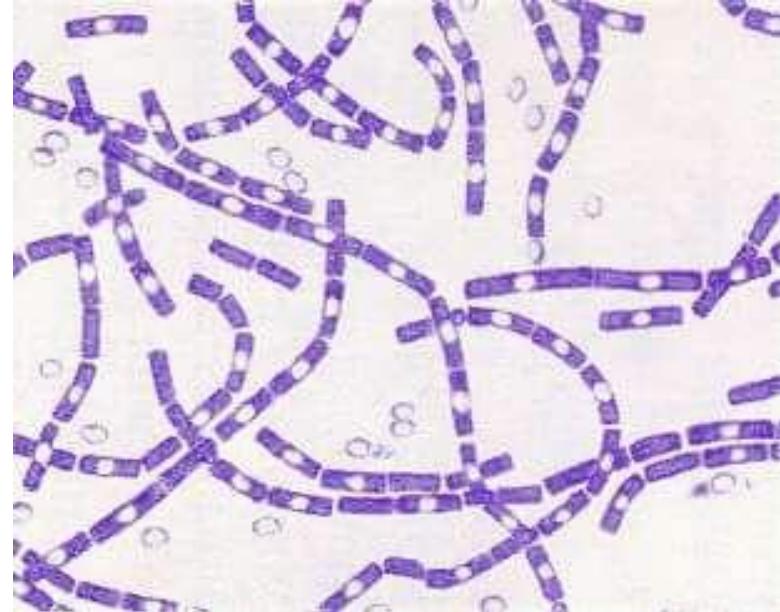
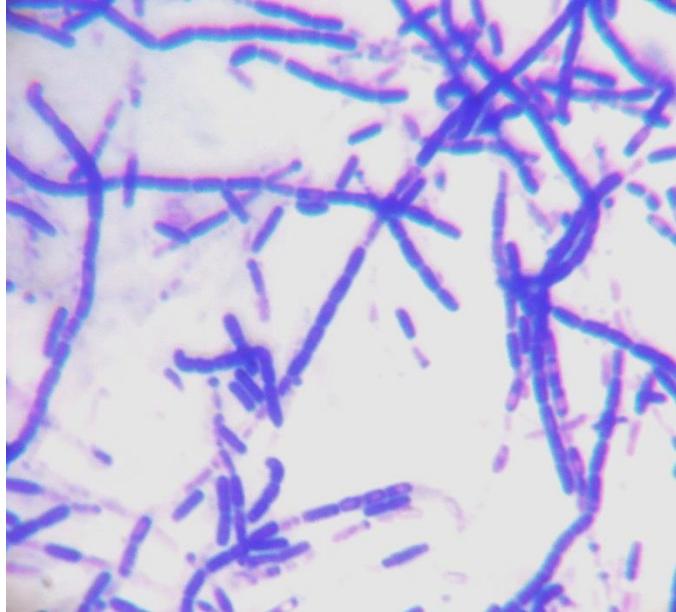
DIAGNOSIS

- Clinical symptoms
- Microscopical examination of peripheral Blood films from dead animals
- Cultural examination
- Bacteriological examination of hair, wool, hide, bone, bone meal & others



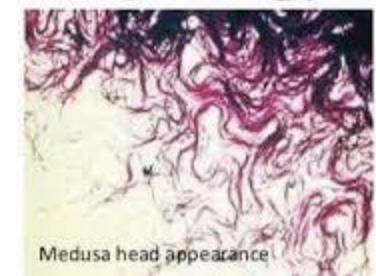
MICROSCOPY

- Gram staining: Anthrax is an aerobic, Gram positive, rod-shaped bacillus occurring in chains with a propensity to form spores under unfavourable conditions
- in high power microscopy it gives boxcar appearance. (Also called bamboo-stick appearance)





Characteristic, 'Medusa head' appearance on Blood Agar



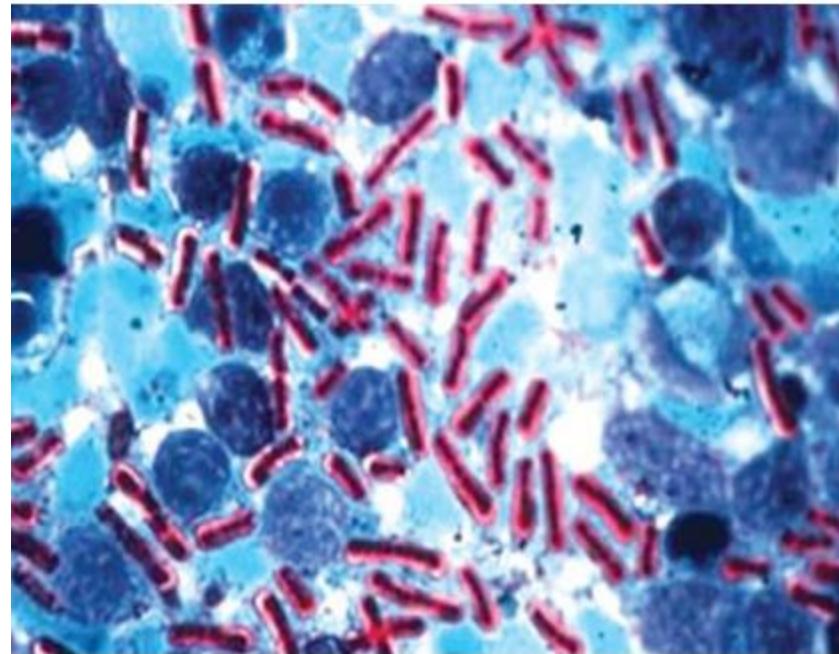
Medusa head appearance



‘Ground glass’ appearance colony

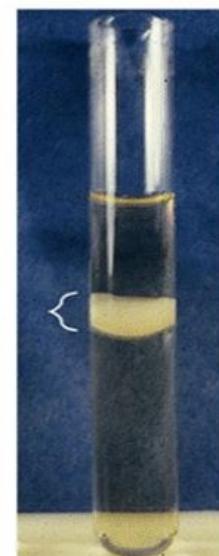
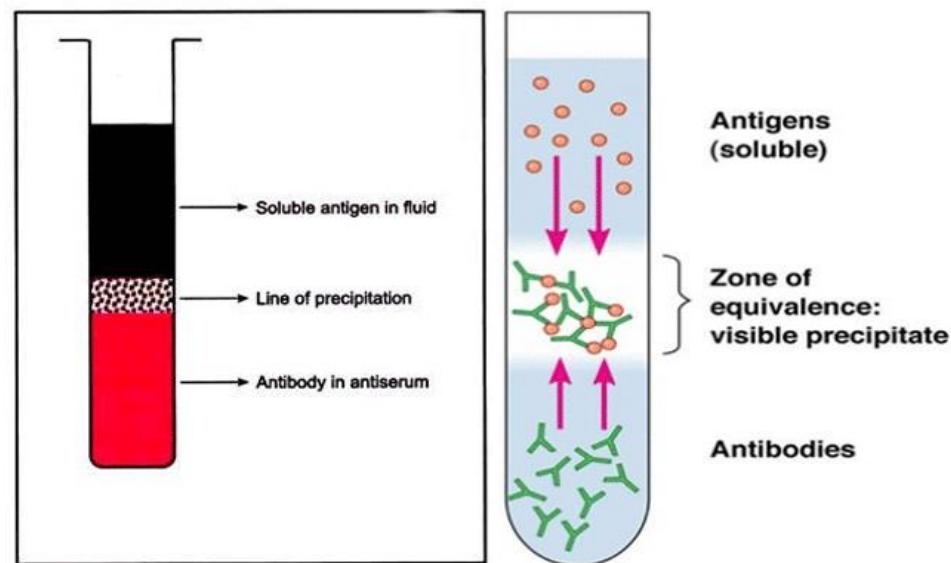


- **Mc'Fadyean reaction:** Giemsa or Polychrome methylene blue stains are used to demonstrate the capsule which is of diagnostic importance. The capsular material is more abundant if the blood smear has been taken from a recently dead animal.
- Polychrome methylene blue-stained smears reveal square-ended, blue rods in short chains surrounded by pink capsular material and is characteristic for *B. anthracis*



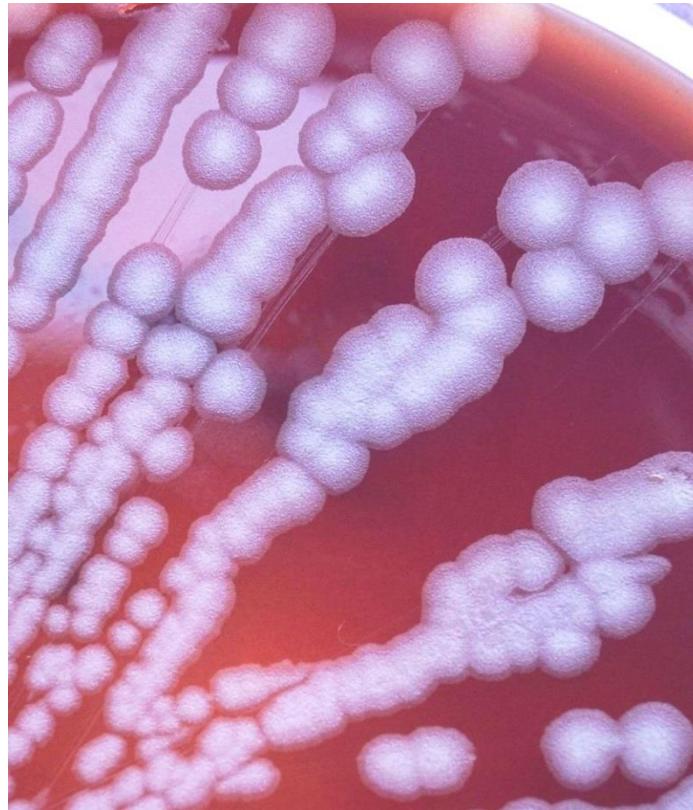
ASCOLI'S PRECIPITATION TEST

- This thermoprecipitation test is used if viable *B. anthracis* can no longer be demonstrated in tissues.
- Boil a piece of tissue (ear piece) in 5 ml of acidified saline (with 1/1000 acetic acid) for 5 minutes.
- Filter the fluid and this will serve as the source of antigen.
- Take 0.5 ml of anthrax antiserum in a narrow tube and add 0.5 ml of the filtrate.
- Development of a distinct ring of precipitate at the interface within 15 minutes indicates positive reaction.



STRING OF PEARL'S TEST

- When *Bacillus anthracis* is grown for 2-3 hours on solid media containing 0.05 to 0.5 IU penicillin / ml, due to impairment of cell wall, the bacilli become spherical in appearance resembling a string of pearls. This test is an adjunct to the rapid recognition of anthrax bacilli.



GELATINE STAB CULTURE

- It forms a characteristic ‘inverted fir tree’ appearance with slow liquefaction commencing from the top.

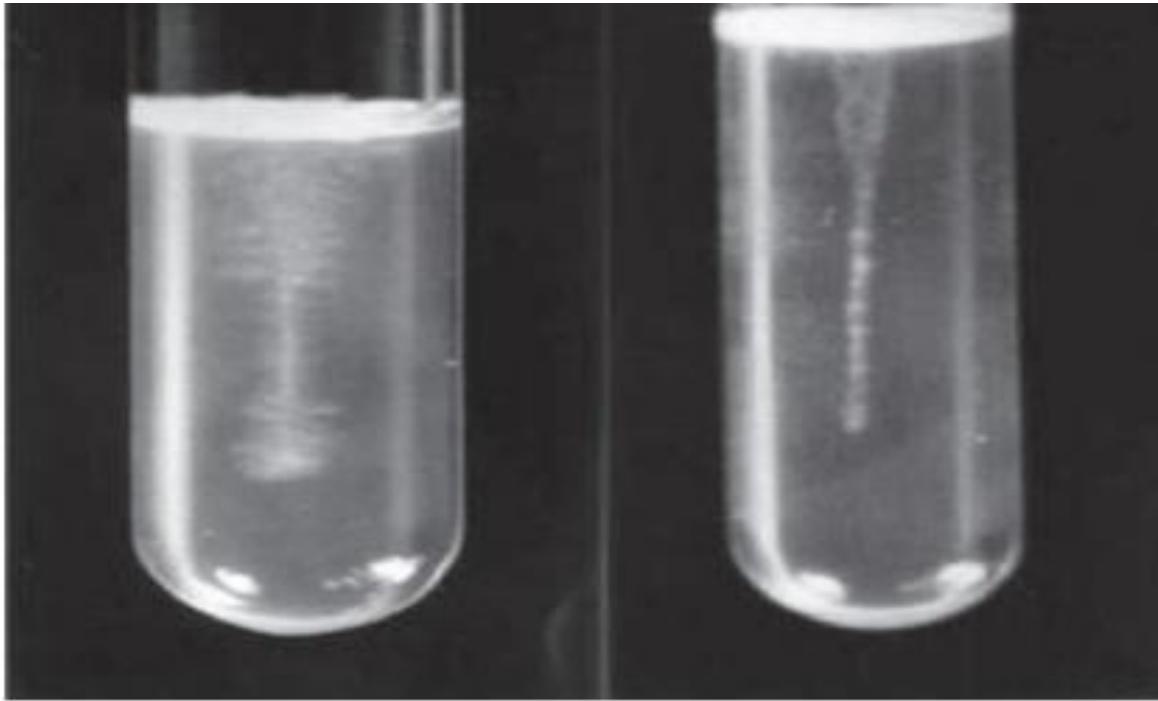
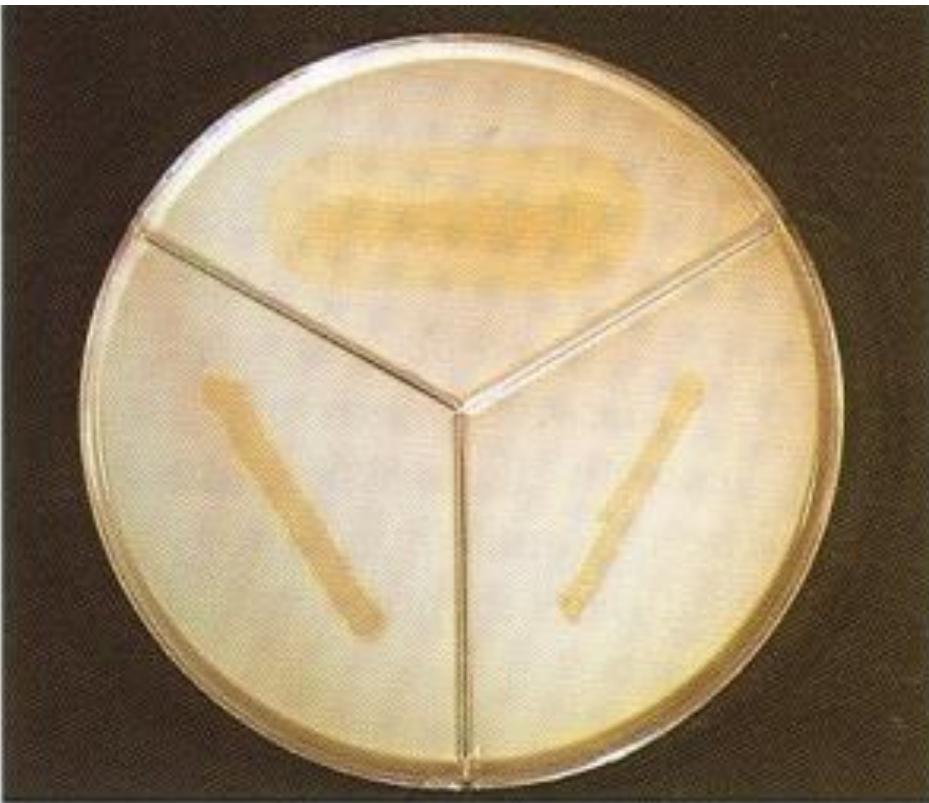


FIG. 28-2. Inverted fir tree appearance of colony of *Bacillus anthracis* in a gelatin stab.

LECITHINASE ACTIVITY TEST



222 Strong lecithinase activity by *B. cereus* (top) on egg yolk agar after 24 hours' incubation. *B. anthracis* (left) gives a weak opaque zone after 48 hours and *B. licheniformis* (right) is unreactive on this medium.

ANIMAL INOCULATION TEST

- Sub-cutaneous inoculation of suspected material into guinea pig or mouse result in death within 48 hours with lesions like gelatinous haemorrhagic oedema at the inoculation site, congested viscera, dark red blood and enlarged darkened spleen.
- Smears from splenic pulp if stained by Gram's method will reveal typical Gram positive bacilli.

PHAGE LYSIS

- Anthrax bacillus is highly susceptible to gamma bacteriophage. This property is used to distinguish anthrax bacillus from other anthracoid bacilli.

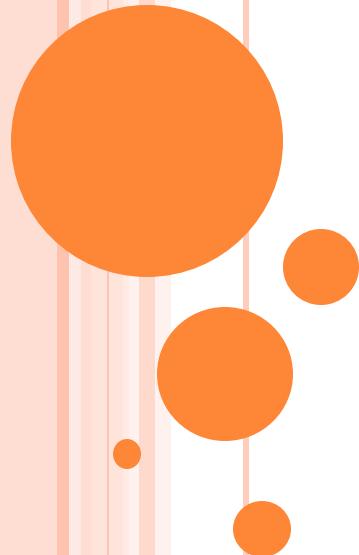
FURTHER READINGS

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease



thank you



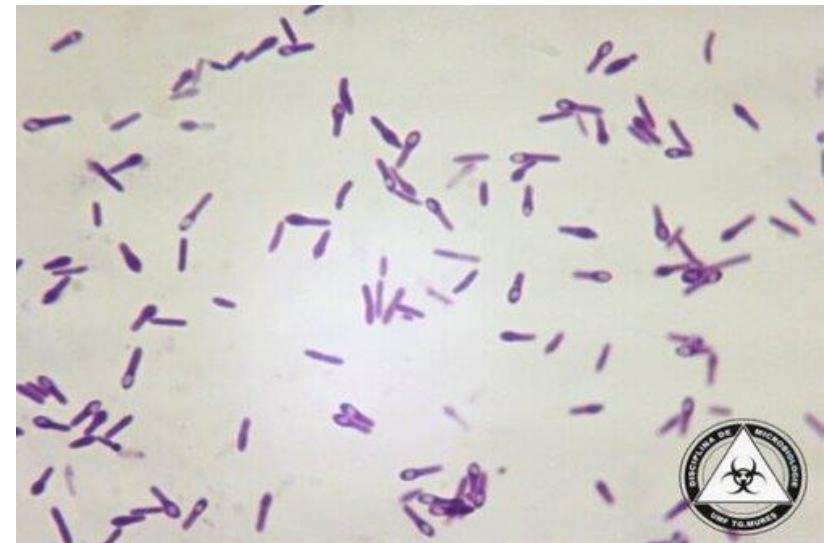


GENUS: CLOSTRIDIA

Dr. Bincy Joseph
Assistant professor
PGIVER, Jaipur

CLOSTRIDIUM

- Kingdom -Prokaryotes
- Division -Firmicutes
- Class -Clostridia
- Order -Clostridiales
- Family -Clostridiaceae
- Genus -*Clostridium*



HISTORY:

- Tetanus has been known from very early times, having been described by Hippocrates.
- But the knowledge of the disease was achieved only in 1884.
- *Rosenbach – 1886* - demonstrated a slender bacillus with round terminal spores in a case of tetanus.
- *Kitasato – 1889* – isolated *C. tetani* in pure culture and reproduced the disease in animals by inoculation of pure culture.
- *C. botulinum* was first isolated by Van Ermengam (1896) from a piece of ham that caused an outbreak of botulism.
- The Greek term “tetanus” which means ‘contracture’ has been taken from the Latin medicine “rigor”.

INTRODUCTION

- The clostridia are large Gram-positive bacteria which are fermentative, **catalase-negative** and **oxidase-negative**, and require **enriched media** for growth.
- They are straight or slightly curved rods and the majority are **motile** by flagella which are peritrichous (**except *C. perfringens***).
- *Clostridium* species produce endospores which usually cause bulging of mother cells
- The size, shape and location of endospores can be used for species differentiation.
- Clostridia are **anaerobic**. *C. odematiens* (*C. novyi*) are strict anaerobes and die on exposure to oxygen.
- *C. histolyticum* and *C. welchii* are aerotolerant and may even grow aerobically.

C. perfringens:
large wide rods
which rarely
form endospores
in vitro

C. tetani: thin rods which
characteristically produce
terminal endospores
('drumstick' appearance)

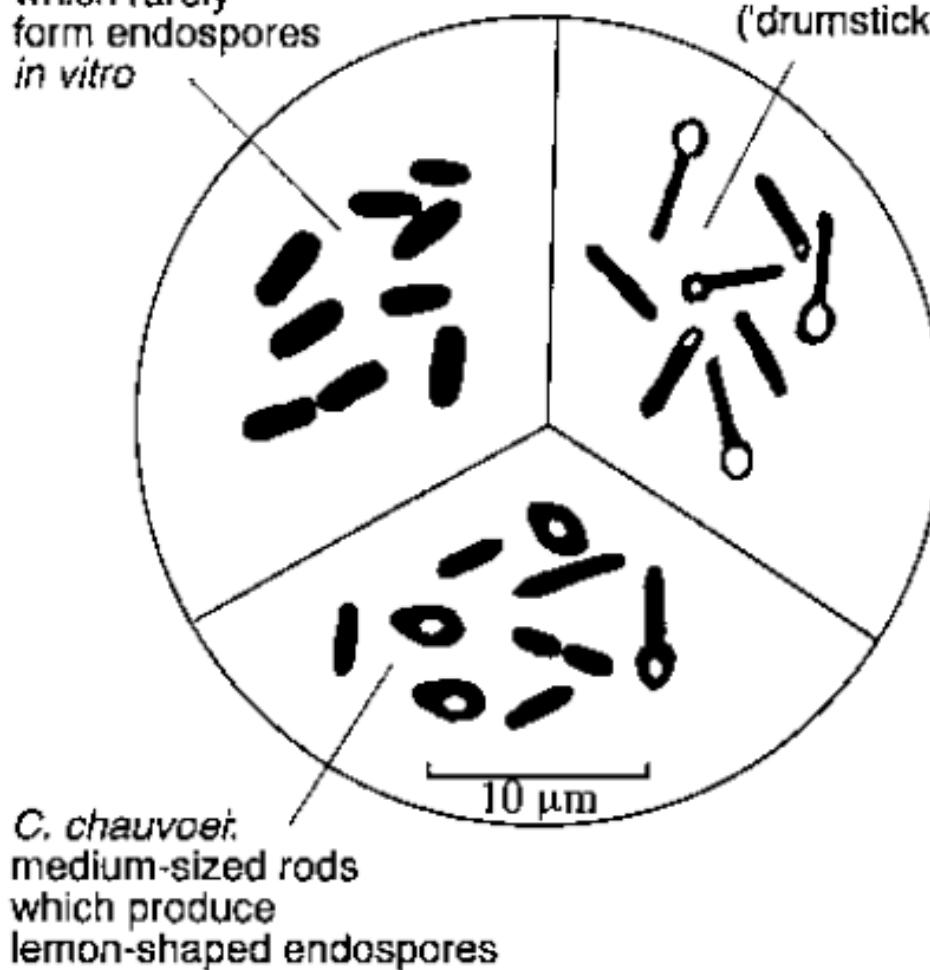


Figure 16.1 Characteristic morphology of some clostridial species.

NATURAL HABITAT

- Clostridia are saprophytes which are found in soil, fresh-water or marine sediments with suitably low redox potentials.
- They constitute part of the normal intestinal flora and some may be sequestered as endospores in muscle or liver. Sequestered endospores, if activated, may produce disease.

Table 16.1 Nomenclature changes of some *Clostridium* species.

Present name	Former name
<i>Clostridium perfringens</i>	<i>Clostridium welchii</i>
<i>Clostridium argentiense</i>	<i>Clostridium botulinum</i> type G
<i>Clostridium haemolyticum</i>	<i>Clostridium novyi</i> type D
<i>Clostridium novyi</i>	<i>Clostridium oedematiens</i>
<i>Clostridium piliforme</i>	<i>Bacillus piliformis</i>

CLASSIFICATION

- These can be grouped in **four categories**, three based on toxin activity and tissues affected and the fourth containing pathogens of lesser importance.
- ***Clostridium tetani*** and ***C. botulinum***, the **neurotoxic clostridia**, affect neuro-muscular function without inducing observable tissue damage.
- In contrast, **histotoxic clostridia** produce relatively localized lesions in tissues such as muscle and liver, and may subsequently cause toxæmia.
- ***Clostridium perfringens*** types A to E, important members of the **third category**, produce inflammatory lesions in the gastrointestinal tract along with enterotoxæmia.
- Clostridia in the **fourth category** are associated with sporadic diseases, usually affecting individual animals.

Pathogenic Clostridium species

Neurotoxic clostridia	Histotoxic clostridia	Enteropathogenic and enterotoxaemia-producing clostridia	Other clostridia
<i>C. tetani</i>	<i>C. chauvoei</i>	<i>C. perfringens</i> (types A-E)	<i>C. colinum</i>
<i>C. botulinum</i> (types A-G)	<i>C. septicum</i>		<i>C. difficile</i>
	<i>C. novyi</i> type A		<i>C. piliforme</i>
	<i>C. perfringens</i> type A		<i>C. spiroforme</i>
	<i>C. sordellii</i>		
	<i>C. haemolyticum</i>		
	<i>C. novyi</i> type B		

Figure 16.2 Pathogenic *Clostridium* species of veterinary importance.

CLINICAL CONDITIONS BY NEUROTOXIC CLOSTRIDIA

- The neurotoxic clostridia, *C. tetani* and *C. botulinum* produce their effects by elaborating potent neurotoxins.

Feature of neuro-toxin	<i>Clostridium tetani</i>	<i>Clostridium botulinum</i>
Site of production	In wounds	In carcasses, decaying vegetation, canned foods. Occasionally in wounds or in intestine (toxico-infections)
Genes which regulate production	In plasmids	Usually in genome (in bacteriophages for types C and D)
Antigenic type	One antigenic type (tetanospasmin)	Eight antigenically distinct toxins, types A to G
Mode of action	Synaptic inhibition	Inhibition of neuromuscular transmission
Clinical effect	Muscular spasms	Flaccid paralysis

TETANUS

- Tetanus is an acute potentially fatal intoxication which affects many species including humans.
- However, Horses and Man are highly susceptible, ruminants and pigs moderately so, and carnivores are comparatively resistant.
- Poultry are not susceptible to tetanus.
- *Clostridium tetani*, the aetiological agent, is a straight, slender anaerobic Gram-positive rod.
- Spherical endospores, which are terminal and bulge mother cells, impart a characteristic 'drumstick' appearance to sporulated organisms



TETANUS

- The endospores are resistant to chemicals and boiling but are killed by autoclaving at 121'C for 15 minutes.
- *Clostridium tetani* has a swarming growth and is haemolytic on blood agar due to the production of tetanolysin.
- Ten serological types of *C. tetani* can be distinguished by their flagellar antigens.
- The neurotoxin, tetanospasmin, is antigenically uniform irrespective of serotype, and antibodies induced by the neurotoxin of any one of the serotypes neutralize the neurotoxins produced by the others.
- Infection occurs when endospores are introduced into traumatized tissue from soil or faeces.



TETANUS

- Common sites of infection include deep penetrating wounds in the horse, castration and docking wounds in sheep, abrasions associated with dystocia in cows and ewes, and the umbilical tissues in all young animals.
- The presence of necrotic tissue, foreign bodies and contaminating facultative anaerobes in wounds may create the anaerobic conditions in which *C. tetani* spores can germinate.
- The clostridial organisms may replicate more readily in the tissues when the haemolytic toxin (tetanolysin), is released.
- Vegetative bacteria multiplying in necrotic tissues produce the potent tetanospasmin which is responsible for the clinical signs of tetanus.

PATHOGENESIS

- Structurally, tetanus toxin consists of **two chains** joined by a disulphide bridge.
- The **light chain is the toxic moiety** and the **heavy chain is responsible for receptor binding** and internalization of the toxin.
- The neurotoxin binds irreversibly to ganglioside receptors on motor neuron terminals and is transported to the nerve cell body and its dendritic processes in the central nervous system in toxin- containing vesicles, by **retrograde intra-axonal flow**.
- Toxin is transferred trans-synaptically to its site of action in the terminals of inhibitory neurons, where it blocks pre- synaptic transmission of inhibitory signals.



PATHOGENESIS

- It does this by **hydrolysis of synaptobrevins**, protein components of vesicles containing neuro-transmitters.
- Because release of **inhibitory neurotransmitters is prevented**, spastic paralysis results.
- Toxin can also be blood-borne, especially when produced in large amounts and can then bind to motor terminals throughout the body prior to transfer to the central nervous system.
- Bound toxin is not neutralized by antitoxin.

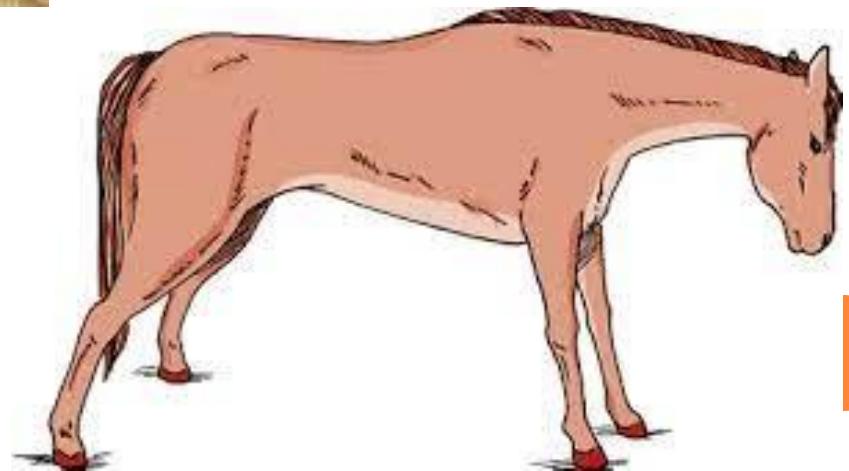


CLINICAL SIGN

- The incubation period of tetanus is usually between 5 and 10 days but may extend to three weeks.
- The clinical effects of the neurotoxin are similar in all domestic animals.
- The nature and severity of the clinical signs are dependent on the anatomical site of the replicating bacteria, the amount of toxin produced and species susceptibility.
- Clinical signs include stiffness, localized spasms, altered heart and respiratory rates, dysphagia and altered facial expression.
- Spasm of mastigatory muscles may lead to '**lockjaw**'. Generalized muscle stiffness can result in a '**saw-horse**' stance especially in horses.



Saw-horse Projection



DIAGNOSTIC PROCEDURES

- The diagnosis of tetanus is usually presumptive and is based on the clinical signs and a history of recent trauma in unvaccinated animals.
- Gram stained smears prepared from material from lesions may reveal the characteristic 'drumstick' forms of *C. tetani*.
- Anaerobic culture of *C. tetani* from necrotic wound tissue may be attempted but is often unsuccessful.
- Serum from affected animals may be used to demonstrate circulating neurotoxin, using **mouse inoculation**.



BOTULISM

- Botulism is a serious, potentially fatal intoxication usually acquired by ingestion of preformed toxin.
- *C. botulinum*, the aetiological agent, is an **anaerobic** Gram- positive rod which produces oval, subterminal endospores.
- The endospores of *C. botulinum* are distributed in soils and aquatic environments worldwide.
- Eight types of *C. botulinum* are recognized on the basis of the toxins (**A, B, C_α, C_β, D, E, F and G**) which they produce.
- These neurotoxins, which are inactivated by boiling for up to 20 minutes, induce similar clinical signs but differ in their antigenicity and potency.



BOTULISM

Table 16.3 Toxins of *Clostridium botulinum*.

Toxin	Source	Susceptible species
Type A	Meat, canned products Toxico-infection Meat, carcasses	Humans Infants Mink, dogs, pigs
Type B	Meat, canned products Toxico-infection Toxico-infection	Humans Infants Foals (up to two months of age)
Type C	Dead invertebrates, maggots, rotting vegetation and carcasses of poultry Ensiled poultry litter, baled silage (poor quality), hay or silage contaminated with rodent carcasses Meat, especially chicken carcasses	Waterfowl, poultry Cattle, sheep, horses Dogs, mink, lions, monkeys
Type D	Carcases, bones Feed contaminated with carcasses	Cattle, sheep Horses
Type E	Dead invertebrates, sludge in earth-bottomed ponds Fish	Farmed fish Fish-eating birds, humans
Type F	Meat, fish	Humans
Type G	Soil-contaminated food	Humans (in Argentina)

BOTULISM

- Some *C. botulinum* types are confined to **particular geographical regions**. Germination of endospores, with growth of vegetative cells and toxin production, occurs in anaerobic locations such as rotting carcasses, decaying vegetation and contaminated canned foods.
- Toxico-infectious botulism, an uncommon form of the disease, occurs when spores germinate in wounds or in the intestinal tract.
- Intestinal toxico-infectious botulism has been recorded in foals (**shaker-foal syndrome**), pups, broiler chickens and turkey poult.



BOTULISM

- *C. botulinum* types C and D cause most outbreaks of botulism in domestic animals.
- Outbreaks of disease occur most commonly in waterfowl, cattle, horses, sheep, mink, poultry and farmed fish.
- Botulism in cattle has been associated with ingestion of poultry carcasses present in ensiled poultry litter used as bedding or spread on pasture
- Poor quality baled silage and silage or hay containing rodent carcasses have been linked to outbreaks of botulism in horses and ruminants.



BOTULISM

- Pica, arising from starvation or phosphorus deficiency in herbivores may induce affected animals to chew bones or carcases containing botulinum toxin.
- The resultant botulism is known as **lamsiekte** in South Africa, **bulbar paralysis** in Australia and **loin disease** in the USA.
- Contaminated raw meat and carcases are often sources of toxin for carnivores.
- Waterfowl and other birds can acquire toxin from dead invertebrates, decaying vegetation or from the consumption of maggots containing toxin.



PATHOGENESIS

- The neurotoxins of *C. botulinum* are the most potent biological toxins known.
- Preformed toxin in food, absorbed from the gastrointestinal tract, circulates in the bloodstream and acts at the neuromuscular junctions of cholinergic nerves and at peripheral autonomic synapses.
- Its structure is similar to that of tetanus toxin and it binds to receptors on nerve endings and enters cells during acetylcholine release.
- As with tetanus toxin, hydrolysis of synaptobrevins causes irreversible interference with the release of the transmitter, acetylcholine in this instance, resulting in flaccid paralysis.
- Death results from paralysis of respiratory muscles.

PATHOGENESIS

- The difference between the effects of tetanus and botulinum toxins is due to their different sites of action.
- Tetanus toxin travels up the nerve axon to the ventral horn whereas botulinum toxin remains at the neuromuscular junction.
- Ingested spores of *C. botulinum* are normally excreted in the faeces.
- In toxico-infectious botulism, however, germination of spores in the intestine, results in toxin production by the vegetative organisms.
- The factors which predispose to toxico-infectious botulism are not known.
- The shaker-foal syndrome, a form of toxico- infectious botulism in foals up to two months of age, has been attributed to the impact of stress on the dam leading to increased corticosteroid levels in the milk.

CLINICAL SIGN

- The clinical signs may develop in 3 to 17 days after ingestion of toxin, are similar in all species.
- Dilated pupils, decreased salivation, tongue flaccidity and dysphagia are in farm animals.
- Incoordination and knuckling of the fetlocks is followed by flaccid paralysis and recumbency.
- Paralysis of respiratory muscles leads to abdominal breathing.
- Body temperature remains normal and affected animals are alert.
- Death may occur within days of the emergence of clinical signs.
- In birds, there is progressive flaccid paralysis which initially affects legs and wings. Paralysis of muscles of the **neck (limberneck)** is evident only in long-necked species.

DIAGNOSTIC PROCEDURES

- Clinical signs and a history of access to contaminated food may suggest botulism
- Confirmation requires the demonstration of toxin in the serum of affected animals. Serum collected from dead animals is unsuitable for mouse inoculation
- The traditional method for demonstrating toxin is by mouse inoculation. **Injected mice develop a characteristic 'wasp-waist' appearance**, a consequence of abdominal breathing following paralysis of respiratory muscles.
- The PCR and nucleic acid probe- based methods have been used for the detection of *C. botulinum* toxin genes.
- Immunological methods using ELISA or chemiluminescent assays are sensitive and specific procedures for toxin detection.
- Toxin neutralization tests in mice, using monovalent antitoxins, can be employed to identify the specific toxin involved if required.



Mouse with classical “wasp waist” sign after intoxication with botulinum toxin



CLINICAL CONDITIONS BY HISTOTOXIC CLOSTRIDIA

- Endospores of histotoxic clostridia are widely distributed in the environment and can persist for long periods in soil.
- The endospores of particular clostridial species are often found in certain localities and in well-defined geographical regions.



PATHOGENESIS

- It is probable that the majority of ingested endospores are excreted in the faeces but some may leave the intestine and become distributed in the tissues where they remain dormant.
- The sequence of events which lead to endospore distribution in tissues is unclear.
- Spores originating in the intestinal lumen may be transported to the tissues in phagocytes.
- Tissue injury leading to reduced oxygen tension is required for spore germination and replication of vegetative bacteria.
- Local necrosis produced by the exotoxins of the replicating bacteria allows further proliferation of the organisms in the tissues with extension of the necrotizing process.

PATHOGENESIS

- Endogenous infections which include blackleg, infectious necrotic hepatitis and bacillary haemoglobinuria result from activation of dormant spores in muscle or liver.
- The anaerobic environment in necrotic tissue is conducive to replication of the clostridia, which are often present together with facultative anaerobes in mixed infections.
- Extension of local tissue destruction results from exotoxin production.



CLINICAL CONDITIONS BY HISTOTOXIC CLOSTRIDIA

<i>Clostridium</i> species	Disease	Toxin	
		Name	Biological activity
<i>C. chauvoei</i>	Blackleg in cattle and sheep	α	Lethal, haemolytic, necrotizing
		β	Deoxyribonuclease
		γ	Hyaluronidase
		δ	Oxygen-labile haemolysin
<i>C. septicum</i>	Malignant oedema in cattle, pigs and sheep Abomasitis in sheep (braxy) and occasionally in calves	α	Lethal, haemolytic, necrotizing
		β	Deoxyribonuclease
		γ	Hyaluronidase
		δ	Oxygen-labile haemolysin
<i>C. novyi</i> type A	'Big head' in young rams Wound infections	α	Necrotizing, lethal
<i>C. perfringens</i> type A	Necrotic enteritis in chickens Necrotizing enterocolitis in pigs Gas gangrene	α	Haemolytic, necrotizing, lethal, lecithinase
<i>C. sordellii</i>	Myositis in cattle, sheep and horses Abomasitis in lambs	α	Lecithinase
		β	Oedema-producing lethal factor
<i>C. novyi</i> type B	Infectious necrotic hepatitis (black disease) in sheep and occasionally in cattle	α	Necrotizing, lethal
		β	Necrotizing, haemolytic, lethal, lecithinase
<i>C. haemolyticum</i>	Bacillary haemoglobinuria in cattle and occasionally in sheep	β	Necrotizing, haemolytic, lethal, lecithinase

BLACK LEG

- Blackleg, an acute disease of cattle and sheep caused by *C. chauvoei*, occurs worldwide.
- In cattle, the disease is most often encountered in **young thriving animals from 3 months to 2 years** of age and infection is usually endogenous, the latent spores in muscle becoming activated through traumatic injury.
- The disease may affect sheep of any age and, in many instances, exogenous infection occurs through skin wounds.
- In both cattle and sheep, **gangrenous cellulitis and myositis** caused by exotoxins produced by the replicating organisms usually lead to rapid death.



MALIGNANT OEDEMA AND GAS GANGRENE

- Malignant oedema and gas gangrene are exogenous, necrotizing, soft tissue infections.
- The bacteria most commonly implicated are *C. septicum* in malignant oedema and *C. perfringens* type A in gas gangrene.
- Malignant oedema manifests as cellulitis with minimal gangrene and gas formation. Tissue swelling due to oedema, and coldness and discolouration of the overlying skin are obvious clinical features.
- Gas gangrene is characterized by extensive bacterial invasion of damaged muscle tissue. Gas production is detectable clinically as subcutaneous crepitation. The clinical features of toxæmia in gas gangrene are similar to those encountered in malignant oedema.

BRAXY

- Braxy, an abomasitis of sheep, is caused by the exotoxins of *C. septicum*.
- The disease, which occurs in winter during periods of **heavy frost or snow**, has been recorded in parts of northern Europe and occasionally elsewhere in the world.
- It has been suggested that ingestion of **frozen herbage may cause local devitalization of abomasal tissue** at its point of contact with the rumen, allowing invasion by *C. septicum*.
- The course of the disease is rapid and most animals die without premonitory signs. Anorexia, depression and fever may be evident immediately before death.

INFECTIOUS NECROTIC HEPATITIS (BLACK DISEASE)

- It is an acute disease affecting sheep and occasionally cattle.
- Rare cases have been described in horses and pigs.
- The hepatic necrosis is caused by exotoxins of *C. novyi* type B replicating in liver tissue which has been damaged by immature *Fasciola hepatica* or other migrating parasites.
- Death is rapid with no premonitory signs and the disease requires differentiation from acute fascioliasis.
- The term 'black disease' relates to the dark discolouration of the skin caused by the marked subcutaneous venous congestion observed at postmortem examination.



BACILLARY HAEMOGLOBINURIA

- It occurs primarily in cattle and occasionally in sheep. In this endogenous infection with *C. haemolyticum*, the clostridial endospores are dormant in the liver, probably in Kupffer cells.
- As in infectious necrotic hepatitis, the main factor which facilitates spore germination and clostridial replication is fluke migration.
- The β toxin, a lecithinase, produced by vegetative cells, causes intravascular haemolysis in addition to hepatic necrosis.
- **Haemoglobinuria**, a major clinical feature of the disease, is a consequence of extensive red cell destruction.



DIAGNOSTIC PROCEDURES

- Histotoxic clostridia contributing to these conditions can be identified by fluorescent antibody techniques.
- *C. perfringens* is cultured anaerobically on blood agar at 37°C for 48 hours. Colonies of *C. perfrigens* type A are up to 5 mm in diameter, circular, flat, greyish and surrounded by a **zone of double haemolysis**.

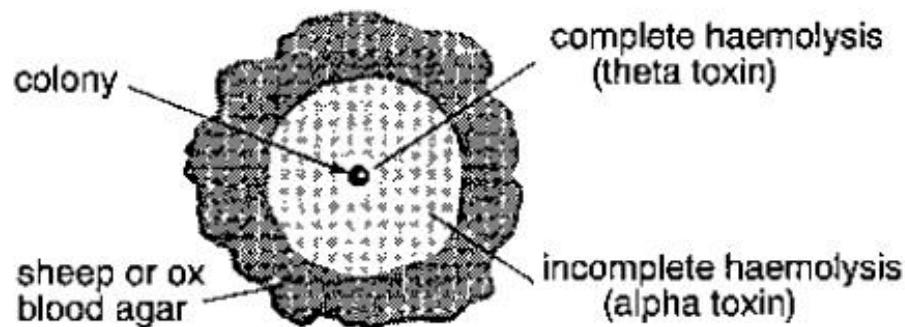


Figure 16.3 Double haemolysis on blood agar around a colony of *Clostridium perfringens*.

- A positive CAMP test occurs with *Streptococcus agalactiae*. A diffusible factor produced by *S. agalactiae* enhances the partial haemolysis of the alpha toxin of *C. perfringens*.
- The pattern of haemolysis is similar to that observed in the *S. agalactiae* reaction with the beta haemolysin of *Staphylococcus aureus*.
- A PCR-based method for the identification of *Clostridium* spp.

- The Nagler reaction, a plate neutralization test, identifies the alpha toxin of *C. perfringens*, which has lecithinase activity

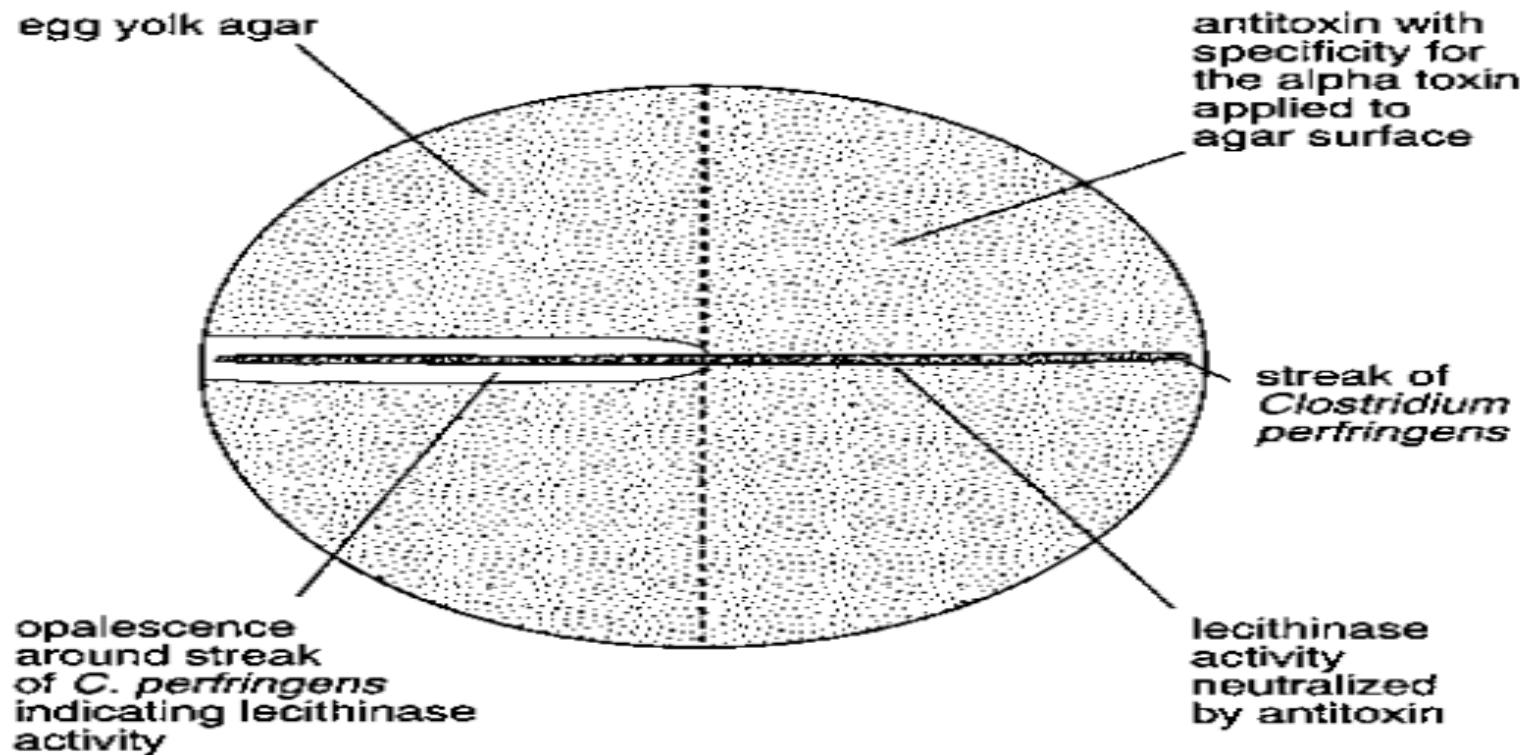


Figure 16.4 Nagler reaction produced by *Clostridium perfringens* growing on egg yolk agar. Antitoxin with specificity for the alpha toxin is applied to the surface of one half of an egg yolk agar plate and allowed to dry. *Clostridium perfringens* is streaked across the plate which is incubated anaerobically at 37°C for 24 hours. Although the organism grows on both halves of the plate, lecithinase activity is evident only on the half without antitoxin.

ENTEROPATHOGENIC AND ENTEROTOXAEMIA-CLOSTRIDIA

- *Clostridium perfringens* types B, C and D are of particular significance in domestic animals.

Usual habitat

- *Clostridium perfringens* is found in soil, in faeces, and in the intestinal tracts of animals and man.
- *C. perfringens* types B, C and D may survive in soil as spores for several months.
- *C. perfringens* type A, which constitutes part of the normal intestinal flora, is widely distributed in soil.



PATHOGENESIS AND PATHOGENICITY

- *C. perfringens* types A to E produce a number of potent, immunologically distinct exotoxins which cause the local and systemic effects encountered in enterotoxaemias.
- A range of minor toxins, some of which may enhance virulence, is also recognized. These include two haemolysins (δ and θ), a collagenase (K) and a hyaluronidase (μ)



Table 16.5 Types of *Clostridium perfringens* and their major toxins.

<i>Clostridium perfringens</i>	Disease	Toxin	
		Name	Biological activity
Type A	Necrotic enteritis in chickens	α (significant toxin)	Lecithinase
	Necrotizing enterocolitis in pigs, Canine haemorrhagic gastroenteritis	Enterotoxin	Cytotoxic
Type B	Lamb dysentery	α	Lecithinase
	Haemorrhagic enteritis in calves and foals	β (significant toxin) ϵ (exists as a prototoxin and requires activation by proteolytic enzymes)	Lethal, necrotizing Increases intestinal and capillary permeability, lethal
Type C	'Struck' in adult sheep	α	Lecithinase
	Sudden death in goats and feedlot cattle	β (significant toxin)	Lethal, necrotizing
	Necrotic enteritis in chickens	Enterotoxin	Cytotoxic
	Haemorrhagic enteritis in neonatal piglets		
Type D	Pulpy kidney in sheep	α	Lecithinase
	Enterotoxaemia in calves, adult goats and kids	ϵ (significant toxin, exists as a prototoxin and requires activation by proteolytic enzymes)	Increases intestinal and capillary permeability, lethal
Type E	Haemorrhagic enteritis in calves	α	Lecithinase
	Enteritis in rabbits	ϵ (significant toxin)	Lethal

PULPY KIDNEY DISEASE

- This disease, caused by *C. perfringens* type D, occurs in sheep worldwide.
- The condition is also described as 'over-eating disease' because gorging on a high grain diet or on succulent pasture predisposes to its development.
- Clinical signs include dullness, opisthotonos, convulsions and terminal coma.
- **Bloating** may be evident in the later stages of illness.
- **Hyperglycaemia and glycosuria** are constant features of the disease.
- Affected adult sheep, which have survived for several days, may exhibit diarrhoea and staggering.



DIAGNOSTIC PROCEDURES

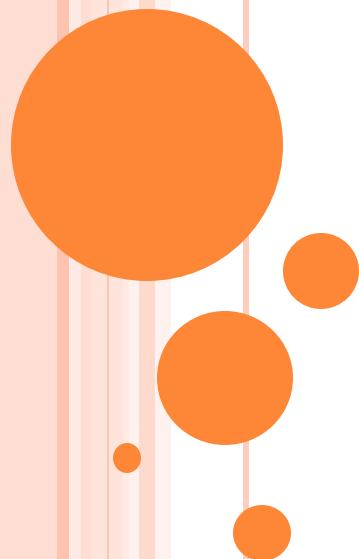
- Direct smears from the mucosa or contents of the small intestine of recently-dead animals, which contain large numbers of thick Gram-positive rods, are consistent with clostridial enterotoxaemia.
- **Glycosuria** is a constant finding in pulpy kidney disease.
- Toxin neutralization tests using mouse and guinea-pig inoculation can definitively identify the toxins of *C. perfringens* present in the intestinal contents of recently-dead animals



FURTHER READINGS

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease



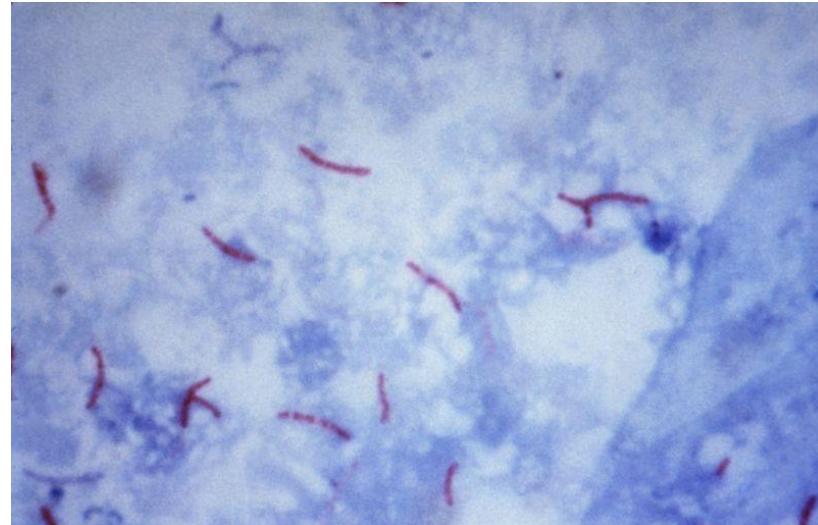


GENUS MYCOBACTERIUM

Dr. Bincy Joseph
Assistant professor
PGIVER, Jaipur.

MYCOBACTERIUM

- Domain - Bacteria
- Phylum - Actinobacteria
- Class - Actinobacteria
- Order - Actinomycetales
- Family - Mycobacteriaceae
- Genus - *Mycobacterium*



HISTORY:

- Generic name mycobacterium (fungus bacterium) was proposed by Lehmann and Neumann (1896).
- First member of this genus - leprae bacillus discovered by Hansen (1868) – Hansen bacillus.
- Koch (1882) isolated the mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying Koch's postulates.
- Acid-fast property of Mycobacterium was discovered by Ehrlich (1882).
- Johne (1895) described Johne's bacillus - *Mycobacterium paratuberculosis*



INTRODUCTION

- Although mycobacteria are cytochemically Gram-positive, the high lipid, waxes and mycolic acid content of their cell walls prevents uptake of the dyes employed in the Gram stain.
- The cell wall lipids bind carbol fuchsin which is not removed by the acid-alcohol decolourizer used in the Ziehl-Neelsen (ZN) staining method.
- Bacilli, which stain red by this method, are called acid-fast or ZN-positive.
- Complex egg-enriched media required for growth of pathogenic species,
- Aerobic, non-motile, non-spore-forming
- Genus includes obligate pathogens, opportunistic pathogens and saprophytes

INTRODUCTION

- Pathogenic species grow slowly, colonies visible after several weeks
- Some mycobacteria produce carotenoid pigments
- Resistant to chemical disinfectants and environmental influences but susceptible to heat treatment.
- Multiply intracellularly and cause chronic, granulomatous infections
- Major diseases include tuberculosis, Johne's disease and feline leprosy.

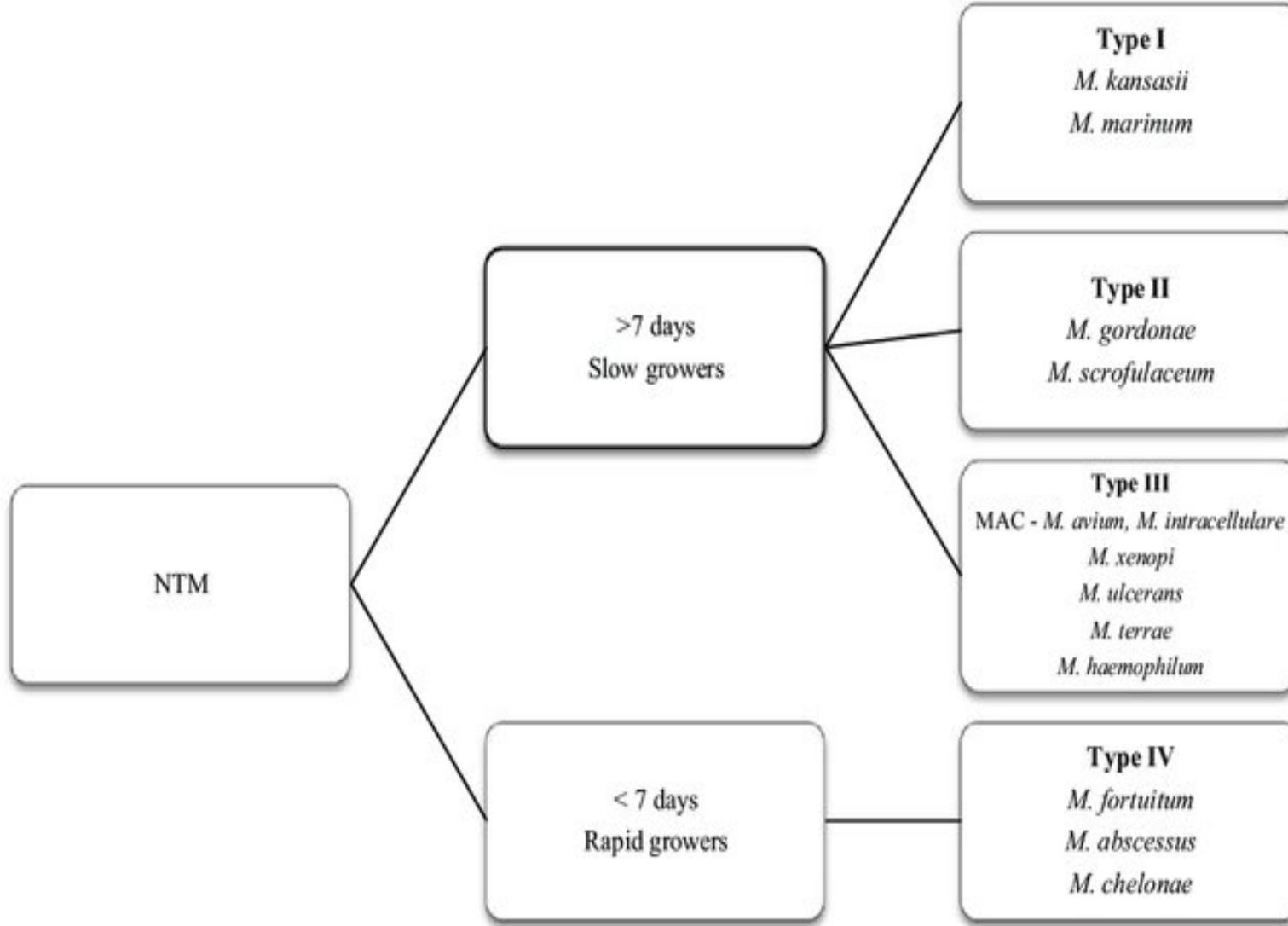


NATURAL HABITAT

- Lipid-rich walls render mycobacteria hydrophobic and resistant to adverse environmental influences.
- Environmental mycobacteria are found in soil, on vegetation and in water.
- *Mycobacterium bovis* is excreted in respiratory discharges, faeces, milk, urine and semen.
- *Mycobacterium avium* and *Mycobacterium paratuberculosis* are shed in faeces
- *Mycobacterium tuberculosis* mainly in respiratory discharges
- Obligate pathogens, shed by infected animals, can also survive in the environment for extended periods



CLASSIFICATION



Classification of non-tuberculous mycobacteria (NTM) (Adapted from Runyon 1959)

CLASSIFICATION

- The Runyon classification of nontuberculous mycobacteria based on the rate of growth, production of yellow pigment and whether this pigment was produced in the dark or only after exposure to light.
- It was introduced by Ernest Runyon in 1959.
- On these bases, the nontuberculous mycobacteria are divided into four Runyon groups.
- The first three groups (Runyon I, II, and III) are classified as slowly growing mycobacteria.
- Runyon IV organisms are rapid growing for mycobacteria



- Runyon I: Photochromogens

Runyon I organisms are slow growing, and produce a yellow-orange pigment when exposed to light.

M. intermedium & *M simiae*

- Runyon II: Scotochromogens

Runyon II organisms are slow-growing and produce a yellow-orange pigment regardless of whether they are grown in the dark or the light.

M. scrofulaceum and *M. szulgai*

- Runyon III: Nonchromogens

Runyon III organisms are slow-growing and never produce pigment, regardless of culture conditions.

M. africanum and *M. bovis*



- Runyon IV: Rapid Growers

Runyon IV organisms are rapid growing for mycobacteria (colonies in 5 days). They do not produce pigment. Some rapidly growing mycobacteria are considered "late-pigmenting"

M. abscessus and *M. chelonae*



DIFFERENTIATION OF MYCOBACTERIA

- The ZN staining method is used to differentiate mycobacteria from other bacteria.
- Differentiation of pathogenic mycobacteria relies on cultural characteristics, biochemical tests, animal inoculation, chromatographic analyses and molecular techniques.
- Pathogenic mycobacteria grow slowly and colonies are not evident until cultures have been incubated for at least three weeks.
- In contrast, the colonies of rapidly growing saprophytes are visible within days.



- *Mycobacterium bovis*, *M. tuberculosis* and *M. avium* subsp, paratuberculosis have an optimal incubation temperature of 37°C.
- *Mycobacteria* belonging to the *M. avium* complex grow in the temperature range of 37 to 43°C.
- Pathogenic species of mycobacteria can be distinguished by their colonial appearance on egg-based media.
- The influence of glycerol and sodium pyruvate on growth rate is used to differentiate pathogenic a species.
- Supplementation of media with mycobactin is required for *M. avium* subsp. paratuberculosis.



- Guinea-pig and rabbit inoculation was used in the past to differentiate *M. tuberculosis* from *M. bovis* and *M. avium*.
- Guinea-pigs are highly susceptible to infection with *M. tuberculosis* and *M. bovis*.
- Rabbits are highly susceptible to infections with *M. bovis* and *M. avium*.
- Chromatographic analyses of the lipid composition of some mycobacterial species are used in specialized laboratories.

- Pigment production and photo reactivity for opportunistic mycobacteria:
 - ✓ Non-chromogens produce colonies devoid of orange, carotenoid pigments.
 - ✓ Photochromogens, when cultured in the dark, produce non-pigmented colonies which become pigmented after a period of exposure to light.
 - ✓ Scotochromogens produce pigment when cultured in the dark or in light.



- Molecular techniques:

- DNA probes, complementary to species-specific sequences of rRNA, are commercially available for the *M. tuberculosis* complex, the *M. avium* complex and *M. kansasi*.
- Nucleic acid amplification procedures, including the polymerase chain reaction, are being developed as sensitive and rapid methods for the detection of mycobacteria in tissue samples.
- DNA restriction endonuclease analyses (DNA fingerprinting) are used in epidemiological studies.



Table 17.1 Mycobacteria which are pathogenic for animals and man.

<i>Mycobacterium</i> species	Main hosts	Species occasionally infected	Disease
<i>M. tuberculosis</i> ^a	Man, captive primates	Dogs, cattle, psittacine birds, canaries	Tuberculosis (worldwide)
<i>M. bovis</i>	Cattle	Deer, badgers, possums, man, cats, other mammalian species	Tuberculosis
<i>M. africanum</i>	Man		Tuberculosis (regions in Africa)
<i>M. avium complex</i> ^a	Most avian species except psittacines	Pigs, cattle	Tuberculosis
<i>M. microti</i>	Voles	Occasionally other mammalian species	Tuberculosis
<i>M. marinum</i>	Fish	Man, aquatic mammals, amphibians	Tuberculosis
<i>M. leprae</i>	Man	Armadillos, chimpanzees	Leprosy
<i>M. lepraeumurium</i>	Rats, mice	Cats	Rat leprosy, feline leprosy
<i>M. avium</i> subsp. <i>paratuberculosis</i>	Cattle, sheep, goats, deer	Other ruminants	Paratuberculosis (Johne's disease)
Unspecified acid-fast bacteria ^a	Cattle		Associated with skin tuberculosis
<i>M. senegalense</i> , <i>M. farcinogenes</i>	Cattle		Implicated in bovine farcy

Table 17.2 Clinical significance, growth characteristics and biochemical differentiation of pathogenic mycobacteria.

	<i>M. tuberculosis</i>	<i>M. bovis</i>	<i>M. avium</i> complex	<i>M. avium</i> subsp. <i>paratuberculosis</i>
Significance of infection	Important in man and occasionally in dogs	Important in cattle and occasionally in other domestic animals and man	Important in free-range domestic poultry, opportunistic infections in man and domestic animals	Important in cattle and other ruminants
Cultural characteristics and requirements				
Growth rate	Slow (3-8 weeks)	Slow (3-8 weeks)	Slow (2-6 weeks)	Very slow (up to 16 weeks)
Optimal incubation temperature	37°C	37°C	37-43°C	37°C
Atmospheric requirements	Aerobic	Aerobic	Aerobic	Aerobic
Colonial features	Rough, buff, difficult to break apart	Cream-coloured, raised with central roughness, break apart easily	Sticky, off-white, break apart easily	Small, hemispherical; some pigmented
Essential growth supplement	None	None	None	Mycobactin
Effect of added glycerol	Enhanced growth (eugenic)	Growth inhibited (dysgenic)	Enhanced growth (eugenic)	
Effect of added sodium pyruvate	No effect	Enhanced growth	No effect	
Biochemical differentiation				
Niacin accumulation	+	-	-	-
Pyrazinamidase production	+	-	-	+
Nitrate reduction	+	-	-	-
Susceptibility to TCH (10 µg/ml) ^a	Resistant	Susceptible	-	Resistant

^a TCH, Thiophen-2-carboxylic acid hydrazine

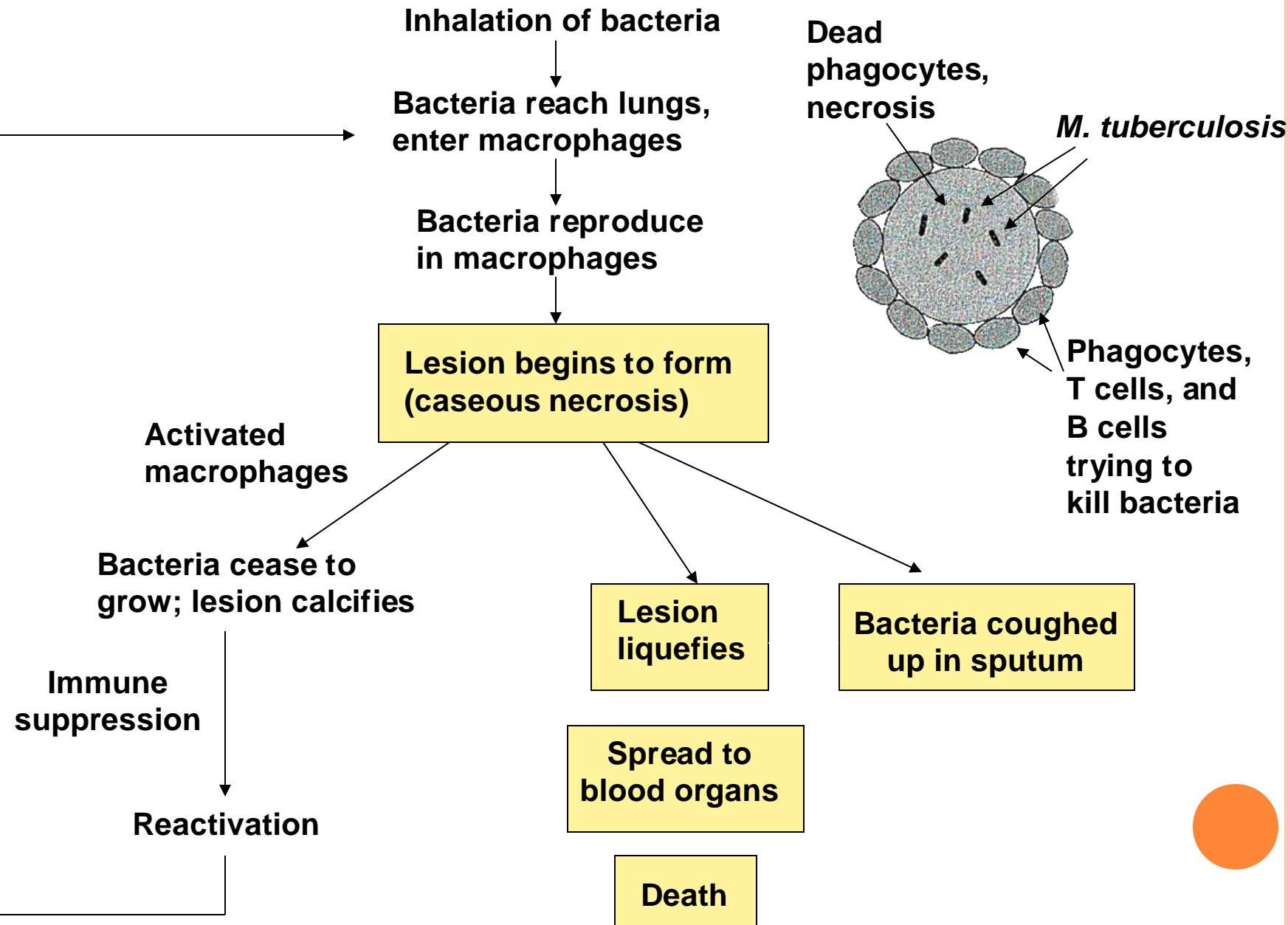
BOVINE TUBERCULOSIS

- Bovine tuberculosis, caused by *M. bovis*, occurs worldwide.
- The virulence of *M. bovis* relates to its ability to survive and multiply in host macrophages.
- Specific toxic factors, contributing to virulence, have not been identified.
- The macrophage accumulation at the primary site of infection is initially a response to the foreign body effect of waxes and lipids in the mycobacterial cell wall.
- Survival within the cytoplasm of macrophages is promoted by interference with phagosome-lysosome fusion and failure of lysosomal digestion.



- Bacilli released from dead macrophages are engulfed by surrounding viable phagocytes.
- Migration of macrophages containing viable mycobacteria can disseminate infection.
- The complex lipid and waxy composition of the mycobacterial cell wall contributes not only to virulence but also, in association with tuberculo proteins, to the immunogenicity on which the development of the host responses and the lesions depends.

Steps in the development of tuberculosis



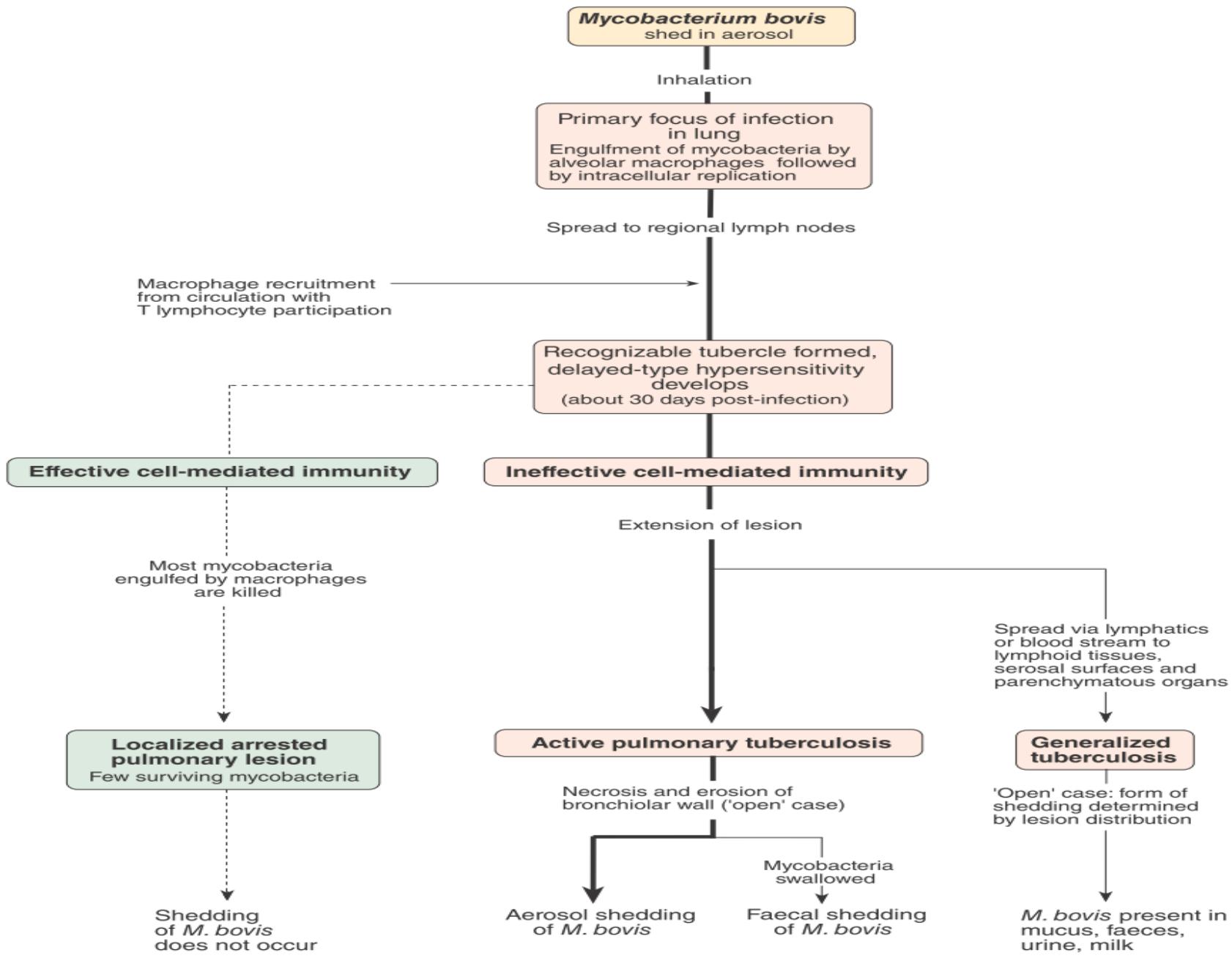
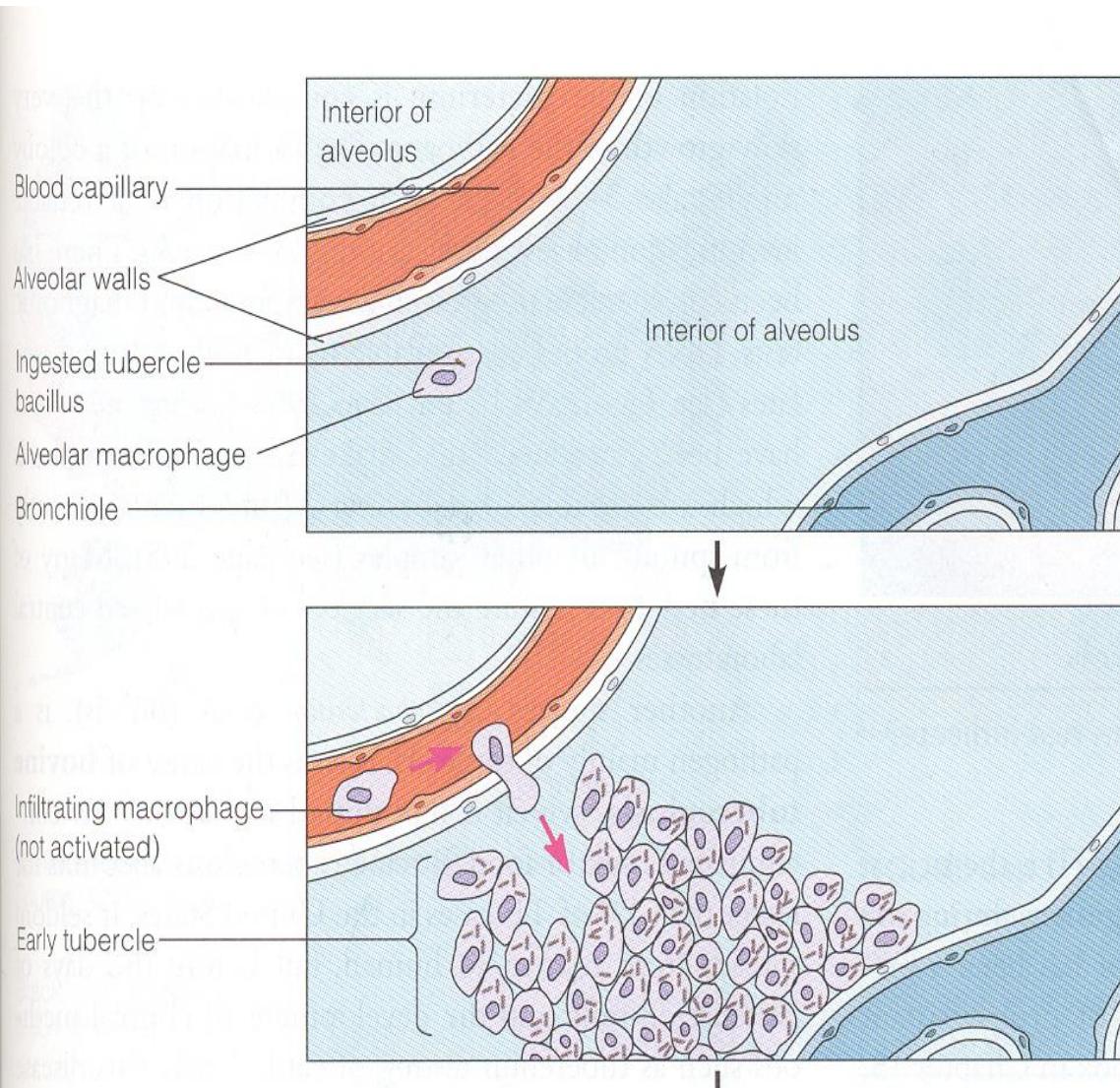


Figure 23.1 The possible consequences of *Mycobacterium bovis* infection in cattle, acquired via aerosols.

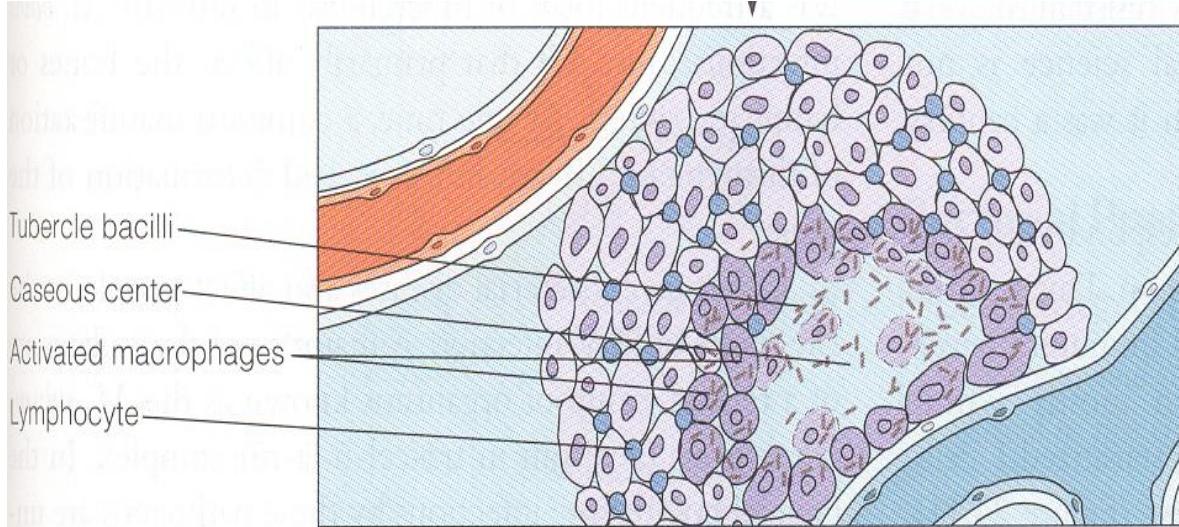
PROGRESSION OF TUBERCULOSIS



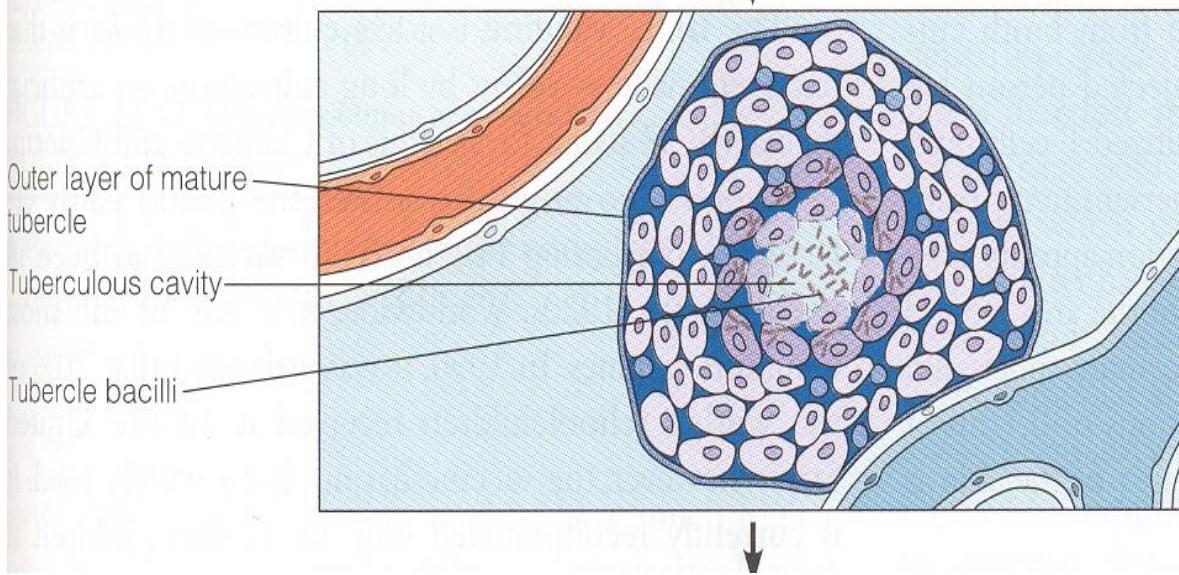
1 Tubercle bacilli that reach the alveoli of the lung (Figure 24.2) are ingested by macrophages, but some often survive.

2 Tubercle bacilli multiplying in macrophages cause a chemotactic response that brings additional macrophages and other defensive cells to the area. These form a surrounding layer and, in turn, an early tubercle. Most of the surrounding macrophages are not successful in destroying bacteria but release enzymes and cytokines that cause a lung-damaging inflammation.

PROGRESSION OF TUBERCULOSIS



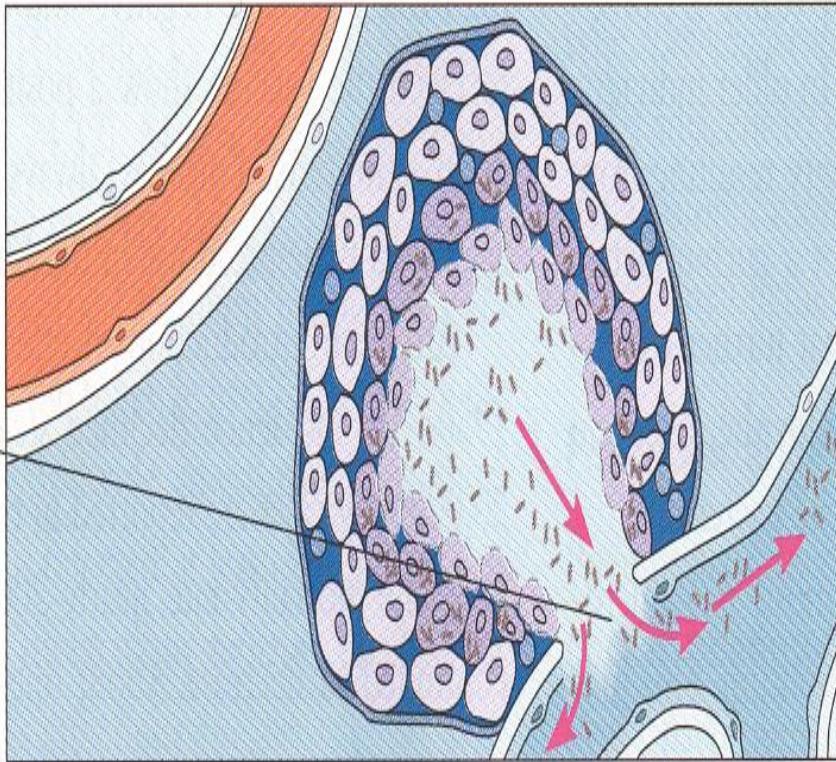
3 After a few weeks, many of the macrophages die, releasing tubercle bacilli and forming a *caseous center* in the tubercle. The aerobic tubercle bacilli do not grow well in this location. However, many remain dormant and serve as a basis for later reactivation of the disease. The disease may be arrested at this stage, and the lesions become calcified.



4 In some individuals, a mature tubercle is formed. The disease progresses as the caseous center enlarges in the process termed *liquefaction*. The caseous center now enlarges and forms an air-filled *tuberculous cavity* in which the aerobic bacilli multiply outside macrophages.

PROGRESSION OF TUBERCULOSIS

Rupture of
bronchiole wall



5

Liquefaction continues until the tubercle ruptures, allowing bacilli to spill into a bronchiole (see Figure 24.2) and thus be disseminated throughout the lungs and then to the circulatory and lymphatic systems.

CLINICAL SIGNS

- Clinical signs are evident only in advanced disease, and cattle with extensive lesions can appear to be in good health.
- Loss of condition may become evident as the disease progresses.
- In advanced pulmonary tuberculosis, animals may eventually develop a cough and intermittent pyrexia.
- In older lesions, fibroplasia produces early capsule formation and there is an area of central caseous necrosis, with the appearance and consistency of soft cheese. The characteristic histological appearance of a typical tubercle



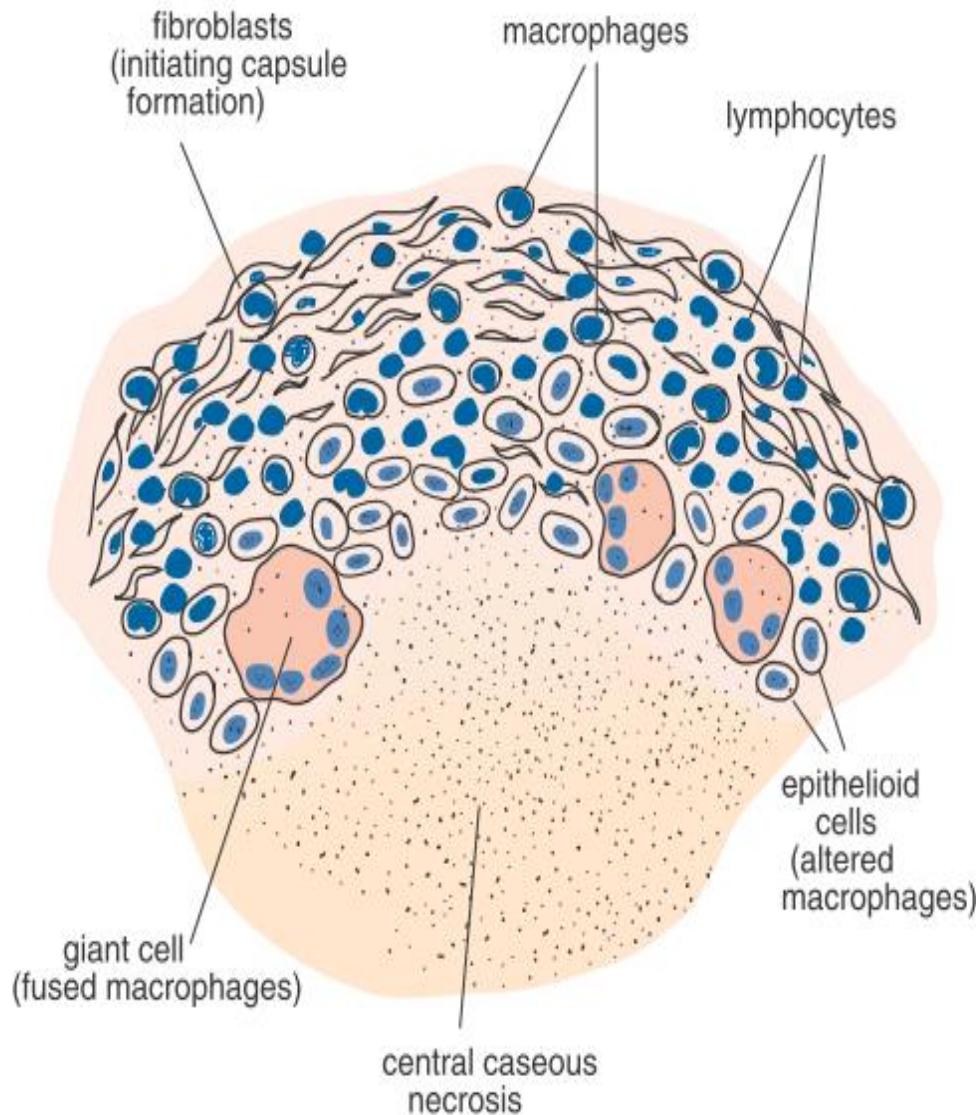


Figure 23.2 Microscopic appearance of part of a typical bovine tuberculous lesion. The tubercle consists of a peripheral zone of mononuclear cells, fibroblasts and giant cells with central caseous necrosis.

DIAGNOSTIC PROCEDURES

- The tuberculin test, based on a delayed - type hypersensitivity to mycobacterial tuberculin, is the standard ante-mortem test in cattle.
- Tuberculin, prepared from mycobacteria and called purified protein derivative (PPD), is injected intradermally to detect sensitization.
 - ❖ In the single intradermal (caudal fold) test
 - ❖ Comparative intradermal test



- ELISA for detecting circulating antibodies.

These tests may be most useful in countries with a high prevalence of bovine TB where there are a large number of animals with chronic disease, and which require low - cost methods of detection

- Gamma interferon assay

This test identifies animals at a slightly earlier stage of infection than the tuberculin test and is approved as a supplementary test for cattle.

- Tissue sections usually reveal typical patterns of tubercle formation



ISOLATION

- Egg based medium (Lowenstein-Jensen)
- Agar and broth based medium (Middlebrook)
- Stonebrinks medium



Lowenstein-Jensen
granular ,rough, dry
colonies. & yellow-
orange colonies.





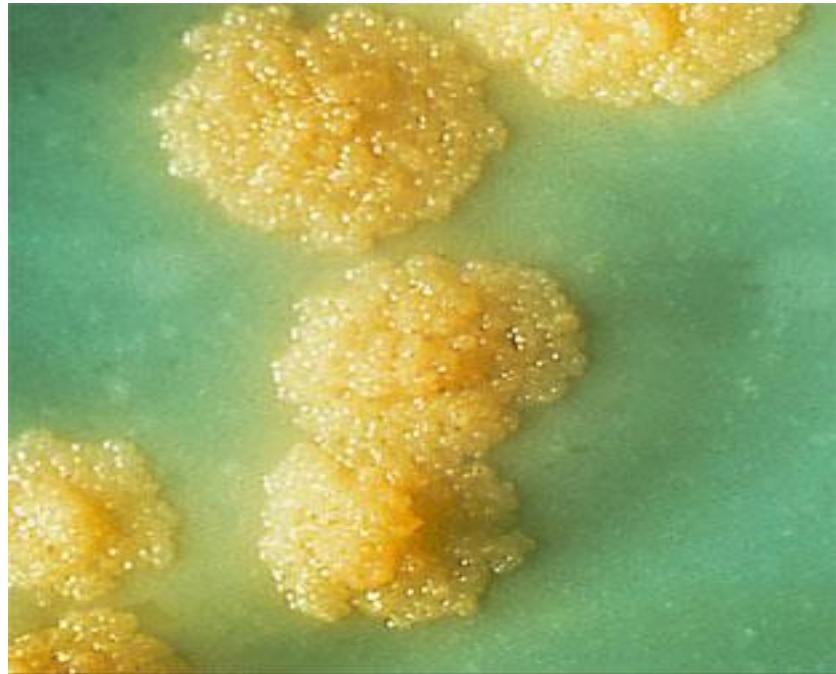
M. bovis Lowenstein-Jensen



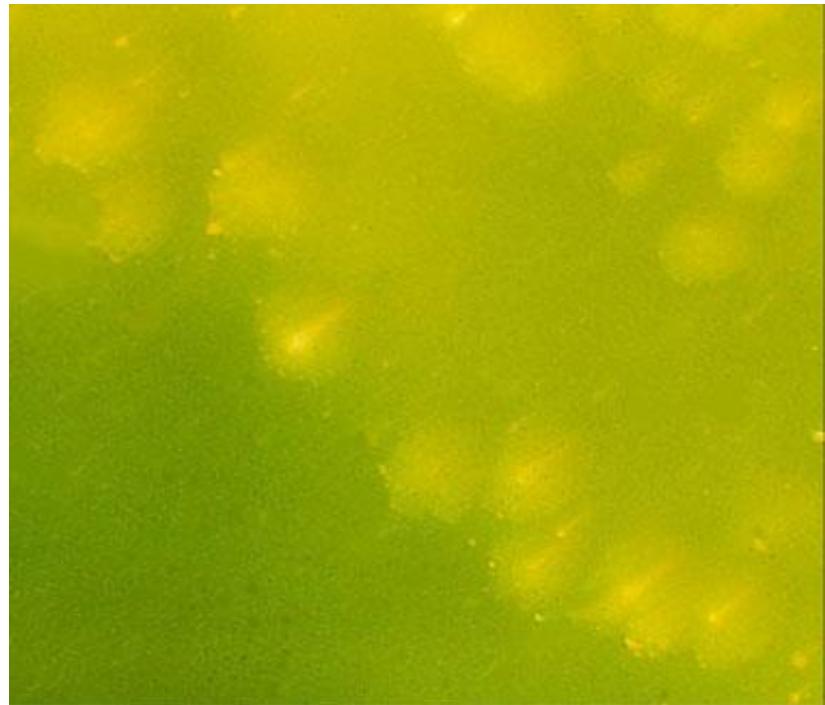
Mycobacterium bovis growth on
Stonebrink Medium

Colonial morphology-

- Rough, tough & buff known as Eugonic
- Smooth, crystal glass appearance known as Dysgonic



EUGONIC GROWTH 14 DAYS



DYSGONIC GROWTH 14 DAYS

TUBERCULOSIS IN POULTRY AND OTHER AVIAN SPECIES

- Avian tuberculosis, which occurs worldwide, is usually caused by members of the *M. avium* complex, serotypes 1 to 3.
- The disease is encountered most often in free-range adult birds.
- Bacilli, excreted in the faeces of birds with advanced lesions, can survive for long periods in soil.



PARATUBERCULOSIS (JOHNE'S DISEASE)

- Paratuberculosis is a chronic, contagious, invariably fatal enteritis which can affect domestic and wild ruminants.
- The aetiological agent, *M. avium* subsp. *paratuberculosis*, is an acid-fast organism formerly referred to as *Mycobacterium johnei*.
- Infection is acquired by calves at an early age through ingestion of organisms shed in the faeces of infected animals.
- Shedding of *M. avium* subsp. *paratuberculosis* in colostrum and milk has been recorded



- There are three major subtypes of *M. avium* subsp. *paratuberculosis*, bovine (type I), ovine (type II) and intermediate (type III) strains: ovine strains have been isolated from cattle and bovine strains from sheep.
- In addition, *M. avium* subsp. *paratuberculosis* has been isolated from many wildlife species, including rabbits, deer, ferrets and mice.
- Molecular typing techniques show that strains infecting wildlife and domestic animals sharing the same environment are frequently indistinguishable.
- The degree to which *M. avium* subsp. *paratuberculosis* strains exhibit host specificity or host preference is unclear at present.

PATHOGENESIS AND PATHOGENICITY

- *Mycobacterium avium* subsp. *paratuberculosis* is an intracellular pathogen and cell-mediated reactions are mainly responsible for the enteric lesions.
- Ingested mycobacteria are taken up by M cells over Peyer's patches.
- Uptake is through the interaction of fibronectin attachment proteins with fibronectin, followed by binding to integrins on the surface of the M cells.
- The organisms cross the intestinal epithelial layer and are engulfed by macrophages in which they survive and replicate.

- Interference with maturation of the phagosome and prevention of phagosome–lysosome fusion appears to be important for intracellular survival of *M. avium* subsp. *paratuberculosis* as is the case for *M. bovis*.
- As the disease progresses, an immune-mediated granulomatous reaction develops, with marked lymphocyte and macrophage accumulation in the lamina propria and submucosa.
- The resulting enteropathy leads to loss of plasma proteins and malabsorption of nutrients and water.
- The macrophages in the intestinal wall and in the regional lymph nodes contain large numbers of mycobacteria.

- Two types of lesion are recognized, multibacillary (lepromatous) and paucibacillary (tuberculoid), which appear to be correlated with host immune response.
- High levels of IL-10 gene expression were detected in cattle with extensive pathological changes and high numbers of bacteria, whereas up-regulation of IFN- γ was recorded in the intestinal tissues of cows with subclinical disease

CLINICAL SIGNS

- Affected cattle are usually more than 2 years of age when signs are first observed. The disease is clinically evident only in mature sheep and goats.
- The main clinical feature in cattle is diarrhoea, initially intermittent but becoming persistent and profuse.
- Progressive weight loss results without loss of appetite, and affected animals seldom survive for more than a year after initial detection.
- In sheep and goats, diarrhoea is less marked and may be absent.



- In cattle, the mucosa of affected areas of the terminal small intestine and the large intestine is usually thickened and folded into transverse corrugations.
- The mesenteric and ileocaecal lymph nodes are enlarged and oedematous.

DIAGNOSIS

- Specimens for direct microscopy from live animals include scrapings or pinch biopsies from the rectum.
- Faeces may be submitted for culture and serum for serological tests.
- Post-mortem specimens for histopathological examination from cattle include tissue from affected regions of the intestines and from regional lymph nodes.
- Specimens for microscopical examination should be stained by the ZN technique



- Isolation of *M. avium* subsp. *paratuberculosis* from faeces or tissues is a sensitive diagnostic procedure but it is difficult and time-consuming.
- After decontamination of the specimen with 0.3% benzalkonium chloride and concentration by centrifugation, slants of Herrold's egg-yolk medium with and without mycobactin are inoculated with the deposit.
- Slants are incubated aerobically at 37°C for up to 16 weeks and examined weekly for evidence of growth.
- Medium containing mycobactin supports growth.

- Serological tests:
 - ❖ Complement fixation tests
 - ❖ Several ELISA tests have been developed for the detection of antibodies to *M. avium* subsp. *paratuberculosis*
- Cell-mediated responses
 - ❖ Johnin, the counterpart of tuberculin PPD, may be used as a field test.
 - ❖ The gamma interferon assay is widely used for early detection of infected animals.
- DNA probes and several real-time PCR methods are highly sensitive, are being used to detect *M. avium* subsp. *paratuberculosis* in faeces.



VIRULENCE FACTORS

- Do not produce any exotoxins or endotoxins.
- Virulence appears to reside in the lipids of the cell wall.
- Cell wall of the mycobacterium is composed of peptidoglycan, arabinogalactan and mycolic acid
- Outer layer of the cell wall has wide range of lipids mycosides (Peptido glycolipids or Phenolic glycolipids).
- Mycosides are responsible for the control of cellular permeability, resistance to action of water-soluble enzymes, antibiotics and disinfectants.
- Cord factor (Trehalose –6,6' dimycolate) and Wax D-inhibits chemotaxis, leukotoxic, responsible for delayed hypersensitivity
- Sulfatides- sulfur containing glycolipids inhibits phagolysosome formation and avoiding exposure to hydrolytic enzymes present in the lysosomes.
- Mycosides, phospholipids and sulpholipids are protecting the tubercle bacilli against Phagocytosis

VACCINATION

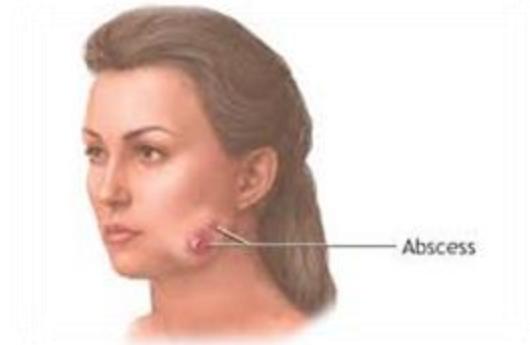
- The French bacteriologists Albert C. Calmette and Guerin notified a loss of virulence of *M. bovis* when cultured in bile containing media.
- A live attenuated vaccine (BCG) is the current vaccine for tuberculosis. It was first used in 1921.



FURTHER READINGS

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GENUS: ACTINOMYCETES

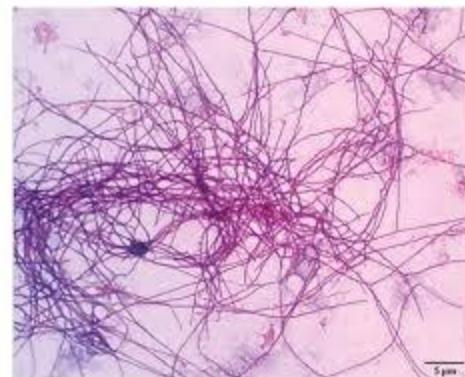
Dr. Bincy Joseph

Assistant Professor

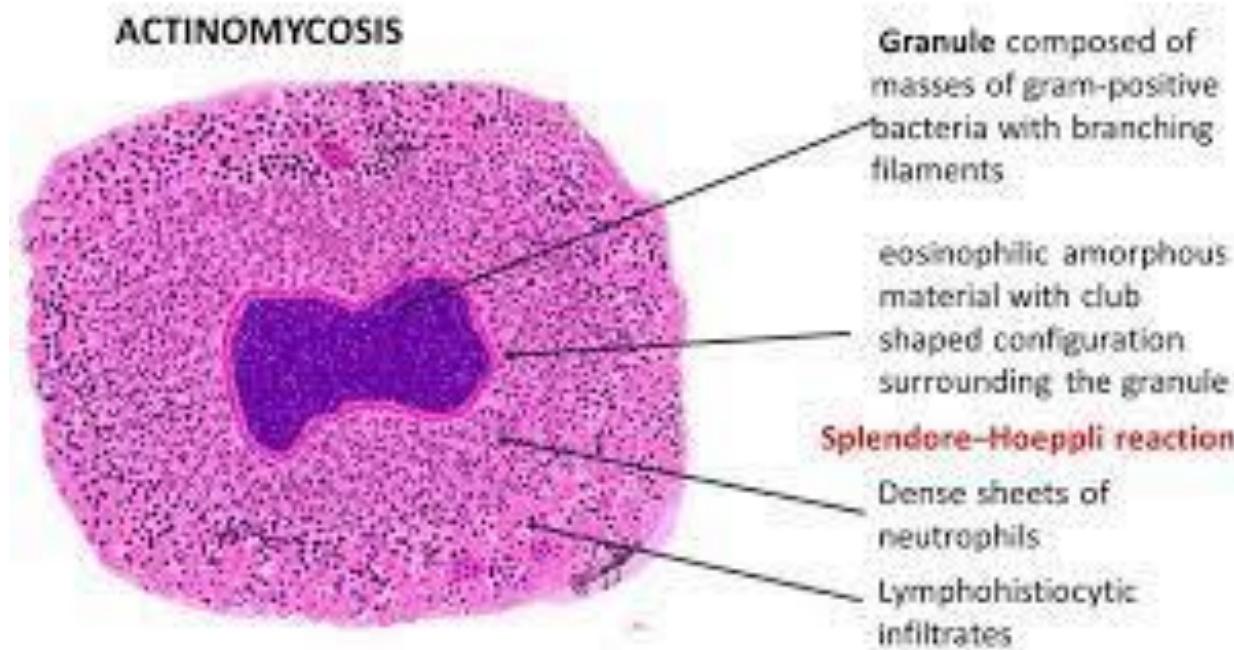
Department of Veterinary Microbiology
PGIVER, Jaipur

ACTINOMYCETES

- The actinomycetes are Gram-positive bacteria
- Grow slowly and produce branching filaments.
- Because of filament formation and granulomatous responses to tissue invasion, these organisms were originally regarded as fungi.
- However, **filaments of the prokaryotic actinomycetes rarely exceed 1.0 um in width, whereas hyphae of the eukaryotic fungi are usually more than 5 um wide.**



- The actinomycetes which cause disease in domestic animals belong to the genera **Actinomyces, Arcanobacterium, Actinobaculum, Nocardia** and **Dermatophilus**



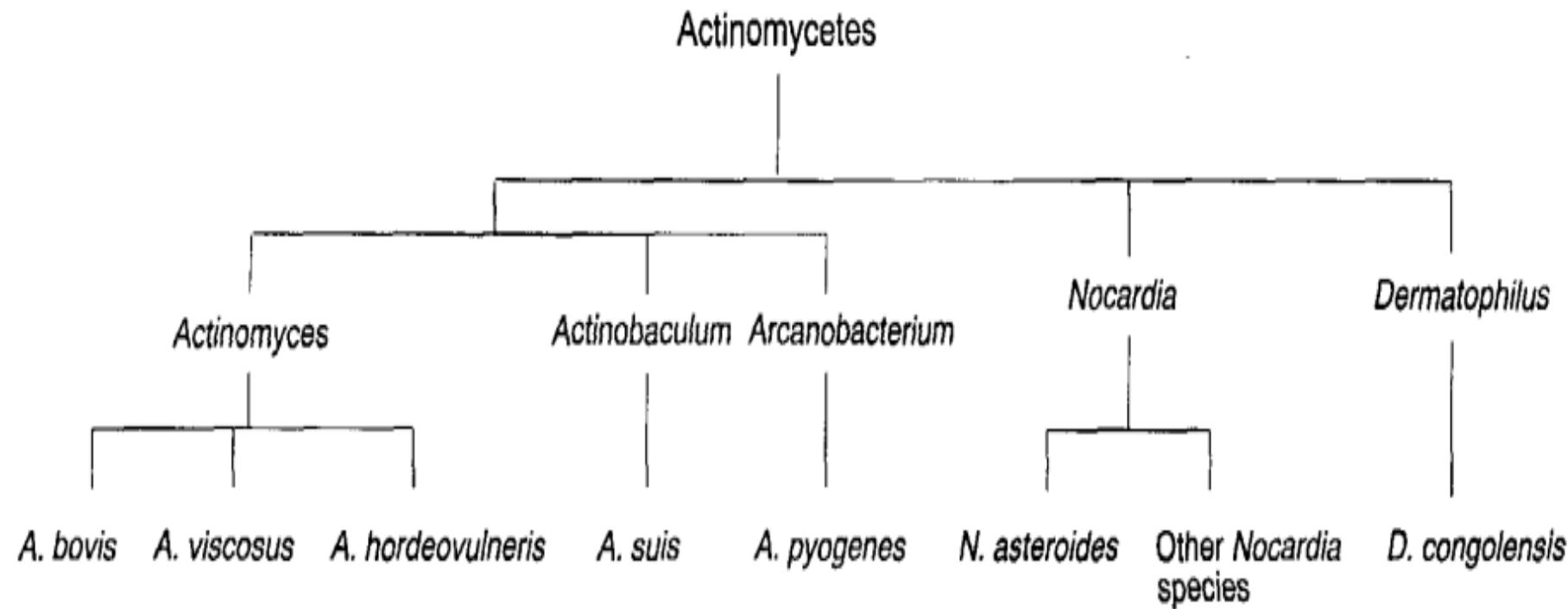


Figure 12.1 Pathogenic actinomycetes of veterinary importance.

- Some thermophilic actinomycetes, such as *Micropolyspora faeni* found in poor-quality overheated hay, produce spores which can induce allergic pulmonary disease in cattle, horses and man – Farmers lung
- The species in these genera are non-motile, non-spore-forming, Gram-positive bacteria which require enriched media for growth.
- *Arcanobacterium pyogenes* has undergone name changes in recent years; it was formerly called *Actinomyces pyogenes* and before that *Corynebacterium pyogenes*.



USUAL HABITAT

- *Actinomyces bovis* is found in the oropharynx of cattle and other domestic animals.
- *Actinomyces viscosus* is a commensal in the oral cavity of dogs and humans.
- *Arcanobacterium pyogenes* is commonly present on the nasopharyngeal mucosa of cattle, sheep and pigs.
- The usual habitat of *Actinobaculum suis* is the preputial mucosa of boars.
- *Actinomyces hordeovulneris* is uncertain, the organism appears to be closely associated with awns in the seed heads of grasses of the genus *Hordeum*.



DIFFERENTIATION OF THE GENERA

Table 12.1 Comparative features of actinomycetes of veterinary importance.

Feature	<i>Actinomyces</i> species	<i>Arcanobacterium pyogenes</i>	<i>Actinobaculum suis</i>	<i>Nocardia</i> species	<i>Dermatophilus congolensis</i>
Atmospheric growth requirements	Anaerobic or facultatively anaerobic and capnophilic	Facultatively anaerobic and capnophilic	Anaerobic	Aerobic	Aerobic and capnophilic
Aerial filament production	—	—	—	+	—
Modified Ziehl-Neelsen staining	—	—	—	+	—
Growth on Sabouraud dextrose agar	—	—	—	+	—
Usual habitat	Nasopharyngeal and oral mucosae	Nasopharyngeal mucosa of cattle, sheep and pigs	Prepuce and preputial diverticulum of boars	Soil	Skin of carrier animals, scabs from lesions
Site of lesions	Many tissues including bone	Soft tissues	Urinary tract of sows	Thoracic cavity, skin and other tissues	Skin

Biochemical reactions

- In routine diagnosis, a presumptive identification of *A. pyogenes* is based on colonial morphology and pitting of a Loeffler's serum slope within 24 hours, which indicates proteolytic activity.
- It also hydrolyses gelatin.
- Urease is produced by *A. suis*



GRANULES IN PUS

- Granules can be detected when pus is diluted with distilled water in a Petri dish.
- In infections caused by *A. bovis*, pinhead-sized, yellowish '**sulphur granules**' are found.
- Whitish, soft, grey granules are demonstrable in pus from animals infected with *A. viscosus*.
- Granules in lesions caused by *A. bovis* contain characteristic clubs.
- Club colony formation is a feature of other chronic infections such as bovine actinobacillosis caused by *Actinobacillus lignieresii* and botryomycosis usually associated with *Staphylococcus aureus*.



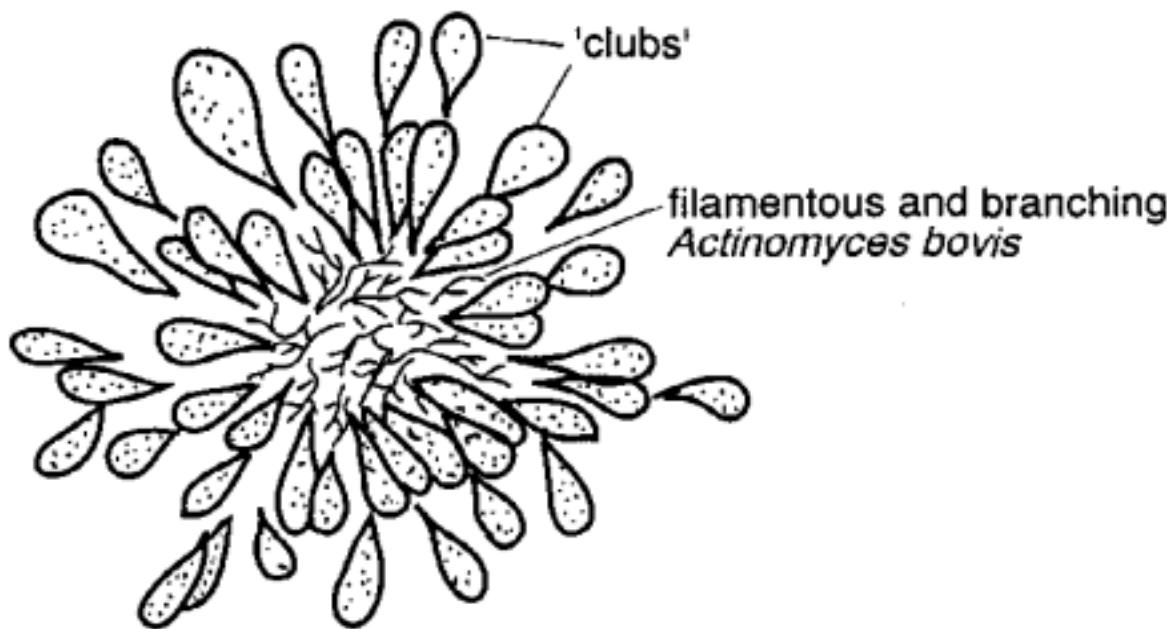


Figure 12.3 A club colony with a core of branching filaments of *Actinomyces bovis* surrounded by club-shaped structures. These structures are part of the host response to this chronic infection.

Table 12.3 Differentiation of *Actinomyces*, *Arcanobacterium* and *Actinobaculum* species of veterinary importance.

Characteristic	<i>Actinomyces bovis</i>	<i>Actinomyces viscosus</i>	<i>Actinomyces hordeovul- neris</i>	<i>Arcanobacterium pyogenes</i>	<i>Actinobaculum suis</i>
Morphology	Filamentous branching, some short forms	Filamentous branching, short forms	Filamentous branching, short forms	Coryneform	Coryneform
Atmospheric requirements	Anaerobic + CO ₂	10% CO ₂	10% CO ₂	Aerobic	Anaerobic
Haemolysis on sheep blood agar	±	-	±	+	±
Catalase production	-	+	+	-	-
Pitting of Loeffler's serum slope	-	-	-	+	-
Granules in pus	'Sulphur granules'	White granules	No granules	No granules	No granules

Table 12.2 Disease conditions produced by *Actinomyces*, *Arcanobacterium* and *Actinobaculum* species in domestic animals.

Species	Hosts	Disease conditions
<i>Arcanobacterium pyogenes</i>	Cattle, sheep, pigs	Abscessation, mastitis, suppurative pneumonia, endometritis, pyometra, arthritis, umbilical infections
<i>Actinomyces hordeovulnaris</i>	Dogs	Cutaneous and visceral abscessation, pleuritis, peritonitis, arthritis
<i>Actinomyces bovis</i>	Cattle	Bovine actinomycosis (lumpy jaw)
<i>A. viscosus</i>	Dogs	Canine actinomycosis: — cutaneous pyogranulomas — pyothorax and proliferative pyogranulomatous pleural lesions — disseminated lesions (rare)
	Horses	Cutaneous pustules
	Cattle	Abortion
<i>Actinomyces</i> species (unclassified)	Pigs	Pyogranulomatous mastitis
	Horses	Poll evil and fistulous withers
<i>Actinobaculum suis</i>	Pigs	Cystitis, pyelonephritis

Table 12.4 Disease conditions produced by *Nocardia* species in domestic animals.

Species	Hosts	Disease conditions
<i>Nocardia asteroides</i>	Dogs	Canine nocardiosis: — cutaneous pyogranulomas — pyogranulomatous pleural lesions and pyothorax — disseminated lesions
	Cattle	Chronic mastitis, abortion
	Pigs	Abortion
	Sheep, goats, horses	Wound infections, mastitis, pneumonia, other pyogranulomatous conditions
<i>Nocardia farcinica</i>	Cattle	Bovine farcy ^a

^a some mycobacteria have also been implicated in bovine farcy

Table 12.5 Differentiation of *Nocardia asteroides* and *Actinomyces viscosus*.

Characteristic	<i>Nocardia asteroides</i>	<i>Actinomyces viscosus</i>
MZN-staining of filaments	+	-
Atmospheric requirement	Aerobic	10% CO ₂
Growth on Sabouraud dextrose agar	+	-
Susceptibility to Penicillin G	-	+
MZN modified Ziehl-Neelsen stain		

BOVINE ACTINOMYCOSIS (LUMPY JAW)

- Invasion of the mandible and, less commonly, the maxilla of cattle by *A. bovis* causes a chronic rarefying osteomyelitis.
- A painless swelling of the **affected bone** enlarges over a period of several weeks.
- The swelling becomes painful and fistulous tracts, discharging purulent exudate, develop.

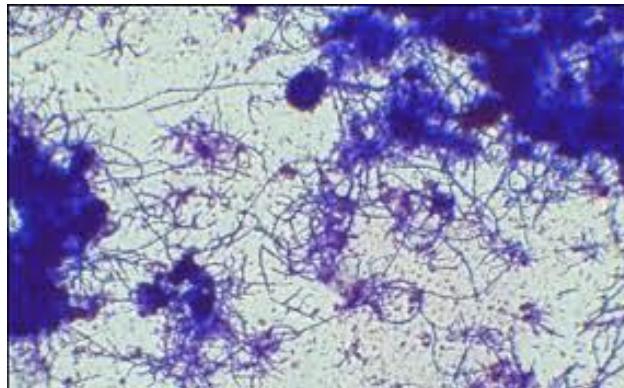


- Spread to continuous soft tissues may occur but there is minimal involvement of regional lymph nodes.
- Lumpy jaw should be distinguished from other conditions which result in swelling of the bones of the jaw and from Actinobacillosis which may involve the soft tissues of the head.

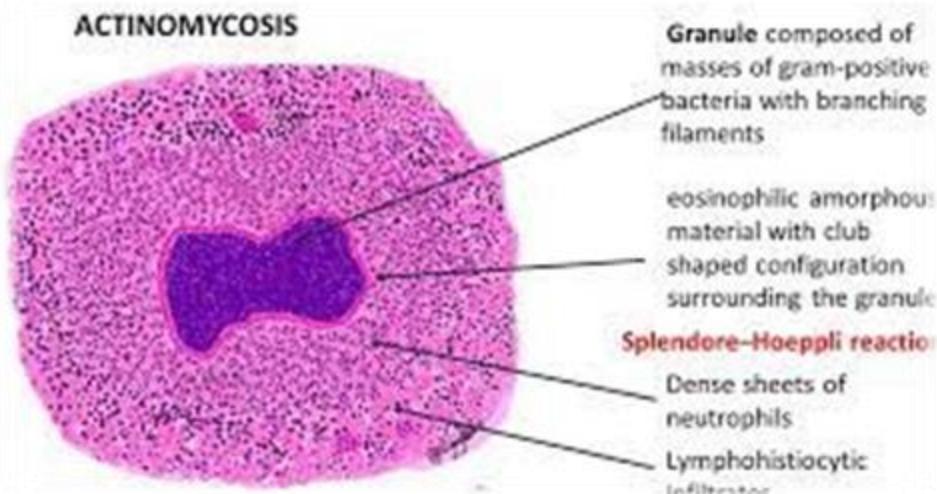


DIAGNOSTIC PROCEDURES

- Clinical presentation, species affected and type and location of lesions may suggest the species involved.
- Gram-stained smears may reveal morphological forms typical of the aetiological agent.
- Unlike *Nocardia* species, these bacteria are modified Ziehl- Neelsen (MZN) negative.



- Histopathological examination of specimens from lesions caused by *A. bovis* reveals aggregates of filamentous organisms surrounded by eosinophilic club shaped structures



- Identification criteria for isolates:
 - ❖ Colonial characteristics
 - ❖ Morphology in stained smears
 - ❖ Presence or absence of haemolysis on blood agar
 - ❖ Absence of growth on MacConkey agar
 - ❖ Absence or presence of growth when subcultured onto Sabouraud dextrose agar
 - ❖ Pitting of a Loeffler's serum slope (*A. pyogenes*)
 - ❖ Urease production (*A. suis*)

FURTHER READINGS

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease

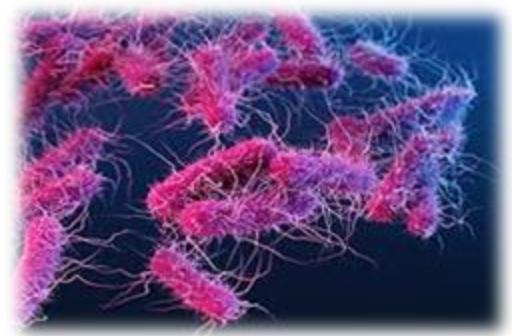




FAMILY : ENTEROBACTERIACEAE I

Dr. Bincy Joseph
PGIVER, Jaipur

FAMILY: ENTEROBACTERIACEAE



General characteristics

- Gram negative non spore forming rods
- Most of them are motile with **peritrichous flagella**
- Some are capsulated and some don't
- Aerobic or **facultative anaerobic**
- All organism ferments glucose
- Organism reduce **nitrate to nitrite** except some strains of *Erwinia*
- Catalase positive except *Shigella dysentiae*
- They are **oxidase negative** (difference from other Gram negative bacteria)
- Organism usually seen in the GI tract of man and animals



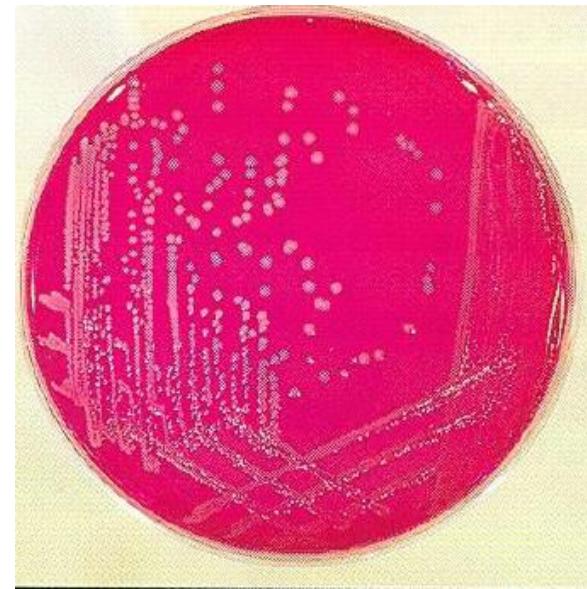
COLONIES ON MACONKEY AGAR

- + Fermentable sugar: lactose
- + pH indicator: neutral red (pale straw at pH 8 and pink at pH 6.8)
- + Inhibitors: bile salts and crystal violet (anti Gram positive)
- + Reactions: if organism can ferment the lactose and acidic metabolic products are produced the medium and colonies are pink (lactose fermenter)
- + If the organism is unable to utilise lactose, then it attacks peptone (nitrogen source) in the medium resulting in alkaline metabolic products and the medium and colonies are pale straw coloured (non lactose fermenter)



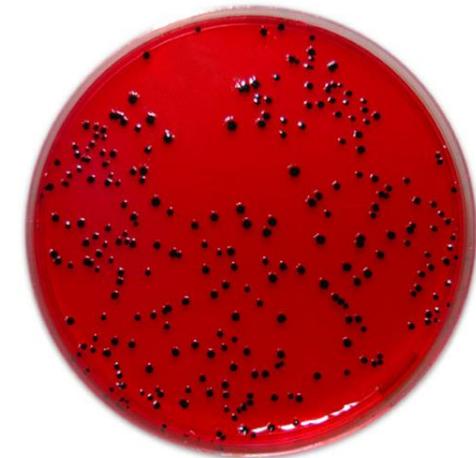
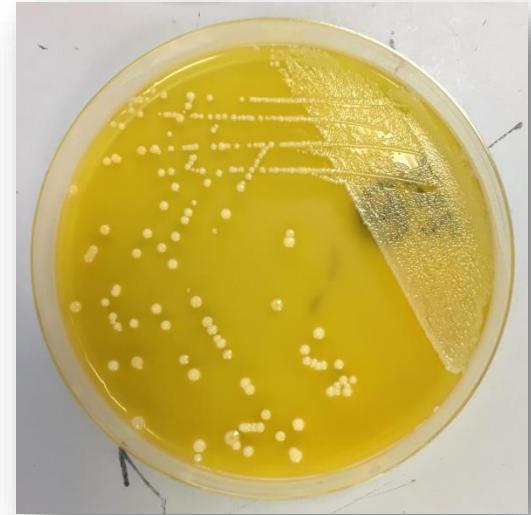
COLONIES ON BGA

- **Brilliant Green agar (BGA) :**
- fermentable sugar : Lactose and sucrose
- PH indicator: Phenol red (red at pH 8.2 and yellow at pH 6.4)
- Inhibitor: brilliant green dye that to some extent inhibits the growth of most enterobacteria except salmonella species
- Reactions: Similar to those occurring on MaConkey agar except that the bacteria may ferment one or both of the sugars with an acid reaction (yellowish green) or unable to ferment either sugar and attack peptone instead with an alkaline reaction and red colonies



COLONIES ON XLD AGAR

- XLD agar (Xylose lysine deoxycholate agar)
- Fermentable sugars: lactose, sucrose and xylose
- pH indicator: phenol red (red at pH 8.2 and yellow at pH 6.4)
- Other substrates: lysine and chemicals for detecting hydrogen sulphide production
- Inhibitors: bile salts (sodium deoxycholate)
- Reactions: Salmonellae will first ferment the xylose creating a temporary acid reaction, but this is reversed by subsequent decarboxylation of lysine with alkaline metabolic products
- Super imposed the red (alkaline) colonies is the production of hydrogen sulphide, so most salmonellae have red colonies with black centre
- The large amount of acid produced by enterobacteria that can ferment either lactose or sucrose or both prevents the reversion back to alkaine even if the bacterium is able to decarboxylate lysine



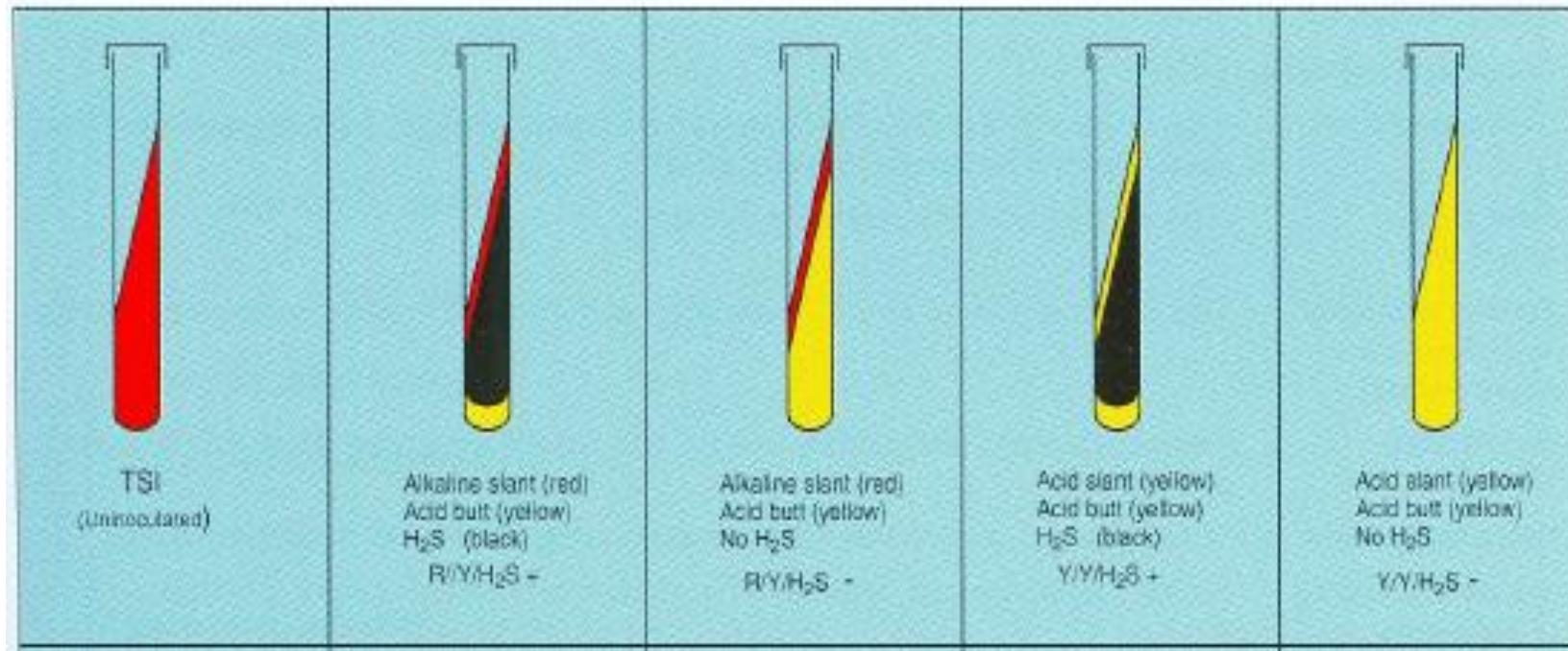
REACTIONS ON TSI AGAR SLANT

- Triple sugar iron agar
- It is an indicator medium and does not contain an inhibitor
- Fermentable sugars: glucose 0.1% lactose 1% and sucrose 1%
- Other substrates: chemicals to indicate hydrogen sulphide (H_2S) production
- PH indicator: Phenol red (red at pH 8.2 and Yellow at pH 6.4)
- Reactions: All members of *Enterobacteriaceae* are capable of fermenting glucose and the small amount will be attacked preferentially and rapidly. So at the early stage both butt and slant will be yellow due to acid production from glucose fermentation

- Some bacteria attack lactose or sucrose in the medium and in this case sufficient acid is produced to maintain both butt and slant in an acid (yellow condition)
- Bacteria that are unable to ferment either lactose or sucrose, after depletion of limited amount of glucose, will utilize the peptones in the medium
- This is less efficient method of generation of energy and occurs mainly at the surface of slant in the presence of atmospheric oxygen
- The metabolites of peptone are alkaline and this causes the slant to revert back to the original red colour

- Some members of Enterobactericeae including most *salmnella* spp. are able to produce hydrogen sulphide
 - .
- This reaction is superimposed over the sugar fermentation and is seen as blackening of the medium
- The general interpretaation of the reactions as follows
- Alkaline (red) slant and acid (yellow) butt: glucose fermentation only
- Acid (yellow) slant and acid (yellow) butt: lactose and/or sucrose attacked as well as glucose
- Blackeningof the medium: hydrogen sulphide production







269 TSI agar slopes showing the range of reactions from the left, uninoculated, R/Y/H₂S+, R/Y/H₂S-, Y/Y/H₂S+, Y/Y/H₂S-. See Diagram 32 for notation.

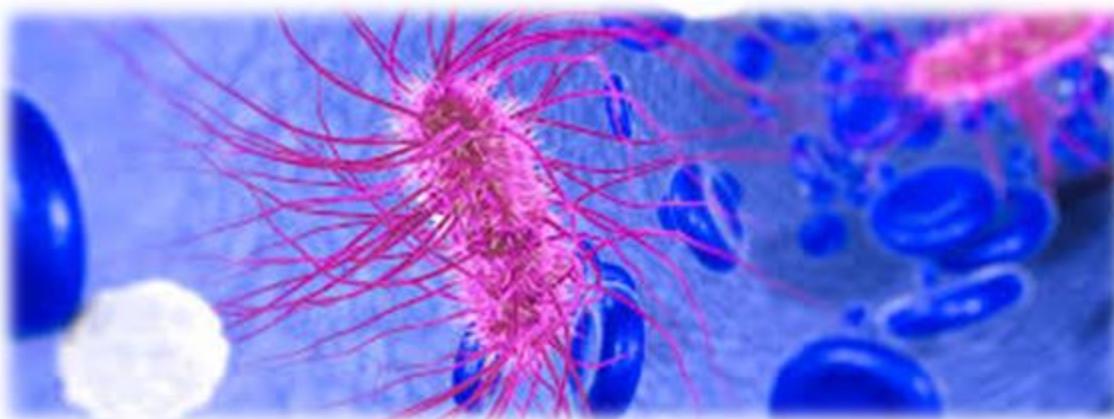


FAMILY : ENTEROBACTERIACEAE II

Dr. Bincy Joseph
PGIVER, Jaipur

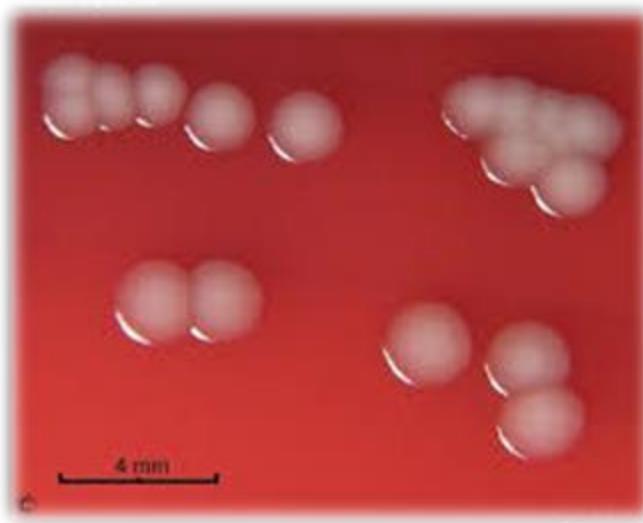
GENUS: ESCHERICHIA

- Type species: *Escherichia coli*
- Isolated by **Theobord Escherich** from feces of new borne baby
- Gram negative bacillus of 2-3 μm length
- Non spore forming organism
- Most of them are motile with **eritrichous flagella**
- Extra intestinal isolates are capsulated



GROWTH REQUIREMENT

- Aerobic or facultative anaerobic
- Temperature for growth is 15-45°C.
- Most of the virulent organism grow at temperature as high as 45°C.
- Organism grow on ordinary medium and colonies are large circular and low convex



GROWTH ON SELECTIVE MEDIA

- Most important is **MaConkey's medium** which contain the inhibitor substance bile, substrate lactose, and indicator is neutral red. Since the organism is lactose fermenting produce rosy pink colonies.



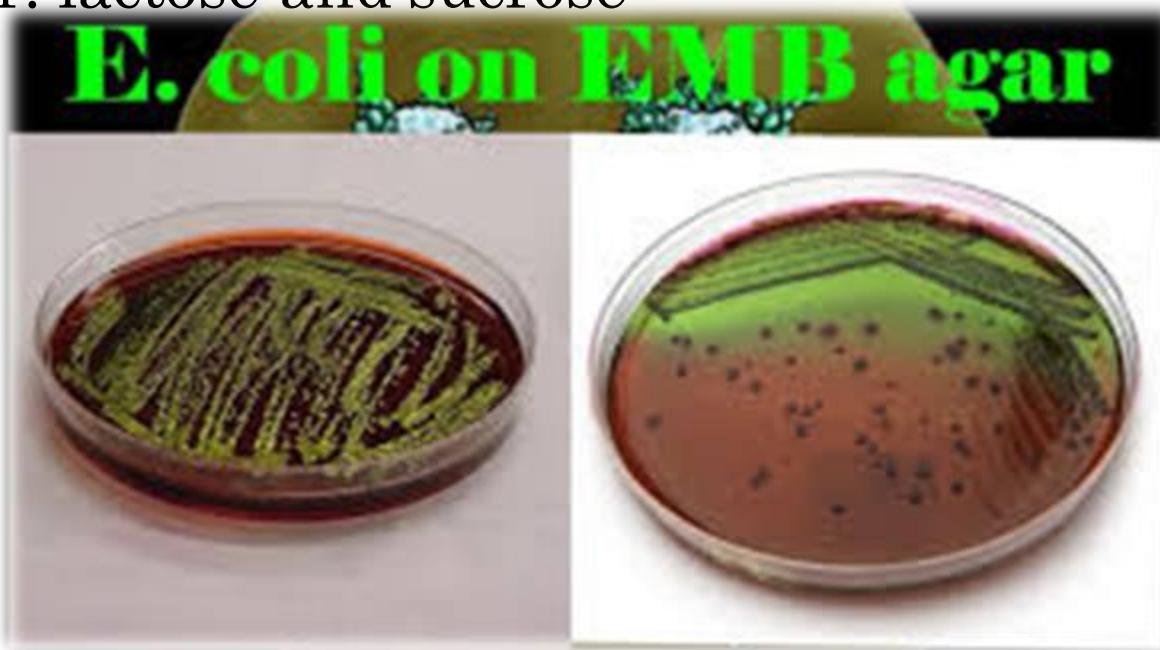
ENDO AGAR

- Endo agar : colonies are red with metallic sheen



EMB AGAR

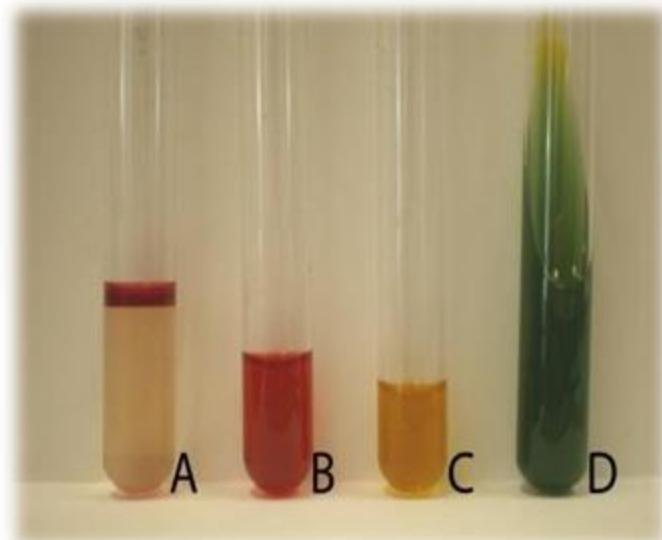
- Eosine methylene blue agar. It will produce brownish black colonies with greenish metallic sheen. It is characteristic appearance of *Escherichia coli*
- Sugar: lactose and sucrose



BIOCHEMICAL CHARACTERISTICS

- Catalase positive and oxidase negative
- **Nitrate reduction** test is positive
- **Imvic test:** Indole, Methyl Red, Voges Proskauer test, Citrate utilization
- *E. coli* ++--

Nitrate Reduction Test



EIJKMAN'S TEST

- It detect the ability of *E. coli* to produce acid and gas from lactose at 44°C. Other organism produce acid and gas at 37°C but not at 44°C
- Eijkman's test is a presumptive test for *E.coli*.
- So E.coli will give Emvic test (++-+-) which stands for Eijkman test, methyl red, vogus Prauskuer test, indole test, and citrate utilization test



GROWTH ON TSI AGAR SLANT

- In TSI agar sugars like sucrose, lactose and glucose present. E. coli can utilise all sugars
- Indicator in the medium is phenyl red.
- It will produce acid butt acid slant (yellow but and yellow slant) and shows gas production. So there will be breakage of the medium
- Organism is negative for urease production



ANTIGENS

- Important antigens are somatic antigen, flagellar antigen, capsular antigen and fimbrial antigen
- Over 165 somatic antigens present and based on somatic antigens over 165 seotypes are present
- Over 100 flagellar antigens present
- 3 types of capsular antigens present **L, A, B.**
- Fimbrial antigen is associated with entero toxin production
- The most significant adhesins in strains of *E. coli* producing disease in domestic animals are K88 (F4), K99 (F5), 987P (F6), F18 and F41
- There is an enterobacterial common antigen **ECA** in all organism of the family, a thermostable somatic antigen

TOXINS

- Important factors associated with virulence of *E. coli* are
- lipid A
- haemolysin
- heat labile and heat stable enterotoxin
- vero toxin (toxin for vero cells)
- CNTF (cytotoxic necrotising factor)



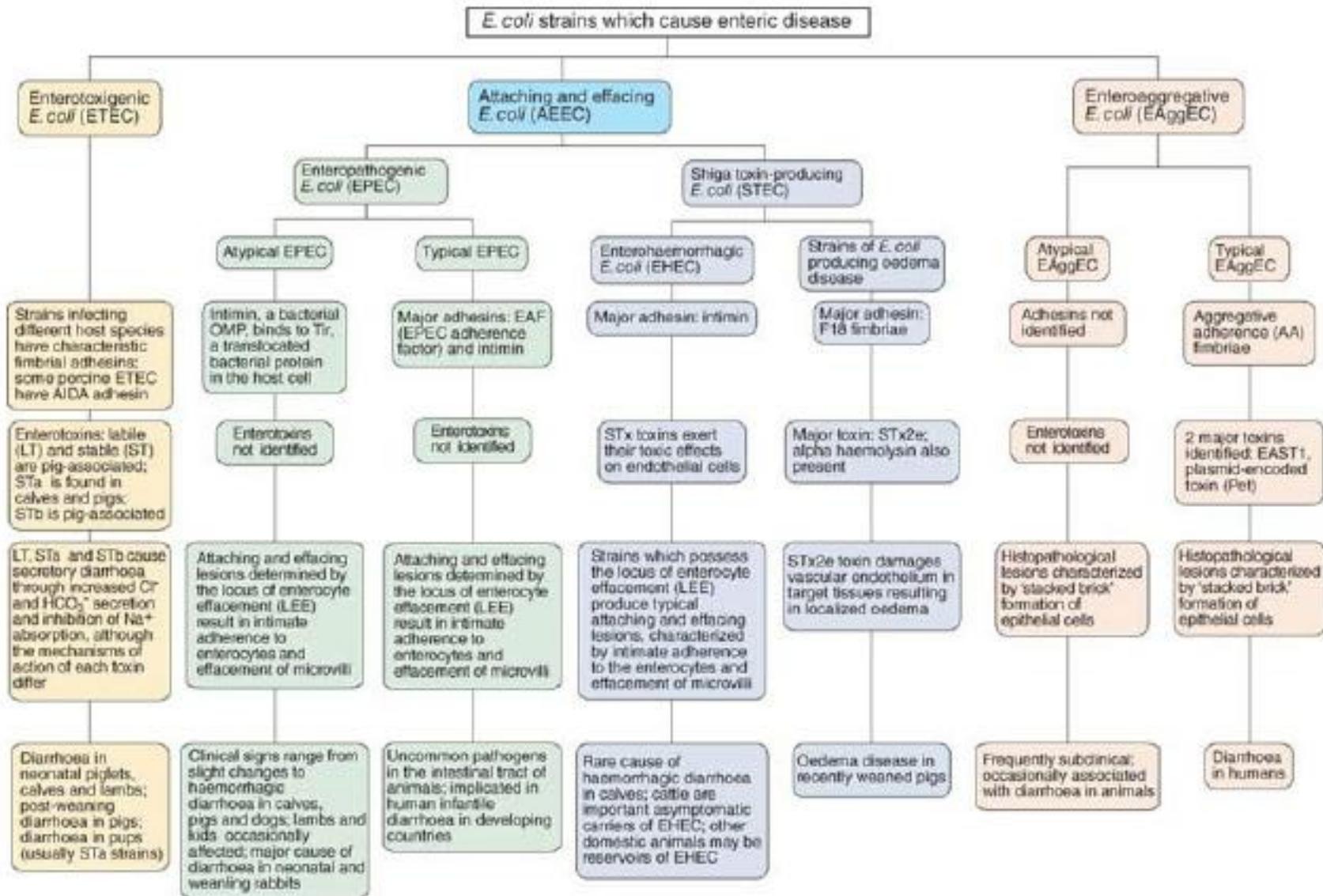
VIRULENCE FACTORS OF E. COLI

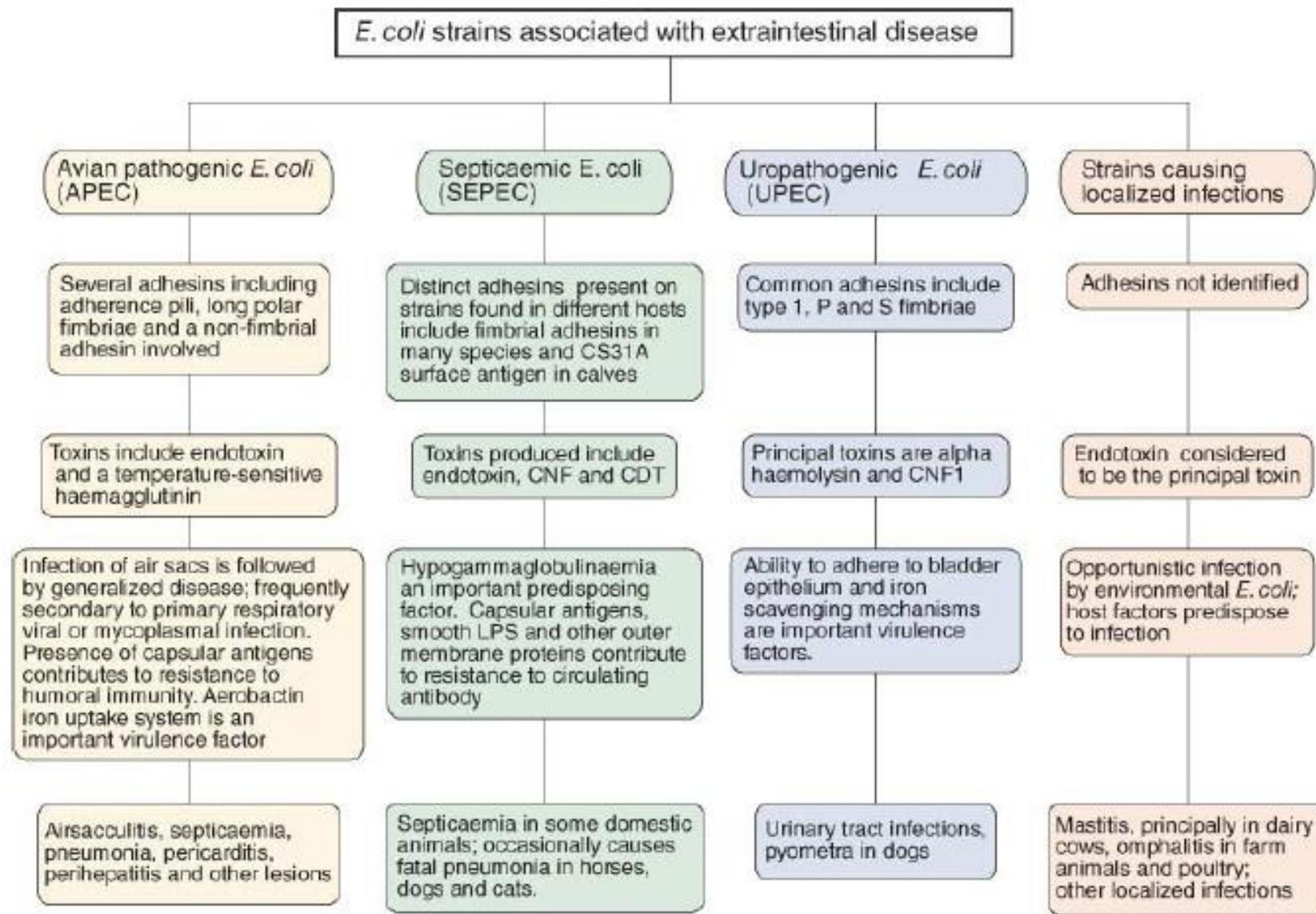
- Capsule: Capsular polysaccharides, which are produced by some *E. coli* strains, interfere with the phagocytic uptake of these organisms.
- Endotoxin, a lipopolysaccharide (LPS) component of the cell wall of Gram-negative organisms is released on death of the bacteria.
- It is composed of a lipid A moiety, core polysaccharide and specific side chains.
- The role of LPS in disease production includes pyrogenic activity, endothelial damage leading to disseminated intravascular coagulation, and endotoxic shock.
- These effects are of greatest significance in septicaemic disease.
- Fimbrial adhesins which are present on many strains of *E. coli* allow attachment to mucosal surfaces in the small intestine and in the lower urinary tract.

VIRULOTYPES/PATHOTYPES OF E.COLI

- Based on virulence E. coli can be classified as
 - Enteropathogenic *E. coli*
 - Enterotoxigenic *E. coli*
 - Enterohaemorrhagic *E. coli*
 - Enteroinvasive *E. coli*
 - Entero aggregative *E. coli*
 - Attaching and effacing *E. coli*
 - CTNF- PEC –cytotoxin necrotising factor positive *E. coli*







DISEASES

- In horse: joint ill, naval ill (i.e. infection affecting umbilical cord) and the condition called **sleepy foal disease**
- In cattle: colisepticaemia, colibacillosis and mastitis
- In calf: **calf scour or white scour**. The organism also produce joint ill or naval ill
- In pig, esp the unweaned pigs: piglet scour
- In weaned piglets: **oedema disease**
- In adult pigs: MMA: **Mastitis metritis agalactia syndrome**

- In sheep and goat: mastitis in adult animals and colibacillosis and colisepticaemia in young
- In poultry: colibacillosis, colisepticaemia, and coligranulaoma (**hjarre's disease**) characterised by granulomatous lesions in visceral organs
- In chicks causes omphalitis; inflammation of yolk sac also known as **mushy chick disease**
- in dogs causes septicaemia, and mortality
- the septicaemic condition known as **fading puppy disease** which causes severe mortality
- In laboratory animals mucoid enteritis
- In human being traveller's diarrhoea and meningitis

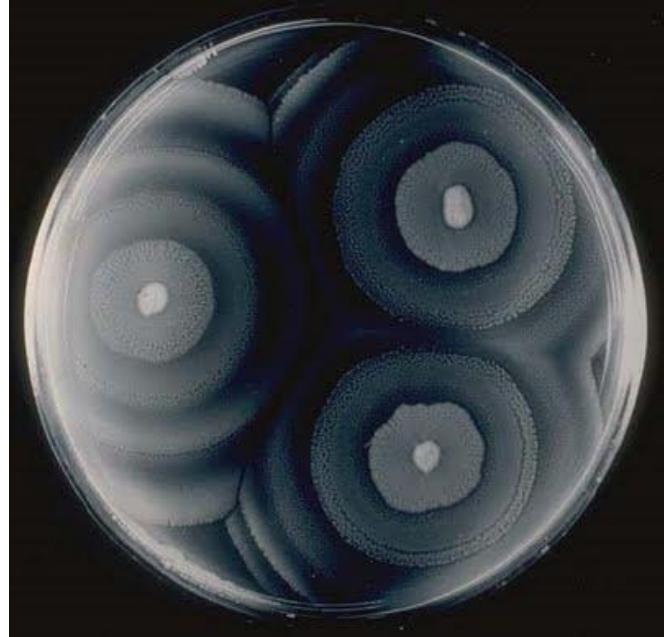
DIAGNOSIS AND CONTROL

- Isolation and identification of organism
- Treatment :Various antibiotics
- Proper hygienic conditions



GENUS: PROTEUS

- Type species: *Proteus vulgaris*
- Produce swarming type of motility
- Culture has fishy smell
- A non lactose fermenting organism
- Imvic test -+-+ based on these the organism simulate Salmonella (in cultural and biochemical characters)
- To differentiate Proteus from salmonella urease test is used, urease is negative for salmonella and positive for proteus
- Another test is phenylalanine deaminase test. Proteus is positive for this test(only organism positive for this test in this family) and Salmonella negative for this test



GENUS: KLEBSIELLA

- *Klebsiella pneumoniae*: Fried lander's bacillus
- Lactose fermenting organism
- Imvic test --++
- Positive for urease test



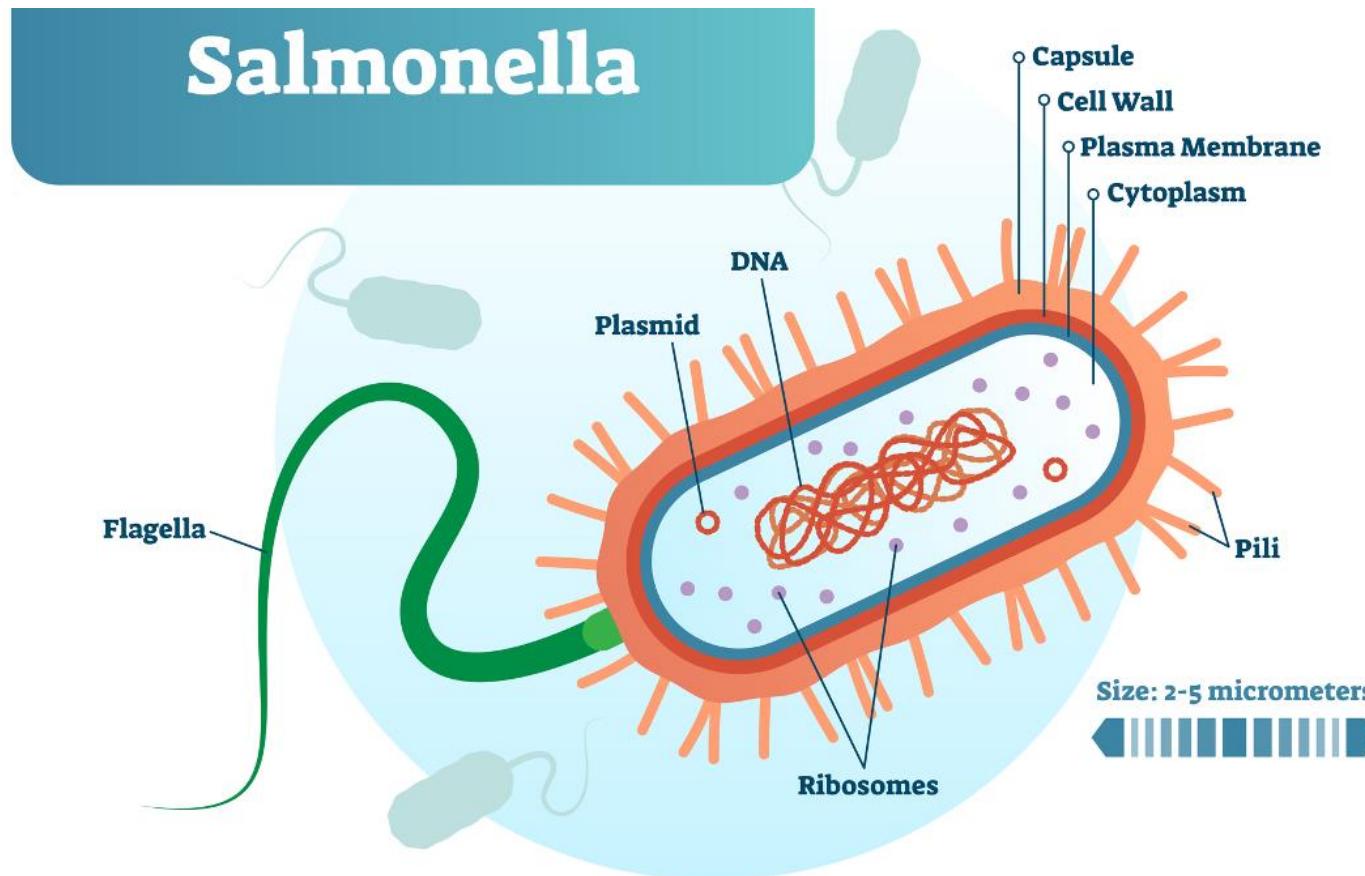
GENUS: ENTEROBACTER

- Type sp. *Enterobacter cloaca*
- Imvic test --++ (similar to Klebsiella)
- but Klebsiella is capsulated non motile organism
Enterobacter is motile
- *E.coli*, Klebsiella and Enterobacter are lactose fermenting organism together known as coliforms



GENUS:SALMONELLA

- Named after E. Salmon who worked on human typhoid



CONTD..

- According to latest classification there are only two species of salmonella
- *Salmonella bongori*
- *Salmonella enterica*
- *Salmonella enterica* is most important and has 6 sub species of these most important is *Salmonella enterica subspecies enterica*
- All other organism other than these two are serovars.
- About more than 2500 serovars are present



CONTD..

- The organism of this genus is highly host specific
- *Salmonella* Dublin: cattle
- *Salmonella* Pullorum, *Salmonella* Gallinarum: poultry
- *Salmonella* Choleraesuis: for pig
- *Salmonella* Abortusovis : goat and ram
- *Salmonella* Abortusequi : for horse
- *Salmonella* Typhi and *Salmonella* Paratyphi: human
- *Salmonella* Enteritidis and *Salmonella* Typhimurium are non host specific affect variety of animals and human being

MORPHOLOGY

- Gram negative bacilli, non capsulated, non spore forming
- All are motile except *Salmonella Pullorum* and *Salmonella Gallinarum*
- Most of them possess type I fimbriae which is mannose sensitive
- Organism also have mannose resistant fimbriae

GROWTH REQUIREMENT

- Aerobes and facultative anaerobes produce smooth colonies
- *Salmonella Pullorum* and *Salmonella Choleraesuis* produce dew drop like colonies



CULTURAL CHARACTERISTICS

- MaConkey agar : colourless colony
- BGA: Pink colony
- XLD agar: Red colonies with black centre because of H₂S production
- Other media are Salmonella Shigella agar (SS agar) DCA Deoxycholate Citrate agar and Bismuth Sulphite agar
- In broth it will produce uniform turbidity

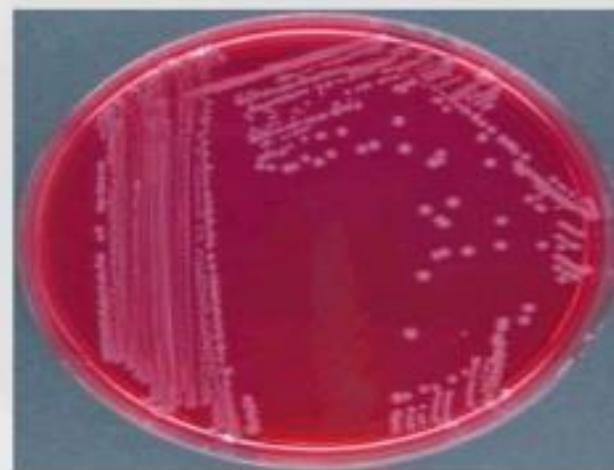


SALMONELLA ON BGA

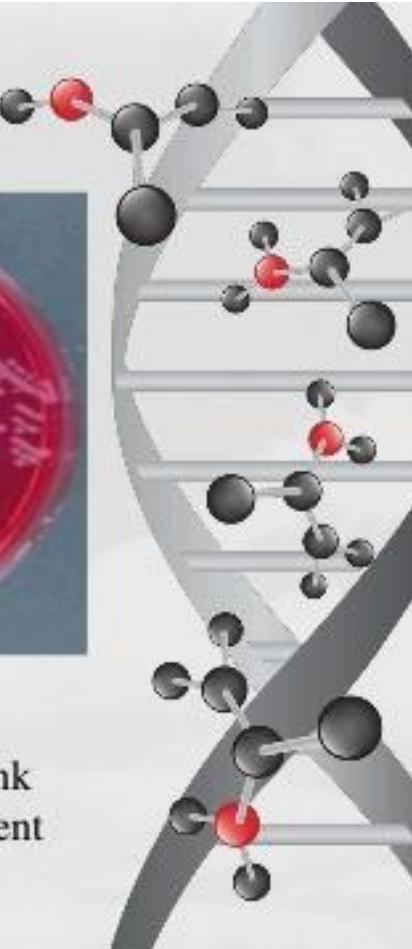
Salmonella on BGA



an uninoculated plate



Salmonella on BGA . The colonies are small, opaque, pink or white. Can also be transparent and colorless.



XLD AGAR

XLD agar:
Red
colonies
with black
centre
because of
H₂S
production



a on XLD



Salmonella

BIOCHEMICAL CHARACTERISTICS

- Organism positive for catalase and negative for oxidase
- Nitrate reduction positive
- Can not ferment lactose
- *Salmonella Gallinarum* is differentiated from *Salmonella Pullorum* by ability to conduct maltose and dglucitol fermentation
- Imvic test is -+-+
- On TSI acid but, alkaline slant and organism produce H₂S.
- The organism is negative for urease test



KAUFFMAN WHITE SCHEME OF SEROTYPING OF SALMONELLA

- Antigens two types : somatic and flagellar antigen
- Somatic antigen designated by arabic numerals from 1 to 67. single organism may possess more than one somatic antigen
- Organism possessing some somatic antigen are included in the same somatic group
- For example *Salmonella Pullorum* important somatic antigen are 9 and 12. for *Salmonella Gallinarum* somatic antigen are 1,9,12. so both in same somatic serogroup

- Then classification is based on flagellar antigen
- Two flagellar antigen as phase I and phase II antigen
phase I flagellar antigen designated by a to z (small letter)
- if number is more than 26 then designated as z1, z2, z3 etc.
- Phase II flagellar antigens designated by arabic numerals. If an organism possess both phase I and phase II antigen , they are known as biphasic or diphasic organism.
- Apart from this antigen *Salmonella Typhi* possess Vi antigen (V indicate virulence) Vi seen only in newly isolated strain and may be lost as a result of subculturing
- Vi antigen may be capsular antigen

- Under Kauffmann white scheme antigenic notation for *Salmonella Pullorum* is 9,12:-----:-----
- Antigenic notation has three part, first part represent somatic antigen, 2nd part phase I flagellar antigen and third part represent phase II flagellar antigen
- Each part is separated by a colon: each component in same part separated by comma
- for *Salmonella Paratyphi* the antigenic notation is 1,4,[5],12:b:12
- underline for '1' indicate this particular antigen is having phage mediated transmission
- [] indicate this antigen seen only in particular strain

PATHOGENICITY

- In human being *S. yphi* : Typhoid
- Most common diagnostic serological test for typhoid is Widal test
- *S. Paratyphi*: paratyphoid in human being
- *Salmonella Enteritidis* and *Salmonella Typhimurium* causes food poisoning in human being
- *Salmonella Abortusovis* : abortion in sheep
- *Salmonella Abortusequi*: abortion in equine
- *Salmonella Choleraesuis*: secondary invaders of hog cholerae or swine fever
- *Salmonella Dublin*: Enteritis, septicaemia and meningitis in calf and abortion in adult animal

PULLORUM DISEASE

- Etiology: *Salmonella Pullorum*
- Bacillary white diarrhoea
- characterized by chalky white pasty feces in birds
below 2 weeks of age
- Transmission by feaco oral route
- Egg transmission (bacteria from feces enter through the holes of egg) and also transovarian transmission
- In adult birds it mainly affect ovary and adult birds act as carrier
- Most important lesion is pedunculate ovary



PEDUNCULATED OVARY IN PULLORAM DISEASE



5/4/2024 Dr. Bincy Joseph

DIAGNOSIS OF SALMONELLA PULLORUM INFECTION

- Tendative diagnosis based on history, symptoms and lesions
- **Confirmatory diagnosis**
- Isolation and identification of organism from feaces, liver and yolk. This material is first inoculated on enrichment medium as selenite broth , tetrathionate broth and incubate at 45oC for 18-24 hours. Then subculture on selective media such as Mcconkey agar, Salmonella Shigella agar, DCA and BGA
- **Whole blood agglutination test :** for flock diagnosis of salmonellosis in field condition. Take one drop of whole blood and 1 drop of crystal violet coloured antigen. In positive cases agglutination in 1-2 minutes

FOWL TYPHOID

- Etiology: *Salmonella Gallinarum*
- Affect mainly adult birds only
- Transmission by feed and water
- There is egg transmission and transovarian transmission
- Two more organism affecting birds are *Salmonella Enteritidis* and *Salmonella Typhimurium* causing condition known as paratyphoid
- Reservoir of salmonellosis in birds is rodents



DIAGNOSIS OF SALMONELLA GALLINARUM

- Tendative diagnosis (history, only adult birds are affected)
- Lesions: brown coloured liver, spleenomegaly and enteritis
- Isolation of organism from liver, intestine and bone marrow
- whole blood test and rapid agglutination
- Crystal violet antigen of *Salmonella pullorum* is used for this also because common 9, 12 antigen

Bronzed Greenish liver



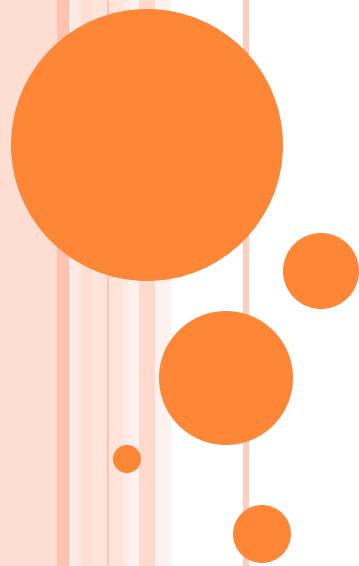
CONTROL

- Periodical conduct of whole blood test
- Slaughter of reactors
- burial of litter properly
- disinfect equipment with 1% phenol or 0.5% cresol
- fumigation of incubators
- Egg dipping
- Egg should not be used for hatching from affected birds
- New stock is introduced after slaughter only after 4-6 months
- feed storage area and poultry shed should be made rat proof





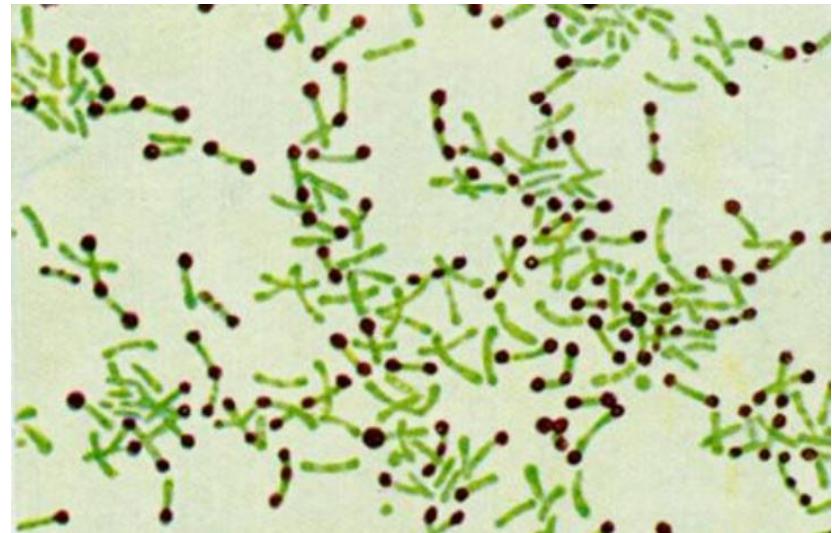
Thank you!



GENUS CORYNEBACTERIUM

Dr. Bincy Joseph
Assistant Professor
PGIVER, Jaipur

CLASSIFICATION



- Kingdom Bacteria
- Phylum Actinobacteria
- Order Actinomycetales
- Family Corynebacteriaceae
- Genus *Corynebacterium*
- Species *Corynebacterium bovis*



HISTORY

- Genus *Corynebacterium* was originally created by Lehmann and Neumann (1896)
- *Corynebacterium diphtheriae* (**Klebs–Löffler bacillus**)

It was discovered in 1884 by German bacteriologists **Edwin Klebs and Friedrich Löffler**

- Various selective media were formulated for *C. diphtheriae*, by the following scientist
 - Frobisher, 1937 - Cystine-tellurite blood agar
 - Hoyle, 1941 - Hoyle's lysed blood tellurite agar
 - Tinsdale, 1947 - Tinsdale agar

INTRODUCTION

- Gram positive
- Coccobacilli with some **metachromatin granules**
- Pleomorphic irregularly stained
- Stained tissue smears reveal groups of cells in parallel (Palisades) or cells at sharp angles to each other “V” or “Y” configurations (**Chinese letter or Cuneiform arrangement**).



HABITAT AND ECOLOGY

- Soil born and often found in manure.
- Survive as commensals on normal mucous membrane and skin of cattle and other domestic animals.
- Chronic cases and adult animals are the common carriers.
- Most species of *Corynebacterium* are opportunistic pathogens.
- Present in the intestines of horses and persist for long periods in the manure and litter of stables.
- As commensals, they can be found on the skin or on mucous membranes of animals



MORPHOLOGY

- Gram-positive slender rod with a tendency to clubbing at one or both ends
- Non-sporing, Non-motile, Non-capsulated , Non-acid fast.
- Palisades or Chinese letter or Cuneiform arrangement



- Small, pleomorphic (club-shaped), Gram-positive rods
- Cell size is 2 to 6 μm long and 0.5 to 1 μm in diameter.
- Modified ZN staining positive
- Straight to slightly curved, often with tapered ends
- Coryne bacterial cell walls contain thin spots which leads to some Gram variability and "ballooning" that produces a "club-shaped" cell.
- Lipid-rich cell wall contains meso-diaminopimelic acid, arabino-galactan polymers, and short-chain mycolic acids



GRANULES

- High energy phosphate granules – polymetaphosphate.
- Strongly Gram positive than the rest of the bacterial cell.
- Stained with Loeffler's methylene blue, the granules take up a reddish purple color and hence they are called metachromatic granules.
- They are called as **volutin** or Babes Ernst Granules.

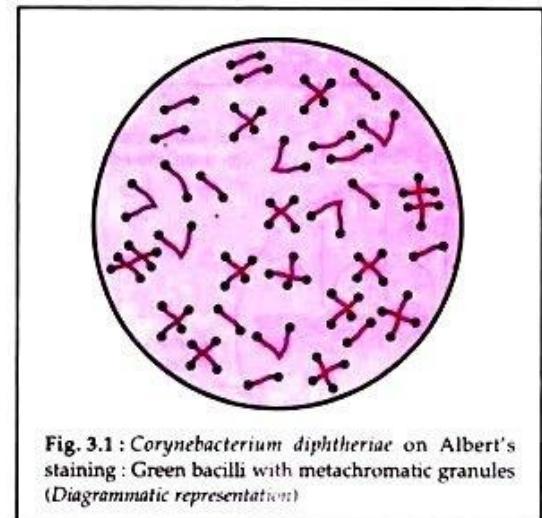
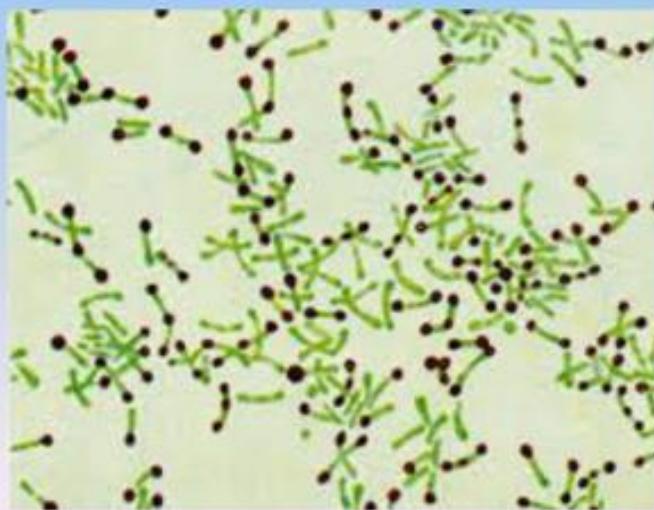


Fig. 3.1 : *Corynebacterium diphtheriae* on Albert's staining : Green bacilli with metachromatic granules (Diagrammatic representation)

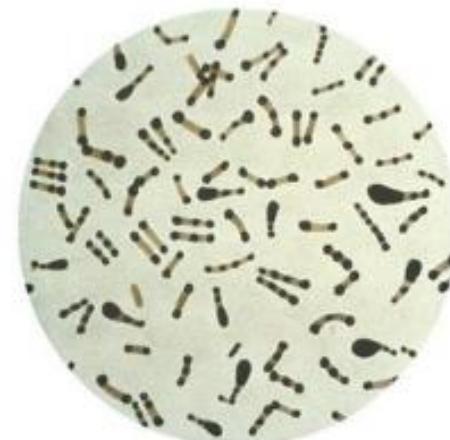
GRANULES

- They are often situated at the poles of the bacilli and are called **polar bodies**.
- Special stains, such as **Albert's**, **Neisser's** and **Ponder's** have been devised for demonstrating the granules clearly.

Neisser's technique



Albert stain



CULTURAL CHARACTERISTICS

- Growth on enriched media (Fastidious) scanty- a gray **pin point** to small colonies.
- Enrichment with blood, serum or egg is necessary for good growth.
- Optimum temperature for growth is 37°C
- Optimum pH is 7.2.
- Aerobe and facultative anaerobe.
- Some of them are beta hemolytic while renale groups are non-haemolytic

- Diptheroids are readily destroyed by heat, 60°C for one hour.
- They are highly susceptible to disinfectants.
- It is more resistant to the action of light, desiccation and freezing.
- *Corynebacterium equi* is resistant to 2.5% oxalic acid for one hour.



- *Corynebacterium bovis* is a lipophilic bacterium which produces small, white, dry, non-haemolytic colonies in the well of plates inoculated with a bovine milk sample.
- *Corynebacteriurn kutscheri* produces whitish colonies. Occasional isolates are haemolytic.
- *Corynebacteriurn pseudotuberculosis* has small, whitish colonies surrounded by a narrow zone of complete haemolysis, which may not be evident for up to 72 hours. After several days, the colonies become dry, crumbly and cream-coloured

- Members of the *C. renale* group produce small non-haemolytic colonies after incubation for 24 hours. Pigment production after incubation for 48 hours is one of the differentiating features of the three species in the group

Table 10.2 Differentiation of bacteria in the *Corynebacterium renale* group.

Feature	<i>C. renale</i> (type I)	<i>C. pilosum</i> (type II)	<i>C. cystitidis</i> (type III)
Colour of colony	Pale yellow	Yellow	White
Growth in broth at pH 5.4	+	-	-
Nitrate reduction	-	+	-
Acid from xylose	-	-	+
Acid from starch	-	+	+
Casein digestion	+	-	-
Hydrolysis of Tween 80	-	-	+

Enhancement of haemolysis test

- The haemolysis produced by *C. pseudotuberculosis* is enhanced when the organisms are inoculated across a streak of *Rhodococcus equi*

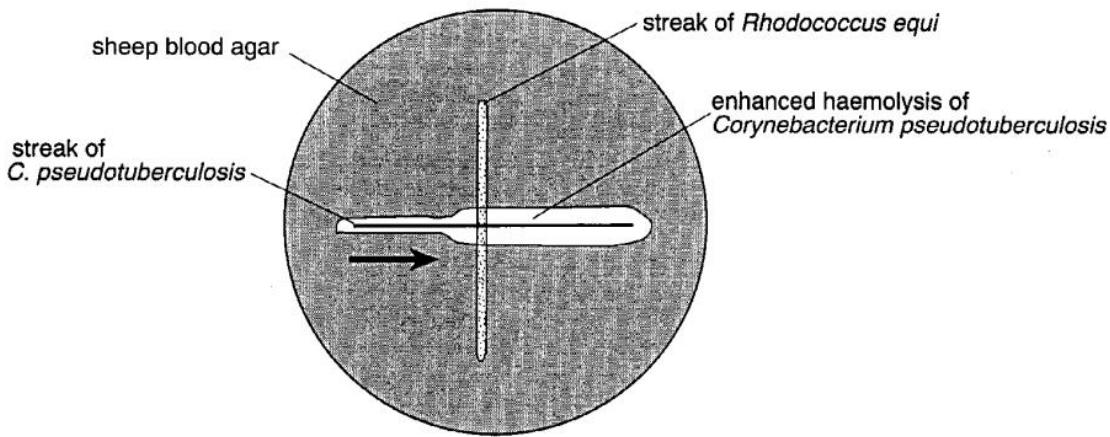


Figure 10.2 Enhancement of haemolysis test for *Corynebacterium pseudotuberculosis*. When a streak of *C. pseudotuberculosis* is drawn at right angles (arrow) across a streak of *Rhodococcus equi*, enhancement of haemolysis occurs.

BIOCHEMICAL PROPERTIES

- Catalase-positive
- Oxidase negative
- Except *Corynebacterium bovis* others are urease positive.
- Renale group is very strong urease positive (less than one hour).
- All diphtheroids ferment sugar except *Rhodococcus equi*.
- *Corynebacterium bovis* and *Corynebacterium renale* ferment both glucose and maltose.
- Two biotypes of *Corynebacterium ovis* are recognized.
- Ovine/Caprine strains lack nitrate-reducing capacity, while the equine/bovine strains usually reduce nitrate.

CORYNEBACTERIUM OF VETERINARY IMPORTANCE

- *Corynebacterium pseudotuberculosis*
(*Corynebacterium ovis* or Preisz Nocard Bacillus)
- *Corynebacterium kutscheri*
- *Corynebacterium renale*
- *Corynebacterium cystitidis*
- *Corynebacterium pilosum*
- *Corynebacterium bovis*
- *Arcanobacterium pyogenes* (*C. Pyogenes*)
- *Rhodococcus equi* (*Corynebacterium equi*)



IMPORTANT ANIMAL DISEASES

Table 10.1 The pathogenic corynebacteria, their hosts, usual habitats and the disease conditions which they produce.

Pathogen	Host	Disease condition	Usual habitat
<i>Corynebacterium bovis</i>	Cattle	Subclinical mastitis	Teat cistern
<i>C. kutscheri</i>	Laboratory rodents	Superficial abscesses, caseopurulent foci in liver, lungs and lymph nodes	Mucous membranes, environment
<i>C. pseudotuberculosis</i>			
Non-nitrate-reducing biotype	Sheep, goats	Caseous lymphadenitis	Skin, mucous membranes, environment
Nitrate-reducing biotype	Horses, cattle	Ulcerative lymphangitis, abscesses	Environment
<i>C. renale</i> group			
<i>C. renale</i> (type I)	Cattle	Cystitis, pyelonephritis	Lower urogenital tracts of cows and bulls
	Sheep and goats	Ulcerative (enzootic) balanoposthitis	Prepuce
<i>C. pilosum</i> (type II)	Cattle	Cystitis, pyelonephritis	Bovine urogenital tract
<i>C. cystitidis</i> (type III)	Cattle	Severe cystitis, rarely pyelonephritis	Bovine urogenital tract
<i>C. ulcerans</i>	Cattle	Mastitis	Human pharyngeal mucosa

IMPORTANT ANIMAL DISEASES

- Bovine pyelonephritis, ureteritis and cystitis is caused by *C. renale* group, previously designated as type I, II, and III. They have been (*C. renale Group*) classified into three species on the basis of their distinct pili and biochemical properties.
 1. *C. renale* Mostly affects cows and causes chronic cystitis, pyelonephritis (Important).
 2. *C. pilosum* <4% and rare cause of pyelonephritis in cows and causes the mildest form of cystitis.
 3. *C. cystitis*. 90% of the bulls are carriers. It can cause severe hemorrhagic cystitis in cows that may lead to chronic pyelonephritis. Transmitted by the bull to the susceptible cow during coitus.



IMPORTANT ANIMAL DISEASES

- Non-nitrate reducing biotype of *Corynebacterium pseudotuberculosis* causes Caseous lymphadenitis (CLA) in sheep and goats
- Nitrate reducing biotype of *Corynebacterium pseudotuberculosis* causes Ulcerative lymphangitis in Horses & Cattle
- *Corynebacterium bovis* causes Subclinical mastitis in cattle
- *Rhodococcus equi* (*Corynebacterium equi*) cause Suppurative bronchopneumonia in foals (2-4 months) and Cervical lymphadenitis in Pigs
- *Arcanobacterium pyogenes* (*Actinomyces pyogenes*) causes *Summer mastitis* – a mixed infection with *Peptostreptococcus indolicus*



CORYNEBACTERIUM PSEUDOTUBERCULOSIS

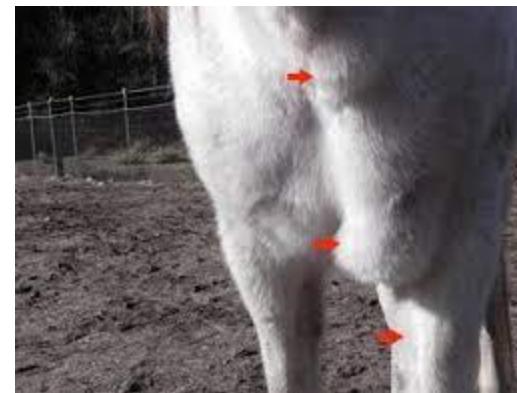
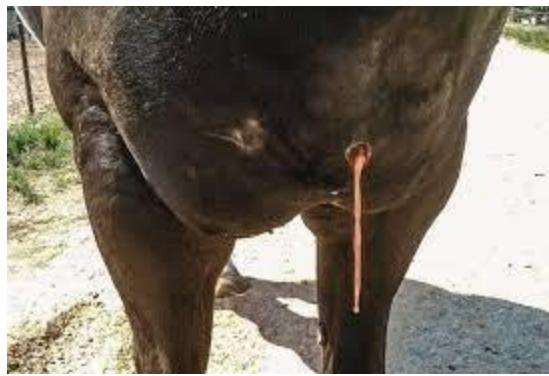
- Non-nitrate reducing biotype causes Caseous lymphadenitis (CLA) in sheep and goats
- Nitrate reducing biotype causes Ulcerative lymphangitis in Horses & Cattle
- Chronic abscessation: peripheral LN
- Thick caseous exudate, slightly greenish



Figure 1 Non-movable and solid mass at



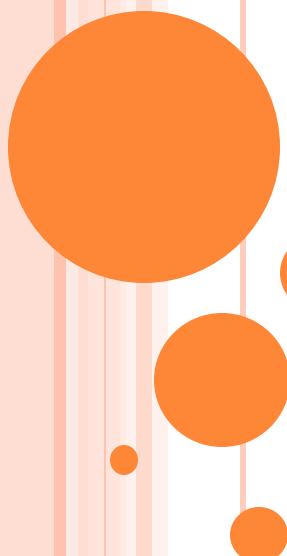
ULCERATIVE LYMPHANGITIS/ PIGEON FEVER



ULCERATIVE BALANOPOSTHITIS

- Ulcerative (enzootic) balanoposthitis (**pizzle rot**), particularly common in Merino sheep and Angora goats, is caused by *C. Renale*
- Characterized by ulceration around the preputial orifice, with a brownish crust developing over the lesion.
- Similar lesions sometimes occur on the vulva in ewes.





STREPTOCOCCUS

Dr. Bincy Joseph
PGIVER, Jaipur

MORPHOLOGY

- Gram positive , non motile except *Lactococcus lactis* non spore forming cocci occurs singly, in pairs or in chains.
- Long chains are observed in *Streptococcus equi*
- *Streptococcus pyogenes* round to ovoid and occur in pairs or chains of varying length
- *Streptococcus pneumoniae* occur as diplococcus and are lancet shaped. Also known as pneumococcus
- In older cultures they will lose Gram positive character.
- Strict anaerobe/ facultative anaerobe (catalase and oxidase negative)



Lactococcus lactis



Streptococcus equi

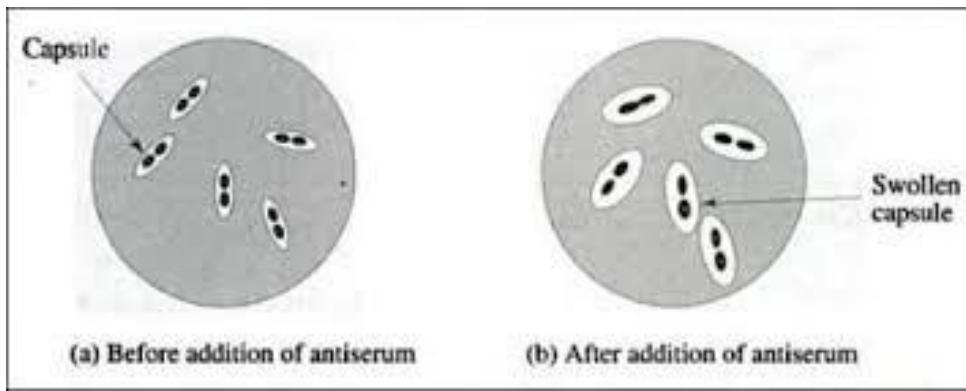


Streptococcus pneumoniae



CAPSULE

- *Streptococcus pyogenes* have hyaluronic acid capsule
- *Streptococcus pneumoniae* have polysaccharide capsule
- *Streptococcus pneumoniae* give **Quellung reaction**, it is a test for capsule demonstration
- In quellung reacton colonies are mixed with hyper immune sera against capsule , there will be swelling of capsule



CLASSIFICATION OF STREPTOCOCCI

- Streptococci can be classified on the basis of
- Growth characteristics
- Haemolysis,
- Serologic specificity



GROWTH CHARACTERISTICS

- Pyogenic streptococci: *Streptococcus pyogenes*
- Oral streptococci: *Streptococcus salivarius*
- Enterococci: *Enterococcus faecalis*
- Lactic streptococci: *Lactococcus lactis*
- Anaerobic streptococci
- Other streptococci



HAEMOLYTIC PATTERN

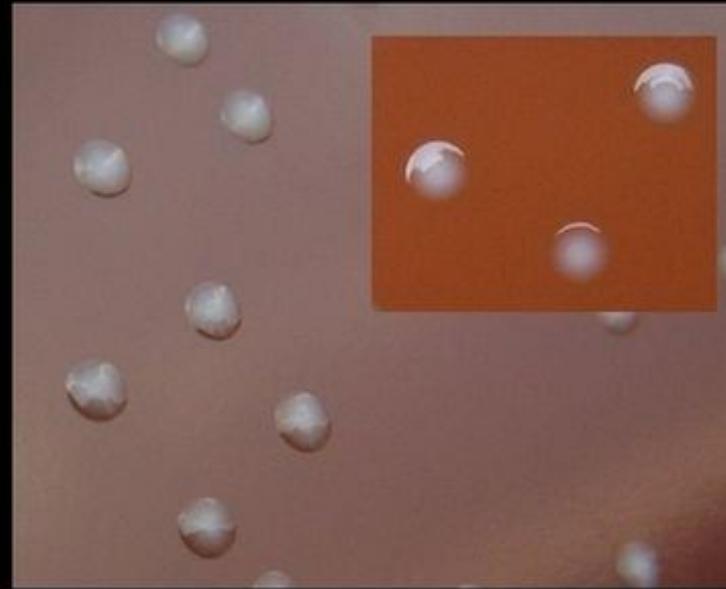
- **α haemolysis:** Partial haemolysis with a zone of green coloration around the colonies.
- **β haemolysis:** complete clear zone of haemolysis around the colonies.
- **γ haemolysis:** No detectable haemolysis
- **α' haemolysis:** a small zone of partial haemolysis followed by a zone of complete haemolysis





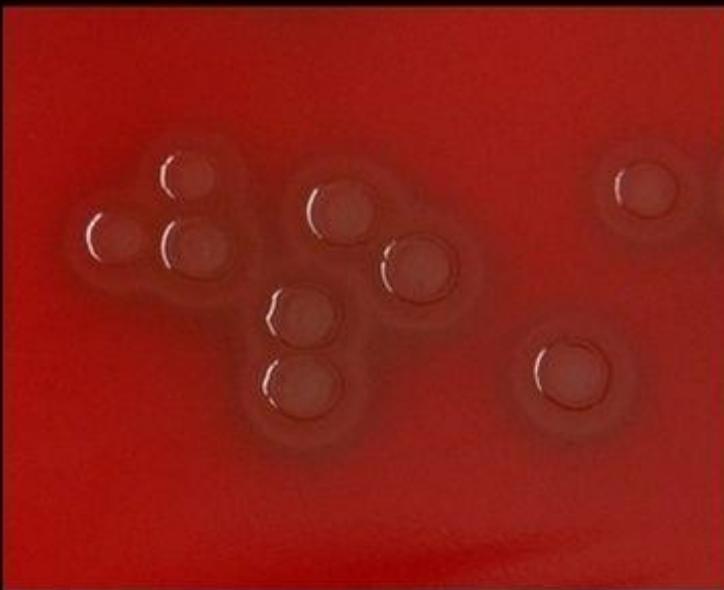
Klebsiella pneumoniae

gamma hemolysis



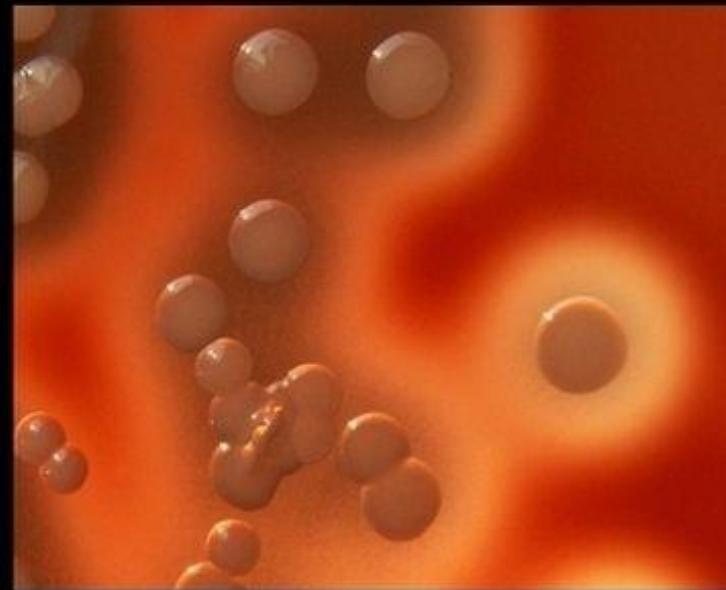
Enterococcus faecalis

gamma hemolysis



Streptococcus pneumoniae

alpha hemolysis



Staphylococcus aureus

beta hemolysis

LANCEFIELD CLASSIFICATION

- by Rabecca Lance field in 1926
- This classification of streptococci is based on the serologic difference in a carbohydrate substance called '**C**' **substance** found on the cell wall of streptococci.
- Here streptococci are divided into different groups and each group is further divided into different types.
- Precipitation test is used to differentiate each group.
- Letters A,B,C used to designate different groups and Arabian numerals used to designate different types
- Typing within the group is based on the serological difference in M protein.

LANCEFIELD CLASSIFICATION

Classification

- Group A - *Streptococcus pyogenes*
- Group B - *Streptococcus agalactiae*
- Group C - *Streptococcus equisimilis*, *Streptococcus equi*, *Streptococcus zooepidemicus*, *Streptococcus dysgalactiae*
- Group D - *Enterococci*, *Streptococcus bovis*
- Group E - *Streptococcus milleri* and *mutans*
- Group F - *Streptococcus anginosus*
- Group G - *Streptococcus canis* and *Streptococcus dysgalactiae*
- Group H - *Streptococcus sanguis*
- Group L - *Streptococcus dysgalactiae*
- Group N - *Lactococcus lactis*
- Group R&S - *Streptococcus suis*
- other *Streptococcus* species are classified as 'non-Lancefield Streptococci'



Lancefield group	Species	Haemo-lysis	Host(s)	Disease	Natural habitat (if known)
A	<i>S. pyogenes</i>	β	Humans	Scarlet fever, septic sore throat, puerperal fever, erysipelas, abscesses and rheumatic fever	Human upper respiratory tract
			Cattle	Mastitis (rare)	
			Foals	Lymphangitis	
B	<i>S. agalactiae</i>	β (α , γ)	Cattle, sheep and goats	Chronic mastitis	Milk ducts
			Humans and dogs	Neonatal septicaemia	Maternal vagina
			Cats	Kidney and uterine infections	
C	<i>S. dysgalactiae</i>	α (β , γ)	Cattle	Acute mastitis	Buccal cavity and genitalia
			Lambs	Polyarthritis	
			Horses	Abscesses, endometritis and mastitis	Skin and vagina
	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>	β	Pigs, cattle, dogs and birds	Various suppurative conditions	
			Horses	Strangles, genital and suppurative conditions, mastitis and purpura haemorrhagica	Equine tonsils
			Horses	Mastitis, abortion, secondary pneumonia and navel infections	Vagina and skin
			Cattle	Metritis and mastitis	

Lancefield group	Species	Haemo-lysis	Host(s)	Disease	Natural habitat (if known)
D	<i>Enterococcus faecalis</i> <i>E. faecium</i> <i>E. durans</i>	α (β , γ)	Many species	Opportunistic infections such as septicaemia in chickens, bovine mastitis, endocarditis in cattle and lambs, and urinary-tract infections in dogs	Intestinal tract of many animals
	<i>S. equinus</i> <i>S. bovis</i>	α	Many species	Opportunistic infections	Intestinal tract of many animals
E (P, U, V)	<i>S. porcinus</i>	β	Pigs	Jowl abscesses and lymphadenitis	Mucous membranes
G	<i>S. canis</i>	β	Carnivores	Neonatal septicaemia. Genital, skin and wound infections	Genital tract and anal mucosa
			Cattle	Occasional mastitis	
N	<i>Lactococcus lactis</i>	α	Cattle	Unknown pathogenicity	Milk, plants and tonsils of pigs fed on whey
Q	<i>Enterococcus avium</i>	α , γ	Many species	Unknown pathogenicity	Faeces of birds and mammals
R(D)	<i>S. suis</i> type 2	α	Pigs (weaning to 6 months)	Meningitis and arthritis	Tonsils and nasal cavity
			Humans	Meningitis and septicaemia	Pigs
S(D)	<i>S. suis</i> type 1	α (β)	Pigs (2–4 weeks old)	Meningitis, arthritis, pneumonia and septicaemia	Tonsils and nasal cavity
Ungroupable	<i>S. uberis</i>	α (γ)	Cattle	Mastitis	Skin, vagina and

CULTURAL CHARACTERISTICS

- The bacteria grow well in ordinary laboratory medium enriched with blood or serum.
- *Enterococcus faecalis* grow at high temperature of 45°C.
- Primary isolation can be done in sheep or ox blood agar
- Blood agar and Edwards medium are the most preferred medium for streptococci
- Small round smooth glistening dew drop like colonies are produced
- Virulent streptococci produce matt colonies and less virulent organism produce glossy colonies



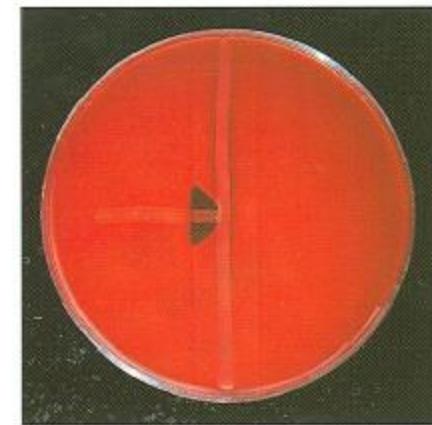
CONTD..

- Produce α, β,γ, and α' haemolysis
- CAMP test is used for identification of *Streptococcus agalactiae*
- ***Streptococcus bovis*, *Streptococcus uberis* and *Enterococcus faecalis*** will grow on Mc Conkey agar.
- In broth organism grow and produce faint growth with uniform turbidity.
- *Streptococcus agalactiae* produce long chains of deposits on the sides of the tube and supernatant will be clear.



CAMP TEST

- A culture of *Staphylococcus aureus* with wide zone of partial haemolysis is streaked across the centre of a sheep or ox blood agar plates
- A streak of suspect group B *Streptococcus* is made at right angles to and taken to within 1 to 1.5 mm of the *Staphylococcal* streak
- The plate is incubated at 37°C for 18-24 hours
- A positive CAMP test is indicated by an arrow head of complete haemolysis
- The group B streptococci produce a diffusable metabolite that complete the lysis of red cells, only partially lysed by beta haemolysisn of the *Staphylococcus*

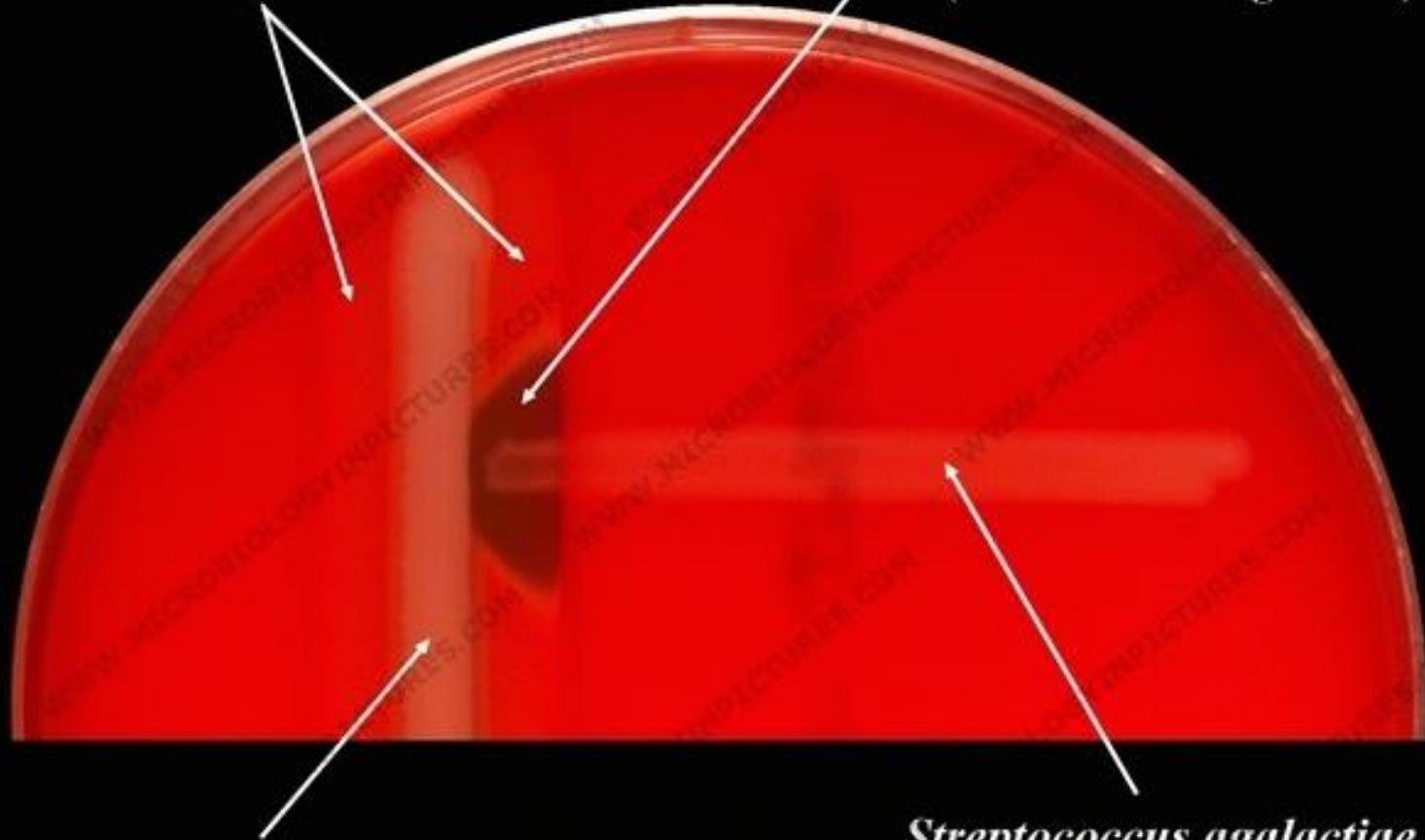


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zone of hemolysis of
S.aureus

an augmentation of the effect of *S.aureus*
β-hemolysin on erythrocytes
(arrowhead configuration)



Hans N.

CAMP reaction

©

BIOCHEMICAL CHARACTERISTICS

- They are **catalase** and **oxidase negative** and fermentative.
- Hydrolyses aesculin and sodium hippurate
- Reduces methylene blue milk
- Able to grow in the presence of 6.5% NaCl
- **Catalase negative** test differentiate Streptococcus from Staphylococcus
- ***Streptococcus agalactiae*** positive for hippurate hydrolysis
- ***Streptococcus uberis* & *Enterococcus faecalis*** positive for aesculin hydrolysis



PATHOGENESIS

- The infection can be either endogenous or exogenous
- Exogenous infection is through inhalation, ingestion or direct contact with fomites.
- The streptococcal infection is characterized by production of pus (pyogenic infection).
- When pyogenic bacteria invade the system, they stimulate inflammatory response that is characterized by vascular dilation and exudation of plasma and neutrophils.
- Neutrophils phagocytose the bacteria and kill the cells.
- But some bacteria can grow in neutrophils resulting in production of toxins which kills phagocytic cells.
- The enzymes liberated by dead phagocytic cells liquefy dead tissues and phagocytic cells.
- The liquefied mass is yellow and thick consistency of pus due to deoxyribonucleoprotein.
- Streptococcal infection is generally localized and rarely it become septicaemic or bacteraemic.

VIRULENCE FACTORS OF STREPTOCOCCI

- Major virulence factor of Streptococci are surface M protein and hyaluronic acid
- Haemolysin : Streptolysin O (oxygen labile haemolysin) and Steptolysin S (Oxygen stable haemolysin) which are toxic for neutrophils and macrophages.
- Streptokinase: Also known as Fibrinolysin. It activates plasminogen to plasmin which prevents the formation of fibrin clots. So it is used in cardiac arrest for dissolving the clots.
- DNases A,B,C and D: Also known as streptodornase. Assist in the production of substance required for growth.
- Hyaluronidase A: A major virulence factor which promotes the spread of organism in tissues.
- Erythrogenic toxins A,B,C: responsible for rashes in Scarlet fever
- NADases: kills the phagocytic cells
- Proteinase, lipoproteinase, Amylase, and Esterase

DIAGNOSIS

- Materials for diagnosis: Pus, joint fluid, milk , organs, blood swab, meningeal swab
- Examination of culture smear by Gram staining method and also milk smear by Newman's staining method.
- Biochemical reaction
 - a) haemolytic pattern
 - b) Differentiation in fermentation of sugars like trehalose, sorbitol, mannose, salicin, lactose, raffinose, inulin, esculin *etc.*
 - c) Hydrolysis of esculin and sodium hippurate
 - d) Reduction of methylene blue milk and preference of 6.5% sodium chloride for growth.



CULTIVATION

- The medium commonly used are nutrient agar, blood agar or **Edward's medium**.
- The Edwards medium contains crystal violet and thallium acetate. Is the selective medium for streptococcus
- Most pyogenic bacteria produce **haemolysis**
- **CAMP test** (Christie, Atkinson, Munch, Peterson): It is a presumptive test for diagnosis of ***Streptococcus dysagalactiae***. The test is based on the ability of streptococcal organism to complete partial haemolysis produced by *Staphylococcus aureus*.
- **Hydrolysis of esculin:** Esculin agar selective or differential media that is used primarily to distinguish faecal streptococci (*Enterococcus* species) from other streptococcal organism.
- *Enterococcus* is the only organism that can hydrolyse esculin
- The hydrolysed esculin complexed with iron to form a dark black brown colour in the tube.



BILE ESCULIN TEST



Name of the test: Bile Esculin test

Example A: Positive - Group D streptococcus (Enterococcus species)

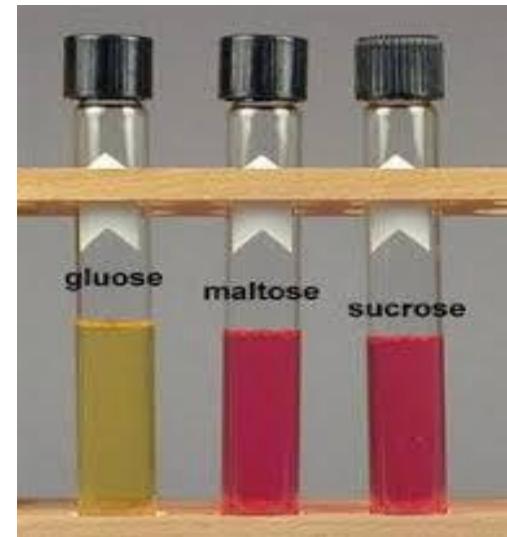
Example B: Negative - Group B streptococcus

Principle: The selective agent **bile**, inhibits most gram positive bacteria.

Esculin in the medium is hydrolyzed to esculetin and dextrose.

The esculetin reacts with ferric chloride in the media to form a black-brown color.

- **Cystine tryptic agar (CTA) sugar fermentation test:** used to identify Streptococci by fermentation reaction.
- The fermentation of carbohydrates in the media produces acids which turns the pH indicator to yellow.
- Colonies obtained from a blood agar culture is stabbed into the media in a CTA tube.
- After incubation a positive fermentation test will give yellow colour.



HIPPURATE HYDROLYSIS TEST

- Hydrolysis of hippurate: distinguishes Group B streptococci from other streptococci



BIOCHEMICAL TESTS

7. **Hippurate hydrolysis test:** Detect hippuricase enzyme production, used to differentiate beta-hemolytic streptococci (*S. pyogenes* & *S. agalactiae*).



➤ **Positive test**

- Deep purple colour
- S. agalactiae*

➤ **Negative test**

- No change in colour
- [Orange box]



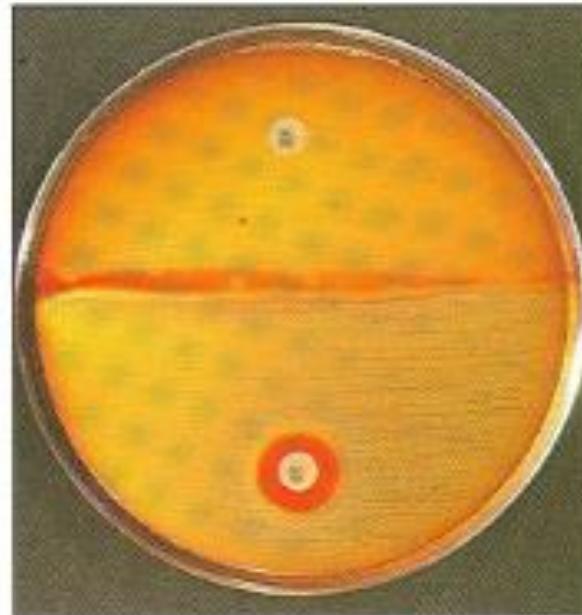
PRODUCTION OF CAROTENOID PIGMENT

- On media such as GBS agar about 97% of group B streptococci produce orange to red pigment when incubated anaerobically



BACITRACIN SUSCEPTIBILITY TEST

- Distinguishes between Group A and Group B streptococci
- Group A Streptococci (*Streptococcus pyogenes*) are susceptible to bacitracin (0.04 U) and group B are resistant



OPTOCHIN SENSITIVITY TEST

- Distinguishes *S. pneumoniae* from other alpha haemolytic bacteria
- It is a disc diffusion test
- The zone of inhibition when using 6 mm disc should be equal to or greater than 14 mm



BILE SOLUBILITY TEST

- Test is conducted by adding suspension of equal amount of *S. pneumoniae* broth culture and 10% solution of Sodium taurocholate
- Within 10-15 minutes pneumococcus will autolyse and the solution become clear



AMYLASE REACTION AND RAPID VP TEST FOR S. SUIS

- *Streptococcus suis* produce an amylase reaction on Columbia agar with starch solution
- *S. suis* is negative for rapid VP (acetoin) test





STRANGLES

STRANGLES

- Highly contagious infection of horses and other equids caused by the bacterium *Streptococcus equi*.
- The disease is characterized by severe inflammation of the mucosa of head and throat with extensive swelling and rupture of the lymph nodes, which produce large amounts of thick creamy pus.
- **Etiology:** *Streptococcus equi subspecies equi*
- Organism isolated from nose and lymph nodes of affected animals and can be readily identified by simple sugar tests.



SYMPTOMS AND LESIONS

- Susceptible horses produce strangles within 3-14 days of exposure.
- Animal shows depression, inappetence, and fever of 39°C-39.5°C.
- Most typically the horses develop nasal discharge (initially mucoid, rapidly thickening and purulent), a soft cough and slight and painful swelling of submandibular lymph node.
- Horses usually keep their head low and extended so as to relieve the head and throat pain.
- With the progression of the disease abscess develop in the submandibular and retropharyngeal lymph nodes.
- The lymph nodes hard very painful and obstruct breathing (Strangles).
- The lymph node abscess will burst in 7-14 days releasing thick pus contaminated with *S. equi*.
- The horses will rapidly recover once abscess have ruptured.

Strangles in Horses

Symptoms:

Nasal discharge
Decreased appetite
Increase in Temperature

Swelling of lymph nodes

-Can obstruct the airway

Puss secreted from abscess

-Abscess ruptures in 7 days and 4 weeks



CONTD..

- The fatal complications of strangles are **bastard strangles** and **purpura haemorrhagica**.
- Bastard strangles which describes the dissemination of infection to unusual sites other than lymph nodes of throat like abdominal or lung lymph nodes.
- A brain abscess may rupture causing sudden death.
- Retropharyngeal abscess may burst in the throat and pus may be inhaled into the lung.



CONTD

- **Purpura haemorrhagica** is an immune mediated acute inflammation of peripheral blood vessels that occurs within 4 weeks of strangles.
- It results from the formation of immune complex between the horse antibody and bacterial components. These immune complex become trapped in capillaries where they cause inflammation visible in the mucus membrane as pin point haemorrhages.
- These haemorrhages leads to wide spread severe edema of the head, limbs and other parts of the body.



- **Diagnosis**
- By culturing pus from nose , from abscessated lymph nodes and from the throat of clinically affected horses
- Sugar tests are done for *Streptococcus equi*
- **Treatment**
- Drug of choice : Penicillin G



MASTITIS

- Mastitis by *Streptococcus agalactiae* , *Streptococcus dysgalactiae*, *S. bovis*, *E. faecalis* and *S. uberis*.
- *S. agalactiae* causes chronic mastitis in cattle, sheep and goat.
- *Streptococcus dysgalactiae* causes acute mastitis and polyarthritis in lambs.
- **Hotis test.** For the identification of mastitis by *Streptococcus agalactiae*.
9.5 ml of milk taken in a test tube and add 0.5 ml of bromocresol purple. Incubate at 37°C for 24 hour. If organism present canary yellow colonies on the side of the test tube.

STREPTOCOCCUS PYOGENES

- *Streptococcus pyogenes* causes scarlet fever, septic sore throat, rheumatoid fever, and erysipelas.
- Dick test is used for *Streptococcus pyogenes*



A vibrant, slightly blurred photograph of a lake or river scene. In the foreground, three small sailboats are visible: a purple one on the left, a red one in the center, and another red one on the right. A faint rainbow arches across the upper left portion of the image. The background is filled with lush green trees and foliage.

8/25/2020 Dr. Dincy Joseph

THANK YOU

RHODOCOCCUS



पशुधन निदेश सर्वोकापकारकम्

**Dr. Bincy Joseph
Assistant Professor
Veterinary Microbiology**

RHODOCOCCUS

- Domain: Bacteria
- Phylum: Actinomycetota
- Class: Actinomycetia
- Order: Mycobacteriales
- Family: Nocardiaceae
- Genus: Rhodococcus

Zopf 1891

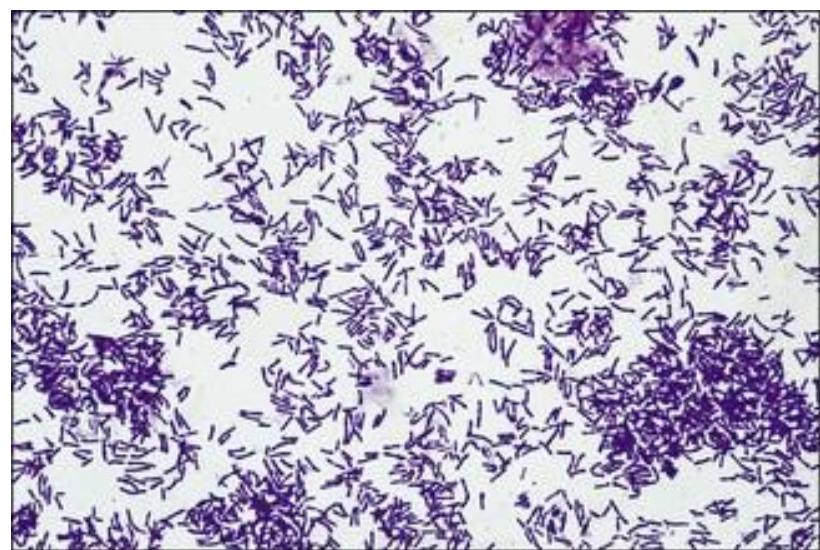
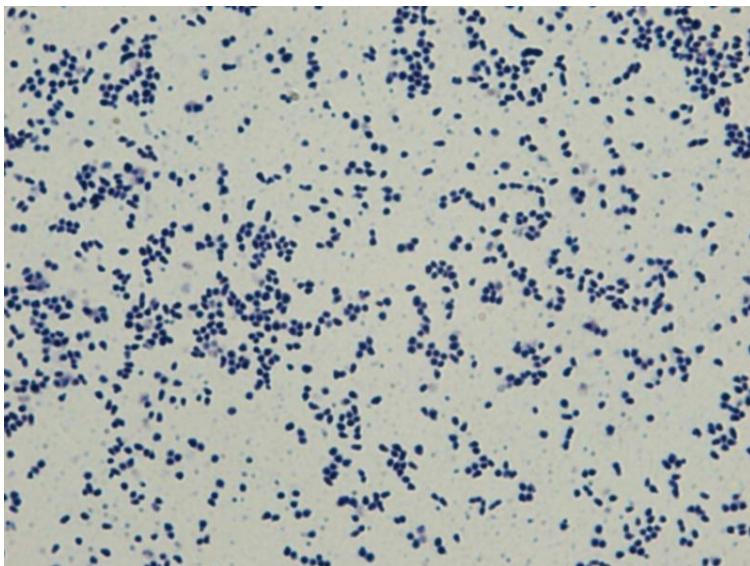


INTRODUCTION

- *Rhodococcus equi* formerly called *Corynebacterium equi* is a Gram-positive coccus or as a rod, aerobic soil saprophyte which occurs worldwide.
- It is capsulated and sometimes weakly acid fast. It is an opportunistic pathogen of foals under 6 months of age.
- Its cell envelope consists of mycolic acids, polysaccharides and glycolipids that form around a unique periplasmic space.
- *Rhodococcus equi*, originally discovered in horses by Magnusson (1923)



- *Rhodococcus equi* grows on non-enriched media such as nutrient agar and produces characteristic mucoid salmon-pink colonies, features reflecting capsule formation and pigment production.
- Some strains of *R. equi* appear as cocci and others as rods up to 5um in length.
- The organism is non-motile, catalase-positive, oxidase-negative and weakly acid-fast.



USUAL HABITAT

- *Rhodococcus equi* is an inhabitant of both soil and the intestinal tracts of animals. It can replicate at warm temperatures in soils enriched with faeces of herbivores.
- It is facultative intracellular.



MORPHOLOGICAL CHARACTERS

- Rhodococcus, implies the coccus shape of the organisms, the bacteria surprisingly show extensive polymorphism.
- At the early stage it is rod-shaped and filamentous, but when grows its breaks into a short rod or coccus



Salmon-pink colonies

CLINICAL INFECTIONS

- Suppurative bronchopneumonia of foals is the major disease caused by this pyogenic organism.
- Superficial abscesses due to *R. equi* have been recorded in horses over 6 months of age.
- Pigs, cats and cattle can occasionally be infected

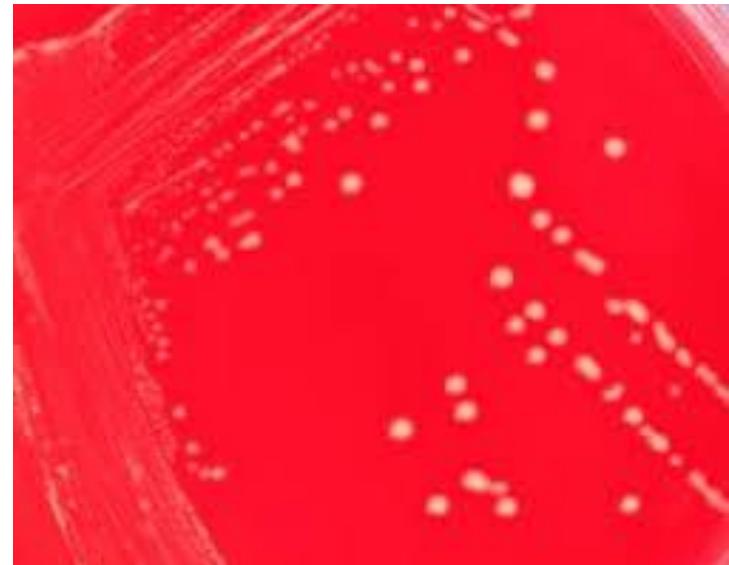
SPECIMENS

- Specimens for laboratory examination include tracheal aspirates and pus from lesions.



CULTURAL CHARACTERS

- Blood and MacConkey agar plates inoculated with suspect material are incubated aerobically at 37°C for 24 to 48 hours.
- Colonies on blood agar are non-haemolytic, salmon-pink and mucoid.
- Absence of growth on MacConkey agar.
- Unreactive in the oxidation-fermentation test and in sugar fermentation tests.
- CAMP-test positive



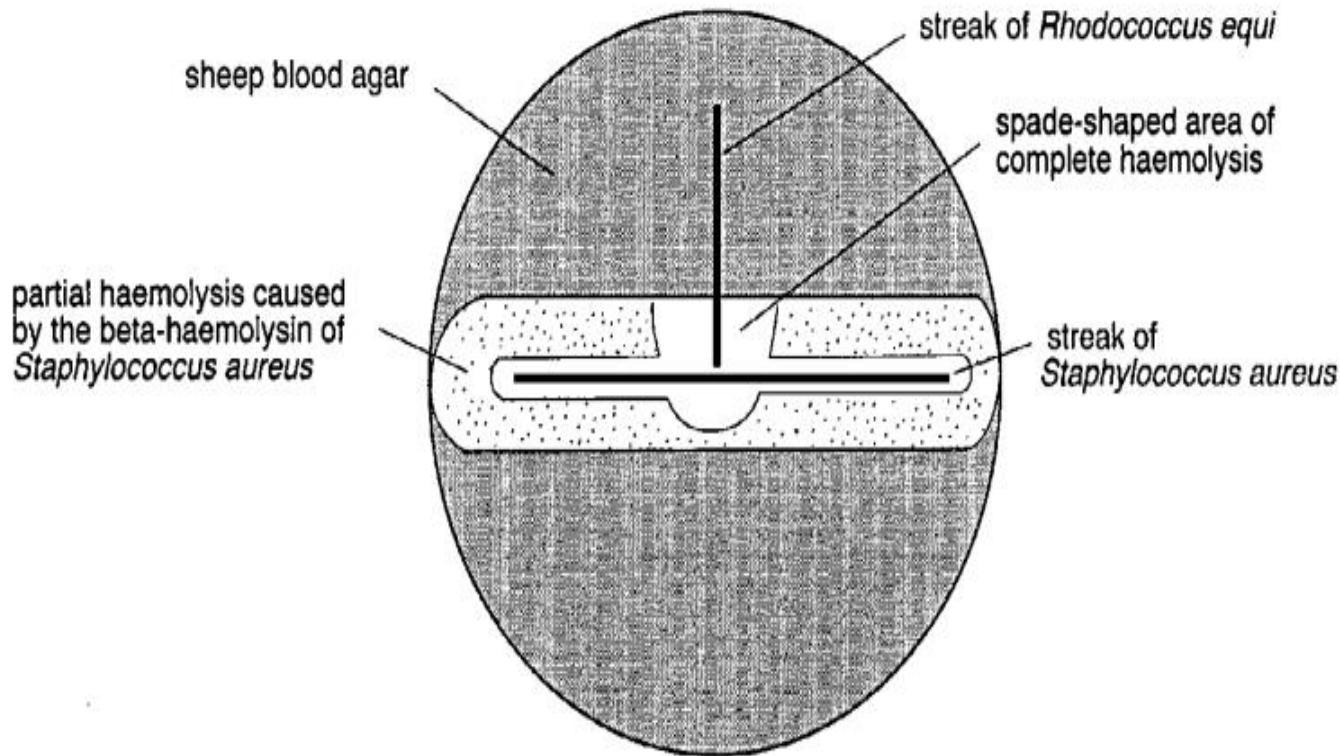


Figure 11.2 CAMP test. *Rhodococcus equi* produces a factor which completely lyses the red cells previously damaged by the beta-haemolysin of *Staphylococcus aureus*, producing a spade-shaped pattern of complete haemolysis which extends across the streak of *S. aureus*.

SUPPURATIVE BRONCHOPNEUMONIA OF FOALS

- It is generally acquired by inhalation of dust contaminated with *R. equi*. The organism is often present in large numbers in the faeces of healthy foals under 3 months of age, and can also be isolated from the faeces of older horses and many other mammals and birds.
- Granulomatous ulcerative enterocolitis and mesenteric lymphadenitis sometimes occur when affected foals swallow sputum containing large numbers of *R. equi*.



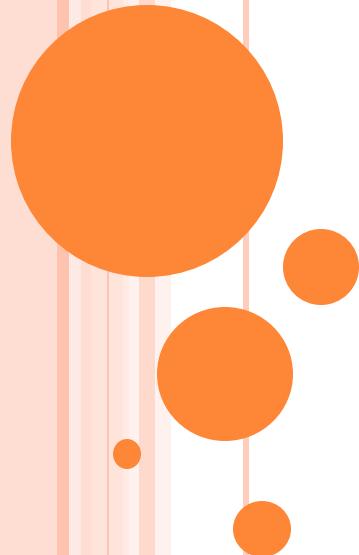
CONTROL

- No commercial vaccines are available.
- On farms where the disease has occurred, foals should be kept under observation and examined clinically twice weekly until they are 4 months of age.
- Hyperimmune serum from the dam, administered to the foal in the first month of life, is claimed to reduce the prevalence of disease on some farms.
- Prevention of a buildup of *R. equi* in the environment of young foals is desirable:
 - Foal manure should be removed from pastures at frequent intervals.
 - Foals and their dams should be moved regularly to fresh pasture.
 - Dusty conditions in paddocks and holding yards should be minimized.

FURTHER READINGS

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease





ORDER : RICKETTSIALES

Dr. Bincy Joseph
Assistant Professor
PGIVER, Jaipur

ORDER RICKETTSIALES

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- It is cultured in **Yolk sac of embryonated egg or in cell culture**
- The organisms are named after **HT Ricketts** who contracted typhus fever and died of this.
- Transmission of **rickettsial disease occur by bite of arthropod** except one disease **Q fever**
- **Q fever or Abattoir fever** caused by **Coxiella burnetti** which is transmitted by ingestion and inhalation

- Coxiella organism can survive flash method of pasteurisation
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- Rickettsial organism stains poorly with aniline dyes
- Important staining methods are **Giemsa**, **Leishman staining**, **Gimenez**, **castanada** and **Machiavello staining**
- Host cell dependence for growth, poor affinity for basic dyes,, presence of invertebrate vector distinguishes them from conventional bacteria and chlamydia

G: RICKETTSIA

- Genus rickettsia have rigid cell wall and cytoplasmic membrane similar to that of E. coli
- They are non capsulated, non motile aerobic organism and multiply by binary fission
- Organism also grow in mice, guinea pig and rabbit
- Inoculation on yolk sac or cell culture also used
- Optimum temperature of growth is **33-35°C**
- Some species like **Rickettsia prowazekii** and **R. typhi** produce lethal toxin harmful to mice



CLASSIFICATION

Order	Family	Genus	Species	Target cells
<i>Rickettsiales</i>	<i>Rickettsiaceae</i>	<i>Rickettsia</i>	<i>R. rickettsii</i>	Vascular endothelium
		<i>Aegyptianella</i>	<i>A. pullorum</i>	Erythrocytes
		<i>Anaplasma</i>	<i>A. bovis</i>	Monocytes, macrophages
			<i>A. marginale</i>	Erythrocytes
			<i>A. ovis</i>	Erythrocytes
	<i>Anaplasmataceae</i>	<i>Ehrlichia</i>	<i>A. phagocytophilum</i>	Granulocytes
			<i>A. platys</i>	Platelets
			<i>E. canis</i>	Monocytes, macrophages
			<i>E. ewingii</i>	Granulocytes
			<i>E. ruminantium</i>	Granulocytes
	<i>Neorickettsiaceae</i>	<i>Neorickettsia</i>	' <i>E. ovina</i> '	Monocytes, macrophages
			' <i>E. ondiri</i> '	Granulocytes, monocytes
			<i>N. helminthoeca</i>	Monocytes, macrophages, lymphoid cells
	<i>Neorickettsiaceae</i>	<i>Neorickettsia</i>	<i>N. risticii</i>	Monocytes, intestinal epithelial cells, mast cells
			' <i>N. elokominica</i> '	Monocytes, macrophages, lymphoid cells



RICKETTSIA

- **R. prowzekii** transmitted by human body louse and the disease is called epidemic typhus
- **Rikcettsia typhi/Rickettsia mooseri**: endemic typhus or murine typhus transmitted by flea
- **Rickettsia rickettsi**: will cause rocky mountain spotted fever where ticks act as vector
- **Rickettsial akari**: rickettsial pox
- **Rickettsia conorii**: indian tick typhus



PATHOGENS OF VETERINARY IMPORTANCE IN ANAPLASMA

Pathogen	Hosts/Vectors	Disease	Geographical distribution
<i>Aegyptianella pullorum</i>	Poultry/Ticks	Aegyptianellosis	Africa, Asia, Mediterranean region
<i>Anaplasma bovis</i>	Cattle/Ticks	Bovine anaplasmosis	Africa, Middle East, Asia, South America
<i>A. marginale</i>	Ruminants/Ticks	Anaplasmosis	Tropical and subtropical regions
<i>A. ovis</i>	Sheep, goats/Ticks	Anaplasmosis	Asia, Africa, Europe, USA
<i>A. phagocytophilum</i>	Ruminants, horses, humans/Ticks	Tick-borne fever, equine and human granulocytic ehrlichiosis	Worldwide
<i>A. platys</i>	Dogs/Ticks suspected	Canine cyclic thrombocytopenia	Americas, Middle East, Mediterranean region
<i>Ehrlichia canis</i>	Dogs/Ticks	Canine monocytic ehrlichiosis	Tropical and subtropical regions
<i>E. ewingii</i>	Dogs/Ticks	Canine granulocytic ehrlichiosis	USA
<i>E. ruminantium</i>	Ruminants/Ticks	Heartwater	Sub-Saharan Africa, Caribbean islands
' <i>E. ondiri'</i>	Cattle/Ticks suspected	Bovine petechial fever	Highlands of East Africa
' <i>E. ovina'</i>	Sheep/Ticks	Ovine ehrlichiosis	Africa, Asia, Middle East
' <i>Neorickettsia elokominica'</i>	Dogs, bears, racoons/Flukes	Elokomin fluke fever	West coast of North America
<i>N. helminthoeca</i>	Dogs, bears/Flukes	Salmon poisoning disease	West coast of North America
<i>N. risticii</i>	Horses/Flukes	Potomac horse fever	North America, Europe



- Orientia: organism is *Orientia tsutsugamushi* (*Rickettsia tsutsugamushi*) transmitted by tick and the disease is called scrub typhus
- Target cells for *Rickettsia* and *Orientia* are vascular endothelial cells

DIAGNOSIS OF RICKETTSIAL INFECTION

- Agglutination test called Weil Felix reaction
- Here patients serum is mixed with *Proteus vulgaris* serotype OX K, OX 2, and OX 19
- in positive cases there will be agglutination
- Other diagnostic test are CFT, ELISA and indirect HA and indirect immunodiffusion test



- Blood or tissue smears stained by the Giemsa technique can be used to demonstrate the morphology of members of the *Anaplasmataceae*. They occur as purplish blue, small, individual organisms, sometimes in clusters, or as morulae up to 4.0 µm in diameter
- Fluorescent antibody techniques can be used to identify *R. rickettsii* and specific members of the *Anaplasmataceae* in smears.
- Some organisms can be isolated in the yolk sac of embryonated eggs or in defined tissue culture cell lines.
- Molecular methods, such as nucleic acid probes and polymerase chain reaction techniques, including real-time PCR techniques, have been developed to detect members of the *Rickettsiales* in host tissues.
- In outbreaks of major diseases such as bovine anaplasmosis, susceptible domestic animals can be inoculated with infected blood or tissue in order to identify an organism or confirm a diagnosis.

ROCKY MOUNTAIN SPOTTED FEVER

Etiology : Rickettsia rickettsii

Vector : Dog tick (*Rhipicephalus*)

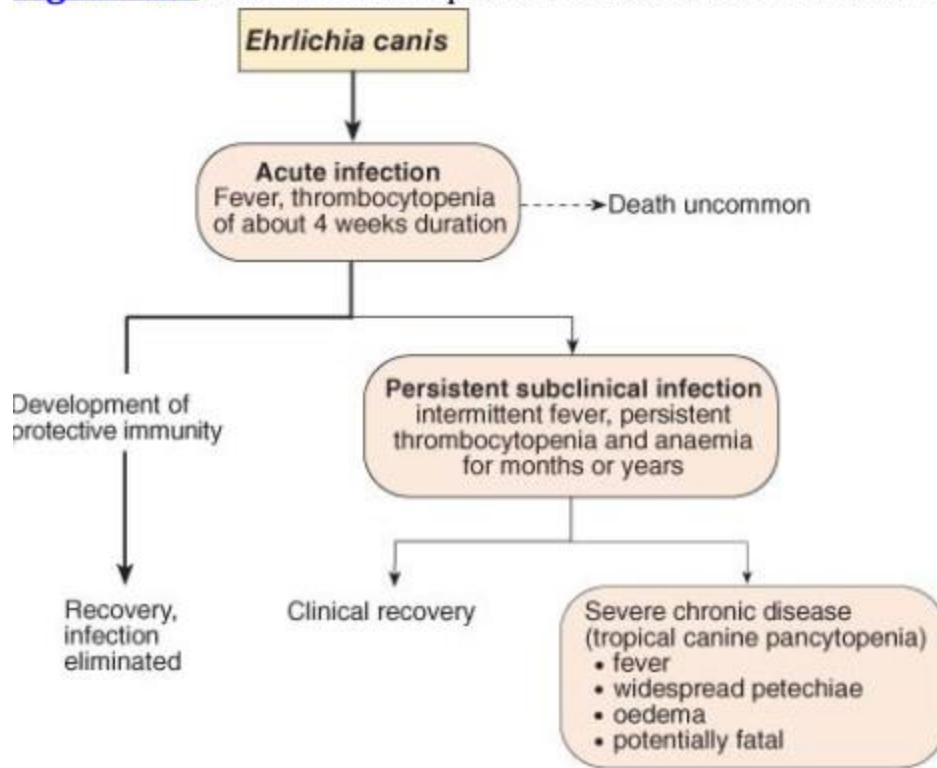
The organisms, which replicate in endothelial cells of infected dogs, produce vasculitis, increased vascular permeability and haemorrhage.



CANINE MONOCYTIC EHRLICHIOSIS:EHRLICHIA CANIS

- Host : dog
- Vector : *Rhipicephalus sanguineus*, the brown dog tick
- Disease is known as Canine Ehrlichiosis, Dog typhus,Tropical canine pancytopaenia
- **Nairobi bleeding disease**
- **Clinical signs:** progressed through subclinical acute and chronic phase
- Acute phase characterised by fever thrombocytopaenia, leucopaenia and anaemia
- Minority of dog later develop severe form of disease characterised by persistent bone marrow depression , haemorrhage , neurological disturbance, petechial haemorrhage oedema and emaciation

Figure 40.2 Possible consequences of infection with *Ehrlichia canis*.



DIAGNOSIS

- Clinical symptoms and histological lesions
- Morulae of organism detected in mononuclear cells by Giemsa staining of buffy coat
- FAT to demonstrate organism in smears
- Supernatant of heparinised blood can be cultured in canine macrophage cell line
- Serum conversion can be demonstrated 3 weeks after infection by indirect immunofluorescence
- **Treatment and control**
- Doxycycline for 10 days, tetracyclin and cloramphaenicol effective
- Fluid replacement therapy and blood transfusion

BOVINE PETECHIALFEVER

- Ehrlichia ondiri
- Cattle host
- Vector : tick
- Clinical signs : Clinical signs include high fluctuating fever, depressed milk yield and widespread petechiation of visible mucous membranes.
- Oedema and petechiation of the conjunctiva produces ‘poached-egg’ eye, a feature typical of severe cases.
- Death often results from pulmonary oedema. Recovered animals, which become carriers, are resistant to reinfection for at least 2 years.



HEART WATER DISEASE

- *Ehrlichia ruminantium*
- Tick belong to Amblyoma are main vector
- Organism replicate in reticuloendothelial cells especially macrophages and endothelial cells of capillaries of CNS
- Clinical signs: sudden onset of fever, neurological signs are common including chewing movement, twitching of eyelids, high stepping gait, circling and recumbency
- Death during convulsions in acute cases
- In sub acute cases lesions are hydropericardium, hydrothorax, pulmonary oedema and congestion, spleenomegaly, extensive mucosal and serosal damage



DIAGNOSIS

- Symptoms as nervous signs
- Post mortem lesions in endemic areas, provide presumptive diagnosis
- Squash preparation off brain tissue stained by Giemsa, so organism seen located close to nucleus
- Nucleic acid probes
- **PCR**
- Antibody detection by indirect immunofluorescence
- ELISA, western blot
- Treatment and control
- Tetracyclin administration during early phase
- Immunisation by inoculating blood from infected animal
- Tick control



SALMON POISONING

- *Neorickettsia helminthoeca*
- Host: dogs
- Vectors are fluke
- Dogs infected by ingestion of raw salmon containing fluke metacercariae.
- Then the organism attach to the blood following attachment of fluke to intestinal mucosa of host
- Replication of bacteria in typhoid tissue result in generalised lymphadenopathy
- *Neorickettsia ristici*: horses are host
- Flukes are suspected vector
- Disease called Potomac horse fever



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BOVINE ANAPLASMOSIS

- Anaplasma marginale: organism appear as purple dot in periphery of RBC
- Host cattle
- Vector Boophilus
- Also called gall sickness
- Important signs are inappetite , depression. decreased milk yield, marked anaemia, jaundice develop in absence of haemoglobinuria and weight loss
- Diagnosis:
- Clinical signs and haematological findings

- Geimsa stained blood smear contain densely stained bodies located near the periphery of RBC. Organism are most numerous at about 10 days after onset of fever when upto 50% of RBC affected
- Identification of organism in blood smear by immunoflourescence
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- PCR
- Serological tests as CFT, Card agglutination test, ELISA, Dot ELSA for detection of antibodies in serum

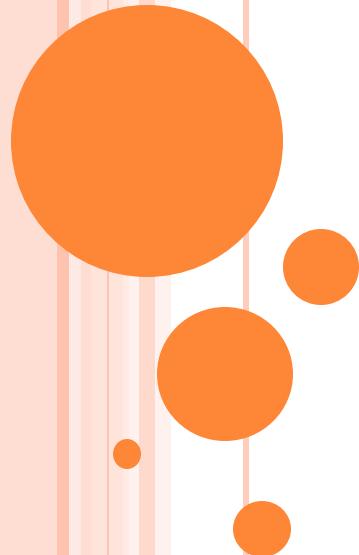
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- Anaplasma platys Dogs host and ticks suspected vector
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- Vector : soft tick of poultry
- Infected birds have ruffled feathers, anorexia, diarrhoea, anaemia, hyperthermia
- Lesions are hepatosplenomegaly, punctiform haemorrhage on serosal surface
- Control of ticks effective and tetracycline therapy



- **Haemobartonella felis**: Now in mycoplasma
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- Signs: In per acute anaemia: overwhelming parasitemia, rapidly result in death
- In acute cases fever , anaemia, depression, weakness and jaundice
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- Diagnosis: Demonstrate organism on the surface of RBC
- by geimsa staining of blood smear
- demonstration of pathogen in blood smear by immunoflourescence
- haematological finding as decreased PCV and regenerative anaemia
- **Treatment**
- Blood transfusion
- Doxycycline for 21 days
- Control of fleas



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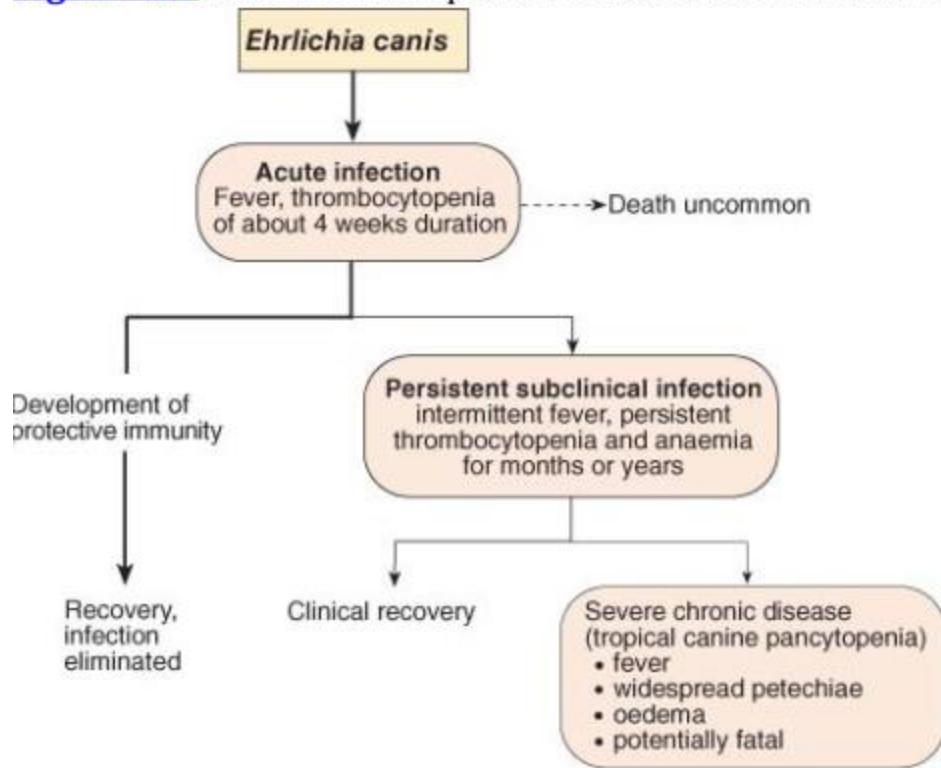
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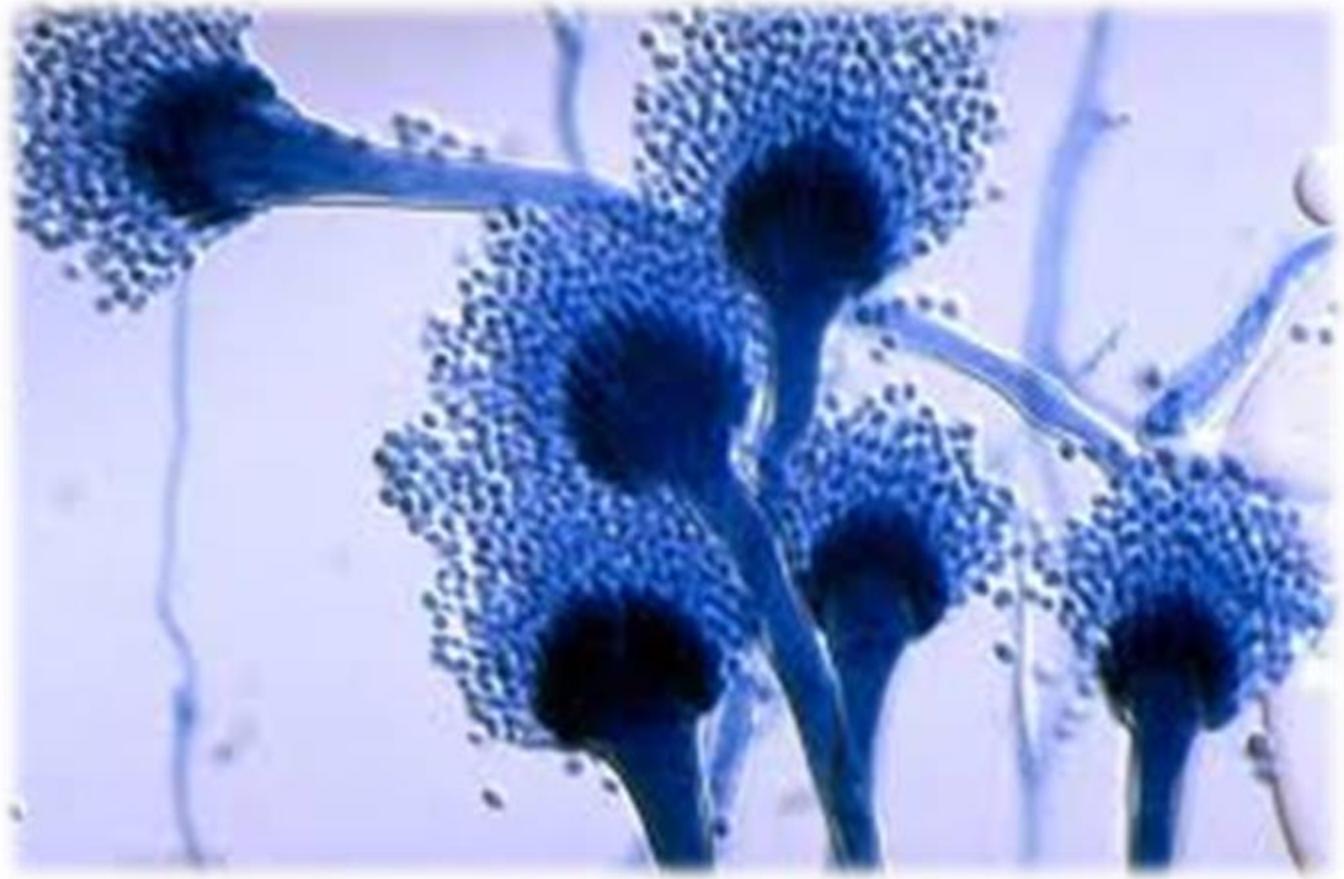
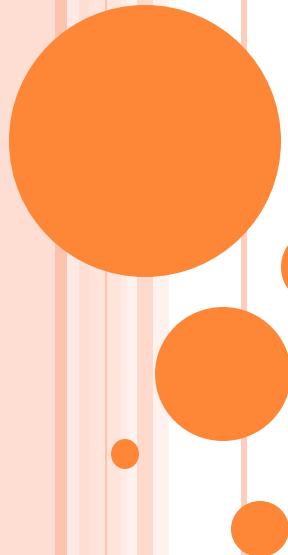
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MYCOLOGY

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MYCOLOGY

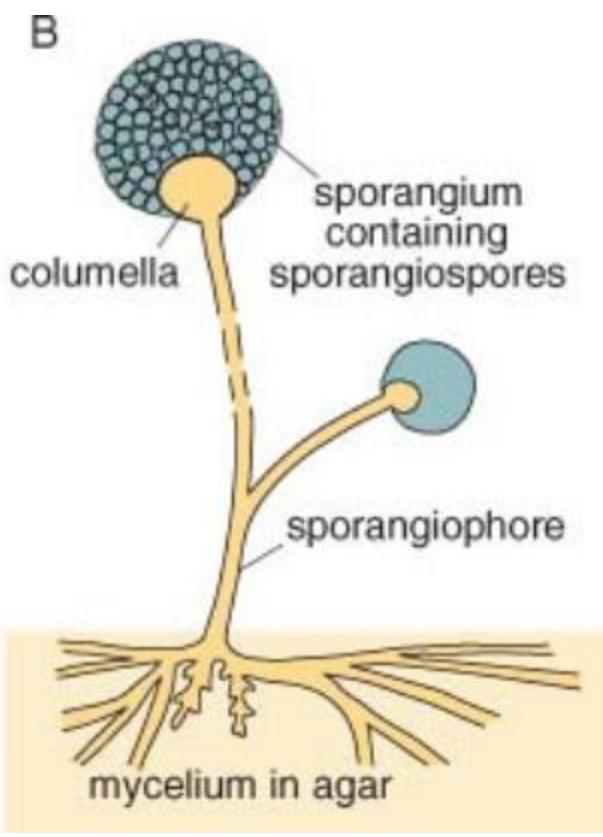
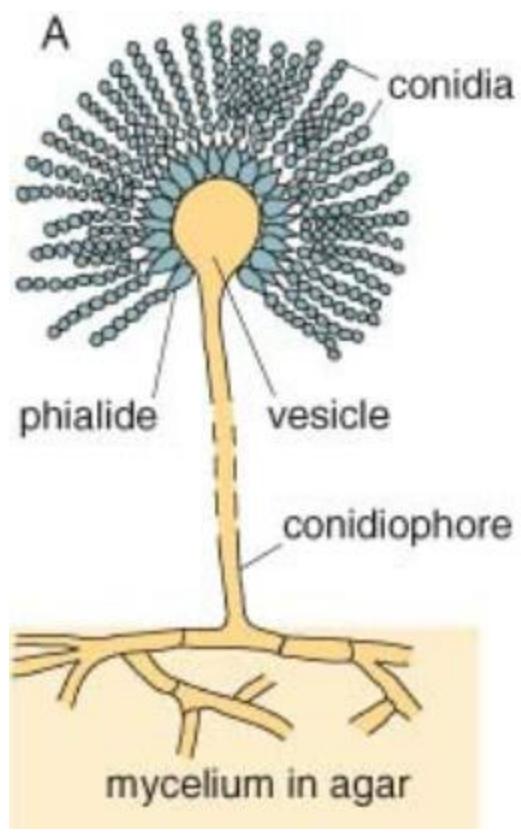
- Fungi are eukaryotic non photosynthetic heterotrophs which produce exoenzymes and obtain nutrients by absorption
- There are four phyla in the Kingdom Fungi
 - Ascomycota (Ascomycetes)
 - Basidiomycota (Basidiomycetes)
 - Zygomycota (Zygomycetes)
 - Fungi imperfecti (deuteromycetes)
- The first three phylum can be distinguished by the characteristics of their sexual form
- In case of fungi imperfecti/ Deuteromycetes the sexual form has not been found
- The sexual form of fungi also known as teleomorphs
- Most fungi of Veterinary importance are under Deuteromycetes





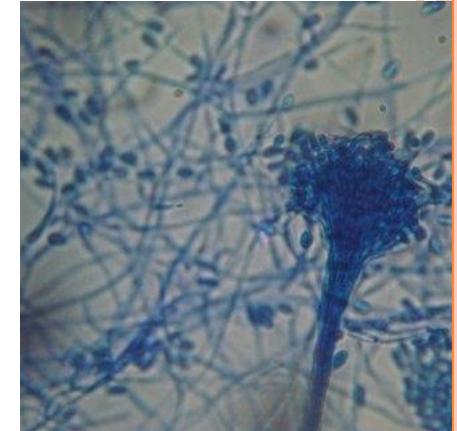
MOULDS AND YEAST

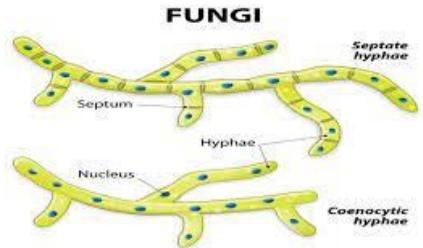
- Two main morphological forms of fungi are molds and yeasts
- Molds grow as branching filaments called hyphae (2-10 μm) diameter whereas unicellular yeasts have an oval or spherical appearance 3-5 μm in diameter
- **Dimorphic fungi** occur in both yeast and mold forms
- Some fungi like *Candida albicans* produce other forms in addition to mold and yeast form, hence they are known as **polymorphic**



CHARACTERISTIC OF FUNGI

- Fungi are **aerobic**
- Reproduction in fungi occur either sexually or asexually by the formation of spores
- A mass of interlacing hyphae form mycelium
- Fungi tolerate high osmotic pressure and **acidic environments as low as pH 5.0**
- Structure
- Hyphal cell wall contain carbohydrate components like **chitin** macromolecules with cellular cross linkages
- In Yeast cell wall contain protein complexed with polysaccharide and lipids
- The predominant sterol is **ergosterol** in contrast to cholesterol which is the predominant sterol in the cell membrane of animals





- Both molds and yeast have well defined nuclear membrane, mitochondria and a network of microtubules
- Septa or cross walls often present on the hyphae
- Septa formed by the inward growth of cell wall have central pores through which nutrients and organelles may pass

GROWTH REPRODUCTION AND COLONIAL FORMATION

- Moulds tend to form large colonies with growth and extension of hyphae at their peripheries.
- In moulds in asexual reproduction two main types of spores are produced
- **Conidia** –produced on conidiophore
- Multicellular conidia called macro conidia and unicellular conidia called micro conidia are produced by Dermatophytes produced on the lateral branches of hyphae
- **Sporangiospores** produced within a sac like structure called sporangium on an aerial hyphae called sporangiophore
- Eg: By fungi in the phylum zygomycota

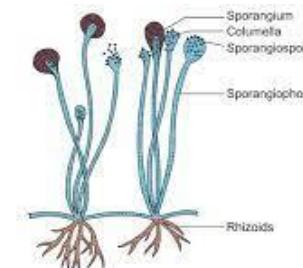
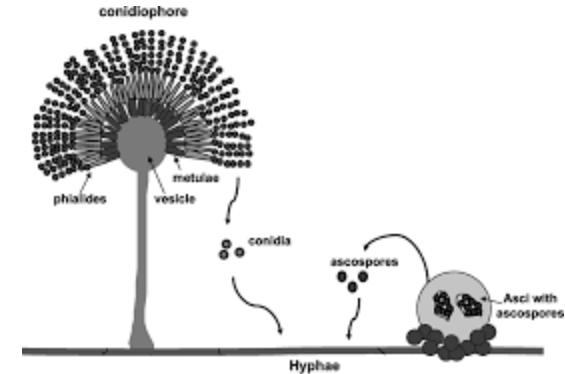
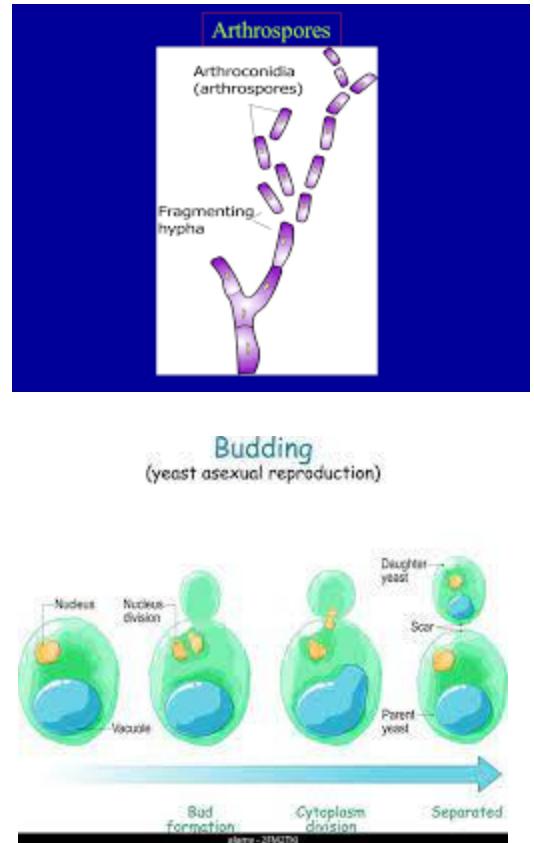


Figure 1.26: *Rhizopus*

- Arthroconidia are the conidia produced by the disintegration of hyphae within keratinised tissues
- In most yeast cells asexual reproduction is by budding
- Daughter cells separate from parent cells after the formation of crosswall at the point of budding
- The colonies of yeast like fungi are soft smooth and round



ASEXUAL SPORES

- **Arthroconidia or Arthospore**
- Spores are produced and are released during the process of fungal fragmentation
- Spores may be produced successively as in dermatophytes or intervening empty cells as in coccidioides
- **Blastoconidia/Blastospore**
- Conidia which are produced by budding
- Eg: *Candida albicans*
- **Chlamydoconidia/ Chamydospores**
- Thick walled resistant spores which contain storage products.
- These are produced by some fungi in unfavourable conditions

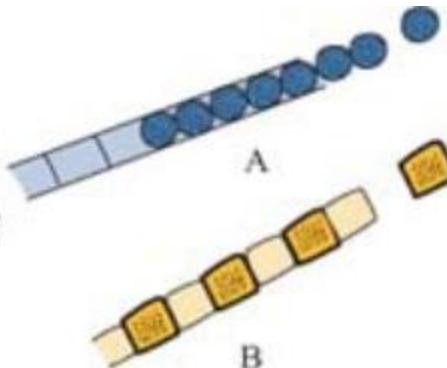


- **Macro conidia**
- Large multicelled conidia which are produced by Dermatophyte in culture
- **Micro conidia**
- Small conidia are produced by certain dermatophytes
- **Phialo conidia**
- Conidia produced from phialids
- The Phialides of Aspergillus species arise from a vesicle
- **Sporangiospores**
- Spore produced by zygomycetes such as rhizopus are released when mature sporangium ruptures



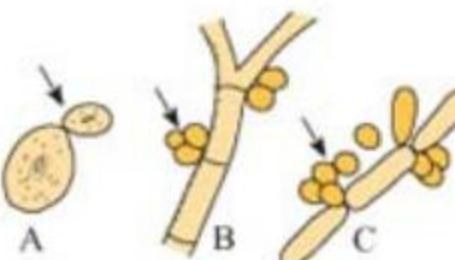
Arthroconidia (arthrospores)

Spores which are formed and subsequently released during the process of hyphal fragmentation. Spores may be formed successively as in dermatophytes (A), or with intervening empty cells as in *Coccidioides immitis* (B)



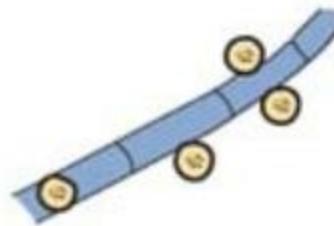
Blastoconidia (blastospores)

Conidia (arrows) which are produced by budding, as in *Candida albicans*, from a mother cell (A), from hyphae (B) or from pseudohyphae (C)



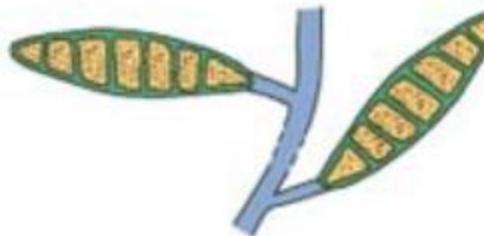
Chlamydoconidia (chlamydospores)

Thick-walled, resistant spores which contain storage products. These structures are formed by some fungi in unfavourable environmental conditions



Macroconidia

Large multi-celled conidia which are produced by dermatophytes in culture



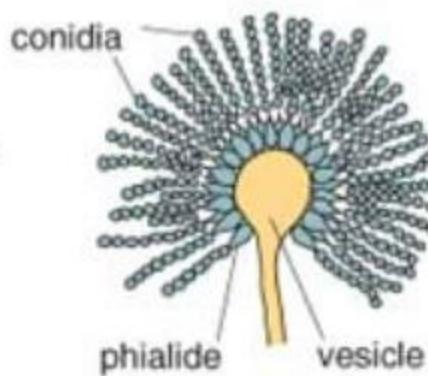
Microconidia

Small conidia which are produced by certain dermatophytes



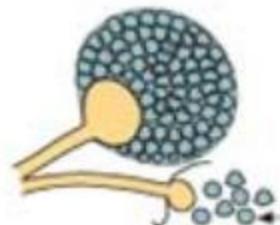
Phialoconidia

Conidia produced from phialides. The phialides of *Aspergillus* species arise from a vesicle



Sporangiospores

Spores (arrow), formed by zygomycetes such as *Rhizopus* species, are released when a mature sporangium ruptures



SEXUAL SPORES

- Sexual spores are produced by fungi in the phyla
- Ascomycota
- Basidimycota
- Zygomycota



- **Ascospores**
- Produced by members of Ascomycota. Ascospores are developed in a sac like structure called an ascus
- Asci are enclosed in a well defined structure termed ascocarp
- **Basidiospores**
- Produced by members of Basidiomycota on a club shaped structure called basidia
- **Zygosporangium**
- Produced by members of zygomycota
- Develop within a thick walled zygosporangium formed by the fusion of side projections of two compatible mycelium



Table 42.2 Sexual spores of fungi in the phyla *Ascomycota*, *Basidiomycota* and *Zygomycota*.

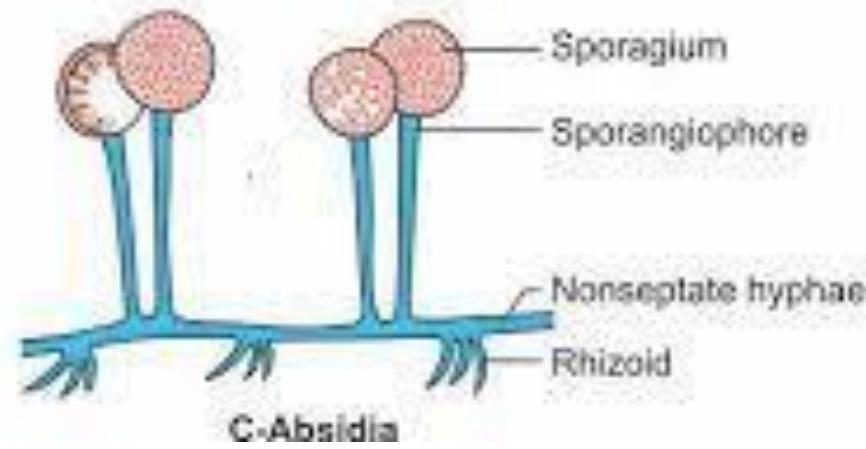
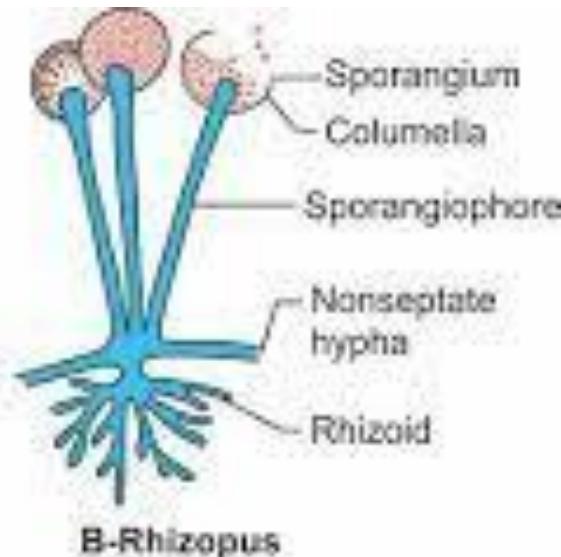
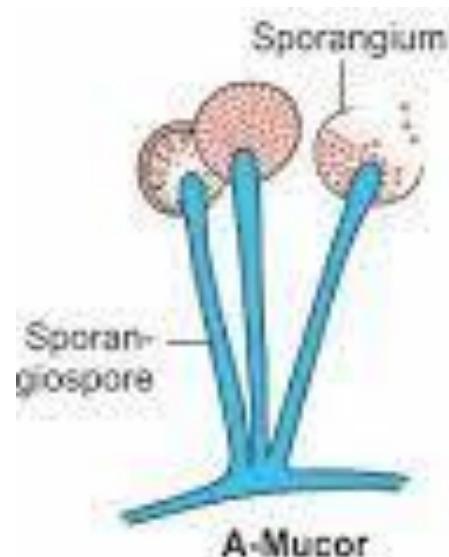
Spores	Comments
Ascospores	Produced by members of <i>Ascomycota</i> ; develop in a sac-like structure called an ascus. Asci may be enclosed in well defined structures termed ascocarps
Basidiospores	Produced by members of <i>Basidiomycota</i> on club-shaped structures called basidia
Zygospores	Produced by members of <i>Zygomycota</i> ; develop in a thick-walled zygosporangium, formed from the fusion of side projections of two compatible hyphae

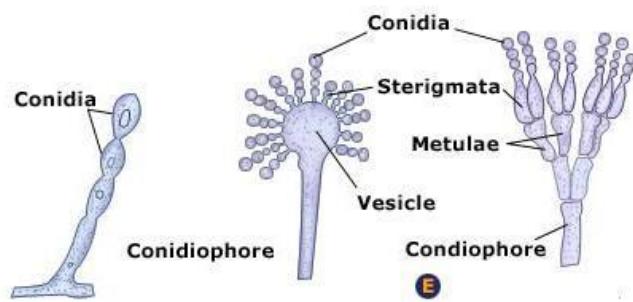
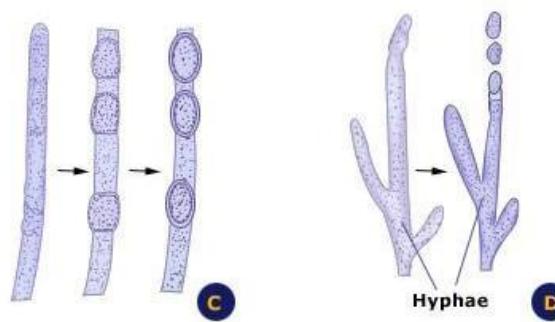
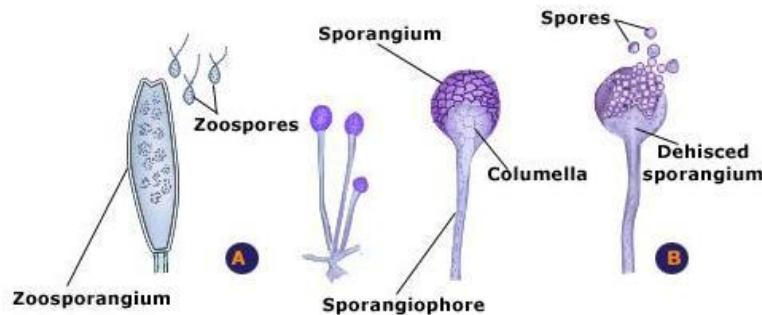
REPRODUCTION IN ZYgomycetes

- Asexual reproduction
- Long non septate aerial filament called **sporangiophore** which have an expended tip called **columella**
- Surrounding which is a sac like closed structure called **sporangium**
- Sporangium contains numerous asexual spores called **sporangiospores**.
- When spores mature, the sporangium ruptures and **sporangiospores** are released to initiate new hyphae and fungus

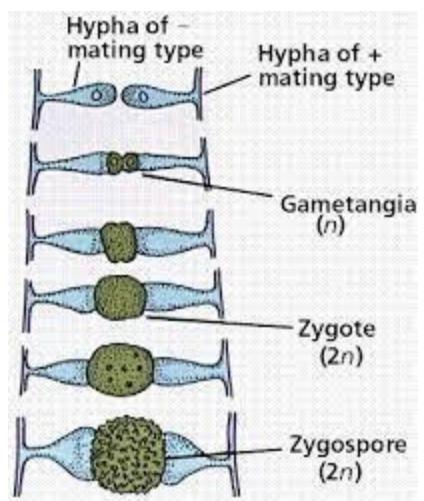


- Sporangiospores are two types
- **Aplanospore**s are non motile containing several nuclei
- **Zoospores** uninucleate and motile with one or more flagella
- All the species have horizontal hyphae which grows along with the surface of the medium
- It has got root like branched hyphae extending into the medium called **rhizoids**
- The horizontal hyphae or runner is known as **stolon**
- In mucor species rhizoids are absent
- In absidia rhizoids are away from sporangiophore and in rhizopus the rhizoids are situated just below sporangiospores





- Sexual reproduction
- Two types
- By fusion of like gametes
- By fusion of unlike gametes
- In the first two suitable adjacent hyphae come together and form short side branches called **suspensors**
- At the point the suspensors meet , a **gametangium** is formed
- The gametangia formed fused to form a **zygote**
- The zygote matures and produce spherical thick walled sexual spore called **zygospore**

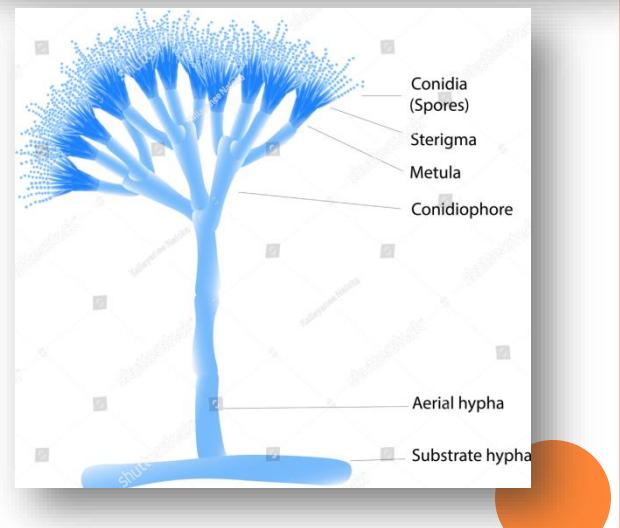
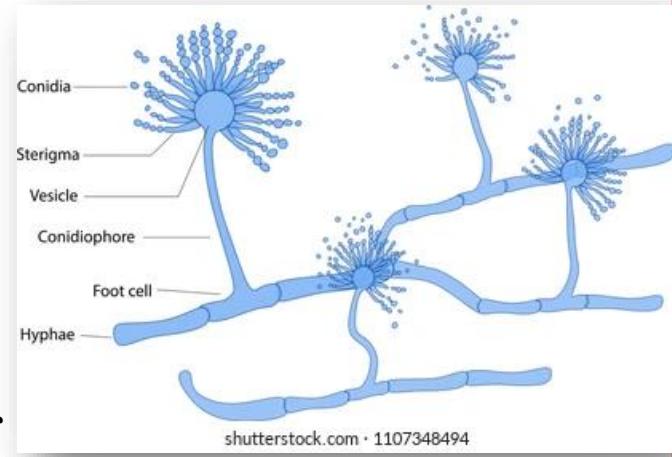


- In the second type there is specialised female structure called **oogonium** fertilised by transfer of nucleus from the male structure called **antheridium**
- The sexual spore formed is called **oospore**. The structure which contain the oospore is called **oosphere**



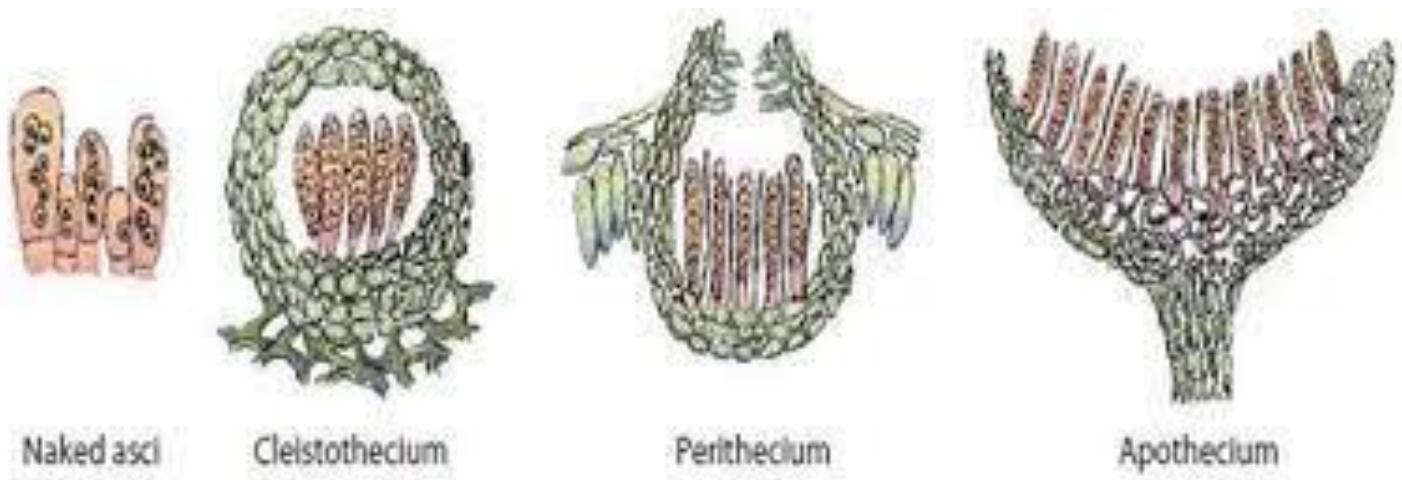
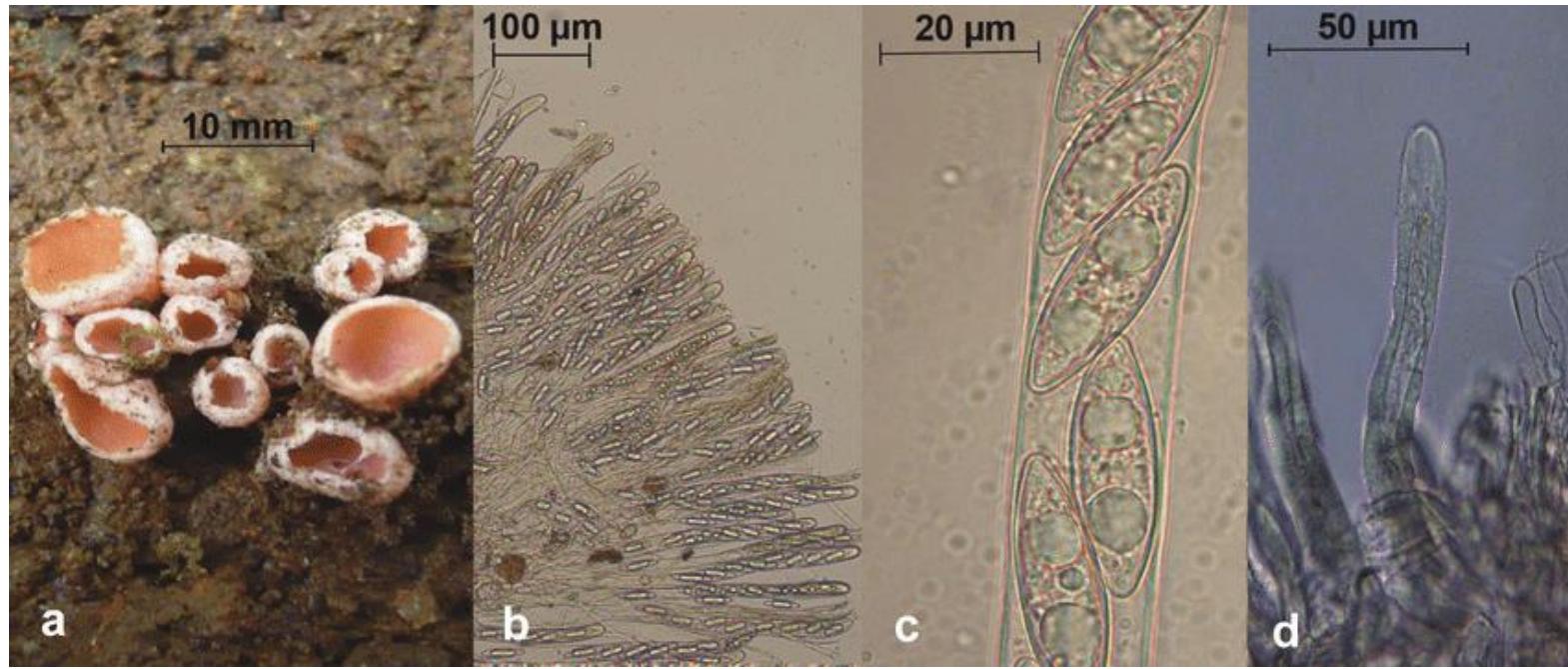
REPRODUCTION IN ASCOMYCETES

- Asexual reproduction
- *Aspergillus spp.* produce long septate aerial modified hyphae known as **conidiophore** which ends in terminal expansion or swollen called **conidiophore vesicle**.
- From surface of vesicle numerous club shaped structures called **sterigmata or phialides** arise.
- From tip of sterigmata, unicellular long chain of asexual spores which may be uninucleate or multinucleate
- In **Penicillium species** the conidiophore vesicle is absent
- It produce lateral side branches called **metullae** from end of which finger like projections (sterigmata) arise which has asexual spores
- This arrangement gives penicillium species a characteristic **brush like appearance**.
- Asexual spores of aspergillus and and penicillium spp. are called **conidia or conidiospores** (phialospores and phialoconidia)



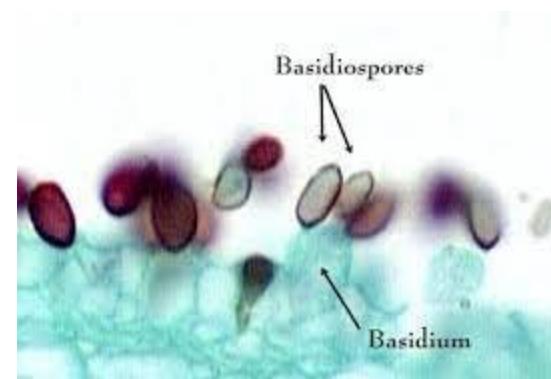
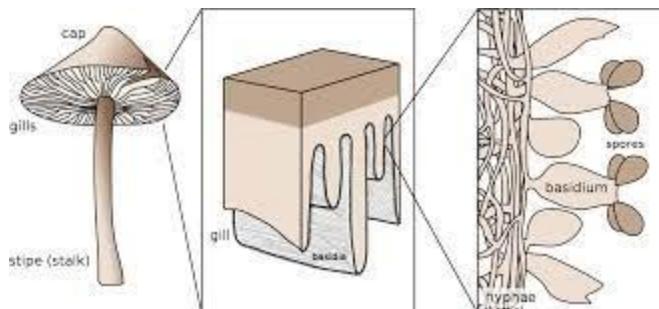
- Sexual reproduction
- Sexual spores are developed **endogenously** in a rounded or elongated sac like structure called **ascus**
- The spores fromed inside are called ascospores
- Usually one ascus has 4 to 8 ascospores.
- Ascus may be formed within a fruiting body called **ascocarp**.
- Ascocarp are of two types
- **Cleistothecium type-** which is largely fairly round closed many celled structure in which asci or ascocarps are formed
- **Peritheциum** which is a large round or pear shaped structure containing small rounded opening.it contains asci and ascospores
- **Apothecium** discoid or cupped body bearing asci on the exposed flat or concave surface.





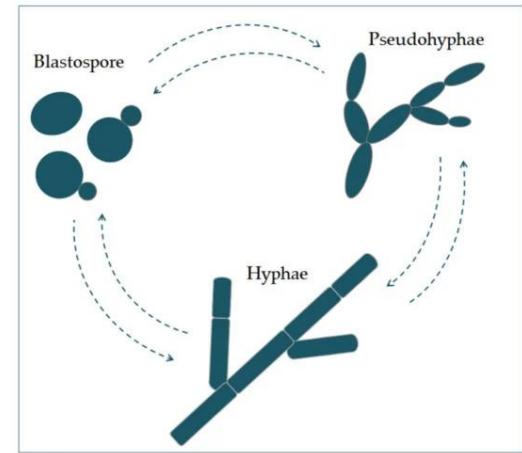
REPRODUCTION IN BASIDIOMYCETES

- Asexual reproduction by production of **conidia**
- Sexual reproduction – sexual spores develop exogenously on a club shaped structure called **basidium**
- Sexual spores are called **basidiospores** usually basidium bears **four spores**

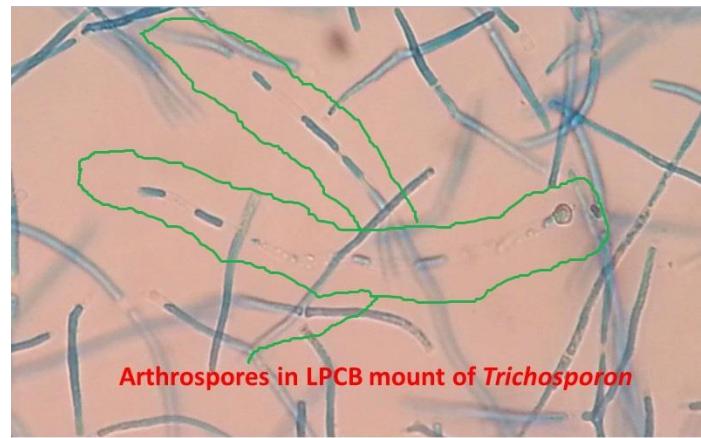
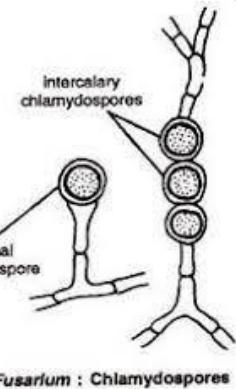
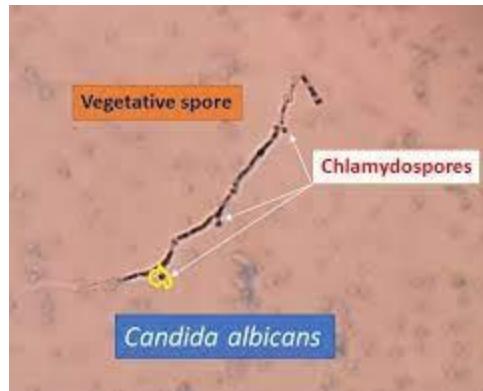


DEUTEROMYCETES- FUNGI INPERFECTI

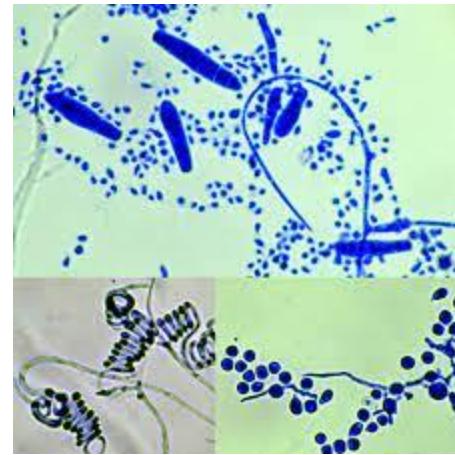
- Only asexual reproduction seen
- Asexual spores of Deuteromycetes are called as **thallospores**
- They are derived from original hyphae itself, not from modified ones
- **Blastospores/bastoconidium in yeast :**
Spores produced from budding process from vegetative cells or from a hyphae
- The bud constrict at the base and detach from mother to reproduce further by budding
- It is seen in *Candida albicans* and *Cryptococcus neoformans*
- **Pseudohyphae** – filaments composed of elongated budding cells that failed to detach in *Candida albicans*



- **Chlamydospores:** Thick walled resistant spores formed by the direct differentiation of hyphae.
- It is seen in *Candida albicans* and *Histoplasma capsulatum*
- **Arthrospheres**
- Formed by fragmentation of hyphae. It is very common in **dermatophytes**
- In addition dermatophytes produce macro conidia and microconidia
- **Microconidia:** very small asexual spores of various shapes and size which may occur on side of hyphae “en thyrse” or in clusters “en grappe”

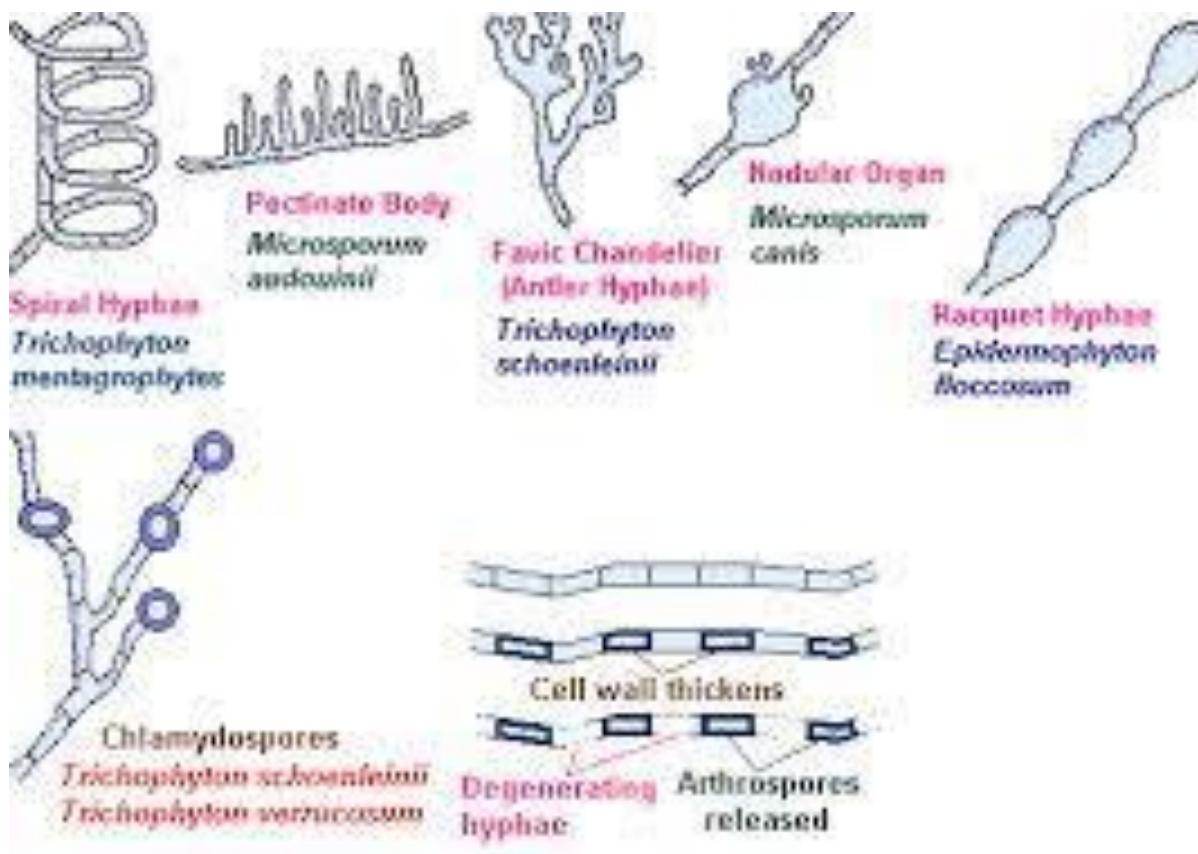


- Macro conidia
- Usually larger and elongated
- In *Microsporum* spp. they are multiseptate, fusiform or spindle shaped some time thick walled or with wrinkled wart like walls
- In *Trichophyton* spp. they are long thin multiseptate, smooth walled and cigar shaped
- In *Epidermophyton* spp. oval or pear shaped and have only few septa



- In addition dermatophytes produce abnormal hyphal forms like
- **Racquet hyphae**
- Composed of chain of individual elongated hyphal cells expanded at one end and resemble a tennis racquet
- **Spiral or coiled hyphae**
- Coiled or cork screw shaped which resemble the shape of plant tendrils
- **Pectinate hyphae**
- Resemble tooth of a comb
- **Favic Chandliers**
- Irregular projections on one or both side of hyphae similar to antlers/ stag horns (very characteristic of *Trichophyton schoenleinii*)
- **Nodular body** : hyphae may intervene and form hard dormant nodular bodies





MYCOSES

- Diseases caused by fungi is called mycoses
- Disease are three types based on the tissues affected
- Superficial mycoses/ Dermatomycoses
- S/c or Intermediary mycoses
- Deep / visceral or systemic mycoses



SPERFICIAL MYCOSES- DERMATOPHYTOSIS

- Caused by Dermatophytes
- generally known as ring worm fungi.
- This require keratin for their growth and affect stratum corneumof skin hair nail etc
- there are three important genera
- *Trichophyton*
- *Microsporon*
- *Epidermophyton*
- This organism are classified under Ascomycetes



- Based on tropism dermatophytes are classified as
- Zoophilic- present in animals
 - *M. canis* and *Trichophyton verucosum*
- Geophilic : Natural habitat is soil
 - *M. gypseum*
 - *M. nanum*
 - *T. simii*
- Anthropophilic: Primarily infect human being
 - *T. rubrum*
 - *E. floccosum*



- The disease produced by Trichophyton and microsporon in human being is called Tinea
- Both microsporon and Trichophyton produce disease in animals and human being
- Trichophyton will affect skin, hair and nail
- Microsporum will affect skin and hair
- Epidermophyton affects nail and skin



IMPORTANT SPECIES ARE

Trichophyton	Microsporon	Epidermophyton
T. verrucosum,	M. nanum	Epidrmophyton
T. Equinum	M. Distortum	floccosum
T. Violaceum	M. Gypseum	
T. Mentagrophytes	M. Canis	
T. Schoenlenii	M. Audouini	
T. Gallinae	M. gallinae	
T. simii		



PATHOGENESIS

- These organism cause hydrolysis of keratin
- As a result of infection the host mount inflammatory response
- Organism can not thrive in an area of intense infection and will move to periphery
 - so it will produce ring like lesions
- Central area will have heated appearance and inflammed periphery
- Lesions are by toxins, allergens and enzymes like keratinase and collaginase



- Disease in four forms
- Subclinical
- Classical ring worm
- Generalised form
- Kerion / tumor like lesions in dogs



CLINICAL SIGNS

- In dogs and cat important organism is *Microsporon canis*
- Main lesion occur in face and extremity
- it will produce crusty alopecia lesion
- in cattle important organism is *Trichphyton verucosum* and *T. mentagrophytes*
- lesions mainly in face and neck region
- Extent of lesion may vary
- it can be mild infection characterised by eruption and alopecia
- Chronic lesions are yellowish brown, very thick asbestose like

- In horse Trichopyton equinum and Trichophyton mentagrophytes lesions are seen in withers, saddle and girth.
- Lesion can be either urticarial eruption or deep ulcerative nodule
- In sheep and goat organism are Trichophyton veerrucosum and T. mentagrophytes
- In pigs
- T. verrucosum
- T. mentagrophytes
- M.nanum
- In pig urticarial lesion on the base of ear and trunk

- In poultry
- *T. gallinae*
- *M.galline*
- disease is known as avian ring worm or favus
- Disease seen as white patchy crust on comb and wattle

DIAGNOSIS

- Symptoms and lesions
- Ring worm appearance
- Examination of infected hair usin Wood's lamp or UV lamp
- If infected hair is shown on wood lamp, there will be apple green florescence due to the production of metabolite of Tryptophan by the organism
- In positve infection by M canis, M. distortum and M. audonii
- False positive with dandruff or application of salicylic acid or petroleum jelly

DIRECT MICROSCOPICAL EXAMINATION OF SKIN SCRAPPING OT HAIR

- Before collection of these , clean the area with 70% alcohol. Scrape skin from old and new lesions with blunt scalpel or razor blade untill blood oozes to come out
- Better to pluck hair
- Take skin scarpping or hair o n a clean slide and add 2-3 drop of 10-20 % KOH (Keratlytic). Gently warm the slide at 60oC for 5-1 minutes. Put coverslip and examine under low and high power
- KOH cause partial digstion of Keratin leading to clearing of specimen



STAIN WITH LACTOPHENOL COTTON BLUE

- In DME of arthrospores of fungi. Most fungal agents of animals, spores can seen on external surface of hair and such arrangement called ectothrix
- *M. canis*
- *T. verrucosum*
- *T. mentagrophytes*
- In some other case large spore arranged lineraly within hair shaft called endothrix
- *T. violaceum*
- *T. schoelleiniialong* with spore. Large number of air bubbles also seen
- *T. gallinae*, *T. simii*, *T.rubrum* will not affect hair



PASTEURELLA

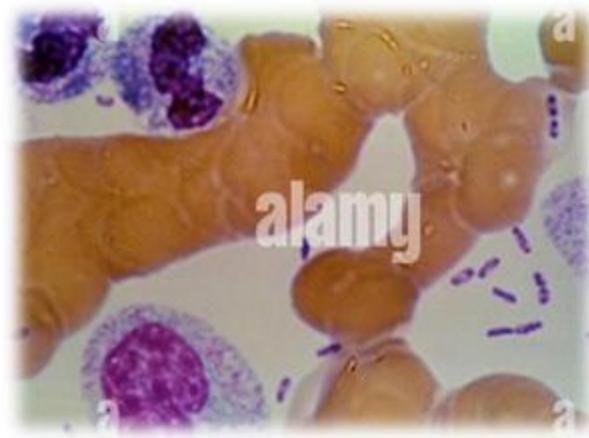
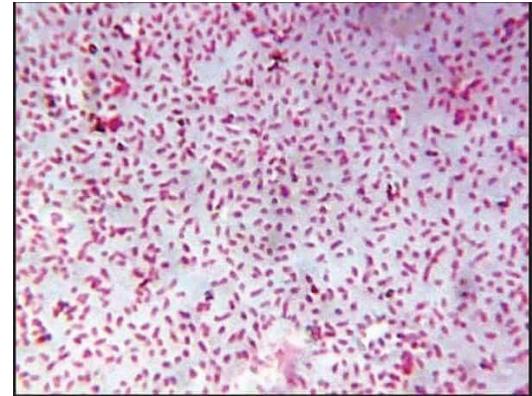
Dr. Bincy Joseph
Assistant Professor
PGIVER, Jaipur

PASTEURELLA

- *Pasteurella multocida* subsp. *multocida*
- The species *Pasteurella multocida* is Gram- negative bacteria that are inhabitants of the upper respiratory tract of many vertebrate hosts including birds, cattle, swine, cats, dogs and rodents.
- Members of this species are responsible for a number of infections that normally are secondary to colonization of the upper respiratory tract
- **Avian cholera/ Fowl cholera** (in waterfowl, chickens and turkeys),
- Respiratory disease and **hemorrhagic septicemia** in ruminants (cattle, sheep, goats and buffalo)
- **Atrophic rhinitis** in pigs
- **Snuffles/septicemia** in rodents (mice & rabbits).
- *P. multocida* is also a rare cause of infection in humans that is normally associated with dog or cat bites or scratches.

MORPHOLOGY

- Members of the Genus *Pasteurella* are Gram-negative cocco-bacilli.
- They are non-motile and non-sporulating organisms.
- They possess a capsule that is composed of **polysaccharide and hyaluronic acid**.
- They are facultative anaerobic organisms, fermentative and **oxidase positive**.
- These organisms are known for **their bi-polar staining property** in tissue smears stained by Leishman's stain or methylene blue



CLASSIFICATION

- The latest classification of *Pasteurella* is based on DNA analysis. Based on this method, the important species of veterinary importance and the diseases produced by them are listed as follows;
 - ***P.multocida* subsp *multocida*** – HS in cattle (type B2 and E2)
 - ***P.multocida* subsp *septica*** - Wound contamination
 - ***P.multocida* subsp *gallicida*** - fowl cholera (type A)
 - ***P.gallinarum*** - respiratory tract infection
 - ***P.canis*** - dog bit wound infections
 - ***P.avium* - *Haemophilus avium***

RESISTANCE

- They are not very resistant organisms
- They are killed by chemical and agents like 0.5% phenol when exposed for 15 minutes
- They are also killed on exposure to sunlight for 3-4 hours and heating at 55°C for 15 minutes.



ANTIGENS AND TOXINS

- Based on differences in capsular substances, *P. multocida* classified into A, B, D, E and F
(carter system of classification)
- These types are further subdivided into 16 subtypes based on differences in somatic antigens (Robertson system of classification)
- These subtypes are assigned numerals
- Thus a serotype is designated by an alphabet followed by a number eg. **Serotype B2 causes HS.**
- A toxin in addition to endotoxin (LPS) is produced by B serotype



CULTURAL CHARACTERISTICS

- They are aerobic or facultative anaerobic organisms and grow at a temperature of 37°C.
- In ordinary media (nutrient agar), three types of colonies – mucoid, smooth (iridescent) and non-iridescent colonies are produced after overnight incubation
- The mucoid colonies are round, flat, mucoid and sticky of 2-3 mm in diameter. Organisms with abundant capsular material produce these types of colonies.
- Organisms with less capsular material produce the iridescent colonies.
- Whereas rough colonies are produced by organisms that have no capsular material
- **Blood agar** is preferred medium for growth
- There is darkening of the medium with no haemolysis and a specific odour (**tender coconut smell**).
- In broth cultures they produce uniform turbidity.



BIOCHEMICAL CHARACTERS

- **Biochemical characters:** HS organisms produce oxidase, catalase and indole, and will reduce nitrates.



HAEMORRHAGIC SEPTICAEMIA

- Haemorrhagic septicemia (HS) is an acute pasteurellosis, caused by particular serotypes of *Pasteurella multocida* and manifested by an acute and **highly fatal septicemia** principally in cattle and water buffaloes the latter are thought to be more susceptible than cattle
- HS occurs infrequently in swine and even less commonly in sheep and goats.
- It has been reported in bison, camels, elephants, horses, and donkeys, and there is evidence of its occurrence in yak.
- Presently, two serotypes are recognized – the Asian serotype and the African serotype
- The Asian strains belong to capsular type B only while the African strains are types B and E.
- Epidemic HS is caused by one of two serotypes of *P multocida*, designated B:2 in Asia and E:2 in Africa



PATHOGENESIS

- Infection is exogenous and animals acquire the infection by contact, inhalation or ingestion
- It is hypothesized that animals become susceptible as a result of various stresses, eg. the inanition seen in cattle and water buffalo at the beginning of the rainy season.
- The heaviest losses occur during the monsoon rains in south east Asia, and it is thought that the organisms, which can survive for hours and probably days in the moist soil and water, are transmitted widely at this time.
- Biting arthropods rarely transmits infection.
- *Pasteurella* organisms are found in the upper respiratory and digestive tract of animals and birds as commensals.



PATHOGENESIS CONTINUES....

- Most of the animals carry these organisms with them without exhibiting any symptoms. The organisms produce infection when they are exposed to stress conditions like extreme weather conditions, transport, immunosuppression etc.
- During these conditions the organisms rapidly multiply and excreted via droplets and through digestive tract. The droplets with organisms are the main source of infection for other animals.
- In *Pasteurella* infection, the morbidity is always higher. *P. multocida* is also a secondary invader in respiratory tract infection. The important virulence factor is the endotoxin (LPS). Besides this a thermostable, cell associated toxin that is released by dying cells also plays a role in pathogenesis.

SYMPTOMS

- The clinical syndrome of HS consists of an initial phase of temperature elevation (often unnoticed),
- A phase of respiratory involvement, and a terminal phase of septicaemia and recumbency leading to death
- The incubation period is usually 1–3 days, and the course of the disease may range from sudden death, with no observable clinical signs, to a protracted course extending up to 5 days
- Buffaloes are generally believed to be more susceptible to HS than cattle, and in this species, the disease course is shorter.
- In endemic areas, most deaths are confined to older calves and young adults.
- In nonendemic areas, massive epizootics may occur.
- Case fatality approaches 100% if treatment is not carried out sufficiently early (in the pyrexic stage).



- Three forms of HS are noticed among animals – acute, sub-acute (oedematous form) and sub acute (pectoral form).
- The important symptoms in acute cases are rise in body temperature, drop in milk yield, abdominal pain, severe diarrhoea and dysentery.
- The respiration becomes rapid before death and the mucous membrane becomes cyanotic.
- Less acute cases are characterised by rise in body temperature and oedema in the head, neck and brisket region.
- A purulent, blood stained nasal discharge is also noticed.
- Occasional cases linger for several days. Recovery is rare
- There appears to be no chronic form.

LESIONS

- The most obvious changes in affected animals are the edema, widely distributed hemorrhages, and general hyperemia.
- In most cases, there is an edematous swelling of the head, neck, and brisket region.
- Incision of the swellings reveals a clear or straw-colored serous fluid.
- The edema is also found in the musculature, and the subserous petechial hemorrhages, which are found throughout the animal, are particularly characteristic.
- Blood-tinged fluid is often found in the pericardial sac and in the thoracic and abdominal cavities.
- The lesions are also coupled with gastroenteritis, marbled lungs, blood stained stools and enlarged and haemorrhagic mesenteric lymph nodes.
- Petechial hemorrhages are seen in the pharyngeal and cervical lymph nodes.
- Gastroenteritis is seen only occasionally and, unlike pneumonic pasteurellosis, pneumonia usually is not extensive.



DIAGNOSIS

- **Based on symptoms and lesions.**
 - **Microscopical examination:**
 - The septicaemia in HS occurs at the terminal stage of the disease.
 - Therefore, blood samples taken from sick animals before death may not always contain *P. multocida* organisms.
 - Also, they are not consistently present in the nasal secretions of sick animals.
 - A blood sample or swab collected from the heart within few hours after death is most ideal.
 - Blood smears from affected animals are stained with Gram, Leishman's or methylene blue stains.
 - The organisms appear as Gram-negative, bipolar-staining short bacilli.
 - No conclusive diagnosis can be made on the basis of direct microscopic examinations alone.



- **Isolation and identification:** Blood in transport medium is the most ideal material for isolation. If the animal has been dead for a long time, a long bone, free of tissue, can also be taken.
- The most ideal medium for isolation of Pasteurella is casein/sucrose/yeast (CSY) agar containing 5% blood (calf blood).
- Freshly isolated *P. multocida* forms smooth, greyish glistening translucent colonies, approximately 1 mm in diameter, on blood agar after 24 hours' incubation at 37°C. Colonies grown on CSY agar are larger.
- Old cultures, particularly those grown on media devoid of blood, may produce smaller colonies.

- **Test to confirm the production of hyaluronidase:**
- HS-causing strains of *P. multocida* has the ability to produce the enzyme hyaluronidase.
- A hyaluronic-acid-producing culture is streaked across the centre of a dextrose starch agar plate.
- The pasteurella culture to be tested for hyaluronidase production is streaked at right angles.
- The plates are incubated at 37°C for 18 hours.
- At the point of intersection, the mucoid growth of the hyaluronic acid producer will diminish into a thin line of growth, indicating the production of hyaluronidase by the test culture.

- **Immunological methods:** Several immunological tests are used for the identification of the HS-causing serotypes of *P. multocida*.
- These consist of a rapid slide agglutination test, an IHA test for capsular typing, the AGID test, and the counter immunoelectrophoresis test (CIEP).



- **Nucleic acid recognition methods:** PCR amplification of specific DNA sequences allows rapid detection and presumptive identification of organisms directly from either clinical specimens or from small amounts of mixed or pure bacterial cultures. Some of the common methods performed to identify *Pasteurella* are as below
 - *Pasteurella-multocida*-specific PCR assay
 - *Pasteurella multocida* multiplex capsular PCR typing system
 - HS-causing type-B-specific PCR assay
 - Genotypic differentiation of isolates
- .
-

- **Serological methods:** Serological tests for detecting antibodies are not normally used for diagnosis.
- **Animal Inoculation:** The mouse usually serves as a biological ‘screen’ for extraneous organisms.
- A small volume (0.2 ml) of eluted blood swabs or a portion of bone marrow in saline is inoculated subcutaneously or intramuscularly into mice.
- If viable *P. multocida* is present, the mice die 24–36 hours following inoculation, and a pure growth of *P. multocida* can be seen in blood smears

- **Treatment:** Various sulfonamides, tetracyclines, penicillin, and chloramphenicol (are effective if administered early).
- Because of the rapid course of the disease and the frequent difficulty of access to animals, antimicrobial therapy often is not practicable.
- **Control:** The principal means of prevention is by vaccination.
- Three kinds of vaccine are widely used: plain bacterin, alum-type precipitated bacterin, and oil-adjuvant bacterin. The most effective bacterin is the oil-adjuvant— one dose provides protection for 9-12 months; it should be administered annually.
- The alum-precipitated-type bacterin is given at 6-months intervals.
- A live vaccine prepared from a B:3,4 serotype of deer origin is being used with reported success in southeast Asia.

FOWL CHOLERA (AVIAN CHOLERA)

- Fowl cholera (avian pasteurellosis) is a commonly occurring avian disease that can affect all types of birds and is often fatal.
- It is contagious and usually occurs as a septicemia of sudden onset with high morbidity and mortality, but chronic and asymptomatic infections also occur.



- Turkeys are more susceptible than chickens, older chickens are more susceptible than young ones, and some breeds of chickens are more susceptible than others.
- Chronically infected birds are considered to be a major source of infection.
- Dissemination of *P multocida* within a flock is primarily by excretions from mouth, nose, and conjunctiva of diseased birds that contaminate their environment.



SYMPTOMS

- These vary greatly depending on the course of disease.
- In acute fowl cholera, dead birds are usually the first indication of disease.
- Fever, depression, anorexia, mucoid discharge from the mouth, ruffled feathers, diarrhea, and increased respiratory rate are usually seen.



LESIONS

- Many of the lesions are related to vascular disturbances
- Hyperemia is especially evident in the vessels of the abdominal viscera.
- Petechial and ecchymotic hemorrhages are common, particularly in subepicardial and subserosal locations.
- Increased amounts of peritoneal and pericardial fluids are frequently seen.
- Livers may be swollen and often develop multiple, small, necrotic foci.
- Pneumonia is particularly common in turkeys.
- In **chronic fowl cholera**, signs and lesions are generally related to localized infections. Sternal bursae, wattles, joints, tendon sheaths, and footpads are often swollen because of accumulated fibrinosuppurative exudate.
- There may be exudative conjunctivitis and pharyngitis.
- Torticollis may result when the meninges, middle ear, or cranial bones are infected.

DIAGNOSIS

Based on the symptoms and lesions

- **Microscopical examination:** . The cells are coccobacillary or short rod-shaped, usually 0.2–0.4 by 0.6–2.5 µm in size, stain Gram negative, and generally occur singly or in pairs. Recently isolated organisms or those found in tissue smears show bipolar staining with Wright or Giemsa stains or methylene blue, and are usually encapsulated.
- **Isolation and identification:** Isolation of the organism from visceral organs, such as liver, bone marrow, spleen, or heart blood of birds that succumb to the acute form of the disease, and from exudative lesions of birds with the chronic form of the disease, is generally easily accomplished.
- *Pasteurella multocida* is a facultative anaerobic bacterium that grows best at 35–37°C.
- Primary isolation is usually accomplished using media such as blood agar, trypticase–soy agar or dextrose starch agar, and isolation may be improved by supplementing these media with 5% heat-inactivated serum.

- **Nucleic acid identification methods:** The most ideal method is the DNA fingerprinting of *P. multocida* by restriction endonuclease analysis (REA).
- **Serological tests:** Serological tests for the presence of specific antibodies are not used for diagnosis of fowl cholera.

◦



- **Treatment:** Sulfonamides and antibiotics are commonly used; early treatment and adequate dosages are important.
- High levels of tetracycline antibiotics in the feed (0.04%) or administered parenterally may be useful.
- **Control:** Good management practices are essential to prevention.
- Adjuvant bacterins are widely used and generally effective
- Autogenous bacterins are recommended when polyvalent bacterins are found to be ineffective
- Attenuated vaccines are available for administration in drinking water to turkeys and by wing-web inoculation to chickens.
- These live vaccines can effectively induce immunity against different serotypes of *P multocida*.
- They are recommended for use in healthy flocks only.

OTHER IMORTANT PASTEURELLA INFECTIONS

- - **Rabbits:** Peracute infection is common in rabbits. In chronic infection coryza like respiratory symptoms are common.
 - **Sheep:** Mastitis (blue bag) and pneumonia
 - **Dogs and cats:** Septic contamination of wounds
 - **Pigs:** Respiratory infection and rhinitis.

ATROPHIC RHINITIS

- An infectious disease of swine characterised by purulent nasal discharge, shortening or twisting of the snout, atrophy of the turbinate (conchal) bones and reduced productivity.
- It may occur enzootically or more sporadically, depending on a variety of factors including herd immunity.
- The most severe progressive form is caused by infection with toxigenic strains of *Pasteurella multocida* type D alone or in combination with *Bordetella bronchiseptica*.

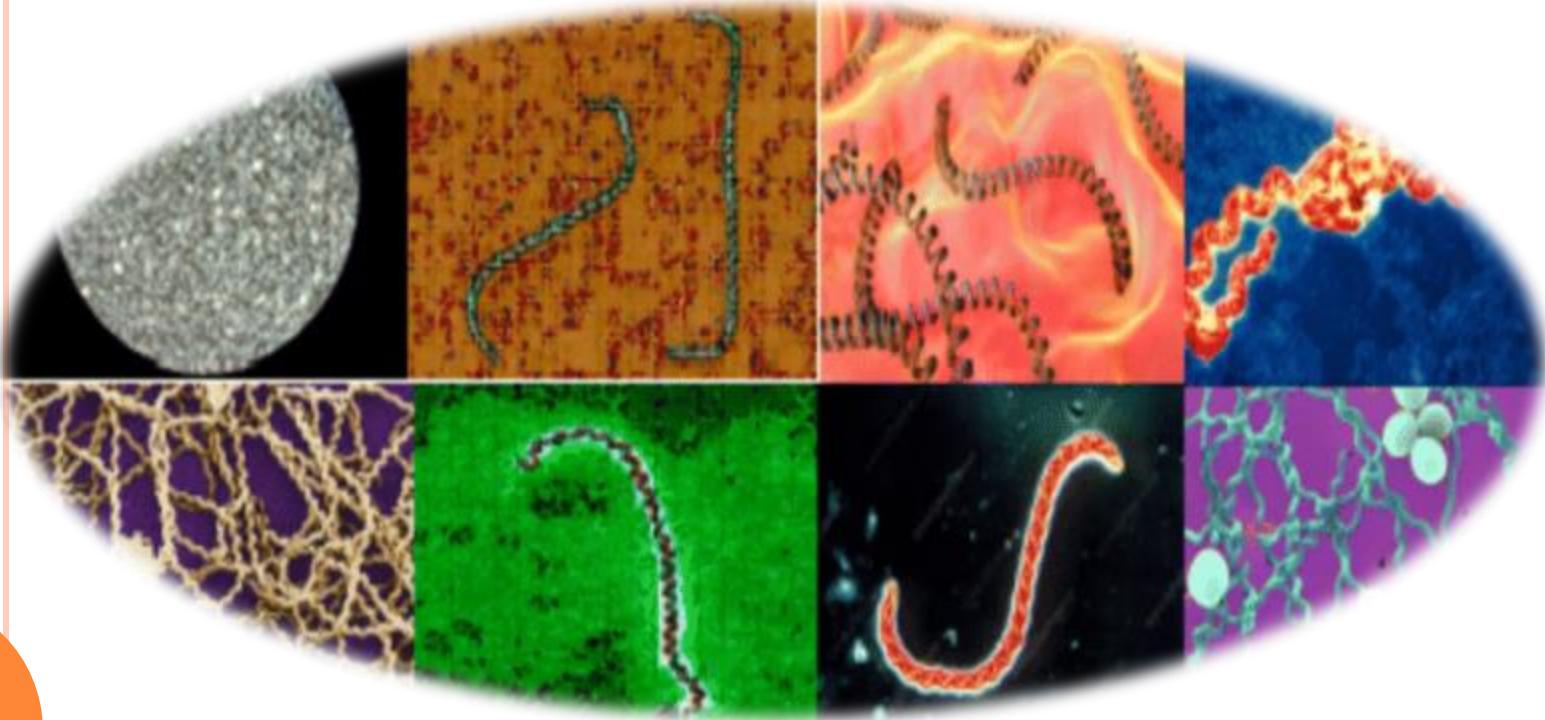


- ***Mannheimia haemolytica* (*P. heamolytica*):**
- Unlike *P. multocida*, it is beta haemolytic.
- Causes transport or **shipping fever** in cattle.
- It is a pneumonic condition with rise in temperature, rapid respiration followed by death with 12-48 hours.
- In less acute cases coughing, debility and death are noticed.
-



RIEMERALLA ANATIPESTIFER **(*P.ANATIPESTIFER*):**

- It is a non-fermenting bacterium.
- It causes infectious serositis (New duck disease) in ducklings.
- Anatipestifer infection causes high mortality, weight loss and condemnation.
- In the acute form, listlessness, eye discharge and diarrhea are commonly seen.
- Ducks show incoordination, shaking of the head and twisted neck. Birds are commonly found on their backs, paddling their legs.
- Typical lesions found in dead birds are infected air sacs, membranes covering the heart and liver, and meningitis.
- Preventive management and vaccination are effective means of control.
- Penicillin, enrofloxacin and sulfadimethoxine-ormetoprim (0.04-0.08% in feed) are effective in reducing mortality.

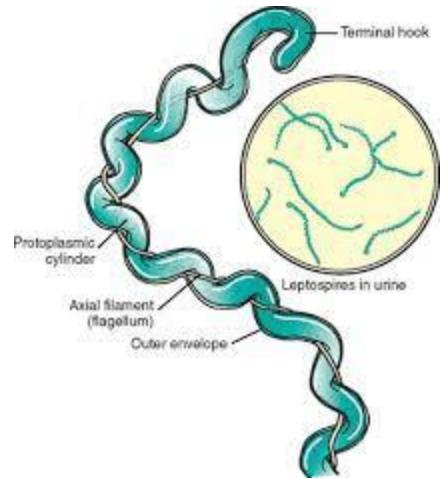


SPIROCHAETES

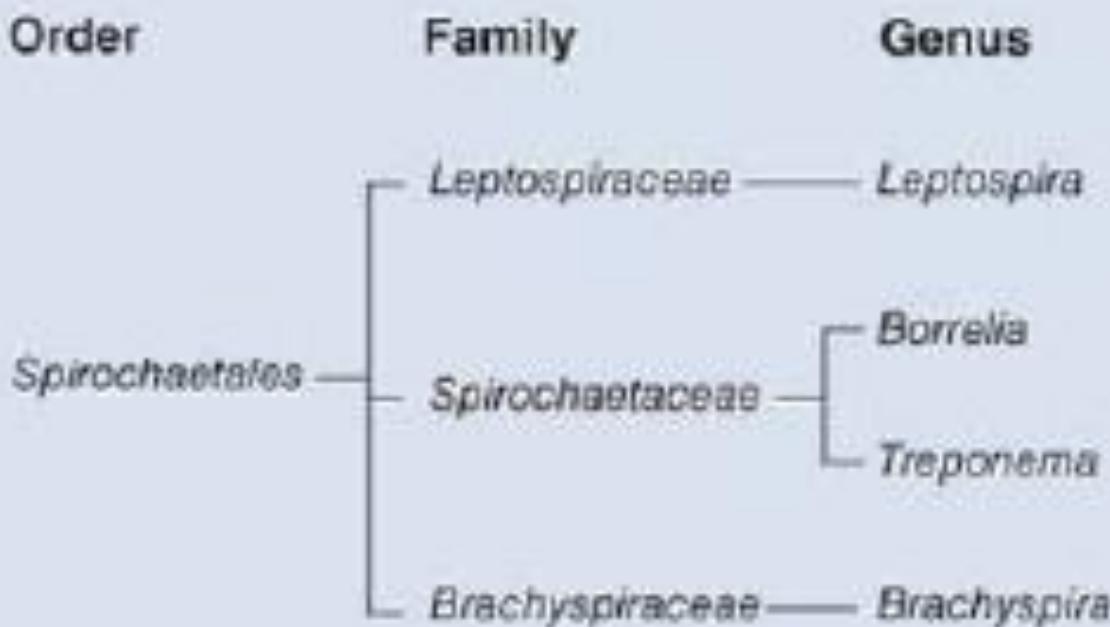
Dr. Bincy Joseph
Assistant Professor
PGIVER, Jaipur

SPIROCHAETES

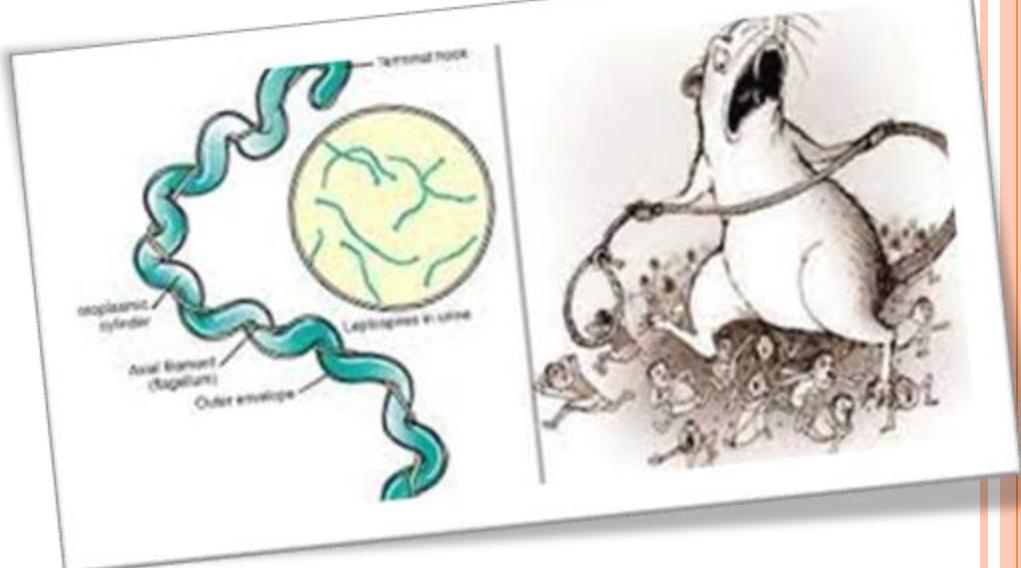
- The order Spirochaetales : Five families
 - Leptospiraceae**
 - Spirochaetaceae**
 - Brachyspiraceae**
- Spiral or Helical bacteria
- Motile by means of endoflagella located within periplasm
- The genus **Leptospira**, **Borrelia**, **Brachyspira**, and **Treponema** contain human and animal pathogen
- Some grow only in liquid media (Korthof broth and Stuarts broth) and most require specialised media for growth
- Many produce zoonotic infections



CLASSIFICATION OF SPIROCHAETES OF VETERINARY IMPORTANCE



LEPTOSPIRA SPECIES



- Lepto means fine and spira means coils
- Leptospires are motile helical bacteria with hook shaped ends
- They have two circular chromosome
- They are Gram negative, but do not stain well with bacteriological dyes
- microaerophilic
- They are visualized by Dark field microscopy
- Silver impregnation and immunological staining technique used to demonstrate leptospires in tissues.
- Leptospires can affect all domestic animals and humans
- Infection ranges from mild infections of the urinary and genital systems to serious systemic diseases.
- Labile in the environment and sensitive to desiccation

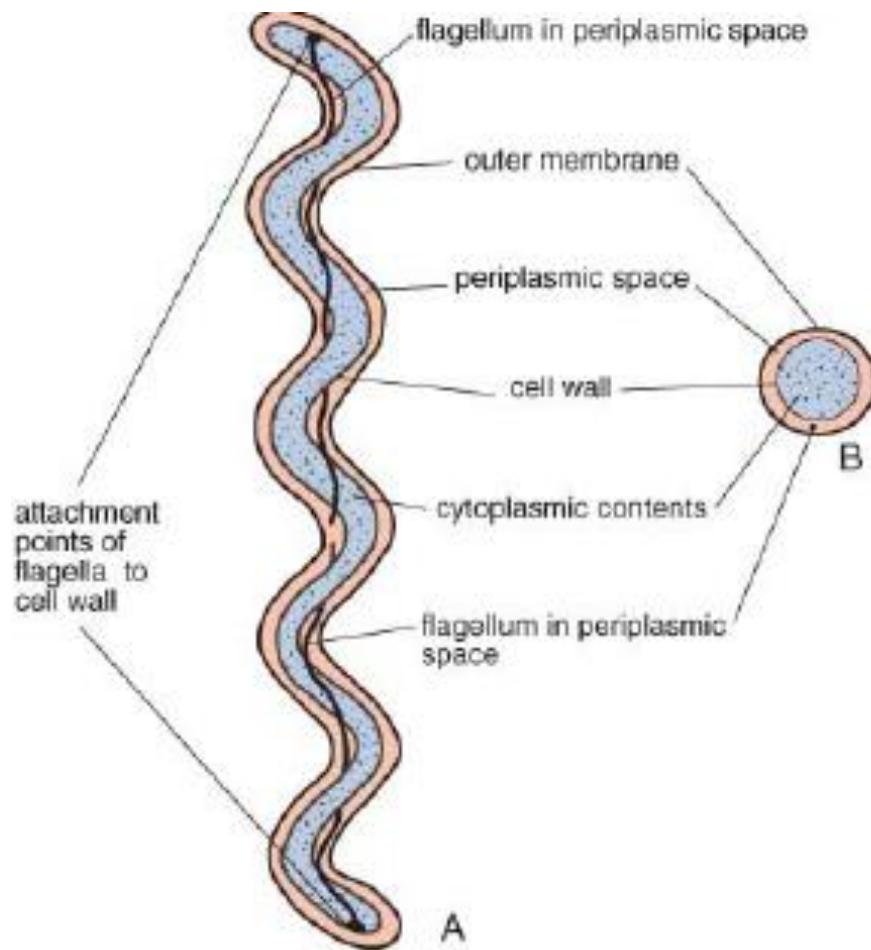
LEPTOSPIRA CONTD.

- Found in aquatic environments
- Produce systemic infections in many species
- The organism responsible for Weil's disease in human being
- Shed in urine of affected species
- Cultured in liquid media aerobically at 30°C
- Darkfield microscopy, Silver staining, Immunofluorescence and molecular techniques used for recognition.



- The organism are thin spiral and slender in size
- One or both ends hooked thus it have S or ? Shape
- It possess an axial filament or endoflagella
- Motility is referred as serpentine / boring/ undulating motility
- The media should be supplemented with 20% rabbit serum
- pH of medium 7.3-7.4
- Temperature of incubation is 30°C
- Growth occur after 6-14 day
- We have to incubate the media for eight weeks before declaring negative
- The growth in liquid media called shot silk growth
- In semisolid media : a ring of growth seen just below the surface and is called Dinger's ring because it is microaerophilic

STRUCTURE OF SPIROCHAETES

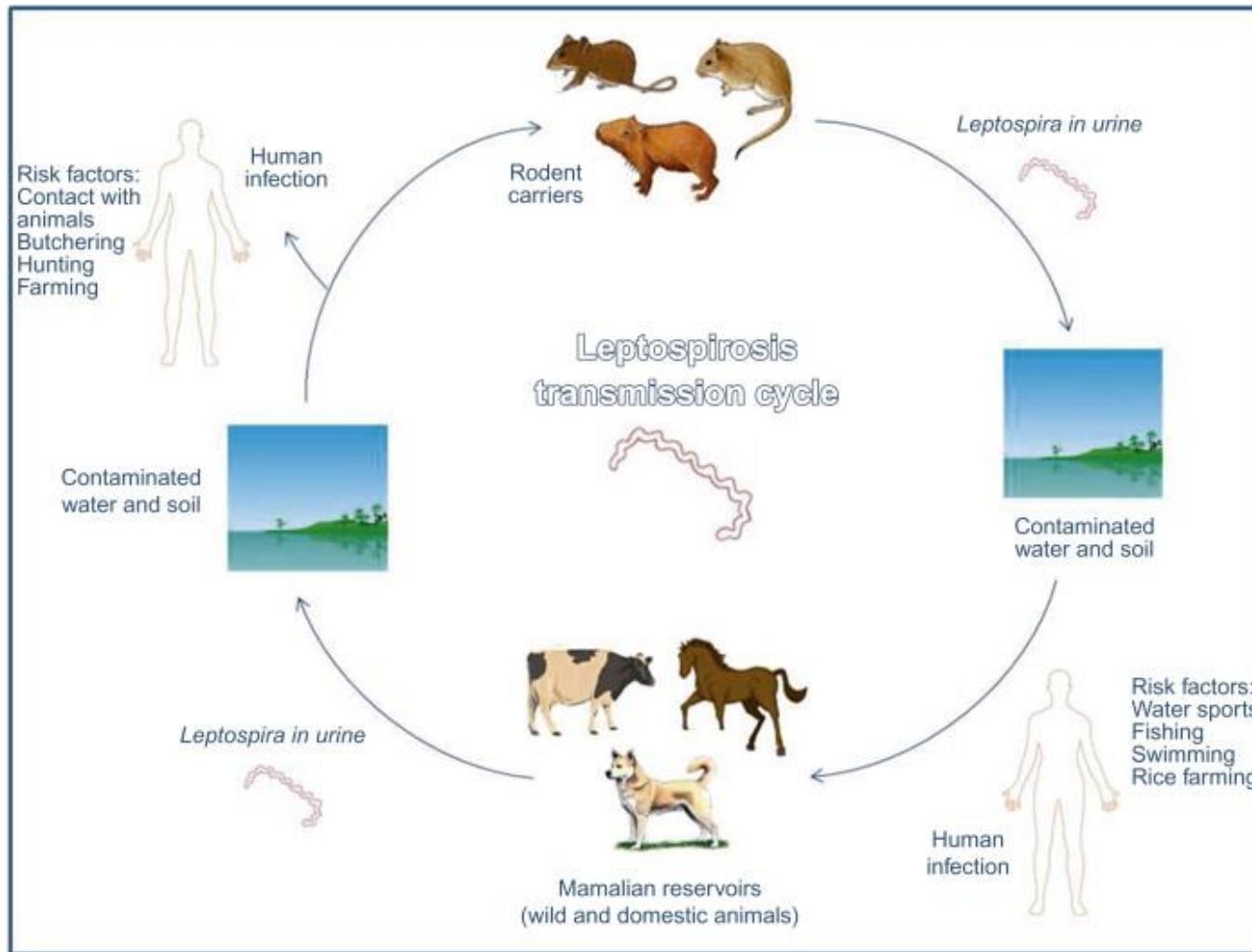


HABITAT

- Ponds, rivers, surface waters, moist soil and mud when environmental temperature is moderate
- Pathogenic leptospires can persist in the renal tubules and genital tract of carrier animals
- Indirect transmission occurs through exposure of susceptible animals to contaminated water sources, food or even bedding
- A suitable habitat for *Leptospira* is slow or stagnant water, resulting in outbreak during periods of flooding



LEPTOSPIROSIS TRANSMISSION CYCLE



LEPTOSPIRA SPECIES

- Formerly leptospires were differentiated by serological reactions
- There were two species
 - Leptospira interrogans* : containing pathogens
 - L. biflexa* : saprophytes
- Now the species of leptospira were classified by DNA homology and within each species various serovars are recognised by serological reactions
- Currently there are 20 leptospiral species:
- More than 250 pathogenic serovars in 24 serogroups
- Serovar Hardjo belongs to two species, *L. borgpetersenii* and *L. interrogans*.
- They share common surface antigen by these genetically distinct organism
- Serological classification remains clinically important because particular serovar tend to be associated with specific host animals and cross immunity between serovars is minimal

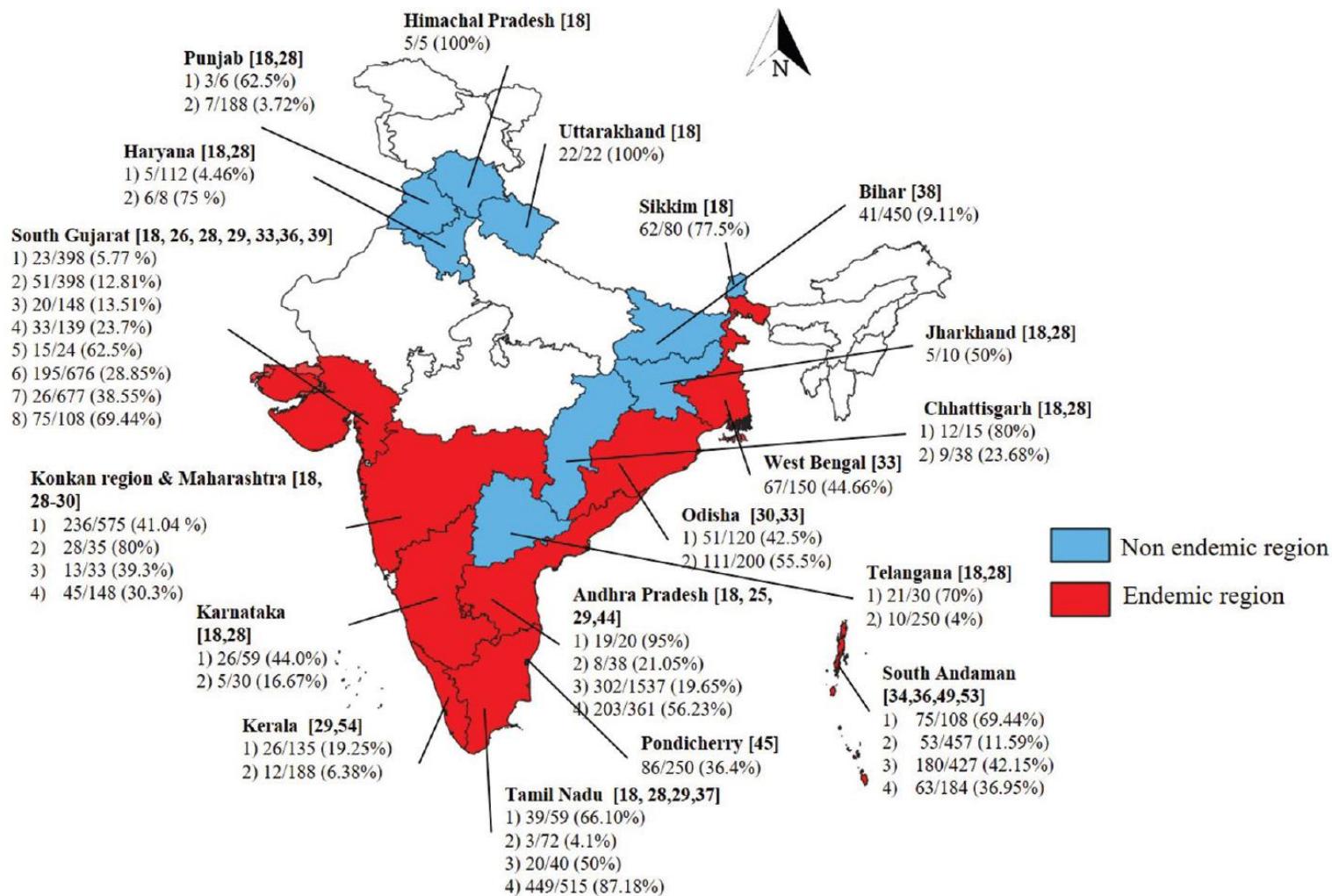
Serovar	hosts	Clinical conditions
<i>Leptospira borgpetersenii</i> serovar Hardjo	Cattle, Sheep	Abortion, still birth, agalactia
<i>Leptospira interrogans</i> Hardjo	Human	Influenza like illness, occasionally liver and kidney disease
<i>Leptospira borgpetersenii</i> serovar Tarassovi	pigs	Reproductive failure, abortions and still birth
<i>Leptospira interrogans</i> serovar Bratislava	Pig, horses, dogs	Reproductive failure, abortion and still birth
<i>L. Interrogans</i> serovar Canicola	Dogs	Acute nephritis in pups. Chronic renal disease in adult animals
	pigs	Abortion, still birth and Renal disease I in young pigs

Serovar	Hosts	Clinical conditions
<i>L. Interrogans</i> serovar Grippotyphosa	Cattle, dogs, pigs	Septicaemic disease in young animals and abortion
<i>L. interrogans</i> serovar Icterohaemorrhagiae	Cattle, sheep, pigs	Acute septicaemic disease in calves, piglets and lambs, abortions
	Dogs and humans	Per acute haemorrhagic disease, acute hepatitis with jaundice
<i>L. interrogans</i> serovar Copenhageni	Domestic animals and humans	Per acute and acute disease ; abortion in animals
<i>L. Interrogans</i> serovar Pomona	Cattle, Sheep	Acute haemolytic disease in calves and lambs; abortions
	Pigs	Reproductive failure; septicaemia in pigs
	Horses	Abortions , periodic ophthalmia

EPIDEMIOLOGY

- Leptospires found world wide
- Some serovars appear to have limited geographical distribution
- Most serovars associated with a particular host species, their maintenance host
- Maintenance host readily acquire infection, usually cause mild or subclinical infection and often associated with prolonged excretion of leptospires in urine.
- MH is main source of contamination of the environment
- Incident host –low susceptibility to infection, develop severe disease and inefficient transmitters of leptospires to other animals
- *L. interrogans* shows prolonged survival in suitable habitats such as surface waters

EPIDEMIOLOGY IN INDIA



[Leptospirosis in Bikaner \(Rajasthan\). A case report - PubMed](#)

by RG Agarwal · 1971 — **Leptospirosis** in Bikaner (Rajasthan). A case report. J Assoc Physicians India. 1971 Jan;19(1):53-4. Authors. R G Agarwal, K D Gupta, T P Bharadwaj.

<https://www.youtube.com/watch> ::

[अब Leptospirosis ने बढ़ाई चिंता ! अब तक सामने आए 26 ... - YouTube](#)

4 days ago — #FirstIndiaNews #Rajasthan About this Video: Now **Leptospirosis** has increased the concern! 26 cases reported so far, 18 cases in Jaipur alone ...

<https://journals.sagepub.com/doi> ::

[Scrub typhus and leptospirosis in rural and urban settings of ...](#)

28-Nov-2019 — Scrub typhus and **leptospirosis** are bacterial zoonotic diseases reported from ... observational study during an outbreak in **Rajasthan**, India.

[Introduction](#) · [Material and methods](#) · [Results](#) · [Discussion](#)

https://www.researchgate.net/post/What_is_the_status_... ::

[What is the status of incidence of leptospirosis in India?](#)

05-Oct-2017 — In some states such as Madhya Pradesh , Chattisgarh , Jharkhand , Bihar , **Rajasthan** & most of states in N.E. India , except Assam , no published ...

<https://m.facebook.com/videos/अ...> · [Translate this page](#) ::

[अब Leptospirosis ने बढ़ाई चिंता ! अब तक सामने आए 26 केस, अकेले ...](#)

4 days ago — अब **Leptospirosis** ने बढ़ाई चिंता ! अब तक सामने ... First India News **Rajasthan** · 50 mins · #FINDVideo #Leptospirosis

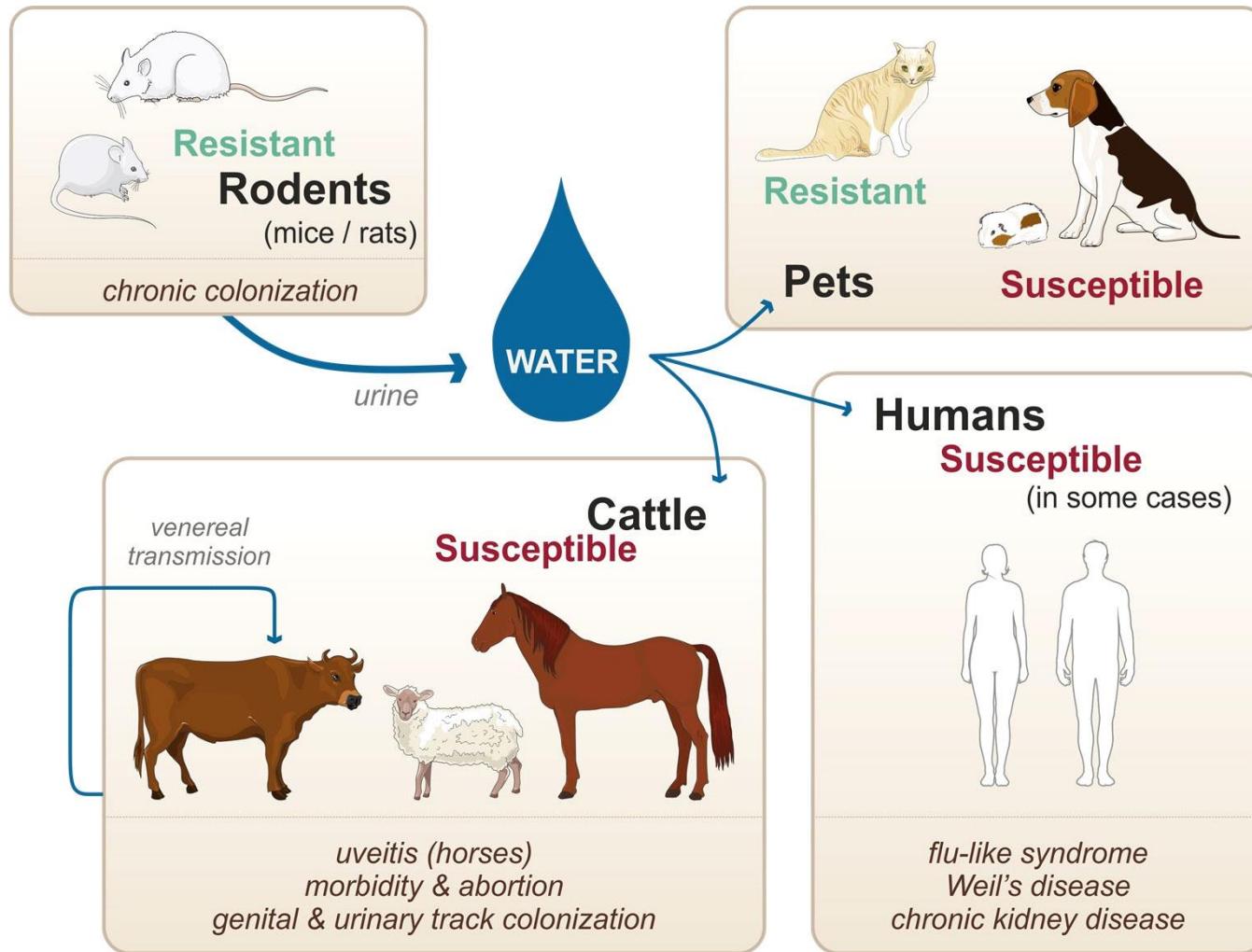
SEROVARS OF VETERINARY IMPORTANCE IN LEPTOSPIRA

Serovar	Maintenance host	Incidental host
Bratislava	Pig, hedgehogs, horses	Dogs
Canicola	Dogs	Pigs, cattle
Grippotyphosa	Rodents	Cattle, pigs, horses, dogs
Hardjo	Cattle (sheep occasionally), deer	Humans
Icterohaemaorrhagiae	Rats	Domestic animals
Pomona	Pigds, Cattle	Sheep, Horses, dogs



PATHOGENESIS AND PATHOGENICITY

- The pathogenicity depends upon **virulence of infecting serovar** and **susceptibility of host species**
- Disease may be severe in **immature maintenance host**, and serious disease occurs most commonly in **incidental hosts**
- Leptospires invade skin through moist , softened skin through mucous membrane, motility may aid tissue invasion
- They spread through body via blood stream , but following appearance of antibodies at about 10 days after infection, they are cleared from the circulation.

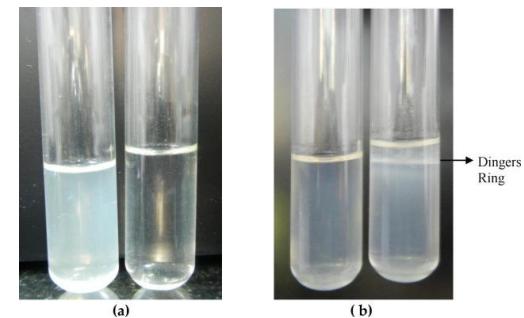


PATHOGENICITY

- The organism evade the immune response and persit in the body, principally in **the renal tubules, but also in the uterus, eye or meninges.**
- Toxic components are cell associated outer membrane proteins
- LPS is less endotoxic than other Gram negative bacteria
- Adherence to host cell surface through fibronectin binding protein
- Leptospires evade phagocytosis by inducing macrophage apoptosis
- The organism damage the cell membranes and endothelial cells along with hepato cellular injury produces haemolytic anaemia

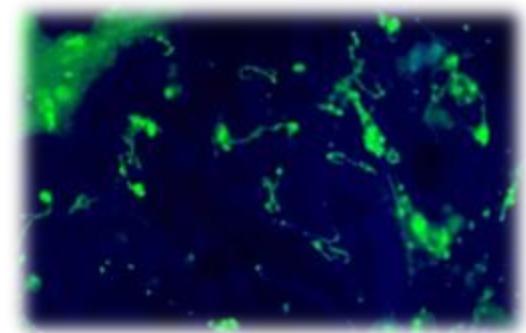
DIAGNOSIS

- Diagnosis in maintenance host by screening of defined population by dark field microscopy or MAT
- Clinical signs along with a history suggestive of exposure to contaminated urine, suggest acute leptospirosis
- Organism can be detected in fresh urine by **darkfield microscopy**
- Leptospires may be isolated from blood in early days of infection and from urine approximately 2 weeks after initial infection
- Inoculation in liquid culture or by animal inoculation



CONTD..

- Slow growing serovars such as Hardjo require 6 months in liquid media at 30° C.
- **EMJH** (Ellinghausen, McCullough, Johnson and Harris) medium, based on **1% bovine serum albumin and Tween 80**.
- Tween provides **long chain fatty acids** as nutrients and albumin absorbs these compounds and release them slowly
- FAT for demonstration of leptospires in tissues such as kidney and liver
- Silver impregnation technique also used for demonstration of leptospires in tissues



- DNA hybridisation, PCR, Magnetic immunocapture PCR and immunomagnetic antigen capture system and Real time quantitative PCR
- The standard serological reference test is **microscopic agglutination test**
- Titres in excess of **1:400 or four fold rise in the titre of paired serum samples**
- Serological diagnosis of host adapted leptospirosis is difficult as titres will not develop
- serovar based ELISA



CLINICAL INFECTIONS CATTLE AND SHEEP

- Cattle, sheep and deer : maintenance host for *L. borgpetersenii* serovar Hardjo
- Cattle: *L. interrogans* serovar Hardjo
- These will not cause severe disease in cattle
- Infection with serovar Hardjo in sheep causes abortions and agalactia
- Infection with serovars pomona, Grippotyphosa and Icterohaemorrhagiae can cause serious disease in calves and lambs
- Infection usually accompanied by pyrexia, haemoglobinuria, (red water disease) jaundice and anorexia
- Extensive renal damage with resultant uraemia often precedes death.
- Vaccination is used for control of serovar Pomona which is important cause of bovine abortion in some countries.

LEPTOSPIROSIS IN HORSES



- Serovar **Bratislava** associated with abortion and still births in horses where horse is the maintenance host
- Clinical infection caused by incidental infection by Serovar **Pomona**
- Clinical signs include abortion in mares and renal disease in young horses
- **Equine recurrent uveitis (periodic ophthalmia, moon blindness)** is manifestation of chronic leptospirosis
- Cross reaction between leptospiral antigens and proteins from the cornea and lens suggest that autoimmune mechanism involved



LEPTOSPIROSIS IN PIGS

- Acute leptospirosis in pigs caused by rodent adapted serovars such as **Icterohaemorrhagiae** and **Copenhageni**
- These serovars sometimes cause fatal infection in piglets
- Principal host adapted serovar is **Pomona (Swine herd disease)**
- Infection results in reproductive failure including abortion and still birth
- Pigs also serve as maintenance host for serovars **Tarassovi** and **Bratislava**



LEPTOSPIROSIS IN DOGS AND CATS



- Serovar **Canicola** and **Icterohaemorrhagiae** (Stuttgart's disease)
- **Grippotyphosa**, **Bratislava**, **Pomona** are emerging as important pathogen for dogs
- Hunting dogs are prone to infection
- Linked to season like heavy rain fall and late summer and early autumn
- Serovar Canicola cause renal disease in pups
- Animals that survive acute phase there will be chronic ureamic syndrome
- Incidental canine infections caused by **Icterohaemorrhagiae**, Copenhageni, signs of renal involvement usually predominate



Signs of Leptospirosis in Dogs



CONTROL

- Vaccination
- As immunity is serovar specific, the vaccine should contain the prevalent serovars



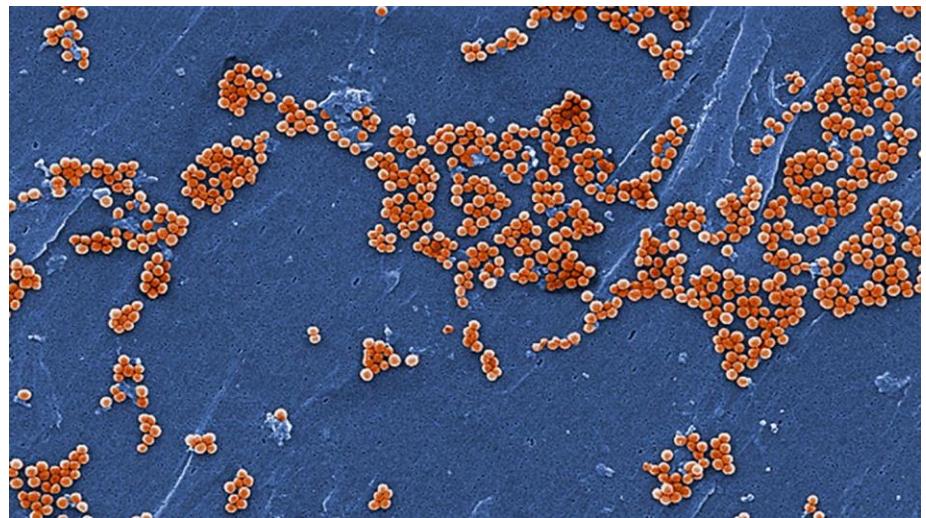
STAPHYLOCOCCUS



**Dr. Sandeep Kumar Sharma
Assistant Professor
Veterinary Microbiology
9414775879
drsharmask01@hotmail.com**

STAPHYLOCOCCUS

- Domain: Bacteria
- Kingdom: Bacteria
- Phylum: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Staphylococcaceae/ Micrococcaceae
- Genus: *Staphylococcus*
- Species: *Staphylococcus aureus*
- Binomial name: *Staphylococcus aureus*
 - Rosenbach 1884

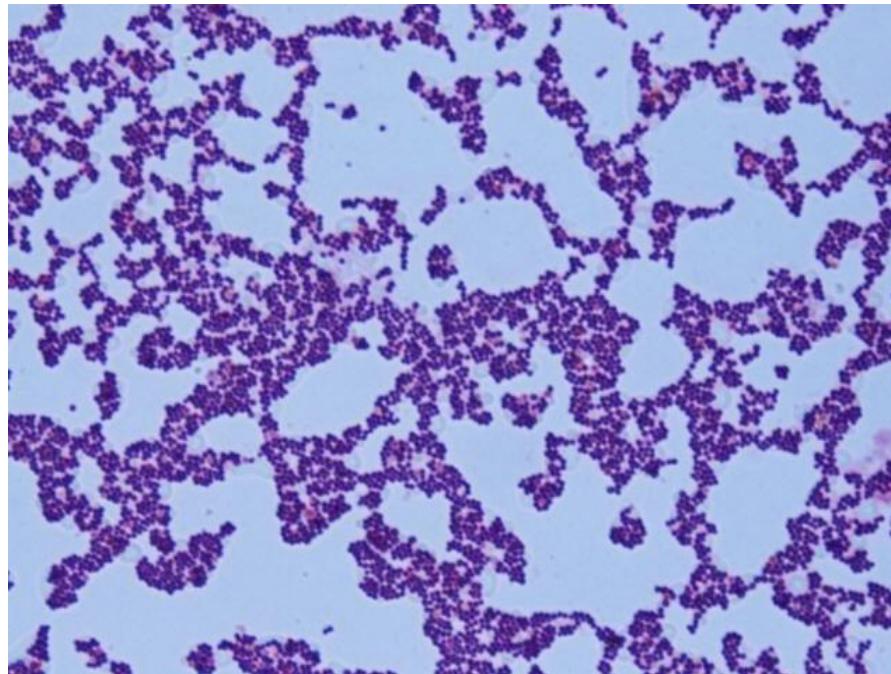


HISTORY

- Staphylococci were first observed in human pyogenic lesions by Von Recklinghausen in 1871.
- Pasteur in 1880 obtained liquid cultures of cocci from pus and produced abscesses by inoculating them into rabbits.
- Sir Alexander Ogston, a Scottish surgeon in 1880 who established conclusively the causative role of the coccus in abscesses and other suppurative lesions.
- He also gave the name Staphylococcus (*Staphyle, in Greek meaning bunch of grapes': Kokkos, meaning a berry*) due to the typical occurrence of the cocci in grape like clusters in pus and in cultures.

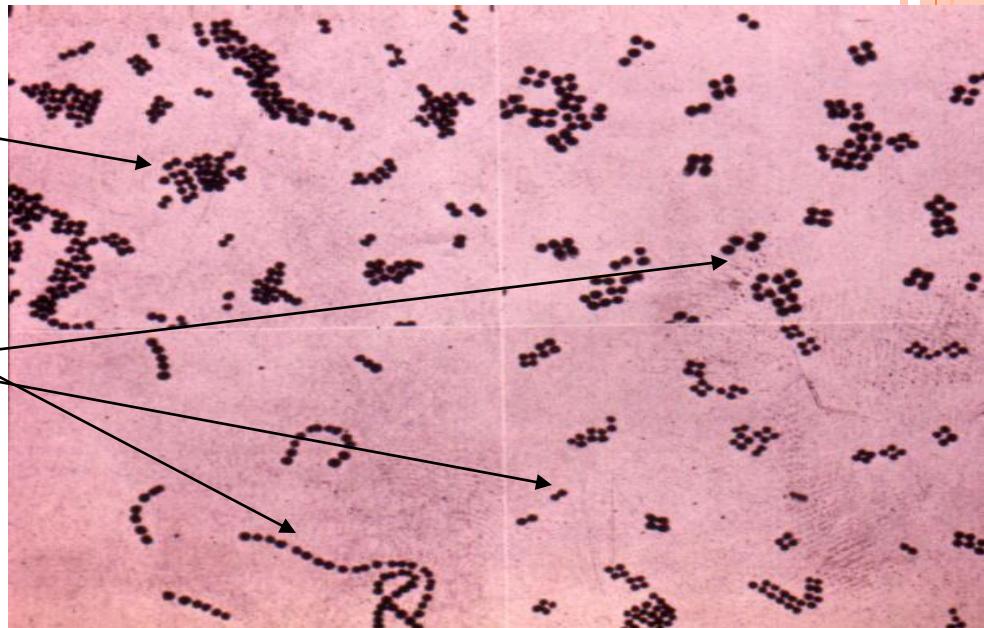


- Ogston had noticed that non-virulent staphylococci were also present on skin surfaces.
- Most staphylococcal strains from pyogenic lesions were found to produce golden yellow colonies, and the strains from normal skin, white colonies on solid media.
- In 1884, Rosenbach named them *Staphylococcus aureus* and *Staphylococcus albus* respectively. Later *S. albus* was renamed as *Staphylococcus epidermidis*

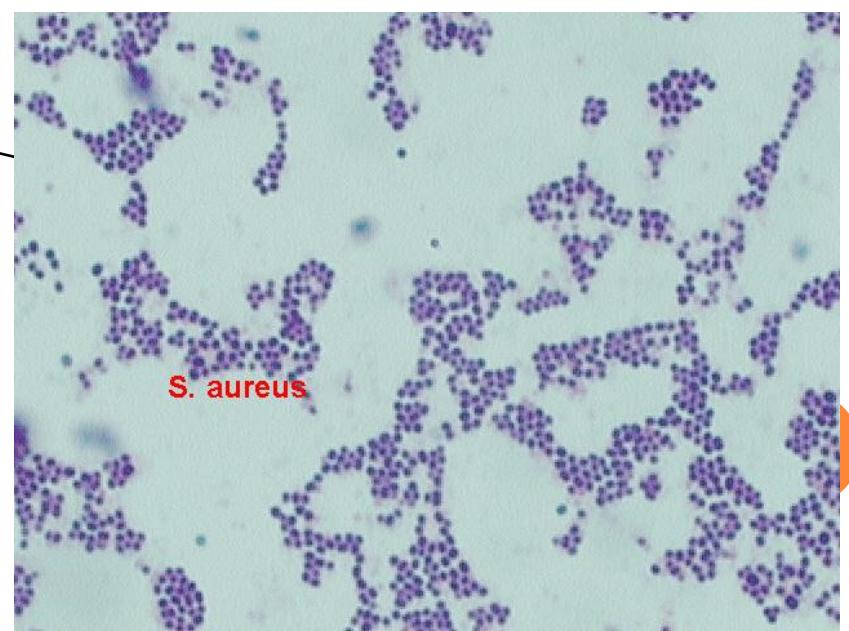


MORPHOLOGY

- *Staphylococcus* sp
- *Streptococcus* sp
- *Diplococcus* sp
- *Micrococcus* sp



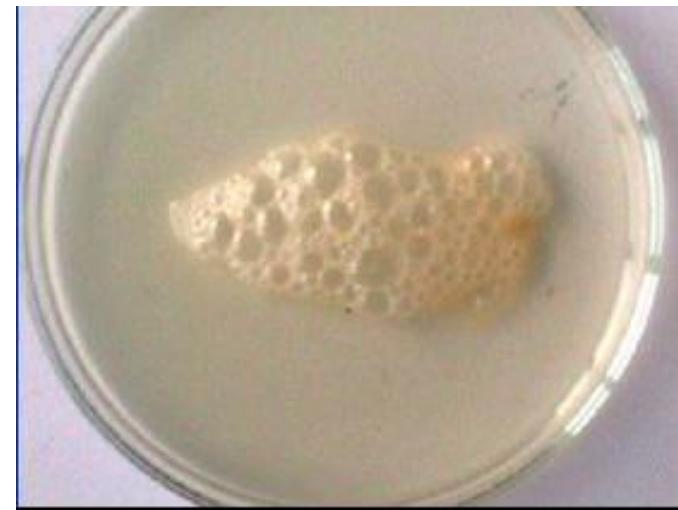
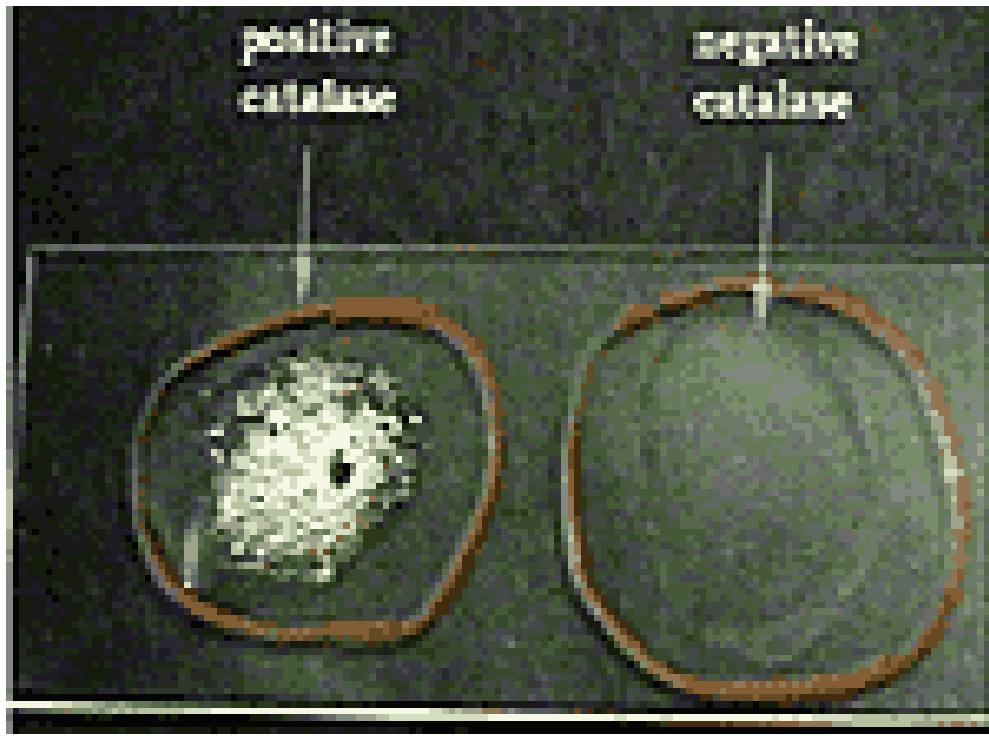
- *Staphylococcus*
colony gram stained



GENERAL PROPERTIES

- Gram positive, 1 μ m size, catalase positive (*Streptococcus* catalase negative), oxidase negative, aerobic/facultative aerobic, non-motile, non-spore forming.
- *S. saccharolyticus* and *S. aureus* subsp. *anaerobius* are anaerobic and catalase negative
- They are capable to divide in any plane so arranged as bunch of grapes
- Generation time is 20 minutes
- Nitrofurantoin susceptibility test (Susceptible: *Staphylococcus*/ Resistant: *Micrococcus*)





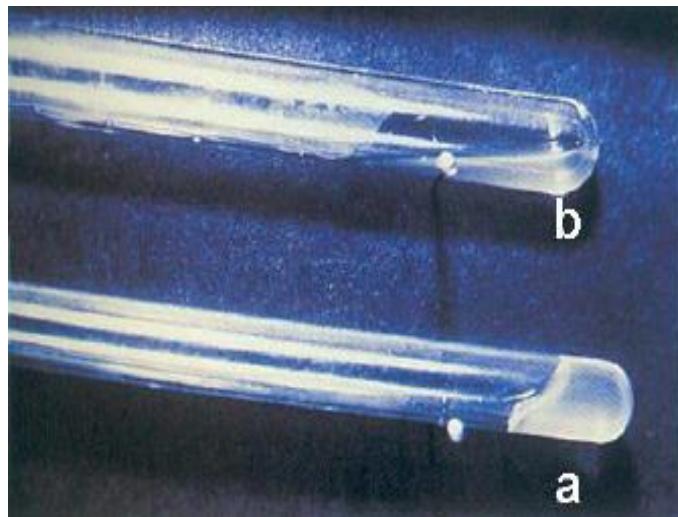
NAME OF SPECIES

- **Coagulase Positive:**

*Staphylococcus aureus, S. intermedicus, S. hyicus,
S. schleiferi*

- **Coagulase Negative:**

*S. arlettae, S. capitis, S. caprae, S. chromogenes, S.
epidemidis, S. equorum, S. felis, S. gallinarum, S.
haemolyticus, S. hominis, S. saprophyticus*



USUAL HABITAT

- Staphylococci are wide spread in nature although they are mainly found living on the skin, skin glands and mucous membrane of mammals and birds.
- They may be found in the mouth, blood, mammary glands, intestinal, genitourinary and upper respiratory tracts of these hosts.
- Skin surface
- Sebaceous glands, sweat glands
- Hair follicles
- Mucous membrane
- Nasal cavity
- 20–30% human population is carrier

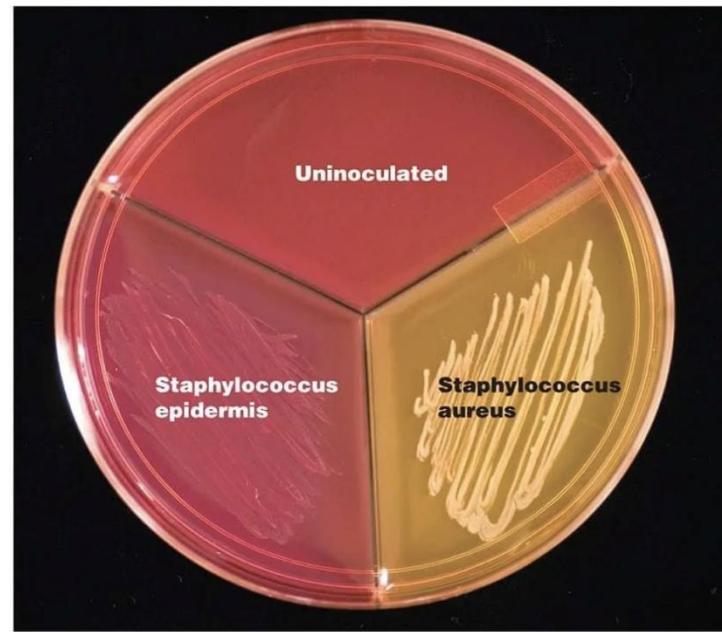


CULTURAL AND BIOCHEMICAL CHARACTERISTICS

- They grow readily on ordinary media within a temperature range of 10-42°C. Optimum temperature is 37°C and pH 7.4-7.6.
- On nutrient agar a typical 24hr *S. aureus* colonies are pigmented, smooth, entire, slightly raised, translucent and hemolytic on routine blood agar. Small colony variants (SCVs) of *S. aureus* produce colonies that are pinpoint in size, non-hemolytic and non-pigmented.
- In liquid medium, uniform turbidity is produced.
- Selective media used for isolating *S. aureus* contain 7.5-10% NaCl like salt-milk agar, ludlam's medium containing lithium chloride and tellurite.

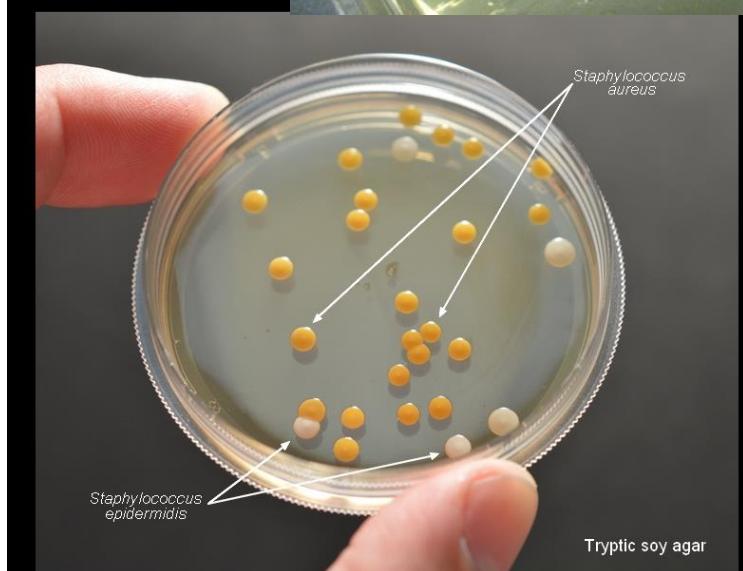


- **Mannitol salt agar:** prepared by 1% Mannitol sugar with high concentration salt (7.5% NaCl) and dye Phenol red. *Staphylococcus* spp. capable to ferment mannitol and produce yellow colonies
- **Maltose purple agar:** prepared 1% maltose with dye bromocresol purple. Use to differentiate maltose fermenting and non fermenting species.



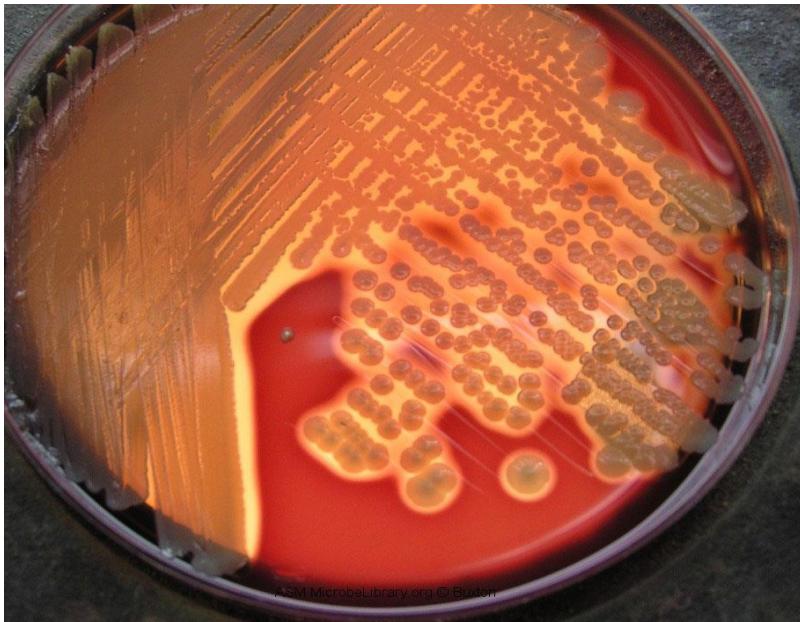
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- Pigmentation: yellow/ golden yellow/ white
- *S. aureus* – yellowish or golden orange pigment.
- *S. albus* - white colonies.
- *S. citreus* - lemon yellow colour pigment

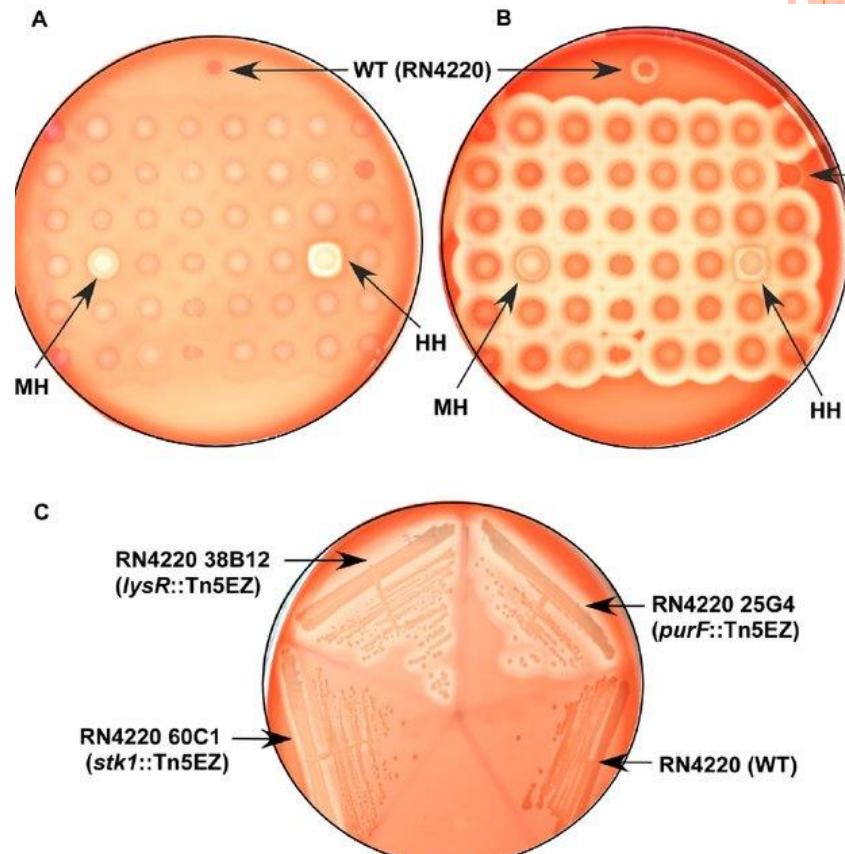
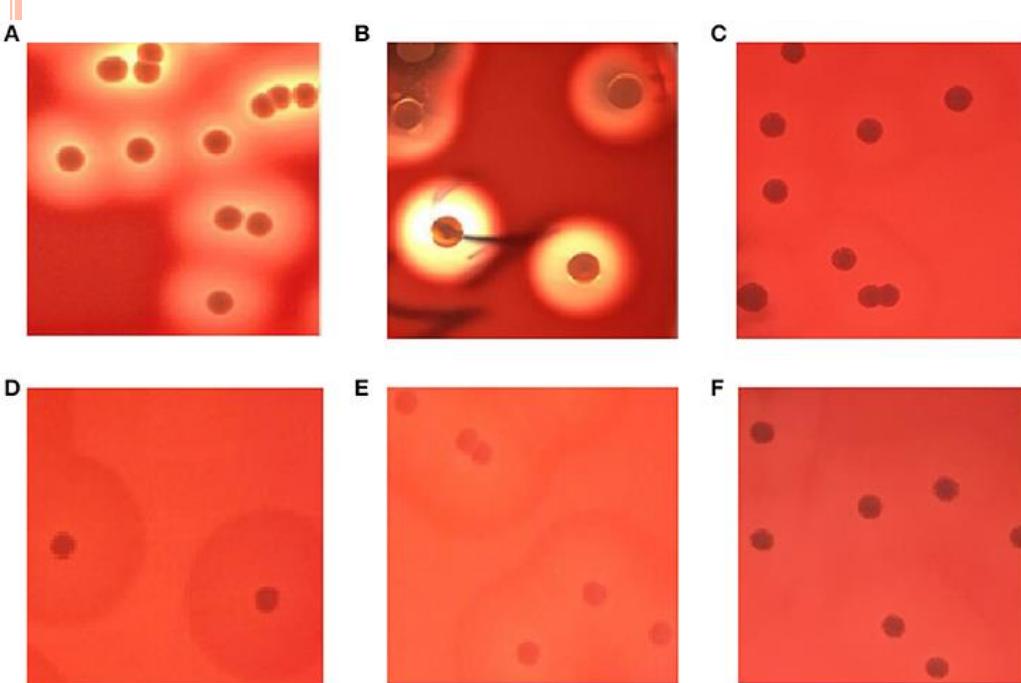


- **Haemolysin:** produce alpha, beta and gamma haemolysin (staphylococcus species showed **double heamolysis phenomena**)

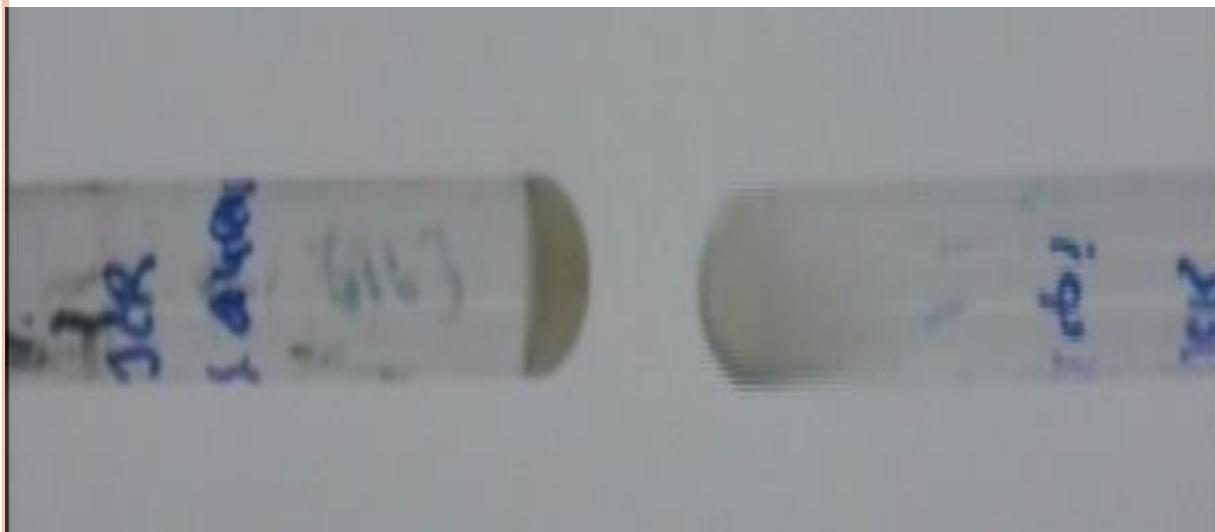
- Alpha haemolysin: produced narrow zone of complete hemolysis
- Beta haemolysin: produced wider zone of partial or incomplete hemolysis
- Gama haemolysin: produced non hemolytic activity



- **Hot- cold lysis phenomenon:** In which beta haemolysin producing strains start to produce alpha haemolysin during refrigeration at 4°C.
- ***S. hyicus* are non hemolytic**
- Capable of liberating ‘V’ factor into the medium, which favours the growth of ***Haemophilus*** organism.

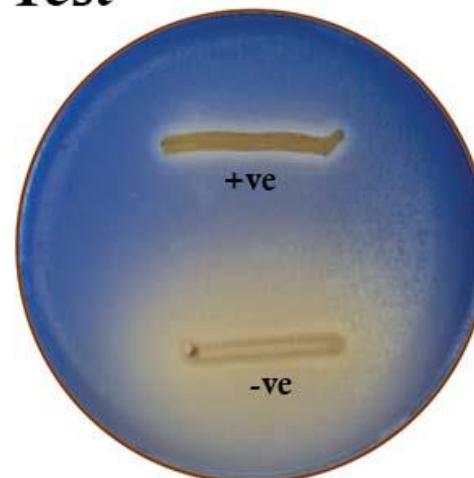
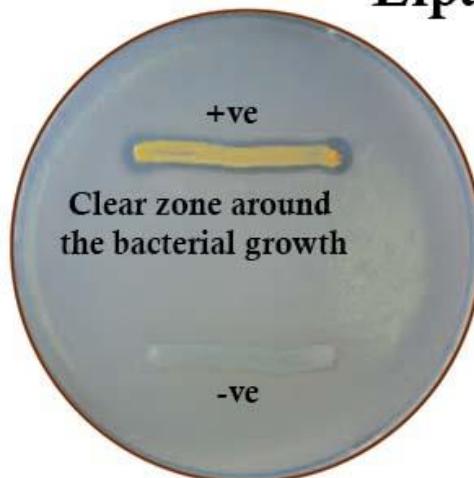


- **Coagulation phenomenon:** *Staphylococcus* spp. produce coagulase enzyme: it is found as two types free and bound coagulase (also known as clumping factor)
- Slide coagulase test can detect bound coagulase and tube coagulase test can detect free coagulase



- Urease positive, they reduce nitrates to nitrites, liquefy gelatin and are MR, VP positive but indole negative.
- They are lipolytic when grown on medium containing egg yolk.
- They produce phosphatase which can be demonstrated by growing on nutrient agar containing phenolphthalein diphosphate.

Lipase Test

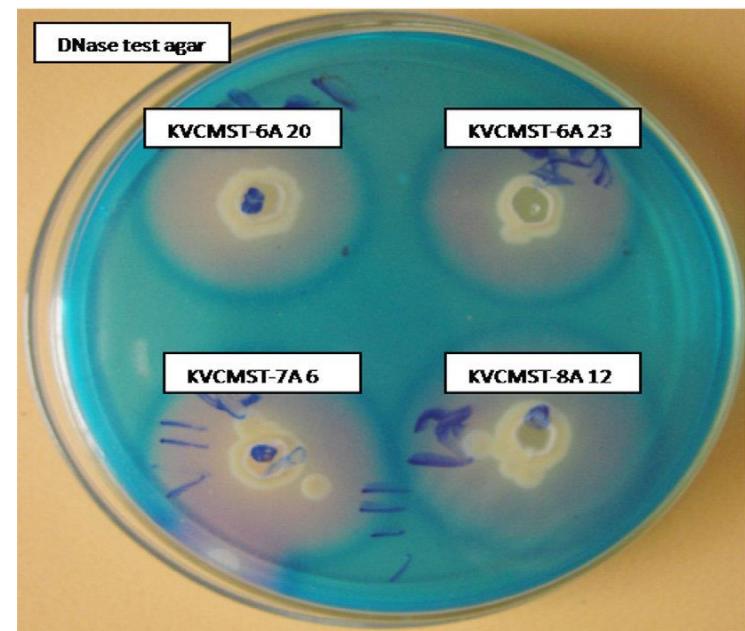
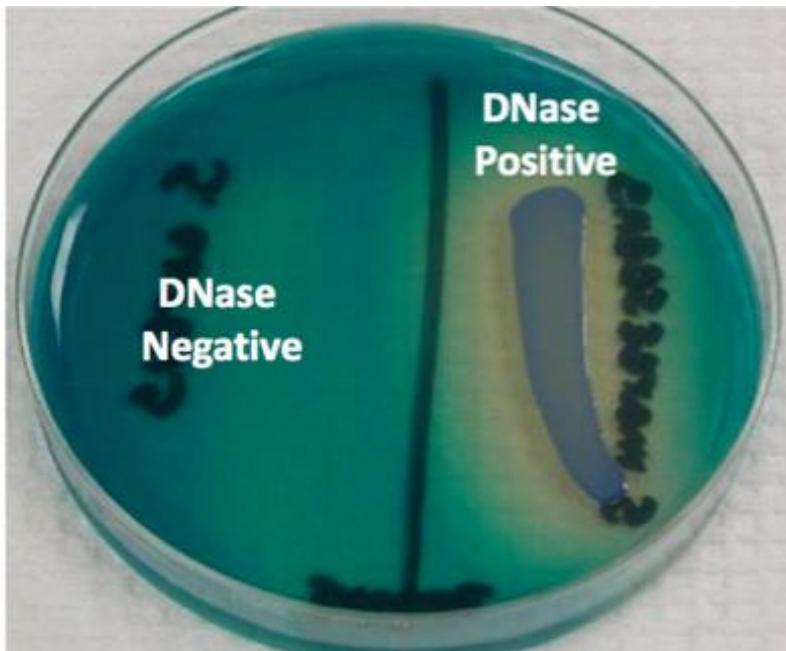


- In a medium containing potassium tellurite, tellurite is reduced and black colonies are produced.

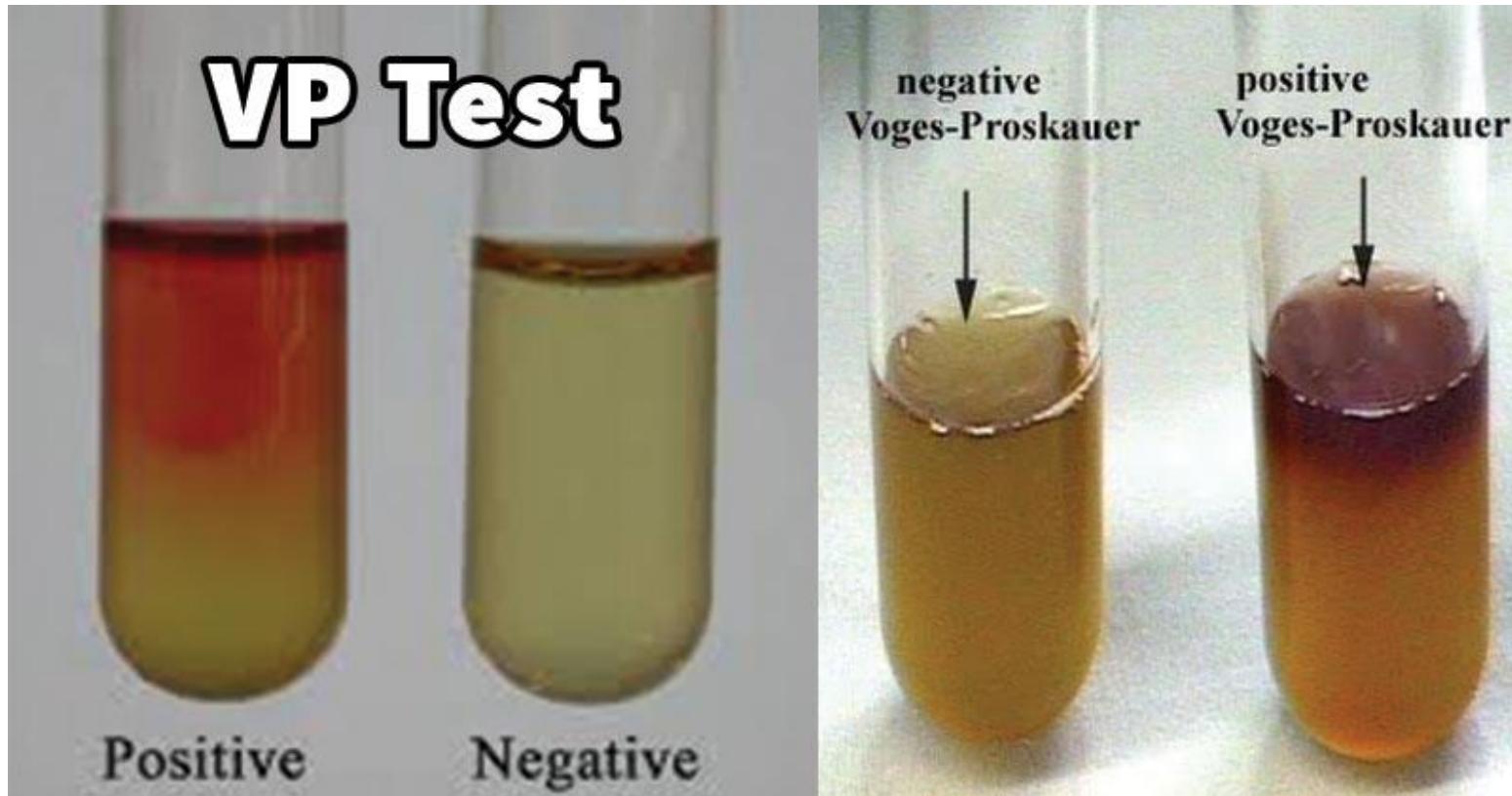


S. aureus Baird Parker Agar

- **Heat Stable Nuclease:** A heat stable staphylococcal nuclease (thermonuclease (TNase)) that has endo and exonucleolytic properties and can cleave RNA or DNA is produced by most strains of *S. aureus*. TNase can be demonstrated by the ability of boiled cultures to degrade DNA in an agar diffusion test or detected by using metachromatic agar diffusion procedure and DNase toludene blue agar.



- **Acetoin Production (acetyl methyl carbinol):** In this biochemical reaction glucose converts in to pyruvic acid which can further be metabolized to produce acetoin (i.e., acetyl methyl carbinol or 3-hydroxybutanone) it can detect by conventional Voges-Proskauer test.



- **Antigens**

Carbohydrates

- The cell wall of *S. aureus* contains ribitol teichoic acid.

S. intermedius contains glycerol teichoic acid.

- Protein A - Present only in *S. aureus*.



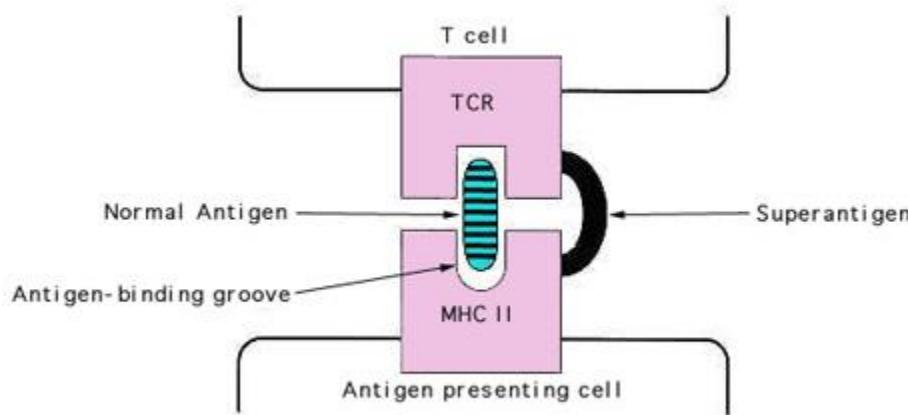
MICROCOCCACEAE FAMILY DIFFERENTIATION

Organism	Appearance in stained smears	Coagulase production	Catalase production	Oxidase production	O-F test	Bacitracin disc (0.04 units)
<i>Staphylococcus</i> spp.	Irregular cluster	+/-	+	-	F	Resistant
<i>Micrococcus</i> spp.	Packets of four	-	+	+	O	Susceptible
<i>Streptococcus</i> and <i>Enterococcus</i> spp.	Chains	-	-	-	F	Resistant

VIRULENCE FACTORS

1. Toxin/ Exotoxin: Toxins can be categorized into 3 groups

A. Pyrogenic toxin super antigens: Super antigen activity that includes toxic shock syndrome (TSS). This group includes TSST-1, which causes toxic shock syndrome and staphylococcal enterotoxins which cause a form of food poisoning. They produce 6 serotypes of enterotoxins which cause diarrhea and vomiting.



B. Exfoliative toxins: are implicated in the disease staphylococcal scalded skin syndrome (SSSS)

C. Membrane damaging toxins: include α toxin, β toxin and γ toxin and the classical Panton-Valentine Leukocidin (PVL) factor.

2. Iron uptake and Plasminogen activator: *Staphlokinase*

3. Immune evasion: chemotaxis inhibitory protein of *Staphylococcus* (chp), staphylococcal binder of immunoglobulin (sbi), staphylococcal complement inhibitor (scn), Protein A (spa)



4. Exoenzyme:

- Aureolysin (Protease; Zinc metalloproteinase)
- Hyaluronate lyase (Spreading factor:
Degradation of hyaluronic acid, contribute to
local dissolution of the extracellular matrix)
- Lipase & esterases (Degrade lipids)
- Staphopain (Cysteine protease)
- Staphylocoagulase
- V8 protease (Serine protease)
- Lysozyme (Hydrolyses the peptidoglycan in the
cell wall of many bacteria)



5. Antiphagocytosis: Microcapsule Produced by over 90% of *S. aureus* strains. Two serotypes (5 and 8)

6. Adherence:

- Clumping factor: Mainly ClfA and ClfB, which bind to different sites in fibrinogen. ClfA binds to the γ -chain whereas ClfB binds to the α -chain
- Collagen binding protein
- Elastin binding protein
- Fibrinogen binding protein
- Fibronectin binding proteins
- Intercellular adhesion



DISEASES CAUSES BY *STAPHYLOCOCCUS* spp

Species	Hosts	Clinical conditions
<i>Staphylococcus aureus</i>	Cattle	Mastitis, udder impetigo
	Sheep	Mastitis Tick pyaemia (lambs) Benign folliculitis (lambs) Dermatitis
	Goats	Mastitis Dermatitis
	Pigs	Botryomycosis of mammary glands Impetigo on mammary glands
	Horses	Scirrhous cord (botryomycosis spermatic cord) , mastitis
	Dogs, cats	Suppurative conditions similar to those caused by <i>S. pseudo-intermedius</i>
	Poultry	Arthritis and septicaemia in turkeys Bumble foot Omphalitis in chicks

DISEASES CAUSES BY *STAPHYLOCOCCUS* spp

Species	Hosts	Clinical conditions
<i>S. pseudintermedius</i>	Dogs	Pyoderma, endometritis, cystitis, otitis externa, and other suppurative conditions
	Cats	Various pyogenic infections
<i>S. hyicus</i>	Pigs	Exudative epidermitis (greasy-pig disease) Arthritis
<i>S. aureus</i> subsp. <i>anaerobius</i>	Sheep	Lymphadenitis
<i>S. schleiferi</i> subsp. <i>coagulans</i>	Dogs	Otitis externa

- Horse: (**Botryomycosis**): Infrequent chronic granulomatous lesions involving the udder of the mare, cow and sow and the spermatic cord of horses.



- **Cattle:**

Mastitis: Staphylococcal bovine mastitis may be chronic, acute and peracute. Gangrenous mastitis due to a toxin is seen in postparturient cows.

- **Sheep:**

Tick pyemia in lambs occurs in 2-5 week old lambs, which is heavily infected with *Ixodes ricinus*.

Periorbital eczema is an infection due to abrasions, Staphylococcal dermatitis due to scratches from vegetation.



Poultry: (Bumble foot)

- A pyogranulomatous process of subcutaneous tissue of foot that can involve the joints.
- Staphylococcal arthritis and septicemia in turkeys, omphalitis – yolk sac infection, wing rot or gangrenous dermatitis infection in poultry.



Pig: (Greasy pig disease)

- Exudative epidermitis (greasy pig disease) is an acute generalized infection of suckling and weaned pigs caused by *S. hyicus*. This disease is characterized by excess sebaceous secretion, exfoliation and exudation.



- Dogs and Cats: Pyoderma is one of the most common skin diseases of dogs.
- In addition to this, Otitis externa and other suppurative conditions are caused by *S. intermedius*.
- Staphylococcal antigens produce intense inflammatory reaction and promote persistence of the bacteria.



OTHER STAPHYLOCOCCAL ORGANISMS

- *S. aureus sub sp.anaerobius* causes caseous lymphadenitis. They are anaerobic and catalase negative.
- *S. caprae* in goat's milk.
- *S. gallinarum* and *S. arlettae* - skin of chickens
- *S. lentus* in skin of sheep and goats.
- *S. equorum* in skin of horses
- *S. simulans* and *S. felis* - clinical specimens in cats
- *S. delphini* in skin of dolphins
- *S. aureus* in **Staphylococcal scalded skin syndrome (SSSS)** and Toxic shock syndrome (TSS) in humans.



ANTIMICROBIAL RESISTANCE

- *Staphylococcus* spp has significant role in context of antibiotic resistance in the form of MRSA in (Methicillin resistant *Staphylococcus aureus*) / VRSA (Vancomycin resistant *Staphylococcus aureus*)



DIAGNOSIS

Staphylococcus spp can diagnosed by various phenotypic and genotypic characteristics

- Primary and secondary biochemical test such as gram staining, catalase, oxidase, O/F, test
- Hemolysis and coagulase properties
- Colony characteristics and Growth on MSA and egg yolk Agar
- Absence of growth on Macconkey agar
- Phage typing



FURTHER READINGS

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease

