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Microbiology

GENERAL BACTERIOLOGY - MICROSCOPY

Measurements :

1 cm = 10 mm.

1 mm = 1000 microns.

1 micron = 1000 nm (nanometre).

Average size of bacteria : 1 micron.

Average size of viruses : 14-15 nm.

Magnification

00:01:30

Factor by which an image appears to be enlarged.

It is a number which is greater than 1 and is usually followed by an "x", as in 10x magnification.

Q. Which of the following best defines magnification?

- A. It is the minimum distance between 2 objects that appear as separate objects.
- B. It is the number of different structures that can be identified in an image.
- C. It is how much bigger the image is than the actual object.
- D. It is calculated by dividing the magnifying power of the objective by the magnification power of the eyepiece.

Resolving power (R.P) :

Definition : Smallest distance (d) between two objects which are seen by the optical system as two separate objects.

Less the d, more the R.P. (inversely proportional).

A microscope with high R.P will allow two small objects, which are close to each other to be seen as two distinct objects.

R.P of naked eye : 0.2 mm (200 microns)

R.P of light microscope : 0.2 micron (200 nm)

R.P of electron microscope : 0.2 - 0.5 nm.

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Simple microscope

00:08:20

Introduced by **Leeuwenhoek**.

He observed bacteria for the first time and termed them **little animalcules**.

uses **1 lens** for magnification.

Not used anymore.

Simple microscope



Compound microscope



Light/compound microscope :

Introduced first by **Janssen duo**.

Contains a set of **2 lenses** : Objective lens

eyepiece/ocular lens.

Can be binocular/unocular microscope.

Objective lens are mounted on a revolving nose piece.



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Final magnification :

magnification of objective lens \times magnification of ocular lens.

Resolving power of light microscope = 0.2 microns.

Best magnification achieved = 1000 times.

λ of visible light = 500nm.

Objective lens	magnification of objective lens	magnification of eye piece lens	Total magnification	Numerical aperture of objective (NA)
Scanning lens	4x	10x	40x	0.12
Low dry objective	10x	10x	100x	0.22
High dry objective	40x	10x	400x	0.65
Oil immersion	100x	10x	1000x	1.25

- Q. Resolving power of a microscope is a function of _____
- Wavelength of light used.
 - Numerical aperture of lens system.
 - Refractive index.
 - Wavelength of light used and numerical aperture of lens system.

$$d = \frac{0.61\lambda}{NA}$$

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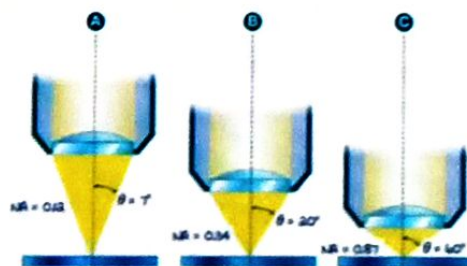
d is inversely proportional to resolving power.

Hence, resolving power of a microscope is :

- Inversely proportional to the wavelength.
- Directly proportional to the numerical aperture (NA).

λ of light = 500nm.

$$NA = n \sin \theta$$



n is the refractive index of the medium. Medium is between the specimen and objective lens.

n of Air = 1

n of Oil = 1.5

θ (theta) is the semi-vertical angle of the cone formed by object at the objective lens.

Increasing the numerical aperture increases the magnification.

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Dark field/ ground microscope

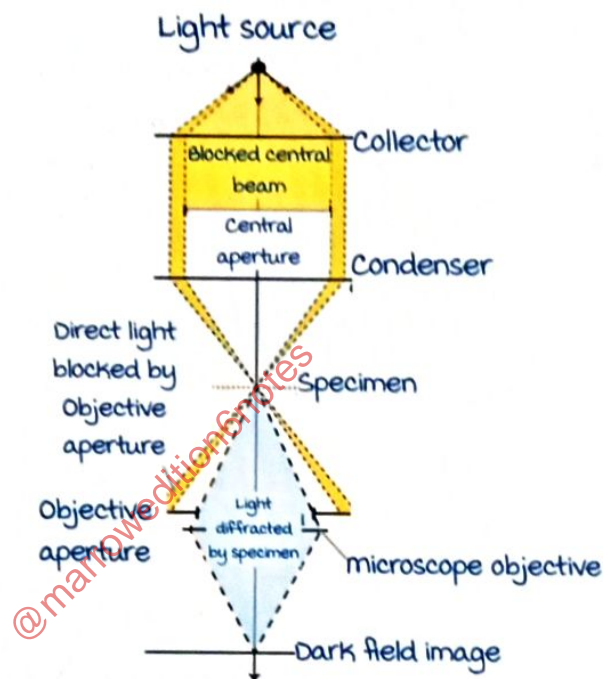
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Principle :

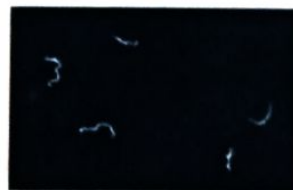
Special **condensing lens** with a **central opaque area (stop)** which illuminates the object on the stage with a hollow cone of light.

Thus, against a dark background, slender micro-organisms are visible.

used for: **Spirochetes** like treponema and Leptospira.



Dark ground microscopy of *Treponema pallidum*



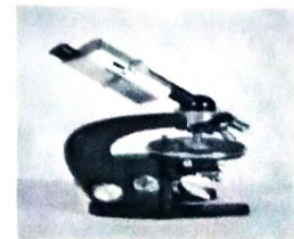
Phase contrast microscope

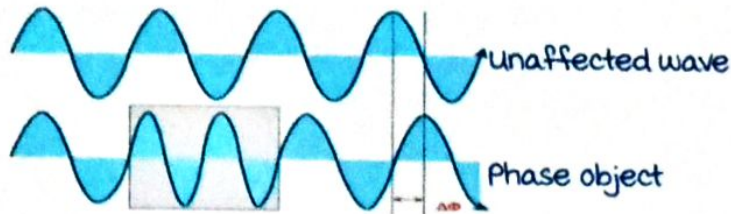
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Light microscope with an annular diaphragm in front of condenser and annular phase plate.

Principle :

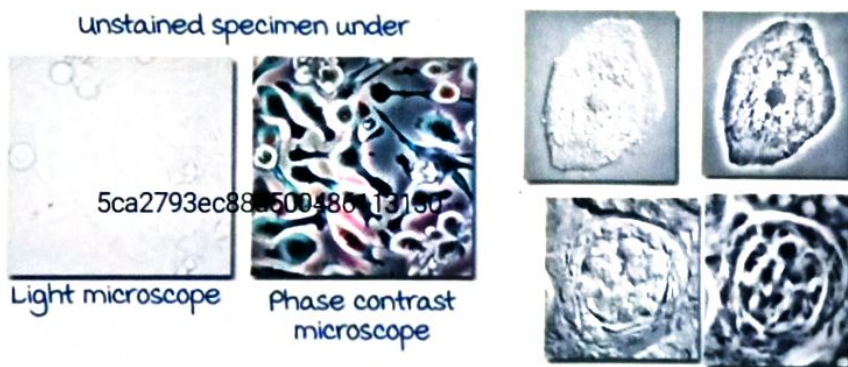
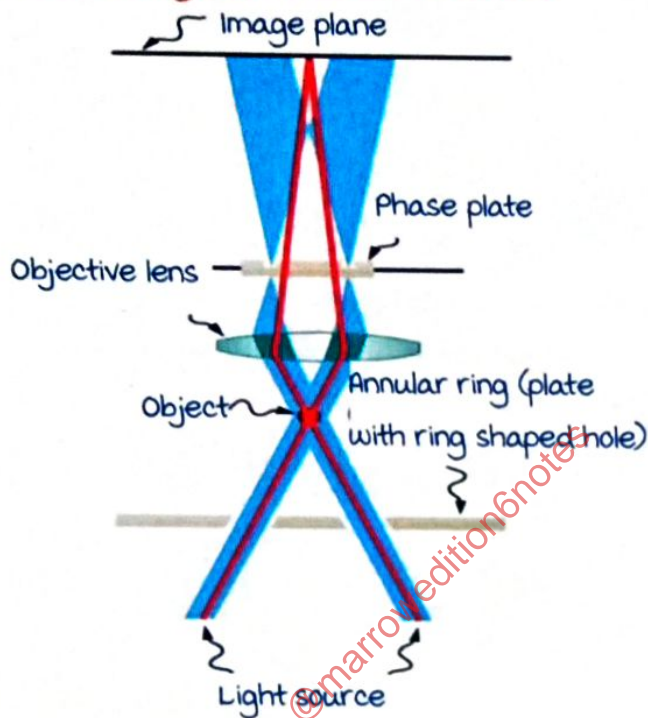
As light passes through the internal structures with different refractive indices, **phase shifts** are produced which are converted into changes in amplitude of light by the phase plate.





Hence, this provides high contrast images and 3-Dimensional effect of unstained cells and micro-organisms.

Use : Live cells or organisms can be visualised.



Fluorescence microscope

00:24:12

Fluorophore is a special chemical which absorbs shorter wavelength light (of UV range) and emits light of longer wavelength (of visible range).

Contains **emission filter**, **excitation filter** and **dichroic mirror**.

Excitation filter allows only the wavelength of light that the specimen absorbs. So shorter wavelength light falls on the

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GENERAL BACTERIOLOGY : STAINING

Need for staining

00:00:27

It provides **contrast** with the background, thus **increasing** visibility of organisms.

Types of stains used in microbiology

00:01:05

- Simple.
- Differential.
- Negative.
- Impregnation.
- Special.

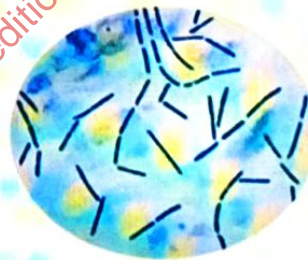
Simple stain

00:01:25

A **single dye** is used after preparing the smear.

It could be any of the following:

- **Basic fuchsin.**
- **Crystal violet.**
- **methylene blue.**



Simple stain

Under microscope, depending on which dye is used, the bacteria and everything else on the smear takes up that **single stain**.

Differential stain

00:02:20

more than one dye is used, so it helps differentiate between organisms based on their interactions with **multiple dyes/** stains.

This aids in choosing antibiotics **specific** to organisms for **treatment**.

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Gram stain

00:03:37

most commonly used differential stain.

Introduced by Danish bacteriologist: **Hans Christian Gram** in 1884.

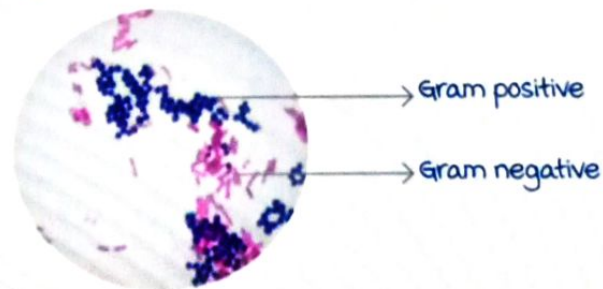
Steps in gram stain:

- Smear preparation → **Heat/methanol fixation**.
 - Primary stain → Crystal violet/methyl violet/Gentian violet [1:1 mixture of crystal violet and methyl violet].
 - Mordant → **Iodine**; fixes the primary stain in the bacterial cytoplasm. Prevents stain from getting washed out.
 - Decolourisation → **Alcohol/Acetone/Alcohol-Acetone mixture (most important step)**.
 - Counter stain → **Safranin/Neutral red/Basic fuchsin**.
- mnemonic for steps: Come In and Stain!

It is the **acidic** cytoplasm that takes up the **basic** dye like crystal violet or safranin, not cell wall.

Based on some principles of gram stain, the organisms can appear:

- Gram positive: **Bluish violet/bluish-purple** in colour.
- Gram negative: **Reddish pink** in colour.



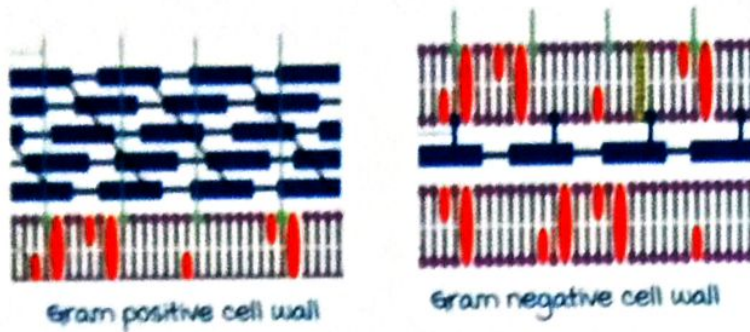
Gram stain

Principles of gram stain

00:08:09

- **Cell wall thickness**: Gram positive cell wall is thicker (80nm) with several layers of peptidoglycan, which helps retain the primary stain/dye-iodine complex better.

Gram negative cell wall has only 2 layers (10 nm thick).



- **Cytoplasm** : Gram positive cytoplasm is more **acidic**; hence, it binds the basic primary stain better and retains it for longer time withstanding **decolourisation/ slower decolourisation**.
- **Cell wall lipids** : Gram negative cell wall has **more lipids** which are dissolved by the decolorising agents leading to **pore formation** causing **rapid decolourisation/faster removal of dye-iodine complex** in these organisms.

(It is essential to **time** the decolourisation process correctly for accurate results).

Exceptions to gram stain

00:13:15

- mycoplasma → Too **small** to be gram stained.
- Chlamydia
- Rickettsia } '**minute intracellular**' bacteria.
- Spirochetes → Too **slender** to be seen under the light microscope, not demonstrated by gram stain.
- ~~brivssprasantly~~ Gram negative → **Lipid rich** cell wall, rendering gram stain reagents unable to stain the cytoplasm.

Classification

00:15:11

Gram positive cocci	Gram negative cocci
Micrococcus	Veillonella
Enterococcus	Neisseria
Staphylococcus	Moraxella
Streptococcus	

Active space

'Gram-negative cocci spread **venom** and gram-positive cocci clean up the **mess**'.

Gram positive rods/bacilli	Gram negative rods/bacilli
Corynebacterium.	Enterobacteriaceae.
Erysipelothrix.	Pseudomonas.
Nocardia.	Burkholderia.
Bacillus.	Vibrio.
Actinomyces.	Legionella.
Listeria.	Bacteroides.
Mycobacterium (as per electron microscope).	
Clostridium.	

'Cory & Ery knocked Back the Actor's List into my Closet'.

Gram negative coccobacilli	Gram negative spirals
Brucella.	Spirochetes (Treponema, Leptospira, Borrelia).
Bordetella.	Spirillum.
Haemophilus.	Campylobacter.
Francisella.	Helicobacter.
Chlamydia.	
Rickettsia.	

Acid fast stain

00:22:12

Introduced by **Paul Ehrlich**.

modified by **Ziehl & Neelsen**.

First introduced for **mycobacteria** but may be used for other bacteriae too.

mycobacterium has several layers of peptidoglycan (PG) following the cytoplasmic membrane, just like all **gram-positive** organisms.

However, the PG layers are followed by several layers of **lipid**

which resist entry of gram stain.

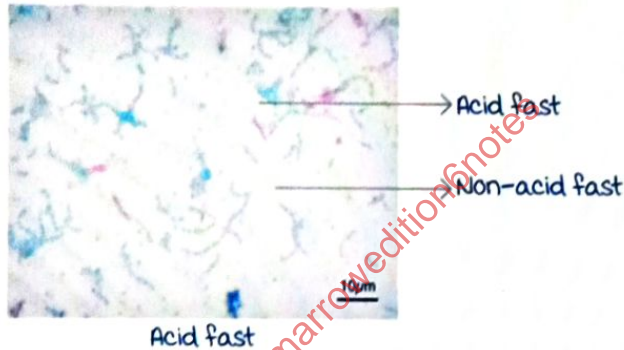
Steps in acid fast stain :

- Smear preparation followed by heat fixation.
- Primary stain → Carbol fuchsin (Basic fuchsin dissolved in phenol) for 3-5 minutes.
- Decolourisation → 20% Sulphuric acid/ H_2SO_4 .
- Counter stain → methylene blue/Picric acid/ malachite green.

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The organisms appear :

- Acid fast → Reddish pink in colour (retains the primary stain with 20% H_2SO_4).
- Non-acid fast → Blue/Yellow/Green in colour based on counter stain used.



Acid fast bacteria

00:28:42

- mycobacterium tuberculosis complex/MTBC.
- Atypical mycobacterium.

MTBC are both acid and alcohol fast i.e., they retain primary stain even if the decolorising agent is 95% alcohol.

Atypical mycobacteria are only acid fast.

Atypical mycobacteria only affect people with decreased cell mediated immunity (CMI) like in HIV.

People with deficient CMI → 3% of acid alcohol [i.e., 3% hydrochloric acid/HCl in 95% alcohol] → Decolorising agent.

Kinyoun/Gabbet stain

00:30:20

It is another acid-fast stain.

Absolutely similar to Ziehl Neelsen stain, except no heating;

Hence also called the cold stain.

The steps include increasing concentration of phenol in carbol fuchsin as well as increasing time of exposure to carbol fuchsin.

Partially acid-fast bacteria/structures

00:34:25

These are structures that retain the primary stain with a lower concentration of H_2SO_4 .

Thus, called modified ZN stain or the modified Kinyoun stain.

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- Acid fast with 5% H_2SO_4 → m. leprae, oocysts of Cystoisospora, Cyclospora & Cryptosporidium.
- Acid fast with 0.5-1% H_2SO_4 → Nocardia, Legionella micdadei & Rhodococcus.
- Acid fast with 0.25-0.5% H_2SO_4 → Bacterial spores & head of sperm.

Negative stain

00:38:10

An acidic dye like India ink or Nigrosin is used.

The acidic cytoplasm of bacteria repels these acidic dyes which get deposited outside the bacterium.

Therefore, helping us visualise outlined bacterium against the dark background of the dye.



Simple stain

Useful in demonstrating :

- Capsule of bacteria.
- Slender bacteria like Spirochetes in fluid specimen : e.g., Exudate of hard chancre caused by Treponema pallidum.
urine specimen in a patient of Leptospirosis.

Impregnation stain

00:39:43

used to deposit something on to the surface of the bacterium or on a structure, making it thick enough to be seen by the

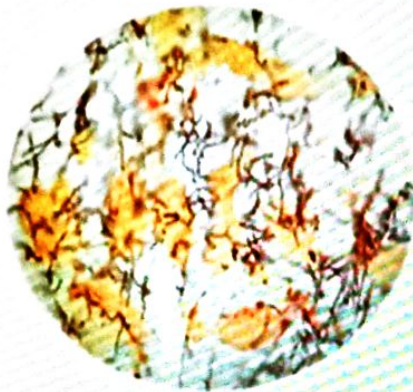
light microscope.

Example → Impregnating silver on Spirochetes which are very slender bacteria, to be seen using light microscope.

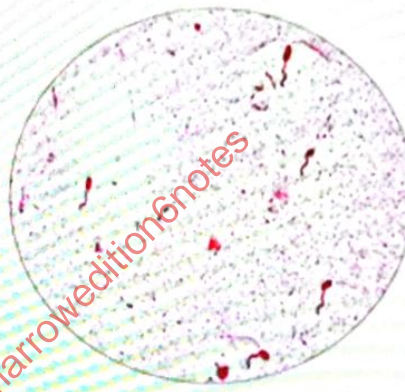
Types of impregnation stain :

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- **Levaditi** silver impregnation stain → used to demonstrate spirochetes in tissue section (**biopsy**).
- **Fontana's** silver impregnation stain → used to demonstrate spirochetes in films of **fluid specimens**.
- **Ashby's stain**/Schaeffer-Fulton stain → used to demonstrate the **flagellum**.



Fontana's stain



Ashby's stain

Special stains

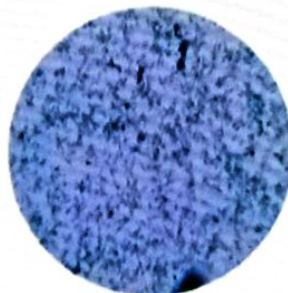
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used to demonstrate **special granules**, that act as **energy stores** present in cytoplasm of bacterium.

Lipid granules → **Sudan Black B**.

Polysaccharide granules → **Iodine stain**.

volutin granules/metachromatic granules/Babes-ernst granules/Polar bodies → **Albert stain (classical)**/Neisser stain/Ponders stain.



Albert stain

Active space

Reagents of Albert stain :

- **Albert A** solution is made of Toluidine blue, malachite green and Glacial acetic acid.

The toluidine blue stains volutin granules purple and the malachite green stains the cytoplasm green.

- **Albert B** solution is made of Iodine and Potassium Iodide; is the mordant that fixes the stain on to the structures we are demonstrating.

Volutin Granules

00:45:15

First demonstrated in *Spirillum volutans*.

Called metachromatic granules because though stained with toluidine blue they appear bluish purple or purplish red in colour [Phenomenon of metachromasia].

These granules are made up of Polymetaphosphate.
metachromatic granules are gram positive in nature.

Bacteria with metachromatic granules :

- *Corynebacterium*.
- *Gardnerella vaginalis*.
- *Yersinia pestis*.
- *Bordetella pertussis*.
- *Mycobacterium* spp.

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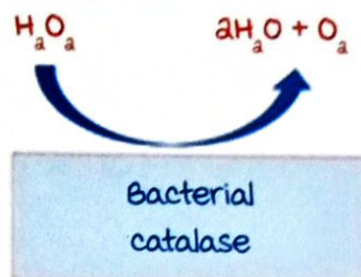
Active space

GENERAL BACTERIOLOGY : BIOCHEMICALS AND TYPING

Catalase test

00:01:30

Detects **bacterial catalase enzyme**. Bacteria produce toxic H_2O_2 during metabolism under **aerobic** conditions. H_2O_2 is eliminated by catalase enzyme.



method :

Take a clean glass slide and divide it into 3 zones : Test, positive control and negative control. using a wooden applicator stick or the corner of a cover slip, place the colonies in their respected zones. Now, add 1 drop of 3% H_2O_2 to each of the colonies in the 3 zones.

Observation : Look for the appearance of gas bubbles.

Inference : oxygen has been produced from the break down of H_2O_2 .

Positive control shows **effervescence of gas/ gas bubbles** and so will the test control. This is a catalase positive test.

Test can also be done in a test tube. Take a few drops of H_2O_2 and a wooden applicator stick is used to add the colonies. Look for the appearance of gas bubbles.

Do **not use** the usual **nichrome inoculating loop** as it is sometimes responsible for a false positive test.

Active space

most pathogenic bacteria are catalase positive except :

St-A-Sh :

- **Streptococcus**, **Pneumococcus**, **Enterococcus**.
- **Anaerobes** (mnemonic : **ABCL**) : **Actinomyces**, **Bacteroides**, **Clostridium** and **Lactobacillus**.
- **Shigella dysenteriae** type I.

Oxidase test :

Detects **bacterial cytochrome C**.

Tetramethyl-para-phenylenediamine dihydrochloride (**TMPD**) in the presence of bacterial cytochrome C gets converted into a **blue compound** : **Indophenol**.

method :

On a clean glass slide, place a filter paper slip and add a few drops of the oxidase reagent.

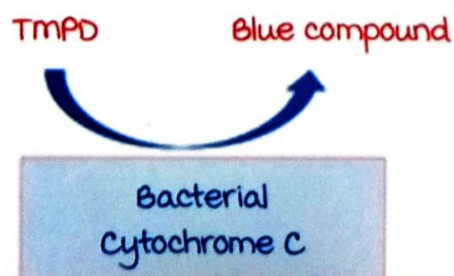
Using the corner of a cover slip, place the colonies on the filter paper impregnated with the reagent.

Observation : Development of a **purple colour** within a **10 seconds** indicate the presence of oxidase.

Do not use the straight wire/ nichrome inoculating wire as they can result in a false positive test.

most pathogenic bacteria are oxidase positive except :

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- **Corynebacterium.**
- **Enterobacteriaceae.**
- **Staphylococcus.**
- **Streptococcus.**

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Utilization of sugars :

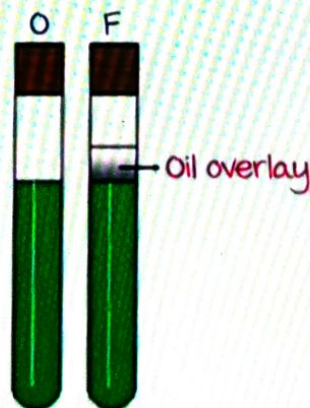
Hugh Leifson oxidative fermentative medium is used to differentiate the utilization of sugars.

Saccharolytic : Breaks down sugars. Can utilize sugars only under aerobic conditions (oxidative utilization) or both aerobic and anaerobic conditions (fermentative utilization).

Asaccharolytic : Does not break down sugars.

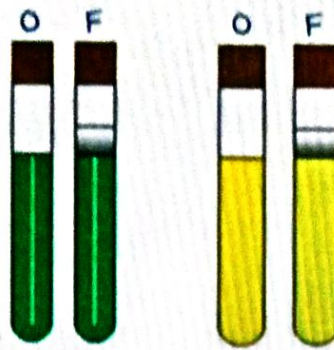
method :

Hugh Leifson oxidative fermentative medium contains glucose and a pH indicator : Bromothymol blue that detects production of acid. Two test tubes containing the above medium are used. Anaerobic condition is produced in one of the test tubes by pouring sterile petroleum jelly on top of it. Bacteria are stab inoculated in both the test tubes and kept under overnight incubation.

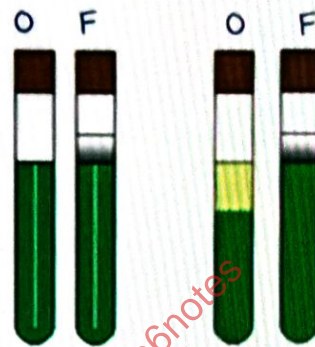


If colour change is seen in both the test tubes, it indicates fermentative bacteria.

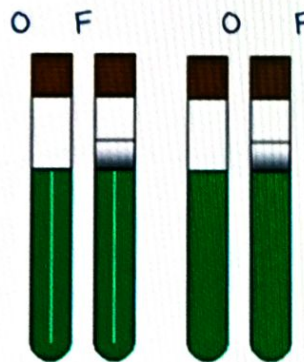
Example : Enterobacteriaceae, Staphylococci, Streptococci, Haemophilus etc.



If colour change is seen in only aerobic test tube, it indicates **oxidative utilization**. Example : Pseudomonas, Brucella, Bordetella, micrococcus etc.



If colour change is not seen in both the test tubes, it indicates **asaccharolytic bacteria**. Use alternative chemicals for metabolism. Example : Moraxella, Acinetobacter, Campylobacter, Helicobacter.



Urease test

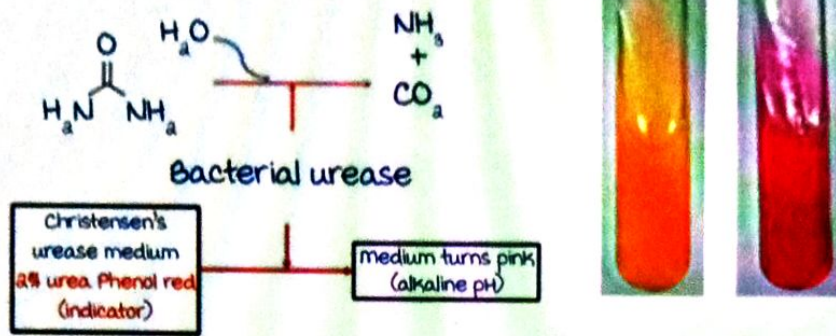
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Bacterial urease breaks down urea into ammonia and CO_2 .

Christensen's urease medium is used.

It contains phenol red as the pH indicator.

Ammonia production due to breakdown of urea by bacterial urease produces alkaline medium and turns the medium pink.



urease positive bacteria: (mnemonic : PUNCH MSKB)

Proteus, **U**reaplasma, **N**ocardia, **C**ryptococcus, **H**elicobacter,
Morganella, **S**taph aureus, **S**taph epidermidis and **S**taph
 saprophyticus, **K**lebsiella pneumoniae, **B**rucella.

maximum urease producing bacterium : Helicobacter.

Rapid urease test done on biopsy specimen :



Other biochemical reactions :

- Coagulase test : Staph aureus.
- Bile solubility test : Streptococcus pneumoniae.
- Optochin sensitivity : Streptococcus pneumoniae.
- Bile resistance (can grow in 40% bile) : Enterococcus.
- Sensitivity to bacitracin : Streptococcus pyogenes.
- Indole positivity : E.coli.
- Indole negativity : Klebsiella.

Bacterial typing

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Intraspecies strain characterization.

Purpose of typing :

- Outbreak studies.
- Identification of virulent and avirulent strains.
- Studies of transmissibility.
- To check if it is a relapse or reinfection.

Typing methods : Phenotypic (character expressed by the pathogen) and **genotypic** (DNA studies -predominant method).

Phenotypic methods :

Serotyping : most commonly used phenotypic method.

Principle of serotyping : Surface antigens are cross reacted with corresponding antibodies to identify the type.

Example :

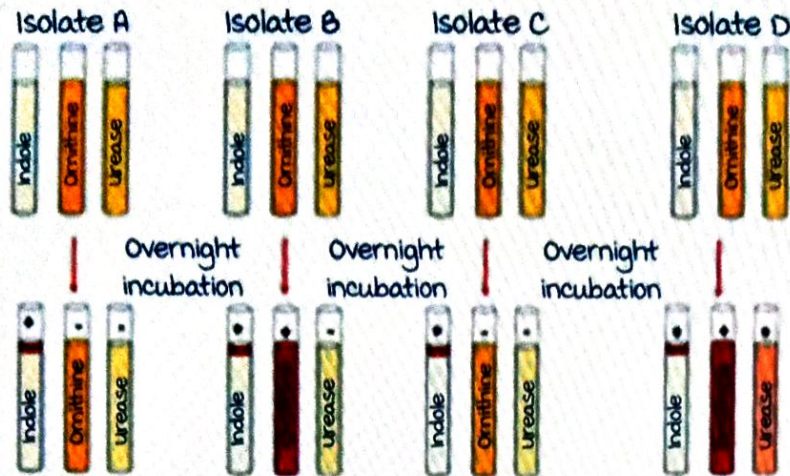
- **Griffith typing** for Streptococcus pyogenes. It is based on **m protein** on the cell wall. There are >120 m types.
- Streptococcus pneumonia is typed based on **capsular antigen**. There are more than 95 serotypes.
- There are more than 170 serotypes of E. coli based on 'o' antigen.

Biotyping :

Principle : On the basis of selected group of **biochemical reactions**.

Example : There are 8 biotypes (1 - 8) of H. influenzae.

To type H. influenzae, Indole, Ornithine and Urease are used.

Biotyping of *H. influenzae* :

Above image shows isolate A and Isolate C are similar to each other.

Corynebacterium diphtheriae has 4 biotypes : Gravis, Intermedius, mitis, Belfanti.

Vibrio cholerae O1 has 2 biotypes : El Tor and Classical.

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Auxotyping

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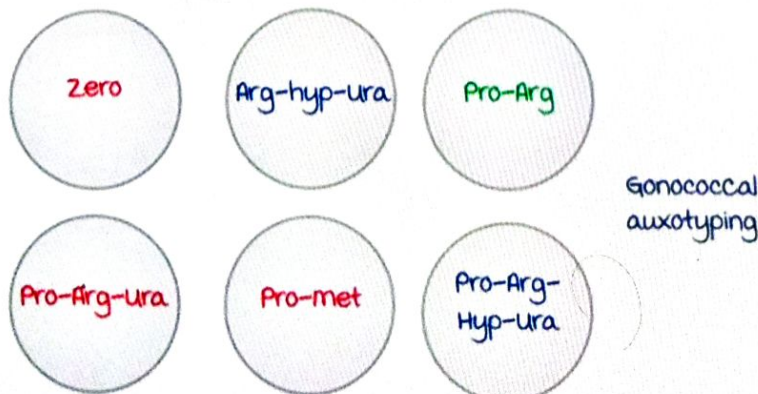
Principle : It is based on the ability of bacteria to utilize or not utilize a specific group of substrates.

Example : *Gonococcus*.

Special defined medium is created. Certain nutrients are removed from it to create different media. Different strains of *Gonococci* are streak inoculated on all these media.

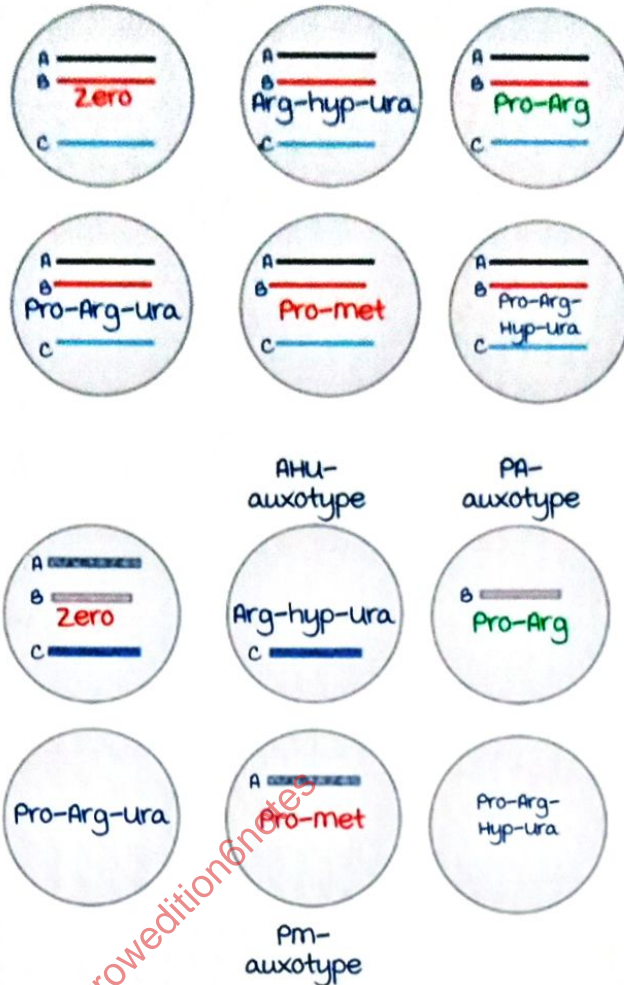
Defined medium : Contains all the nutrients essential for the growth of *Gonococcus*.

Defined medium



Active space

Defined medium



Strain C is growing in the absence of arginine, hypoxanthine and uracil. It is AHU - auxotype.

Strain B is growing in the absence of proline and arginine. It is

PA - & AHU - auxotype.

Phage typing :

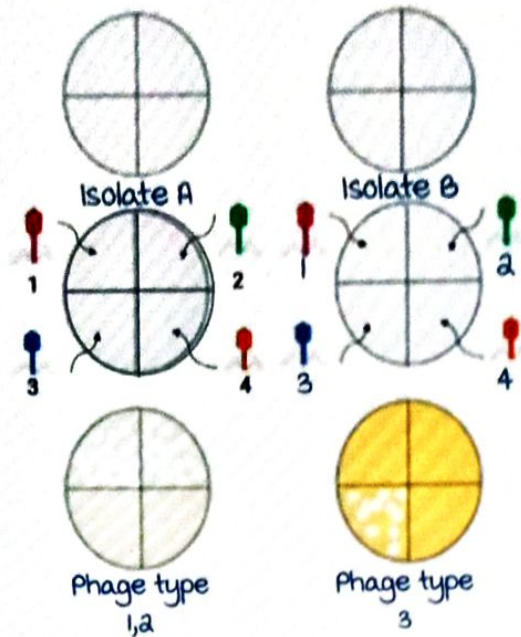
Principle : Based on susceptibility to standard set of bacteriophages (viruses that infect bacteria).

Phage typing is done for *Corynebacteria*, *Staph aureus*, *Salmonella*, *Vibrios*.

Corynebacterium phage typing :

Culture plate is divided into 4 quadrants. *Corynebacterium* is swab inoculated onto the whole petriplate. 4 different bacteriophages are put on 4 different quadrants. Zones of lysis are seen if infected by bacteriophage which shows

Corynebacterium is susceptible to bacteriophage infection.



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Image shows phage type 1,2 susceptible Corynebacteria and phage type 3 susceptible Corynebacteria.

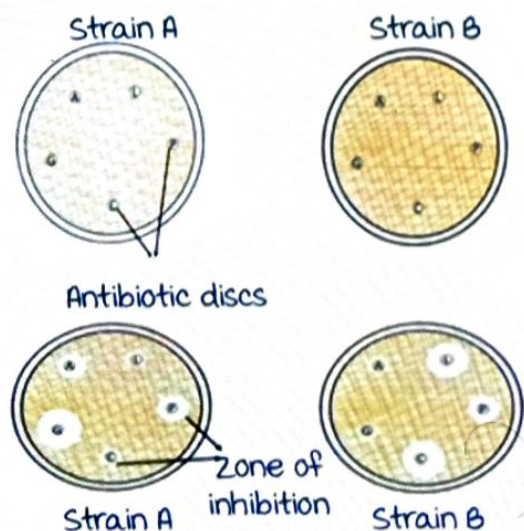
Antibiogram typing :

Principle : Based on susceptibility to antibiotics.

Bacteria are swab inoculated on two culture plates. Antibiotic discs impregnated with standard concentrations of antibiotics are placed on each culture plate.

After overnight incubation, zones of inhibition are compared. If they are different, it indicates two different strains of bacteria.

Smaller zone of inhibition : Bacteria resistant to that antibiotic.



Active space

Other methods :

Bacteriocin typing :

Comparison of susceptibility of standard strains to the bacteriocins secreted by isolates.

Bacteriocin : Antibiotic like protein which kills related bacteria. Produced by all bacteria and gives a survival advantage.

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Whole cell protein typing :

Relative mobilities of whole cell proteins under electrophoresis compared by banding patterns.

Multi locus enzyme electrophoresis (MLEE) :

Relative mobilities under electrophoresis of specific intracellular enzymes.

Phenotypic methods change with environmental conditions. Though the strains of a bacteria are the same, growth in different environmental conditions causes different phenotypic expression.

Genotypic methods

00:38:10

Superior to phenotypic methods (no change with environmental conditions).

Principle : DNA based analysis.

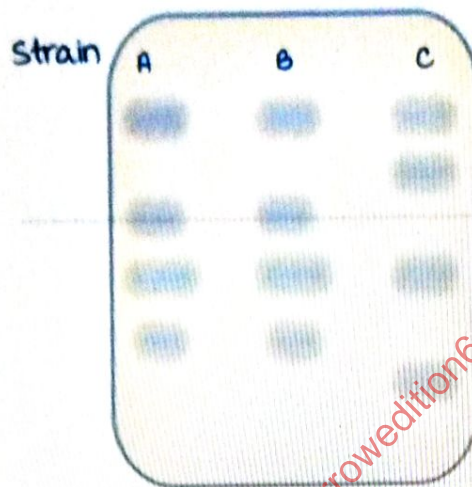
Genotypic methods :

- **Plasmid profile analysis** : Plasmid is extracted.
- **Ribotyping** : Part of DNA coding ribosomal RNA is extracted.
- **Pulsed field gel electrophoresis** : Whole genomic DNA is extracted.
- **Restriction fragment length polymorphism (RFLP)** : Whole genomic DNA is extracted.

Extracted DNA is exposed to restriction endonucleases that recognise particular DNA sequences and cleave them.

Gel electrophoresis is done on fragments of DNA.

If two strains of a bacteria are the same, restriction endonucleases generate absolutely the same fragments. Gel electrophoresis shows the same banding patterns if both the bacteria are the same strains (A & B).



In **pulsed field gel electrophoresis**, rare cutting restriction enzymes are used (very few recognition patterns & so larger fragments are generated).

In **RFLP**, frequent cutting restriction enzymes are used (smaller fragments are generated).

Use of restriction enzymes are variable since it can identify only if the desired recognition sequence is present.

Best method for typing of any pathogen (Gold standard) :
Nucleic acid sequencing.

Multi locus sequence typing (MLST) :

Smaller version of nucleic acid sequencing. In this method,

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GENERAL BACTERIOLOGY : CULTURE TECHNIQUES

Bacteria are categorized into :

Phototrophs : Can utilize light as a source of energy.

Chemotrophs : Need to undergo chemical reactions to synthesize energy.

Chemotrophs are further divided into :

Autotrophs : Can utilize inorganic sources of carbon and nitrogen in the form of atmospheric gases.

Heterotrophs (chemo organotrophs) : Require preformed organic compounds to undergo chemical reactions. most pathogenic bacteria and human commensals are heterotrophs.

Cultivation of bacteria

00:04:38

Louis Pasteur :

- First person to grow bacteria in a laboratory.
- **Father of medical microbiology.**
- Established bacterial growth requirements.
- Used liquid media (broths) to grow bacteria.

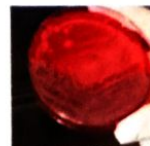
Liquid media (Broths) :

- Advantage : Bacteria multiplies **rapidly.**
- Appearance of turbidity indicates bacterial growth.
- **Cannot determine the type/ number of bacteria in the specimen.**



Solid media :

- Robert Koch introduced the concept of solid media.
- Advantage : **Different bacterial colonies** can be seen.
- **Slower growth** compared to broths.



Active space

Solidifying agents :

Gelatin :

- Derived from animal bone/ hide.
- **15% concentration** is used to solidify a liquid medium.
- Disadvantages : Bacteria can proteolyse gelatin.
Gelatin liquifies **above 24 °C**.

Agar agar/Chinese grass :

- Derived from sea weeds and red algae.
- **1-2% concentration** is used to solidify a liquid medium.
- **Polysaccharide** in nature.
- Provides no nutrition to bacteria.
- No effect on bacterial growth.
- Not hydrolysed by bacteria.
- **Liquifies at >98 °C** and **solidifies at <42 °C**.

Soft agar :

- To check the **motility** of bacteria.
- Concentration of agar : **0.2 to 0.5%**.
- Semi solid in consistency.

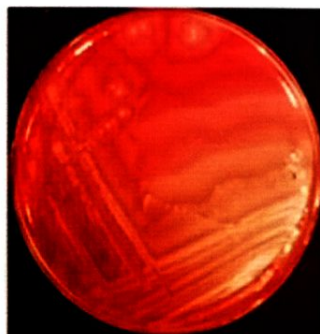
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Firm agar :

- Concentration of agar : **5 - 6%**.
- Used for **inhibition of swarming**.
- Hard in consistency.



Individual colonies on firm agar



Swarming

Swarming is seen with :

Gram positive : Clostridium tetani, Bacillus cereus.

Active space

Gram negative : *Vibrio alginolyticus*, *Proteus vulgaris*, *Proteus mirabilis*.

Constituents of culture media :

- Peptones.
- meat/yeast extract.
- Casein hydrolysate.
- Electrolytes.
- Water.

Types of culture media

00:12:42

Chemically defined medium : Exact composition is known.

Complex medium : Exact composition is not known.

Based on the use in laboratory :

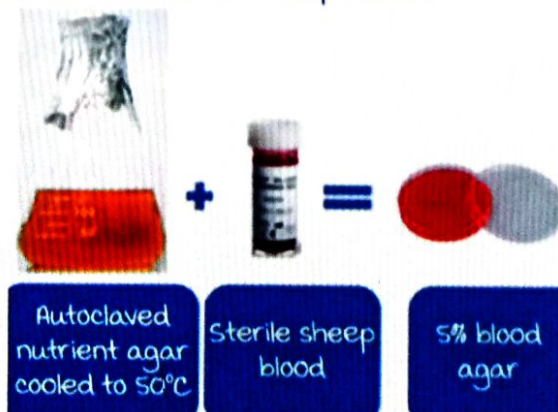
1. Simple medium (basal medium/basic medium) :

- Only **non fastidious bacteria** will grow.
- Just a source of carbon and nitrogen is provided.
- Example : Peptone water, nutrient broth (meat extract + peptone dissolved in water), nutrient agar (nutrient broth + agar in 1 - 2% concentration).

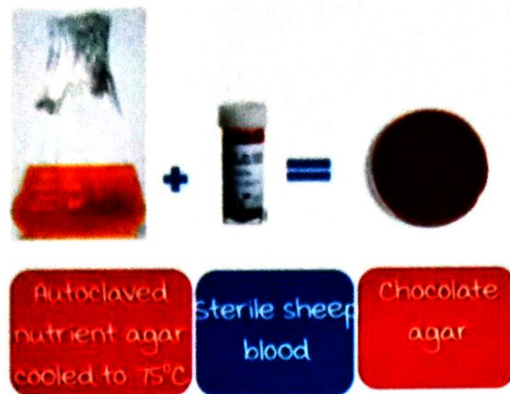
2. Enriched medium :

- used to grow **fastidious bacteria**.
- **egg/blood/serum** added to provide extra nutrition to bacteria.
- Example :

Blood agar : Autoclaved nutrient agar cooled to 50°C + 5% sterile sheep blood.



Chocolate agar : Autoclaved nutrient agar cooled to 75 °C + 5% sterile sheep blood.



Loeffler's serum slope.

Lowenstein Jensen medium.



Loeffler's serum slope.



Lowenstein Jensen medium.

3. Selective medium :

- Addition of a component to **inhibit unwanted bacteria**.
- Solid in consistency.

Examples :

- **macConkey medium** : mildly selective for **gram negative bacteria**.
Contains sodium taurocholate that inhibits gram positive bacteria (except staphylococcus, enterococcus).
- **eosin methylene blue (EMB) agar** : mildly selective for **gram negative bacteria**.
- **Salt agar** : Selective medium for **Staphylococci** (grows in the presence of **7-10% salt**).
- **Crystal violet blood agar** : Selective medium for group A streptococcus (*Streptococcus pyogenes*).
- **modified Thayer martin medium** : Selective medium for **Neisseria**. Contains antibiotics like Vancomycin, Colistin & Nystatin.

- Thiosulphate citrate bile salt sucrose (TCBS) agar : Selective medium for **vibrio**.
- Potassium tellurite (0.03 - 0.04%) : Selective medium for **Corynebacteria**.
- Ceftrimide agar : Selective medium for **Pseudomonas**.
- Polymyxin Lysozyme EDTA thalious acetate (PLET) medium : Selective medium for **Bacillus anthracis**.
- mannitol egg yolk phenol red polymyxin (MYPA) agar : Selective medium for **Bacillus cereus**.

4. Enrichment medium :

Liquid medium which is selective in nature.

Example :

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- Alkaline peptone water : Enrichment medium for **Vibrios**.
- Selenite F (feces) broth : Enrichment medium for **Salmonella, Shigella**.
- Tetrathionate broth : Enrichment medium for **Salmonella**.

Difference between enriched and enrichment medium :

Enriched medium	Enrichment medium
Any medium that contains egg, blood or serum	Liquid medium that is selective in nature.

Differential medium

00:23:12

Colony morphology/colour differentiates bacteria.

Examples :

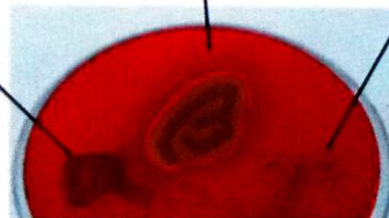
Blood agar :

Beta : Complete hemolysis; **Gamma :** Non hemolytic.

Alpha - hemolytic

Beta hemolytic

Gamma hemolytic



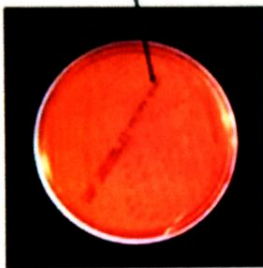
5. Indicator medium :

Special component is added to indicate growth of a particular group of bacteria.

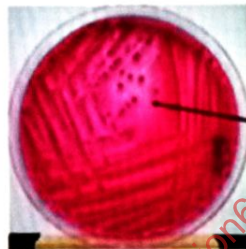
medium	Indicator component	Changes to	Type of bacteria
mc Conkey agar	Neutral red	bright	Lactose fermenting
mannitol salt agar	Phenol red	Yellow	mannitol fermenting
TCBS medium	Bromothymol blue	Yellow	Sucrose fermenting

macConkey agar :

Non-lactose fermenting



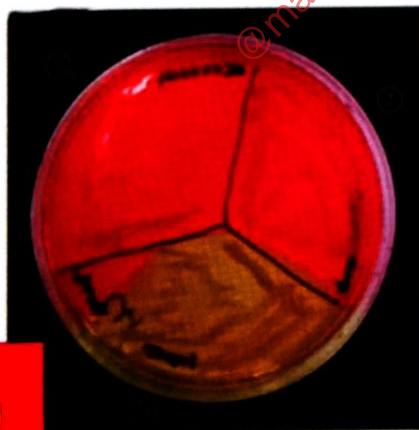
Neutral red



Lactose fermenting

mannitol salt agar :

Phenol red



mannitol fermenting

Non-mannitol fermenting

TCBS medium :

Sucrose fermenting

Bromothymol blue



Non-sucrose fermenting

Active space

Transport medium

00:28:48

maintains the original count of bacteria in a clinical specimen.

example :

- Venkatraman Ramakrishnan medium : *Vibrios*.
- Pike's medium : *Streptococcus pyogenes*.
- Stuart's medium : *Neisseria*.
- Amie's medium : *Neisseria*.
- Thioglycollate broth : *Anaerobes*.
- Cary Blair medium : *universal stool transport medium*.

MCQs :

1. EMB agar is a medium used in the identification and isolation of pathogenic bacteria. It contains digested meat proteins as a source of organic nutrients. Two indicator dyes, eosin and methylene blue, inhibit the growth of gram positive bacteria and distinguish between lactose fermenting and non lactose fermenting organisms. Lactose fermenters form metallic green or deep purple colonies, whereas the non lactose fermenters form completely colorless colonies. EMB agar is an example of which of the following :
 - A. Selective medium only.
 - B. Differential medium only.
 - C. Selective medium and a chemically defined medium.
 - D. Selective medium, differential medium and a complex medium.



EMB plates - Left : Proteus spp. Right : E. coli

5.

2. Not true about agar:

- A. Source of nutrition.
- B. Long chain polysaccharide.
- C. melts at 95 - 98 °C.
- D. Solidifies around 40 °C.

3. State true or false :

- A. Streptococci grow well on mannitol salt agar : **False.**
- B. Thayer-martin agar contains gentamicin, amongst other antibiotics : **False.**
- C. Lactose fermenters produce yellow colonies on cysteine lactose electrolyte deficient (CLEO) agar : **True.**
- D. On MacConkey, non lactose fermenting bacteria will use peptone as their energy source instead : **True.**

MacConkey agar contains **PLANT** : Peptone, lactose, agar, neutral red, taurocholate.

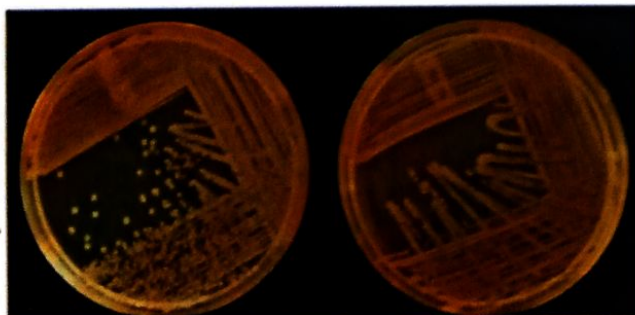
- **Taurocholate** inhibits gram positive bacteria.
- **Neutral red** is an indicator, gives characteristic colour to lactose fermenters.
- **Agar** is a solidifying agent.
- **Lactose** is the **source of energy**.
- If bacteria is a non lactose fermenter, it uses **peptone** as its source of energy.

Culture techniques

00:38:45

Streak culture :

Used to obtain **pure isolated bacterial colonies**.

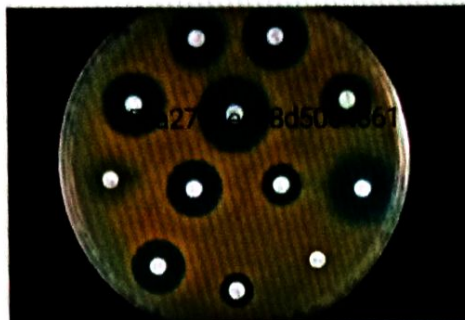


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Lawn culture :

Used for antibiotic sensitivity testing by disk diffusion method and bacteriophage typing.



Stroke culture :

Used for biochemical tests (example : Urease test) and maintaining stock cultures of bacteria in the laboratory.



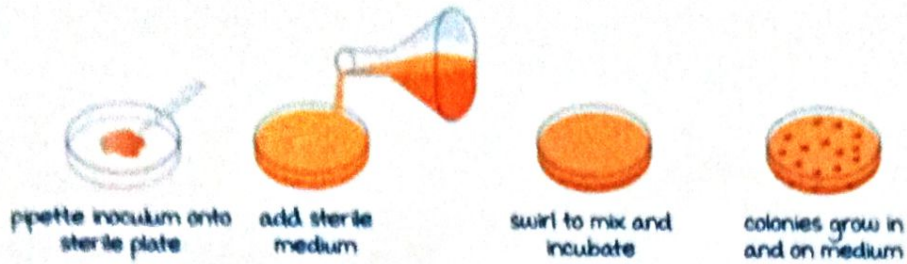
Stab culture :

Used in biochemical tests (example : Triple sugar iron test) and to check motility of bacteria in a motility test agar (soft agar).



Four plate culture :

Used for quantification of bacteria in a specimen. Pipette inoculum (few drops) onto sterile plate. Add sterile medium (molten medium), Swirl to mix and incubate overnight. Colonies grow in and on medium.



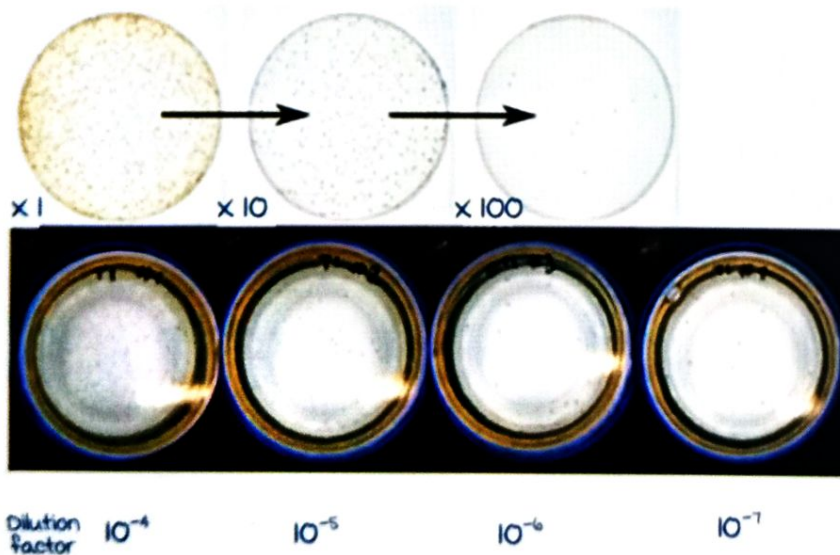
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MCQs :

1. Which of the following culture methods is used for calculating 'significant bacteriuria'?
 - A. Streak culture.
 - B. Lawn culture.
 - C. Stroke culture.
 - D. Pour plate method.

Quantification of bacteria :



Serial dilutions of patient's urine specimen (in sterile water) is prepared. Separate pour plate culture is prepared for each dilution. Number of colonies multiplied by dilution factor gives the number of bacteria/ml.

Active space

GENERAL BACTERIOLOGY : ANTIBIOTIC SENSITIVITY AND RESISTANCE

Purpose of AST

00:00:36

Guideline to the physician in choosing the right antibiotic.
To assess susceptibility patterns in a hospital or community
for empirical use of antibiotics.

Definitions :

minimal Inhibitory Concentration (MIC) : Lowest
concentration of the antibiotic that just inhibits the growth of
the pathogen in the medium.

minimal Bactericidal Concentration (MBC) : Lowest
concentration of an antibiotic that kills all bacteria in medium.

$MBC \gg MIC$

MIC is calculated from sensitivity testing in dilution methods.

Methods of AST :

- Dilution method.
- Disc Diffusion method.
- E-Test : Combination of dilution & disc diffusion methods.
- Automated methods : Dependent on micro broth dilution method.

Examples : microScan WalkAway, Vitek -2, Phoenix

Standardization of AST :

To control the impact of environmental factors, the conditions
for susceptibility testing are extensively standardized by the
Clinical Laboratory Standards Institute (CLSI) in the US.

- Anti-microbial content to be added in disc/medium
should be absolutely the same.
- **Standard inoculum** of test isolate must be prepared for
all bacteria.
- **Reliable medium** for the sensitivity testing : **Mueller**

Hinton agar/broth.

- Standard temperature of incubation : $35-37^{\circ}\text{C}$.
- Standard duration of incubation : minimum 16 - 18 hours.
- **Quality control strains** : Strains of **ATCC number specific bacteria** are kept for which quality control should be done **regularly**.

mueller Hinton agar/broth : very basic medium that contains **Casein hydrolysate agar/broth**.

Bacteria that wouldn't grow on mueller Hinton medium would require additional nutrition supplementation.

Bacteria	Special nutrition + mueller Hinton medium
Pneumococcus	Lysed horse/sheep blood.
Staphylococcus aureus	Never add mueller Hinton agar alone. Always add 2-4% salt as it enhances expression of mecA gene , responsible for MRSA .

Standard inoculum preparation :

Bacterial suspension in **peptone water** or **nutrient broth** that has a **turbidity of 0.5 mcf** (mac Farlands, unit of bacterial suspension).

Procedure :

Example : Carry out AST for a urine sample of *E. coli*.

- Take sterile peptone water, add a few colonies of *E. coli* from the culture plate and gently shake it.
- Incubate for a few hours as the *E. coli* enters a log phase and then starts multiplying.
- After some time, measure the turbidity using a **spectrophotometer** or using special standardised liquid media, called **mac Farlands standard media**. These are sealed media of which turbidity has been standardised **0.5 mac Farlands = 1.5×10^8 cfu/ml**.
- Standardisation is done to improve reliability and comparability of the result.

Dilution method

00:13:32

Reference method for AST.

Procedure :

Serial dilutions of antibiotics in muller Hinton agar/broth +
Fixed amount of standard inoculum.

35 - 37 °C.

Overnight for 16-18 hours.

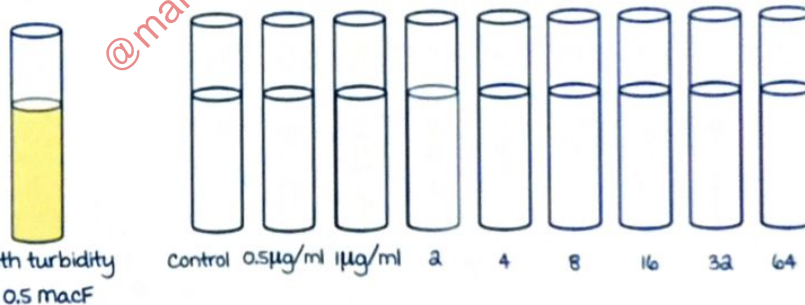
MIC

Types of dilution methods :

Broth dilution :

micro broth	macro broth
Done in 96 well micro titre plates.	Done in test tubes.
Saves amount of medium, commonly used.	Requires lots of culture media.

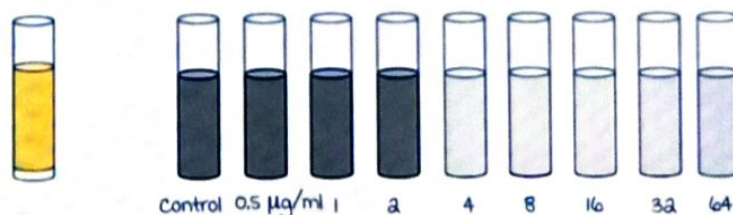
macro broth dilution method :



Procedure :

Control has no antibiotic.

1 ml of standard inoculum is added to each test tube and incubated overnight.



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Serial dilutions of antibiotic after overnight incubation with turbidity.

Observation : First 3 test tubes are turbid indicating microbial

growth.

At 4 $\mu\text{g/ml}$, bacterial growth is inhibited. For the tested bacterial sample, this is the MIC of the antibiotic.

This is compared with break point concentration laid down by CLSI and determine if the strain is sensitive or resistant.

microbroth dilution :

Done in a 96 well microtiter plate.

Serial dilutions of 8 antibiotics can be added to each set of test tubes (wells) and turbidity can be observed to determine its sensitivity or resistance.

microbroth dilution



Agar dilution method :

Procedure :

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Within the solid culture medium, serial concentrations of an antibiotic are prepared and the agar plate is divided into smaller areas. Each small area will be inoculated with samples from different patients.

Observation :

Absence of growth in a particular small area shows that the bacteria is unable to grow at that particular concentration of antibiotic.

Similarly, each agar plate will have serial dilutions of different antibiotics and samples from different patients will be inoculated and tested for sensitivity.



Agar dilution method.

Dilution methods are cumbersome and are done only in reference laboratories.

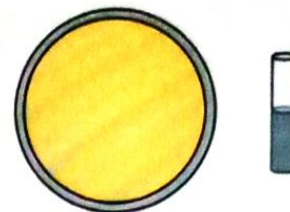
Disc diffusion method

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Kirby Bauer disc diffusion method : mc used worldwide.
uses **antibiotic discs** impregnated with a single standardised concentration of antibiotic as laid down by CLSI.

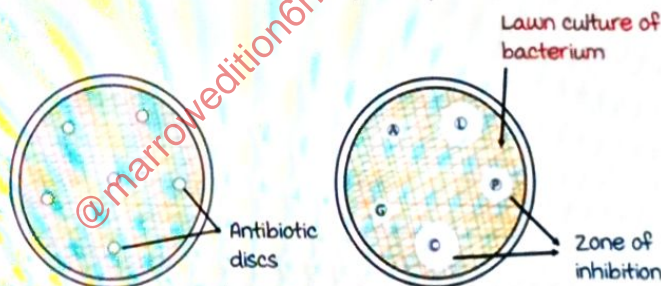
Procedure :

- Take a sterile cotton swab, dip it into the standard inoculum once and swab inoculate the solid mueller Hinton agar plate in multiple directions.



mueller Hinton agar and standard inoculum.

- Next morning, agar plates will have lawn cultures or semi-confluent growth of bacteria over the media.
- using specialised disc dispensers or sterile forceps, **Antibiotic discs of different antibiotics** are placed firmly over the swab inoculated petri plate.



- Each antibiotic disc must be placed at distance of **at least 24-25mm** to see the zones of inhibition clearly. Close the petri plate and incubate over night at a standard temperature of **35 - 37 °C**.
- Observation : **Zone of inhibition** around each antibiotic disc.
- Use a ruler or a caliper to measure the diameter of the zone of inhibition.
- Refer to the **standardised tables** provided along with the antibiotic disc dispensers that include the diameter of zones of inhibition for different bacteria. Categorize the bacteria into **sensitive, intermediate sensitive and resistant**.



Example :

E.coli and other enteric gram negative tods			
	Resistant	Intermediate	Susceptible
Amikacin (30 µg)	≤ 14 mm	15-16 mm	≥ 17 mm
Ampicillin (10 µg)	≤ 13 mm	14-16 mm	≥ 17 mm
Cefazolin (30 µg)	≤ 14 mm	15-17 mm	≥ 18 mm
Gentamicin (10 µg)	≤ 12 mm	13-14 mm	≥ 15 mm

Interpretation :

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Sensitive	Intermediate	Resistant
Organism is inhibited by the recommended dose of the antimicrobial agent at the infection site.	Organism may require a higher dose of antibiotic for a longer period of time to be inhibited.	Organism is not inhibited by the recommended dose of the antimicrobial agent at the infection site.

For example in the case above, if zone of inhibition around the amikacin disc is ≤ 14 mm, bacteria is resistant to amikacin

Pros : Easy procedure and easy to interpret.

Cons : It is **only qualitative**. Does not quantify MIC.

Stoke's disc diffusion method

00:28:48

used only in few countries of Europe.

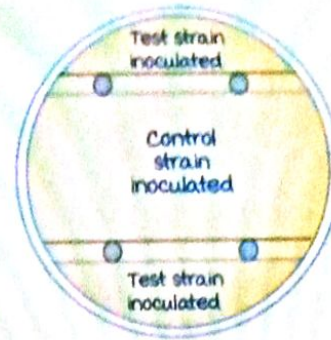
Test and control strains are inoculated in the same petri plate.

Procedure :

- Prepare the standard inoculum of the test strain and control strain.
- Before adding the inoculum, make **parallel lines** on the under surface of the petri plate. This is where **antibiotic discs** will be placed.

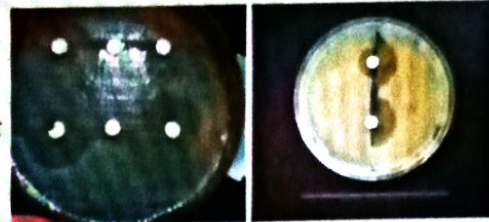
Active space

- **Control strain** is inoculated in the **centre** and the **test strains** are inoculated at the **sides**.
- Then, antibiotic discs are placed which diffuses into the medium and inhibit the growth of both test and control strains.



Observation :

Compare the zones of inhibition between the test and control strains and determine the sensitivity/resistance of the antibiotic based on certain guidelines.

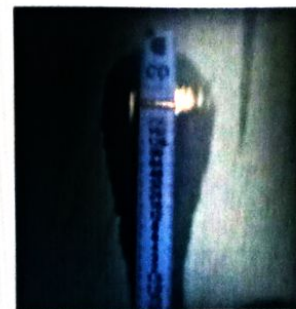
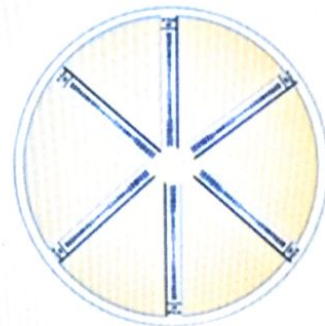


Epsilonometer Test (E-Test) :

Combination of dilution and disc diffusion method.

Procedure :

- In Muller Hinton agar plate, swab inoculate the standard inoculum of the test bacteria.
- Now, instead of discs, plastic strips impregnated with graded concentrations of antibiotics on its under surface is placed over the medium.
- Overnight, the antibiotic diffuses into the medium and inhibits growth of bacteria around it.
- We can test **six antibiotics** on the same plate.



Observation :

Zone of inhibition around the plastic strips.

The concentration at which zone of inhibition intercepts the plastic strip is the **MIC of the antibiotic** for that bacterium.

Pros : Quantitative test.

Cons but sometimes gives erroneous/conflicting results as zone of inhibition may be different on either side of the disc.

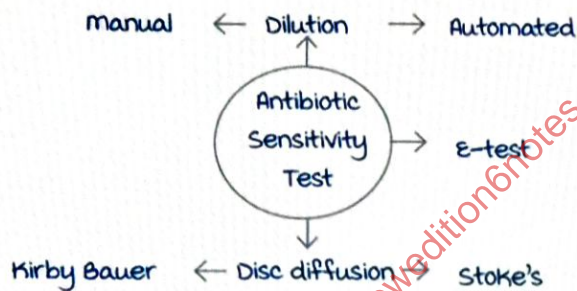
Automated systems

00:36:12

- Includes microScan WalkAway, Vitek -2, Phoenix
- Based on **micro broth dilution method**.
- Uses preformed petri plates that contain standard concentrations of the antibiotic.



Several strains and antibiotics can be tested simultaneously.



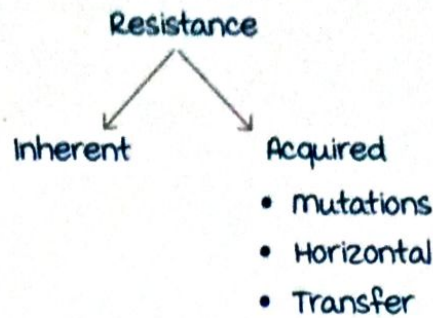
Antibiotics and its mechanism of action

00:40:32

Antibiotics	mechanism of action
Beta-lactams	Binds to Transpeptidases/Penicillin binding proteins and inhibit cell wall synthesis.
Glycopeptides E.g., Vancomycin	Binds to cell wall precursors (D-ala D-ala of pentapeptide of Nacetyl muramic acid) and inhibit cell wall synthesis.
Aminoglycosides Tetracyclines	Bind to 30s ribosomal subunits and inhibit protein synthesis.
macrolides Chloramphenicol	Binds to 50s ribosomal subunits and inhibit protein synthesis.
Fluoroquinolones	Binds to DNA gyrase/ topoisomerase IV and inhibits DNA replication.
Sulphonamides & trimethoprim	Inhibit folate synthesis.

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Inherent antibiotic resistance :

Innate ability of the bacterium to resist killing by the antibiotic due to its structural/ functional characteristics.

mechanisms :

1. **Reduced Entry** : Bacteria is impermeable to the drug.
2. **Efflux pumps** : Extrusion of the drug by inherently encoded transporters.
3. **Inherent enzymes** that inactivate the antibiotic.

Examples :

Bacteria	Inherently resistant to	mechanism of Resistance
Enterococci & anaerobes	Aminoglycosides.	Cannot enter the cytoplasm.
Gram positive bacteria	Aztreonam.	Bacteria lacks Penicillin Binding Proteins to which the drug binds
Gram negative bacteria.	Vancomycin.	Huge 1400D molecule. Cannot pass through the outer membrane of the bacteria

Acquired antibiotic resistance

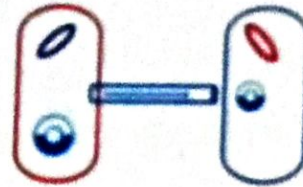
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Via mutations and horizontal transfer.

mechanisms of horizontal transfer :

Conjugation : m/c mode of antibiotic resistance spread.

R plasmid : Part of F plasmid that has r determinants (antibiotic resistance genes) which forms conjugation tube and facilitates transfer of the resistance genes between bacteria.



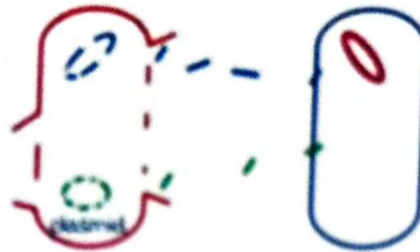
R plasmid : r determinants in F plasmid

Transformation : Uptake of soluble DNA fragments containing antibiotic resistance genes.

After a death of a bacteria, its antibiotic resistance

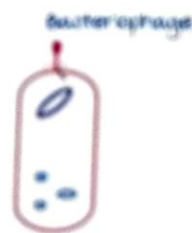
genes are taken up directly by other bacteria through its cell wall and incorporated into its genome.

Eg **Pneumococcus** : m/c mode of antibiotic resistance.



Transduction : Via **bacteriophages**, viruses that infect one bacterium, acquires resistance genes and causes lysis of the host. Once released, infects another bacterium and transfers the resistance gene.

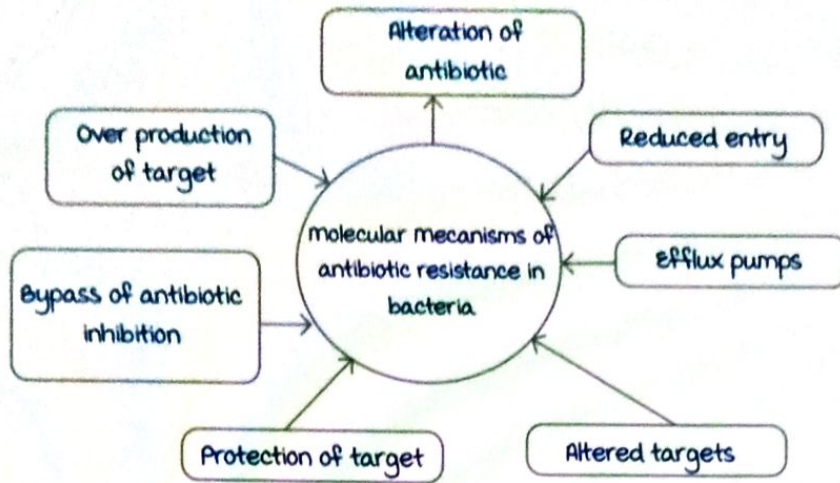
Eg **Staphylococcus** : m/c mode of antibiotic resistance.



modes of Antibiotic resistance :

mode of Resistance	Examples
Inactivating enzymes m/c mode	mnemonic : ABCs Aminoglycosides : Inactivated by phosphorylation, N- Acetylation. Beta-lactams : Inactivated by beta-lactamases Chloramphenicol : Inactivated by Acetyl transferases.

<p>Reduced permeability : Absence of porins or outer membrane proteins that do not allow the antibiotic to enter the cell.</p>	<p>Resistance to beta -lactams in gram negative bacteria. Aminoglycosides. macrolides. Fluoroquinolones.</p>
<p>Antibiotic efflux : Antibiotics pumped out through transport channels.</p>	<p>m/c mode of resistance to Tetracyclines. Also seen in macrolides, fluoroquinolones and beta lactams.</p>
<p>Altered target site</p>	<ul style="list-style-type: none"> • Altered ribosomes : Resistance to Aminoglycosides & macrolides. • Altered cell wall precursors : Vancomycin. • Altered enzymes : Altered Penicillin Binding Proteins e.g. MRSA. DNA Gyrase : Fluoroquinolones.
<p>Protection of target sites : Bacteria synthesizes proteins that binds to the target sites and protects it from antibiotics.</p>	<p>Tetracyclines through ribosomal protection proteins.</p>
<p>Over production of target sites : Target sites in excess to antibiotic administered.</p>	<p>Beta-lactam resistance in gram positive bacteria. Sulfonamide resistance.</p>
<p>Bypass of antibiotic inhibition</p>	<p>Sulfonamide resistance. Bacteria starts producing folic acid through alternate pathways.</p>

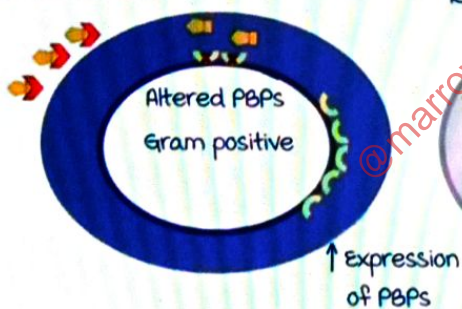


Modes of Resistance to common antibiotics 00:57:55

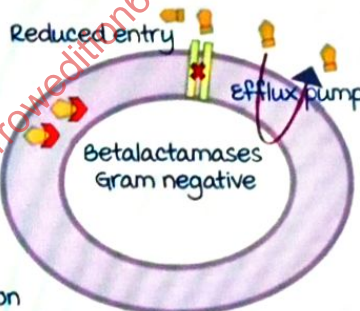
1. Beta lactam antibiotics :

Gram positive bacteria.

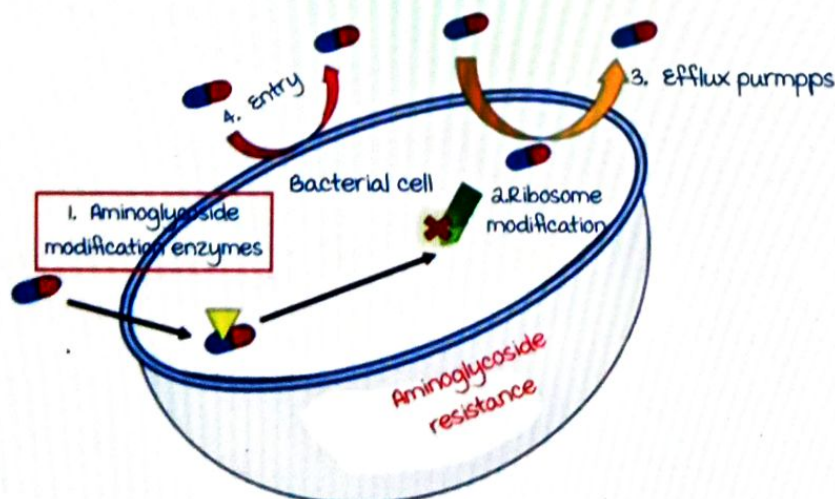
Betalactamases



Gram negative bacteria.

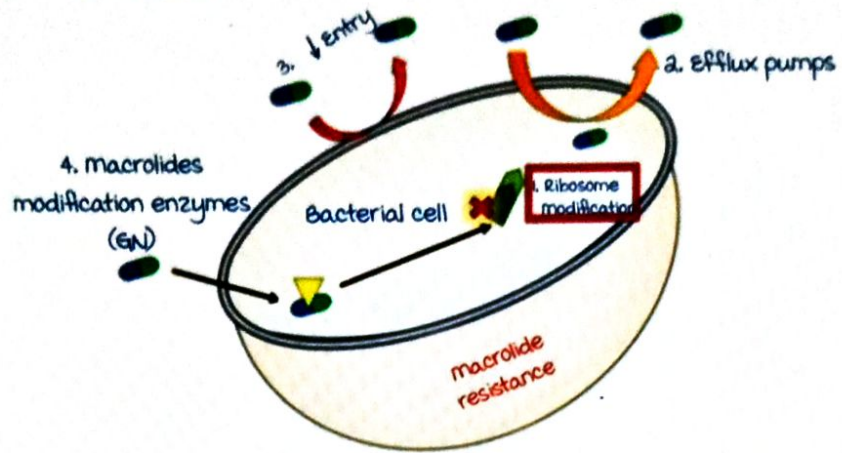


2. Aminoglycosides :

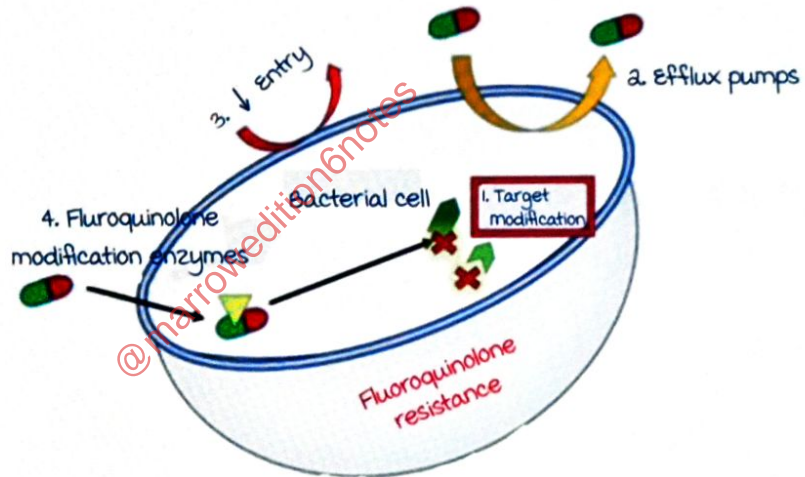


Active space

3. macrolides :



4. Fluoroquinolones :



m/c modes of resistance to common antibiotics :

Antibiotics	m/c mode of resistance
Beta lactams	Inactivating enzymes
Aminoglycosides	
Chloramphenicol	
macrolides	Altered drug targets
Fluoroquinolones	
Tetracyclines	Efflux pumps

Active space

GENERAL BACTERIOLOGY - BACTERIAL GENETICS

Bacteriophages

00:00:39

viruses that infect bacteria.

They have varied morphology (filamentous, polyhedron etc.,).

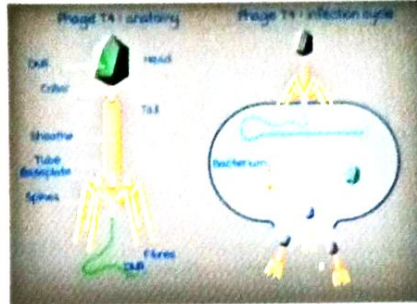
Either DNA virus or RNA virus (less common).

Bind to a certain receptor on the bacterial cell wall.



Specific to certain bacteria.

Inject genetic material into bacterial cytoplasm.



Cycle of phage: Follow either lytic cycle or lysogenic cycle.

Lytic cycle.	Lysogenic cycle.
Phage DNA injected into bacterial cytoplasm	Phage DNA injected into bacterial cytoplasm
↓	↓
Inhibits bacterial metabolism	Phage DNA integrates with the bacterial chromosome: Prophage. Bacterium is called lysogenic
↓	↓
Phage components synthesized	Vertical transfer of prophage
↓	↓
Assembly of daughter phages	On exposure to UV light or nitrogen mustards
↓	↓
Release by lysis	Induction of prophage (phage DNA disintegrates from the bacterial chromosome)
↓	↓
Infect new bacteria	Enters lytic cycle

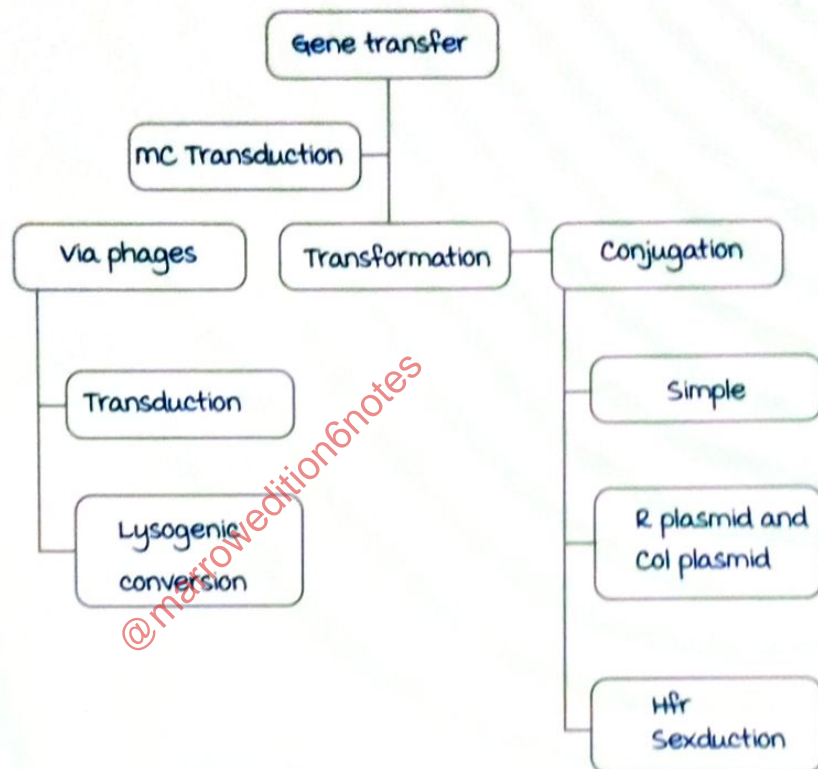
Two types of phages :

1. **Virulent phages** : Follow only lytic cycles.
E.g. T₁, T₂ phage of E. coli.

2. **Temperate phage** : Can follow both lytic and lysogenic cycles.
E.g. λ phage of *E. coli*.

mechanisms of Gene Transfer

1. Transduction
 2. Lysogenic conversion
 3. Transformation
 4. Conjugation
- bnvssprasanth7@gmail.com
- } **Via Phages.**

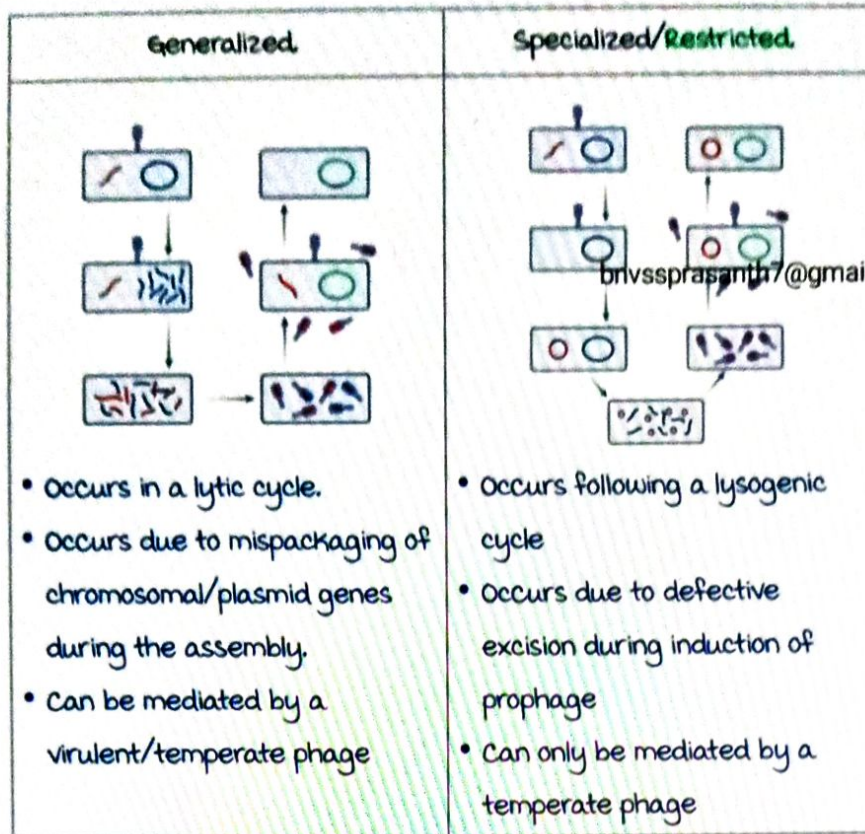


Via Phages : Transduction

00:10:38

Transfer of bacterial genes from one to another via phage.
most common mechanism.

1st demonstrated on *Salmonella typhimurium*.
Two types : **Generalized** and **Specialized**.
MC mechanism of multidrug resistance in *S. aureus*.

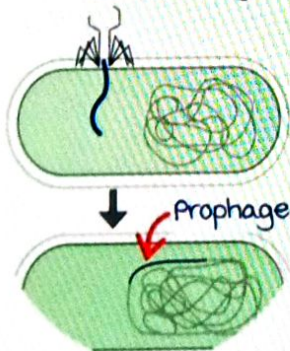


Via Phages: Lysogenic Conversion

00:17:59

Prophage genes (in a lysogenic cycle) provide genes for a new property to the lysogenic bacteria.

Classical E.g. **Toxin production** → Phage mediated toxins.



- Pyrogenic toxin A & C of *Strep. pyogenes* (Group A β hemolytic)
- Botulinum toxin C/D
- Cholera toxin
- Diphtheria toxin
- Shiga like toxin/verocytotoxin (EHEC)

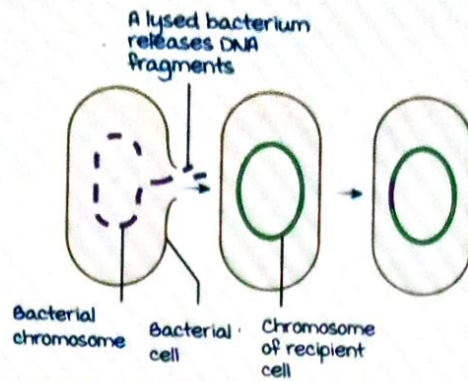
MCQ. Which of the following statements is true about bacteriophage? Allms.

- It is a bacterium.
- It helps in transformation.
- It imparts toxigenicity to bacteria.
- It transfers only chromosomal gene.

Active space

Transformation

00:21:55



uptake of soluble DNA fragments in the environment **directly** through the cell wall.

Ability to take up DNA through a cell wall : **Competence**.

1st shown on Pneumococcus by Griffith.

used in **genetic engineering**. Eg. Insulin production by artificial transformation.

Plasmid vector with specific gene b (like insulin) is incubated with E. coli. Heat is provided to increase the permeability of E.coli, that takes up the plasmid.

MC mechanism of multidrug resistance through plasmid in **Pneumococcus**.

MCQ. multidrug resistance through plasmids is transferred by

A. Transduction.

B. Transfection.

C. Conjugation.

D. Recombination.

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Conjugation

00:25:30

MC method of multi-drug resistance through plasmid in bacteria.

Except for S. aureus : Transduction

Pneumococcus : Transformation.

1st demonstrated by Lederberg and Tatum on E. coli K12 strain.

Ability to form Sex pilus/Conjugation tube.



encoded on F plasmid/Fertility plasmid/Sex plasmid/transfer factor.

- F⁺ bacteria : F plasmid **present**.
- F⁻ bacteria : F plasmid **absent**.

method : When an F⁺ bacteria conjugates with F⁻ bacteria.

↓
One complete strand of F plasmid transferred to F⁻

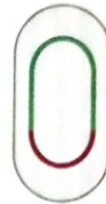
↓
F⁻ gets converted to F⁺ → 2F⁺.

Hfr bacteria cell : High frequency recombinant bacterial cell.

F plasmid gets integrated into chromosomes of bacteria.

It is a **reversible state**.

Hfr cell



1. Hfr cell conjugation with F⁻ :

The sex pilus breaks before the complete transfer of strand.

Only some chromosomal genes are transferred (not the F plasmid genes).

Hfr + F⁻ → Hfr + **Recombinant F⁻**.

2. F' (F prime) Bacterium :

When F plasmid is disintegrating from bacterial chromosome, it sometimes carries away some chromosomal genes next to the site of integration.

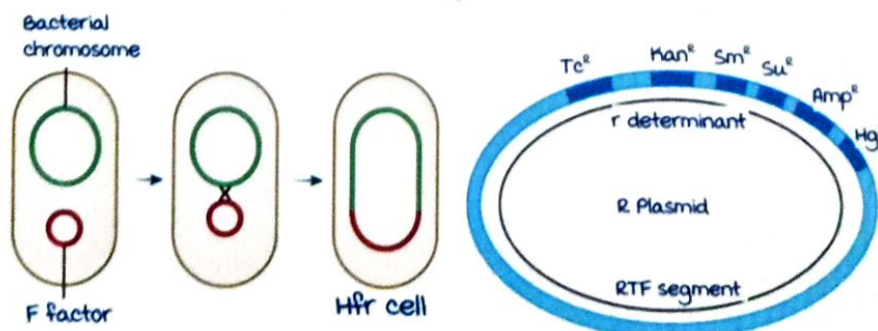
↓
This independent F plasmid → F' plasmid in F' bacteria.

3. F' cell conjugation with F⁻ :

Complete transfer of F' plasmid.

F' + F⁻ → 2 F'.

Sexduction : Transfer of F' plasmid to F⁻ bacterium.



Active space

R Plasmid : most common mode of spread of antibiotic

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resistance in bacteria.

First shown on **shigella**.

F plasmid + Antibiotic resistance genes.

↓ ↓
Resistance transfer factor. r determinants.

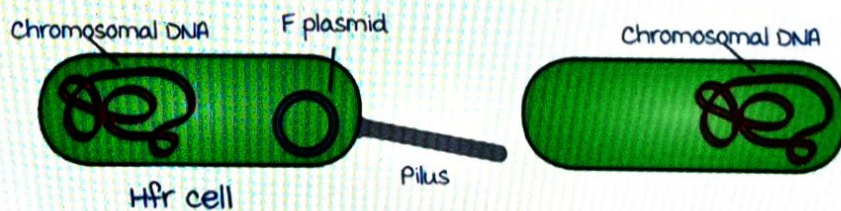
Col Plasmid: F plasmid + Bacteriocin genes (antibiotic like proteins, kill related bacteria).

Summary:

- F+ (donor) × F- (recipient) = 2F+
- R+ (donor) × F- (recipient) = 2R+
- C+ (donor) × F- (recipient) = 2C+
- Hfr (donor) × F- (recipient) = Hfr + Recombinant F-
- F' (donor) × F- (recipient) = 2F'

M.C.Q. Shown in the figure is conjugation occurring between 2 bacterial cells. What will be transferred from Hfr bacterium during conjugation?

- F plasmid.
- Chromosomal genes.**
- F plasmid and resistance genes.
- F plasmid and chromosomal genes.



Restriction Endonuclease

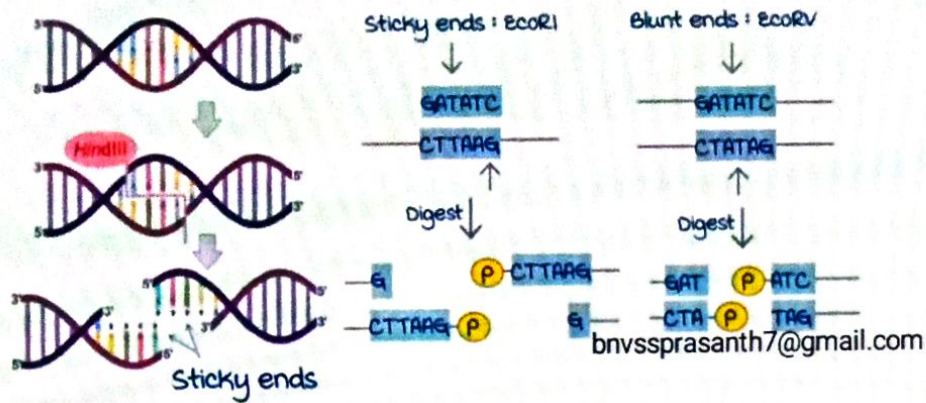
00:39:23

Property: **Break DNA** somewhere in the middle (not near ends).

Do so by identifying recognition sequences (**Palindrome** or **not palindromic**).

Restriction site: Breaking of DNA at a specific site.

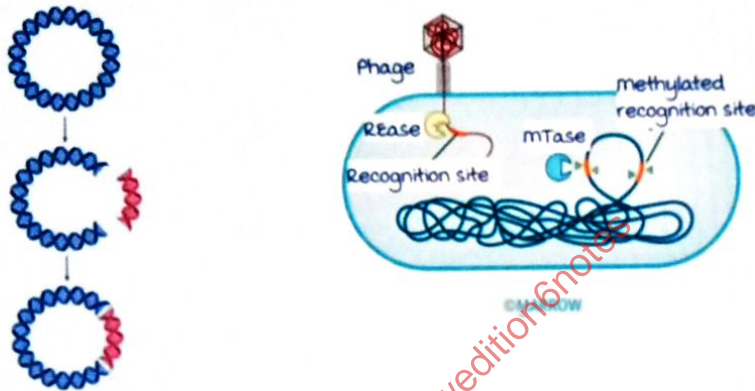
maybe a part of the recognition sequence or several nucleotides away from it.



4 types of restriction endonucleases :

Type II MC used for genetic engineering.

Type II gives staggered ends or cohesive ends.



Restriction modification system : methylation to protect its own genome.

MCQ. The restriction endonuclease is a defence mechanism in the bacterial system againsta foreign DNA such as viruses. But how is it able to protect its own DNA?

- By methylation of bacterial DNA by restriction enzyme.
- By methylation of foreign DNA by restriction enzyme.
- By phosphorylation of bacterial DNA by restriction enzyme.
- By phosphorylation of foreign DNA by restriction enzyme.

MCQ. False about restriction enzyme:

- Type I & 3 cut away from recognition site.
- Type 2 cuts within recognition site.
- All require Mg^{++} as co factor.
- Produced by bacteriophages.

mCQ. Function of CRISPR is

A. Bacterial genome editing to protect against infecting virus.
 B. Bacterial genome editing to protect against human immune system.

C. mechanism used by virus for reassortment.

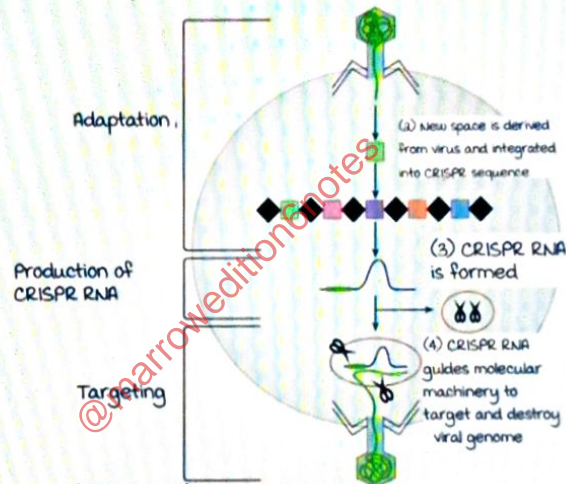
C. Genotypic mixing seen in virus for genetic reactivation.

CRISPR array :



CRISPR

00:45:45



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CRISPR → Clustered regularly interspaced short palindromic repeat Adaptive immune response of a bacteria.

Bacterial genome editing method to protect against infecting viruses.

Some segment of the bacterial chromosome is composed of absolutely the same repeats.

- 20-45 nucleotide long repeats
- Repeats separated by **variable separators**
- Repeats were fragments of phage DNA (which had infected bacteria)
- Acted as memory bank for bacterium

CRISPR Cas-9 complex used for gene editing.

MCQ. A 16 year old cystic fibrosis patient was admitted to the hospital. A sputum culture yields *Burkholderia cepacia*. Subsequently, there are two other patients with *B. cepacia* bacteremia and the organism is cultured from the sputum of four additional patients. During this nosocomial outbreak, 50 environmental and seven patient isolates are being typed to identify the source of the outbreak, which of the following techniques would be most useful in this endeavour?

- A. Culture.
- B. Ribotyping.
- C. Nucleic acid sequencing.
- D. Antimicrobial susceptibility testing.

Typing

00:50:30

Intraspecies strain characterization.

Used in epidemiological studies.

Characterized into :

1. **Phenotypic typing** : Compares characters expressed by the bacterium.

Inferior (liable to environmental change).

- a. Serotyping : **MC method**. Uses particular antigens.

Capsular typing in *S. pneumoniae*

- b. Biotyping : Uses biochemical reactions. 5ca2793ec88d500486113130

Indole, Ornithine & Urease are used to identify 8 biotypes of *H. influenzae*

- c. Antibigram typing : Compares susceptibility to antibiotics.

- d. Bacteriocin typing.

- e. Phage typing : Susceptibility to standard phages.

- f. Whole-cell protein electrophoresis : Extract proteins of test strains on a gel electrophoresis and compare the banding patterns.

- g. Multilocus enzyme electrophoresis. : Extract specific enzymes from the bacteria, do a gel electrophoresis and compare the banding patterns.

Active space

a. Genotypic :

Principle : Extract a particular DNA of the bacterium, expose them to restriction enzymes, generate fragments, do a gel electrophoresis and compare the banding patterns.

If there are same bacteria, the banding patterns will look the same.

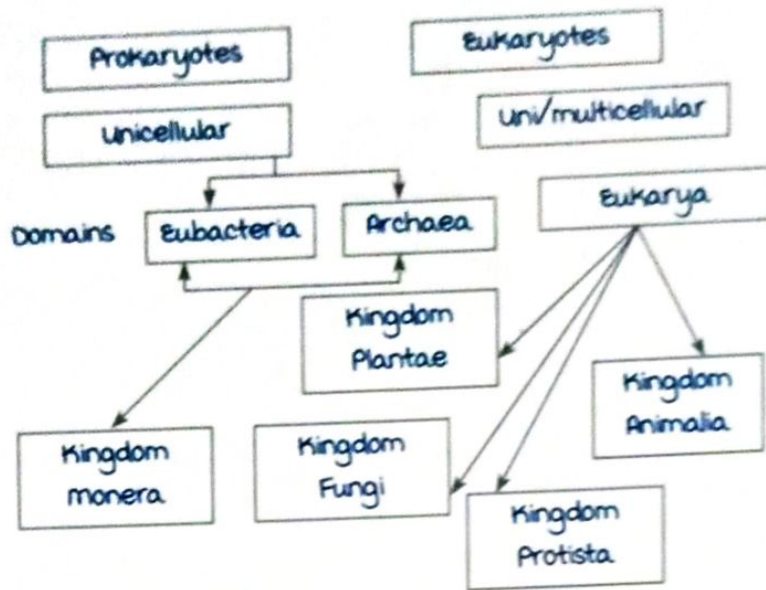
- Plasmid profile analysis
- Pulsed-field gel electrophoresis [PFGE]
- Restriction fragment length polymorphism [RFLP]
- Ribotyping. bnvssprasanth7@gmail.com
- Nucleic acid sequencing (most superior)

but whole genome sequencing is difficult

multilocus sequence typing [MLST].

MLST : Smaller version of nucleic acid sequencing in which only 7 house keeping genes are sequenced and compared.

BACTERIAL ANATOMY AND PHYSIOLOGY : PART 1



Difference between prokaryotes and eukaryotes

00:03:08

	Prokaryotes	Eukaryotes
Nucleus	Nucleoid : No well defined nuclear membrane. Nucleolus : Absent. Histones : Absent.	Nucleus : Well defined nuclear membrane.
Chromosome	Single, circular ds DNA	Multiple, linear chromosomes.
Extra chromosomal DNA	Plasmids.	In mitochondria.
membrane bound organelles	mitochondria, endoplasmic reticulum, golgi bodies, lysosomes are absent.	mitochondria, endoplasmic reticulum, golgi bodies, lysosomes are present.
Cell wall	muramic acid present.	Cell wall : Absent. If present, muramic acid is absent.
Cell membrane	Sterols : Absent.	Sterols : Present.
Ribosomes	70S (30S + 50S) ribosome.	80S (40S + 60S) ribosome.
mesosomes (Unique invaginations of the cytoplasmic membrane that are sites for respiratory enzymes)	Present (more prominent in gram positive than gram negative).	Absent.

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Active space

Exceptions :

- Prokaryote lacking a cell wall : **mycoplasma**. Hence, highly pleomorphic.
- Prokaryote having sterols in cell membrane : **mycoplasma**.
- Hence, mycoplasma are called "Jumping jokers" of microbiology.
- Prokaryote having 2 chromosomes : **Vibrio**.
- Prokaryote that lacks muramic acid in its cell wall : **Chlamydia**.

Eubacteria & Archaea (domains) are classified in prokaryotes under the **kingdom monera**.

Archaea :

- They are non pathogenic bacteria.
Example : **Thermus aquaticus** from which taq polymerase enzyme is obtained for PCR tests.
- Also called **extremophiles**, because they can survive in extremes of conditions (high salt, low pH, high temperature). This is enabled by isoprenoid lipid in the cell membrane.

	Eubacteria	Archaea
Peptidoglycan in cell wall	Present.	Absent.
Isoprenoid lipid in cell membrane	Absent.	Present.
Introns	Absent.	Present in some genes.
Characteristic rRNA sequence	Absent.	Present.

Eubacteria (domain) :

Contains Eubacteria & cyanobacteria (blue green algae).
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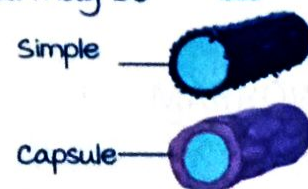
Glycocalyx

00:14:41

Outermost layer around the cell wall that may be present in some bacteria.

Types of glycocalyx :

- Slime.
- Capsule.



Glycocalyx - slime :

- A loose, ill defined, polysaccharide layer around cell wall.
- When a colony of slime producing bacteria grow together, it will lead to a collection of slime around them, known as **biofilm**. Biofilms enclose the bacteria, stick to the surfaces of instruments and cause disease.

Examples :

1. Streptococcus mutans (MC cause of dental caries).
2. Pseudomonas aeruginosa.
3. Staphylococcus epidermidis.

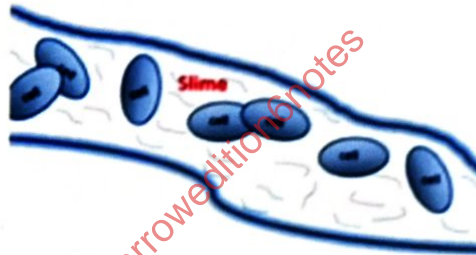
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Role of biofilm in pathogenicity :

1. Adhesion.
2. Anti phagocytic.
3. Reduces the entry of antibiotics.



Slime



Biofilm

Glycocalyx - capsule :

well defined/ demarcated layer around the cell wall.

- mostly polysaccharide **except Bacillus anthracis & Yersinia pestis** (polypeptide capsule).
- Anti phagocytic.
- **No net charge** : It cannot be gram stained.
- Capsule can stained by **copper salts** (not routinely available).
- Easy method to demonstrate capsule : **Negative staining** (India ink or nigrosine).
- Antigenic : Induce antibodies.
- **Quellung/ Neufeld reaction** : Capsule with specific anti capsular antibodies leads to **swelling** of capsule.



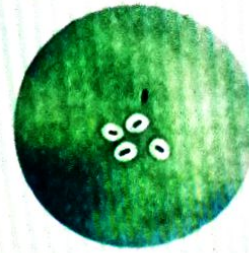
Capsule

This reaction is used in capsular typing of bacteria.

India ink negative stain



Quellung/ Neufeld reaction



Bacteria having capsules :

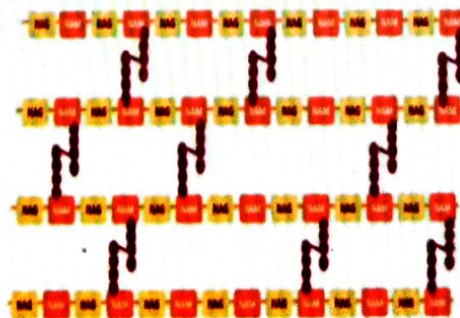
mnemonic : Yes, Some Bacteria Have Very Killer And mean CCapsules.

- *Yersinia pestis* (polypeptide capsule).
- *Streptococcus pneumoniae*.
- *Staphylococcus aureus* (microcapsule, zwitter ionic capsule).
- *Bordetella pertussis* (non antigenic capsule)
- *Bacteroides fragilis* (zwitter ionic capsule : Able to form abscess).
- *Haemophilus influenzae*.
- *Vibrio parahemolyticus*.
- *Klebsiella pneumoniae*.
- *Bacillus anthracis* (polypeptide capsule).
- *Meningococcus* (*Neisseria meningitidis*).
- *Clostridium perfringens* (MC cause of gas gangrene).
- *Cryptococcus* (*Cryptococcus neoformans* & *Cryptococcus gattii* : Only capsulated fungi).

Bacterial cell wall

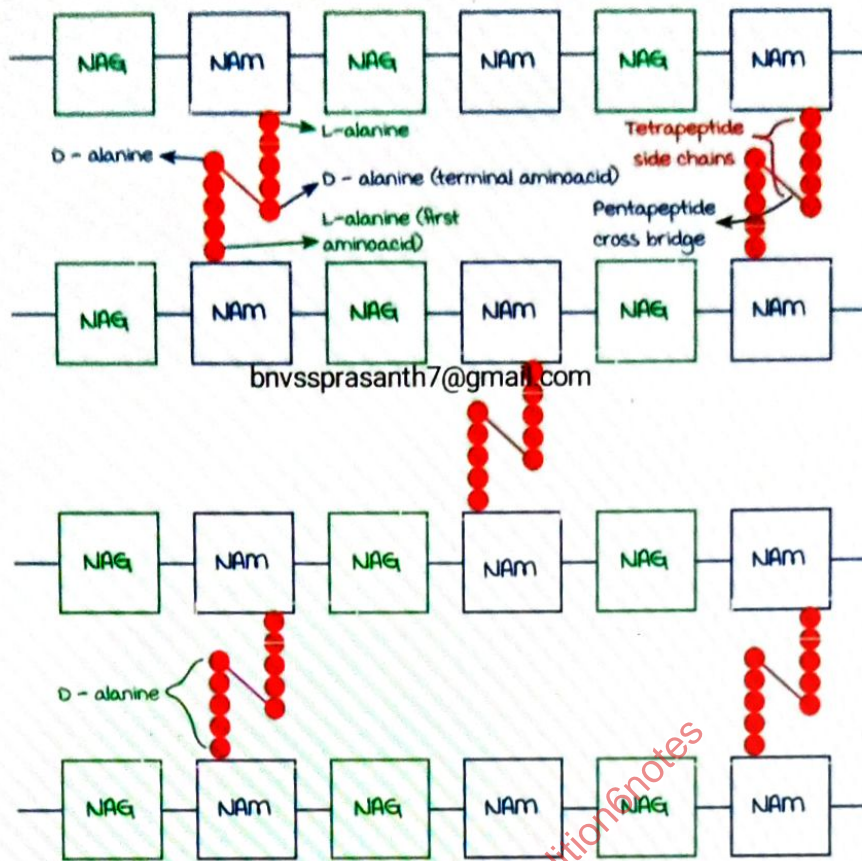
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Gram positive cell wall :

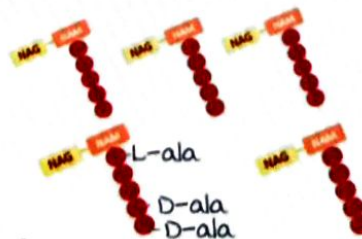


One chain of murein monomer : made of 2 carbohydrates.

N - acetyl glucosamine & N - acetyl muramic acid

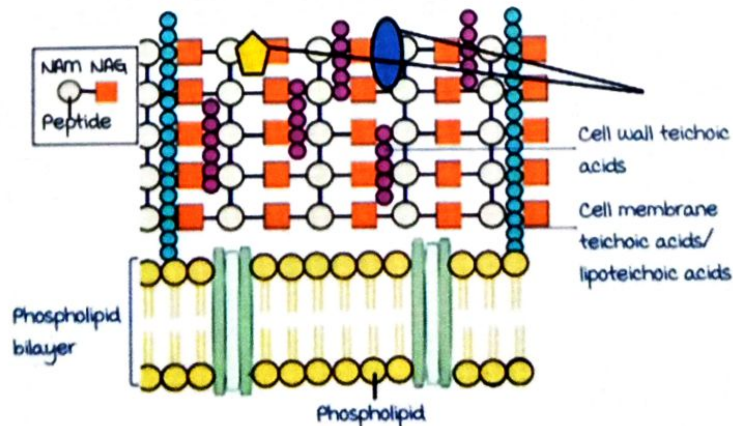
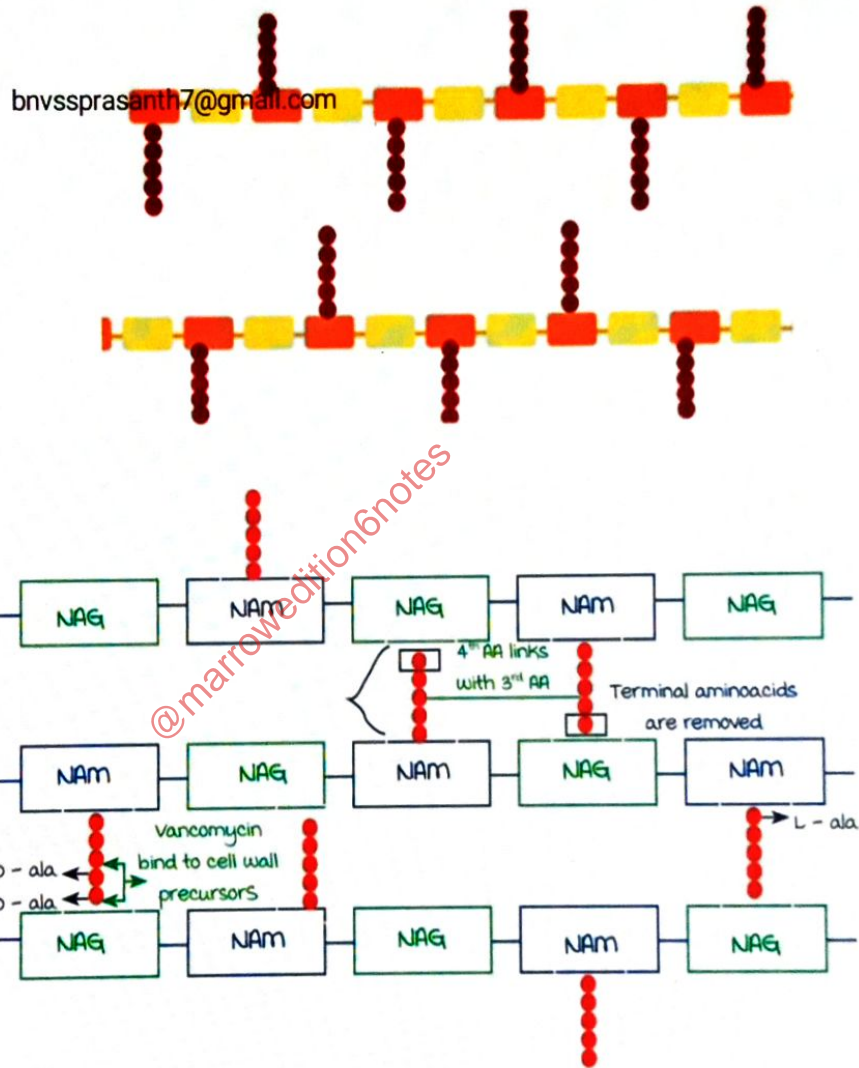


- There are 50 - 100 layers of murein monomers in gram positive cell wall that are cross linked to each other by tetra peptides attached to N-acetyl muramic acid (NAM).
- These tetrapeptides are cross linked to each other by pentapeptide cross bridges.
- The 3rd amino acid of the tetrapeptide side chain is cross linked to the 4th amino acid of the adjacent tetrapeptide side chain.
- Constant : 1st amino acid of the tetrapeptide : L-alanine, Terminal amino acid : D-alanine.
- This cross linking between murein monomers in gram positive bacteria are very important for cell membrane integrity. Any interference within the cross linking can lead to lysis of the bacteria.



Active space

- Cross linking brought about by enzymes :
Transpeptidases &
Transglycolases.
- All beta lactam antibiotics bind to transpeptidases & inactivate the enzymes (prevent crosslinking of cell wall). Hence, transpeptidases are called **penicillin binding proteins**.



Active space

Teichoic acids inhibit cell wall synthesis by binding to enzymes (transpeptidases) responsible for cross-linking
teichoic acids (wall teichoic) inhibit cell wall synthesis by binding to cell wall precursors (horizontal to side to side).

Cell wall and cell membrane teichoic acids are present in gram positive cell wall.

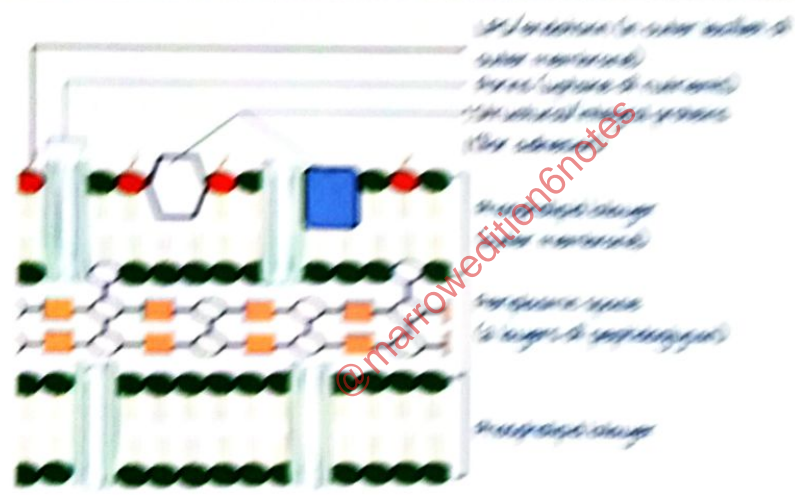
Cell wall teichoic acids are polymers of ribitol phosphate.

Cell membrane teichoic acids are polymers of glycerol phosphate.

Role of teichoic acids: Exact role is not clear, probably in bacterial adhesion.

Gram negative cell wall

58/61/11



	Gram positive cell wall	Gram negative cell wall
Thickness	~ 80 nm	10 - 25 nm
Layers of peptidoglycan	50 - 100	2 (in periplasmic space)
Amino acids in peptidoglycan	serine, threonine, glutamic acid, aspartic acid, glycine, alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, tyrosine, histidine, lysine, and ornithine	All types are present
Teichoic acids	Present	Absent
Outer membrane	Absent	Present
Lipid content	5 - 10%	30 - 50%
Porins, periplasmic space	Absent	Present
LPS	Absent	Present

Endotoxin/LPS

00:47:58

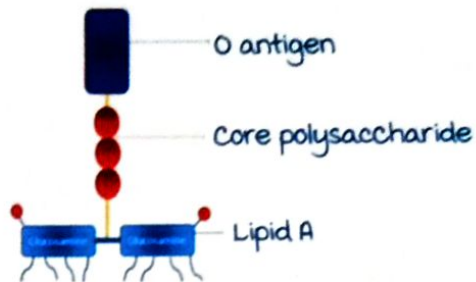
Endotoxins are unique to gram negatives.

1 exception with LPS in its cell wall : *Listeria monocytogenes* (gram positive rod).

Structure of LPS molecule :

Lipid A :

- Embedded in the outer membrane.
- Has actual **endotoxic activity**.



Core polysaccharide :

- Short chain of 6 - 10 amino acids.
- Has unique amino acid : **KDO** (keto deoxy octanoic acid).

O antigen :

- Outermost part.
- Polysaccharide in nature.
- most **variable part** in LPS molecule.

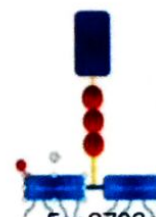
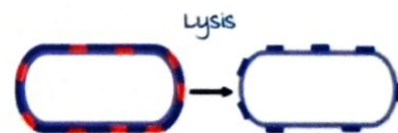
Action of endotoxin :

LPS is released only on lysis of bacteria.

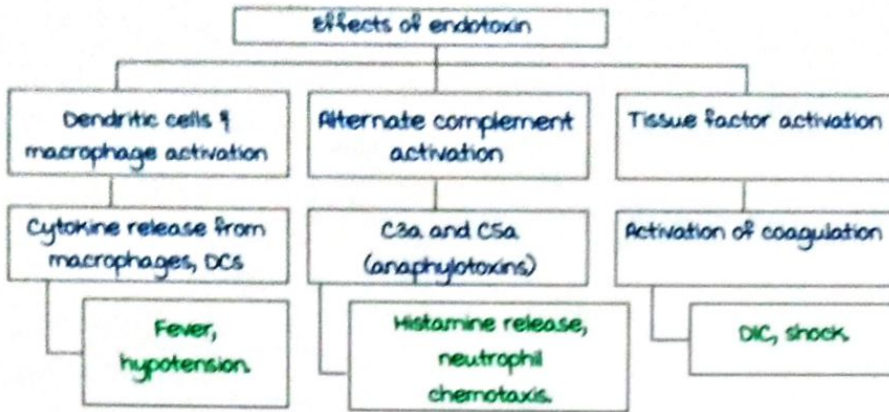
Lipid A part of LPS is recognised by **TLR 4** (pattern recognition receptors present on dendritic cells and macrophages like toll like receptors/TLR that are part of the innate immunity).

Binding of these 2 leads to activation of nuclear transcription factor kappa B (NFkB).

Cytokine release: **IL 1, IL 6, TNF α , IL 8, IL 12.**



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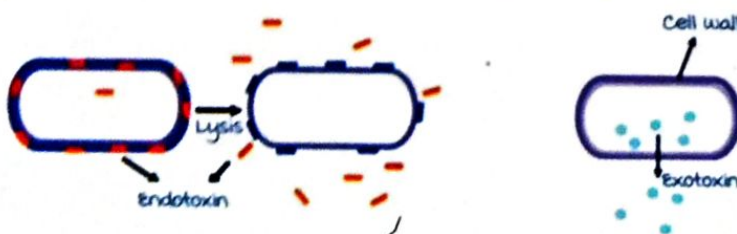
Symptoms of patient with endotoxemia :

- High grade fever.
- Severe hypotension.
- Increased vascular permeability.
- DIC, shock.
- multi organ failure.

All these effects of endotoxin except fever are seen only when LPS is present in large amounts.

Endotoxin	Exotoxin
LPS in nature.	Protein in nature.
Released only on lysis.	Actively secreted except botulinum toxin (which is released on lysis).
Unique to gram negative bacteria.	Gram positive & gram negative bacteria.
Heat stable.	Very heat labile except Staph aureus enterotoxins & Bacillus cereus emetic toxin (they are relatively heat stable).
Low antigenicity.	Highly antigenic.
Cannot be toxoided.	Can be toxoided (often used as vaccines).
Large amount is needed to mediate action in humans : Low toxicity.	Small amounts can mediate the action : High toxicity.
Constant effects.	Variable effects.

Toxoid : Has antigenicity but no pathogenicity.



LAL Assay

01:01:26

Limulus Amebocyte Lysate (LAL) assay:

- Limulus : Name of "horse shoe crab" and its body fluid has cells called **amebocytes**.
- Ameobocytes lysed to form lysate.
- Lysate + drop of clinical specimen (with endotoxin) = lead to **gelling of lysate**.
- Very sensitive assay for LPS detection.



Horse shoe crab

L forms :

- They are **cell wall deficient bacteria**.
- First discovered by Kleinberger Nobel in Lister institute (L forms) on a gram negative bacillus **Streptobacillus moniliformis** (cause of rat bite fever & Haverhill fever).
- Can be formed by both gram positive & gram negative bacteria.
- Form **spontaneously** in the presence of cell wall inhibitors like **penicillin** in the presence of **lysozymes** (cleaves bond between N-acetyl muramic acid & N-acetyl glucosamine leading to cell wall deficiency).

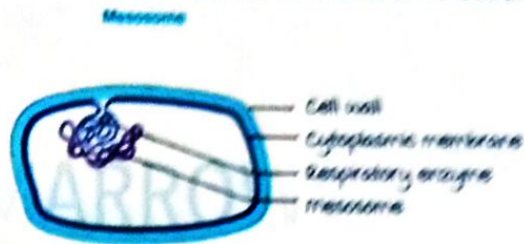
Gram **positive** bacteria on exposure to lysozymes in the presence of **osmotically protected medium** lead to the formation of **protoplasts (L forms)**.

Gram **negative** bacteria on exposure to lysozymes in the presence of osmotically protected medium along with **EDTA** (disrupts outer membrane first) lead to the formation of **spheroplasts (L forms)**.

- L forms can be cultivated on special media & they form "**tiny colonies**". Can be seen only with a microscope.
- Non pathogenic.
- 2 types : Stable (remain cell wall deficient through out life) and unstable (can revert back to having a cell wall).

- Can be responsible for persistence and recurrence/relapse of infections despite giving antibiotics.

Mesosomes : Present in bacteria. Absent in eukaryotes.



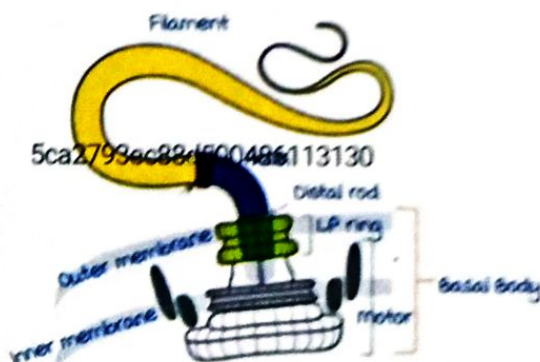
- Mesosomes are the invaginations of cytoplasmic membrane.
- They are the sites for respiratory enzymes.
- Site of mesosomes is the site where bacteria undergo binary fission.
- Gram positive bacteria have more mesosomes than gram negative bacteria.

Flagellum

01:14:09

- Function : Locomotion.
- Composition : Protein subunits called 'flagellin'.
- Antigenicity : +++ (4 antibodies are induced against flagellar antigen).
- Length : 3 - 20 μ .
- Thickness : 0.01 μ .
- Seen only under electron microscope. (Because in light microscope, only up to 0.2 μ can be seen.)




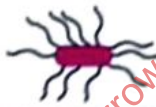

Structure of flagellum :



3 parts of flagellum : Filament, hook and basal body

Basal body :

- made up of rings that are constantly rotated by proton pump.
- In gram positive bacteria : 2 rings (M & S).
- In gram negative bacteria : 4 rings (M, S, P & L).

Flagellar distribution	Examples
Monotrichous : Single flagellum at 1 pole. 	Vibrio, Pseudomonas.
Lophotrichous : > 1 or tuft of flagella at 1 pole. 	Helicobacter, Campylobacter.
Amphitrichous : 1 or tuft of flagella at both poles. 	Campylobacter, Spirillum (pathogenic species : <i>Spirillum minus</i> - causes fever).
Peritrichous : Flagella all over the surface. 	Enterobacteriaceae, Clostridium, Bacillus, Listeria (Flagella forms only around 25° - 28°C & hence is non motile at 37°C).
Atrichous : No flagella present. 	All pathogenic cocci Some bacilli (Corynebacterium, Bacillus anthracis, Clostridium perfringens).

Endoflagella :

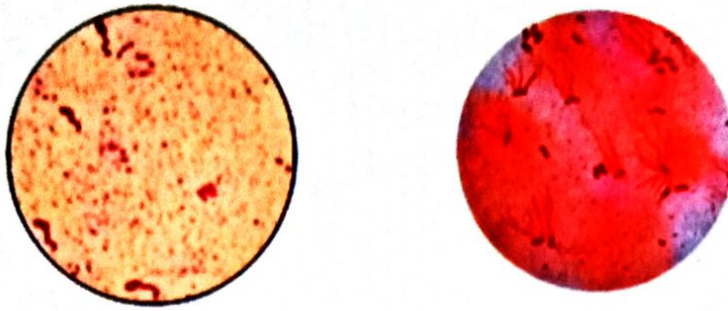
- Flagella in periplasmic space.
- Present in *spirochetes* (Treponema, Leptospira, Borrelia).

Demonstration of flagellum

01:22:24

Direct methods : Electron microscope, Leifson & Ryu's impregnation stain.

Indirect methods : Demonstrates motility of bacteria.



Flagella seen under light microscope after impregnation with tannic acid (Leifson & Ryu's impregnation stain)

Demonstration of motility :

1. **Wet mount** : Easiest method. Take a clean slide, drop of bacterial suspension, cover slip and visualize it.
2. **Hanging drop preparation** : Special slide with a depression. Sterilize the cover slip & add the bacterial suspension to it. Invert the slide onto that cover slip.

Now turn the slide

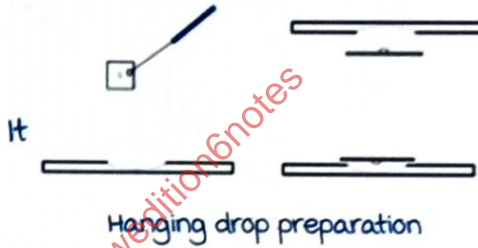
back.

appears as if the bacterial suspension is hanging off

the cover slip. Focus the microscope to edge of drop & change resolution. motile bacteria can be seen at the edge. use plasticine if depressed slide is not available.

3. **Soft agar (motility test agar)** :

- **Semisolid transparent medium (0.2-0.5% agar)**.
- Stab inoculated & incubated overnight.
- Opaque line of growth just along the stab inoculate : Non motile.
- Transparent medium turns **translucent** all over : **motile**.
- If triphenyl tetrazolium chloride (**TTC**) is incorporated into the semisolid medium : Colour change (**red**) happens when it comes across bacterial metabolites. Color change along the inoculate (non motile)/ color spreads all over (motile).
- **Craigie's tube** :



Soft agar is taken in a broad tube, capillary tube is placed in it, bacterial colonies are inoculated through the capillary tube. After overnight incubation, subculture is done from broad outer tube.

If bacterial colonies are **recovered** from subculture : **motile**.

If non motile : Bacterial growth is seen just at the site of inoculation (not recovered).

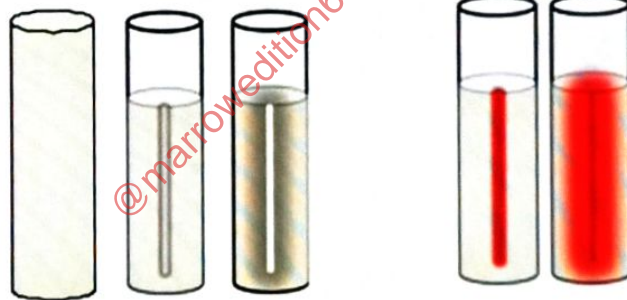
- **U - tube :**

Inoculate bacterial colonies at 1 end.

After overnight incubation, subculture is done from the opposite end.

If bacterial colonies are recovered from subculture : motile.

Not recovered : Non motile.



Plain motility test agar

motility test agar with TTC



Craigie's tube

Inoculation through capillary tube
capillary tube
Soft agar
Sample taken for subculture

Inoculated at one end

Subculture from other end



U-Tube

Active space

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Types of motility

01:31:08

Types of motility	examples
Corkscrew	Treponema pallidum.
Darting/shooting star	Vibrio, Campylobacter.
Gliding	Mycoplasma.
Stately (slow & purposeful)	Salmonella, Clostridium.
Tumbling/end on end	Listeria.
Twitching	Eikenella corrodens.

Swarming :

- It is not a type of motility.
- It is a phenomenon shown by certain bacteria.
- Waves of growth on the surface of the medium.
- Inhibition of swarming : By using firm agar (5 - 6% agar).



Swarming

Inhibition is done to form individual colonies of the bacteria since swarming might hide other bacteria.

Examples :

- Gram positive : Clostridium tetani, Bacillus cereus.
- Gram negative : Proteus vulgaris, Proteus mirabilis, Vibrio alginolyticus, Vibrio parahaemolyticus.

Differential motility :

- motile at room temperature 25° - 28°C, non motile at 37°C.
- Example : Listeria, Yersinia enterocolitica, Yersinia pseudotuberculosis.

Pili/fimbriae :

- made up of protein subunits : Pilin.
- Protein in nature.
- Divided into common pili and sex pili.



Common Pili

Common pili :

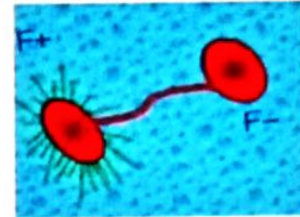
- Help in adhesion.

Active space

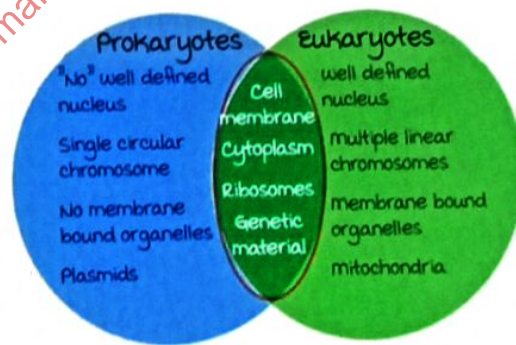
- Only on gram negative bacteria.
- Length : 1 - 1.5 μ .
- Thickness : 0.01 μ .
- Seen only on electron microscope.
- Type IV pili on certain bacterium gives **flagella independent twitching motility** (in *Neisseria* and *Pseudomonas aeruginosa*).

Sex Pili :

- mediate **conjugation**.
- Formed by both gram positive & gram negative bacteria.
- Should have **F plasmid/tra genes** that give the bacteria the ability to form tube/ bridge like connection through which F+ bacteria can transfer genes to F- bacteria. This is called conjugation.

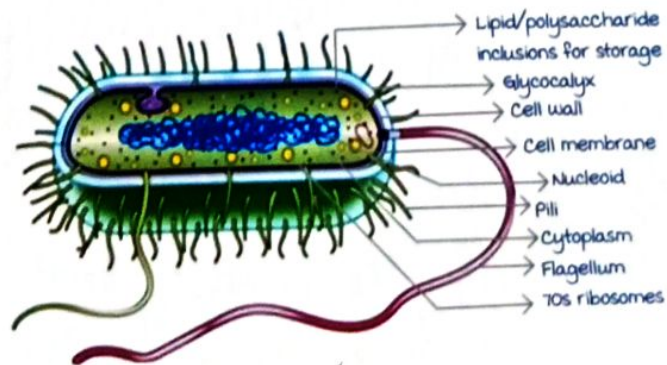


Summary :



"PRO" rhymes with "NO"

"EU" rhymes with "DO"



Structure of bacteria

MCQs :

Question 1 : Which of the following is true of endotoxins ?

- a) are lipopolysaccharide secreted by the bacteria.
- b) may be converted to toxoids.
- c) are only present in gram negative bacteria.
- d) are tissue destroying enzymes.

Question 2 : Which of the following is false ?

- a) Endotoxins can increase the virulence of a non invasive bacterium.
- b) Endotoxins are required in large amounts to cause a disease.
- c) Endotoxins cause the bacteria cell to lyse.
- d) Endotoxins are generally heat sensitive.

@marroweditionsnotes

Active space

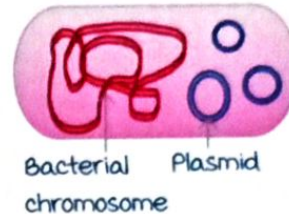
BACTERIAL ANATOMY AND PHYSIOLOGY : PART 2

Plasmids

00:00:36

Plasmids are **extrachromosomal circular ds DNA** molecules.
1 - 40 plasmids/ bacterium.

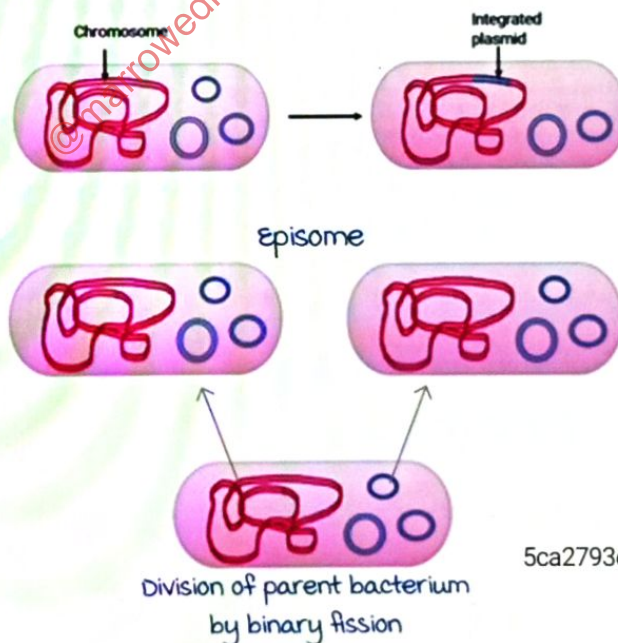
Not essential for bacterial survival
(does not encode gene).
Replicate independent of
chromosomes.



Plasmids integrated with bacterial chromosome are called **episomes**.

Division : **Binary fission**.

Vertical transfer of 1 copy of all plasmids to each daughter cell.



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Active space

Horizontal transfer :

Donor bacterium is connected to recipient bacterium by **sex pilus/conjugation tube**.

One strand of plasmid from donor bacterium is transferred through sex pilus to the recipient bacterium.

Transferred plasmid strand : **Template** for synthesis of complementary strand in recipient.
 Plasmids with **tra** (transfer) **genes** undergo **horizontal transfer**.
 These gene encodes for sex pilus.



Horizontal transfer of plasmid

Types of plasmids (based on mode of transfer) :

- **Conjugative** : Transferred **both vertically & horizontally** (tra gene +).
 Example : F plasmid (fertility plasmid), R plasmid (Fertility plasmid + antibiotic resistance genes), Col plasmid (F plasmid + genes encoding bacteriocins).
- **Non conjugative** : **vertical transfer only**.

Classification of plasmids based on encoding genes

00:07:28

1. **Virulence plasmids** : Encodes special virulence factors (exotoxin, capsule).
2. **Resistance plasmids** : Encodes antibiotic resistant genes.
3. **Fertility plasmids** : Encodes sex pilus genes.
4. **metabolic plasmids** : Encodes enzymes to metabolize special substrates (urease, protease breakdown).
5. **Col plasmids** : Encodes **bacteriocin production** (antibiotic like small proteins secreted by one bacterium killing other related bacteria).

Bacteria	Bacteriocin
E. coli	Colicin
Klebsiella	Klebocin
Pseudomonas	Pyocin

Active space

Transferred plasmid strand : **Template** for synthesis of complementary strand in recipient.

Plasmids with **tra** (transfer) genes undergo **horizontal transfer**.

These gene encodes for sex pilus.



Horizontal transfer of plasmid

Types of plasmids (based on mode of transfer) pnvssprasanth7@gmail.com

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00:07:28

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Bacteria	Bacteriocin
E. coli	Colicin
Klebsiella	Klebocin
Pseudomonas	Pyocin

Active space

Bacterial spore/ endospore : Formed under environmental stress (extreme heat/ nutrient depletion/ dryness).

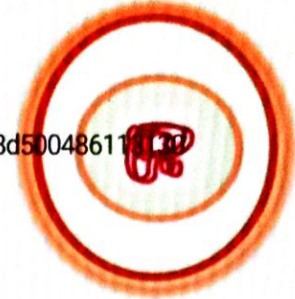
Hibernation of bacteria.

Yellow : Core of the spore contains single circular chromosome.

Chemical in the spore is **dipicolinic acid**.

Helps survive extreme heat.

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Bacterial spore

Inner orange : Spore wall.

White : Spore cortex.

Red : Spore coat (keratin like protein).

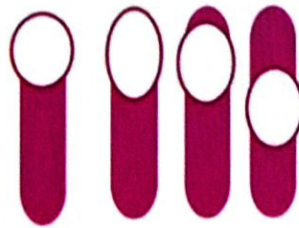
Provides chemical disinfectant resistance.

Outer orange : Exo sporium.

Spore forming pathogenic genera

00:15:14

Features	Clostridium	Bacillus
Spore	Bulging (terminal, sub terminal, central).	Non bulging.
Presence	Soil, culture, human body.	Soil and culture only.
Exception	<i>C. perfringens</i> (does not form spores).	



Bulging spores of clostridium



Non bulging spores of Bacillus genera

Spore demonstration :

Bacterial spores do not take up **gram stain**. Appears empty.

Stained by **Ashby stain/ Schaeffer Fulton stain** (modified Ashby).

Bacterial physiology :



Bacterial cytoplasm : Safranin.
Spores : malachite green.

Bacteria are differentiated into 3 types based on oxygen requirement.

Strict aerobes : Cannot survive without O_2 .

(mnemonic : **N**agging **P**ests **m**ust **B**reathe **F**or **L**ife).

Strict anaerobes : Cannot survive in O_2 . These lack catalase peroxidase, superoxide dismutase enzymes.

(mnemonic : **L**osers **C**hoked **B**y **A**ir). These enzymes eliminate the oxygen radicals produced in O_2 metabolism.

Facultative anaerobes : Can survive with/without oxygen.

Strict aerobes	Strict anaerobes	Facultative anaerobes
<p>Nocardia.</p> <p>Pseudomonas aeruginosa.</p> <p>Mycobacterium TB.</p> <p>Micrococcus (oral flora).</p> <p>Brucella.</p> <p>Bordatella.</p> <p>Francisella.</p> <p>Legionella.</p>	<p>Lactobacillus.</p> <p>Clostridium.</p> <p>Bacteroides.</p> <p>Actionomyces.</p> <p>Porphyromonas.</p> <p>Prevotella.</p> <p>Bifidobacterium.</p> <p>Eubacterium.</p>	<p>Staphylococci.</p> <p>Streptococci.</p> <p>Enterobacteriaceae.</p> <p>Corynebacterium.</p> <p>Hemophilus.</p>

Strict aerobes utilize sugars through oxidative metabolism.

Facultative anaerobes utilize sugars through fermentation.

Special types of bacteria

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00:24:21

Aerotolerant : **Anaerobes**, but can grow in small amount of O_2 .

microaerophilic : **Aerobes** requiring small amount of oxygen 2 - 8 %.

- Campylobacter.
- Helicobacter.
- mycobacterium bovis.
- Cultivable spirochetes (leptospira, borrelia).

Bacterial classification based on PH :

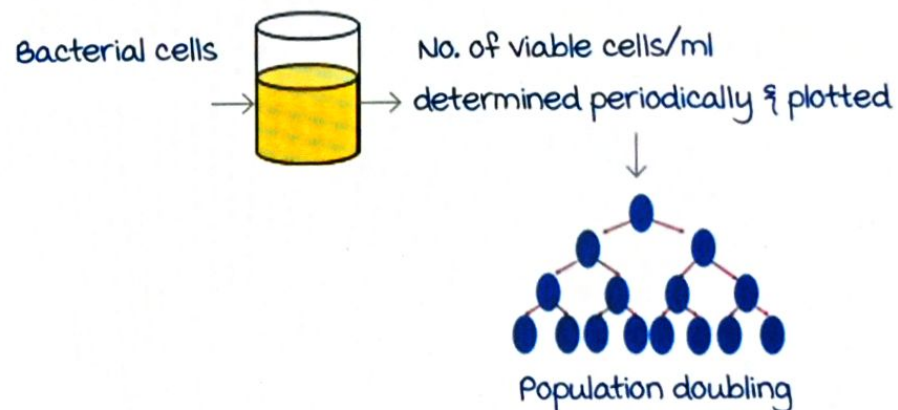
1. **mesophiles/ neutrophiles** : majority of pathogenic bacteria.
Grow at pH 6 - 8 (optimum pH : 7.2 - 7.4).
2. **Acidophile** : At pH < 6 (Lactobacillus).
3. **Alkaliphile** : At pH 8 - 10 (Vibrio, Alkaligenes).

Based on temperature requirement :

1. **mesophiles** : most of the pathogenic bacteria grow between 20 - 40°C (optimum 35 - 37°C).
2. **Psychrophiles** : most are saprophytes. Grows best < 20°C.
3. **Psychrotrophs** : Best growth seen between 20 - 40°C.
Growth seen even at < 20°C.
Examples : Listeria, Yersinia (isolated by cold enrichment).
4. **Thermophiles** : Best growth at 55 - 80°C.
Example : Thermus aquaticus (taq polymerase enzyme is derived from the bacteria).

Generation time/ population doubling time : Time required for binary fission of bacterium.

Example : E. coli (20 min), m. TB (20 hrs), m. leprae (20 days).



Bacterial growth curve

00:33:38

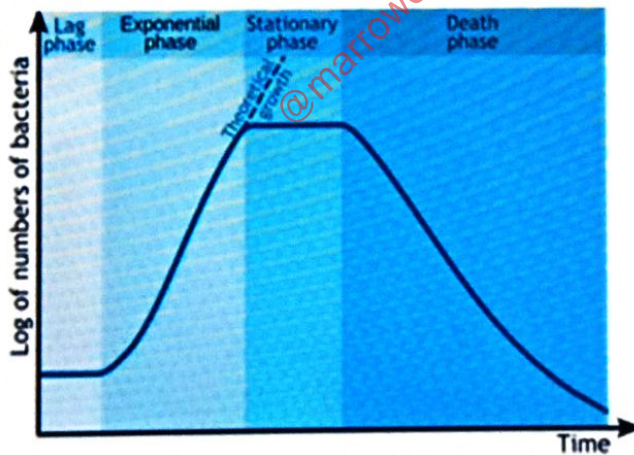
Bacterial growth curve :
Has 4 stages viz.,

Lag phase :

- Bacterium adapts to nutrients in the medium (metabolically active).
- No growth is seen.
- Viable count (live bacteria) and total count (live + dead bacteria) remains constant. (No replication)
- Size of bacterium maximum towards end of lag phase.
- Variable in different bacteria.

Log/ exponential phase :

- Stage of active binary fission.
- Exponential increase in number (in multiples of 2).
- Both viable and total count increases.
- Size of bacteria remains small.
- Stains uniformly.
- Antibiotics added to the medium maximally acts in this phase (maximum metabolic activity).



viable bacteria on the Y axis.

Stationary phase :

- Nutrients in the culture medium decreases.
- Toxic metabolites increase (due to bacterial growth).
- multiplying bacteria = Dead bacteria (curve to plateau).
- Viable count becomes constant. Total count increases.
- Sporulation occurs.
- Bacterial antibiotics and exotoxins are produced.

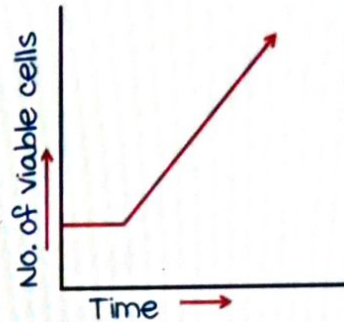
Declining/ death phase :

- Total nutrient depletion.
- No binary fission.
- most of the bacteria dies.
- Viable count decreases, total count becomes constant.
- Pleomorphic involution forms seen (bacteria with very low metabolic rate).

Batch culture :

A closed system with all components added to the medium **at the beginning**. Closed system refers to a culture medium with **fixed amount of nutrients**.

No nutrients are added in between.



Continuous bacterial culture.

Continuous culture :

Nutrients are **added** at **regular intervals**.

Toxic metabolites are **removed** at regular intervals.

Used in pharma industries for manufacturing antibiotics.

Stationery and death phases are absent.

Chemostat

00:45:38

Apparatus using **continuous bacterial culture**.

Special nutrient regulates the inflow of fresh nutrients and outflow toxic metabolites.

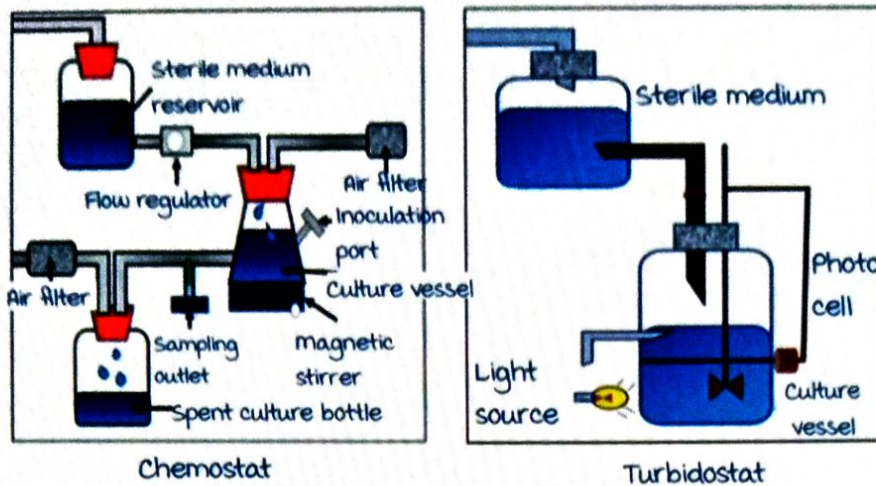
Turbidostat :

uses **continuous bacterial culture** as principle.

Turbidity of the medium acts as a regulator.

(Turbidity threshold = Inflow of nutrients & outflow of toxic metabolites).

Turbidity is monitored by a photo electric cell.

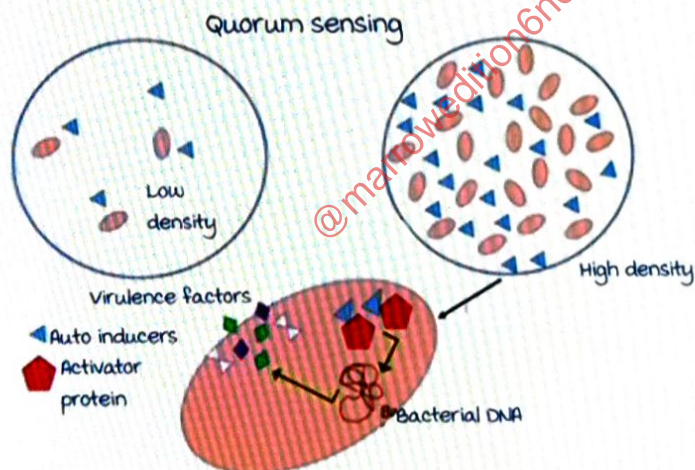


Quorum sensing :

Process that allows bacteria to **co ordinate** their gene expression within themselves.

Coordinated by chemicals called **auto inducers**.

First demonstrated on *Vibrio Fischeri* (bacteria in symbiotic relationship with squid : Help in light emission).

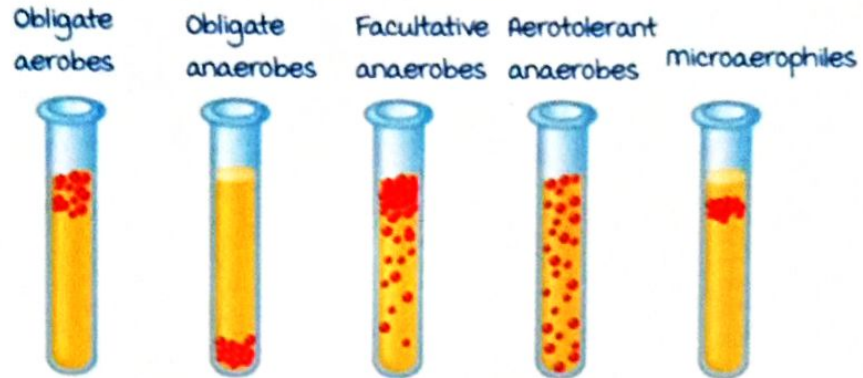


- Q. The average size of the cells in the exponential phase is
- Larger than the initial size.
 - Smaller than the initial size.**
 - Equal to the initial size.
 - maybe smaller or larger than initial size.

Q. Quorum sensing is used by bacterial cells to determine which of the following?

- The size of the population.
- The availability of nutrients
- The speed of water flow.
- The density of the population.**

Q. An uninoculated thioglycolate medium culture tube shows dense growth at the surface and turbidity throughout the rest of the tube. What is your conclusion?



- A. The organisms die in the presence of oxygen.
- B. The organisms are facultative anaerobes.
- C. The organisms should be grown in an anaerobic chamber.
- D. The organisms are obligate aerobes.

Q. A soup container was forgotten in the refrigerator and shows contamination. The contaminants are probably which of the following?

- A. Thermophiles
- B. Acidophiles
- C. mesophiles.
- D. Psychrotrops.

STAPHYLOCOCCUS : PART 1

Nosocomial and community-acquired pathogen.

Important cocci which cause diseases in human :

Gram negative cocci (mnemonic : VENOM) :

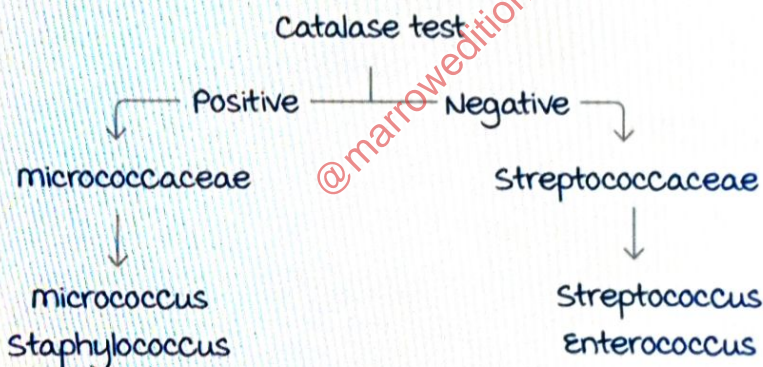
- Veillonella.
- Neisseria.
- Moraxella.

Gram positive cocci (mnemonic : MESS) :

- Micrococcus.
- Enterococcus.
- Staphylococcus.
- Streptococcus.

Pathogenic gram-positive cocci

00:02:08



Family : **Micrococcaceae**

- Genus : Staphylococcus.
- Genus : Micrococcus.

Parameters	Staphylococcus	Micrococcus
Arrangement	Clusters	Tetrads
Gas requirement	Facultative anaerobe	Strict aerobe
Catalase	+	+

Active space

Oxidase	-	+
Sugar utilization	Ferments sugars	Oxidize sugars
Lysostaphin	Sensitive	Resistant
Bacitracin sensitivity test	Resistant	Sensitive (zone of inhibition present)

Difference between Staphylococcus and Streptococcus

	Staphylococcus	Streptococcus
Arrangement	Clusters	Chains or pairs
Gas requirement	Facultative anaerobe	Facultative anaerobe
Catalase	+	-
Oxidase	-	-
Sugar utilization	Ferments sugars	Ferments sugars

Staphylococcus : general characters

00:07:34

Gram stain : Gram positive cocci arranged in **clusters**.

Motility : Non-motile (**atrichous** / no flagella).

Gas requirement : **Facultative anaerobes**.

Capsule : +/- .

Biochemical reactions :

- Catalase test : Positive.
- Oxidase test : Negative.
- Sugar utilization test :
Ferments sugars.

They grow on simple mediums as they are **non-fastidious**.

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Selective medium : **Salt agar**



Catalase positive

Positive



Negative



Oxidase negative

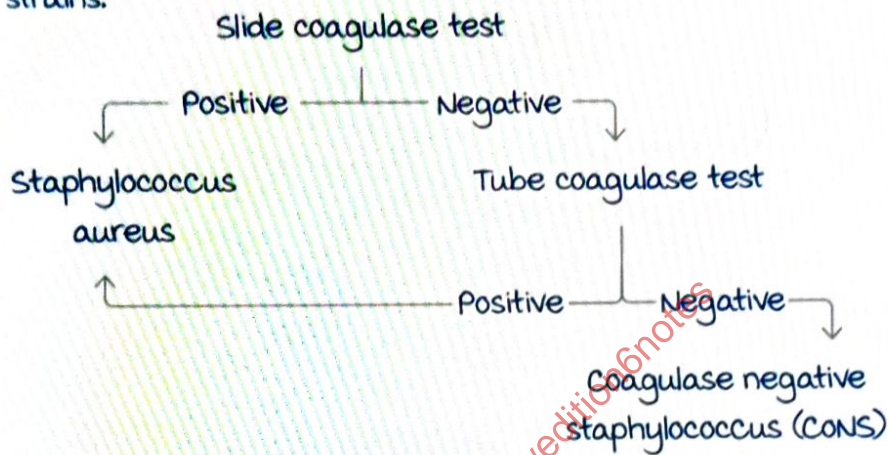
because they are resistant to 7-10% salts.

Classification of Staphylococcus : Based on :

- **Slide coagulase test** : Detects the bound coagulase / clumping factor.
Can be done within 2 minutes.
- **Tube coagulase test** : Detects the free coagulase.
This test takes 4 hours.

12% of the Staphylococcus aureus strains are negative for bound coagulase enzymes.

Free coagulase enzyme is present in 100% of S. aureus strains.



Special characteristics of Staphylococcus aureus :

- **Golden yellow pigment** is produced.
- **β -hemolytic** on blood agar.
- **mannitol fermentation** test positive.

CONS	Characteristics
S. saprophyticus	Resistant to Novobiocin
S. xylosus	
S. hemolyticus	Only CONS which is β -hemolytic
S. lugdunensis	Bound coagulase positive
S. schleiferi, subspecies schleiferi	
S. intermedius	Free coagulase positive.
S. schleiferi, subspecies coagulans	
S. hyicus	

bnvssprasanth7@gmail.com
S. epidermidis is sensitive to Novobiocin.

Staphylococcus aureus

00:21:07

Normal carriage sites : 30-50% of humans are normal carriers.

Nose (most common), skin, oropharynx, vagina, hair, perineum.

It is the **most common nosocomial** pathogen overall.

Elimination of carriage :

- Nasal carrier : Ointment **mupirocin** BD for 7 days.
- Other sites of carriage : **Chlorhexidine** washes.

MC mode of spread is via **hands** of

health care personnel.

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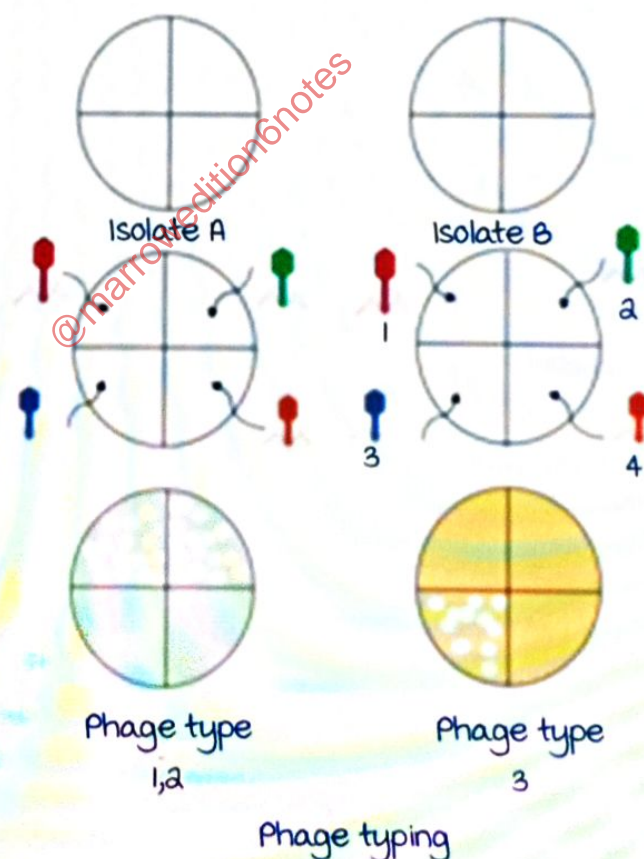


Logo for the Global hand washing day

Global hand washing day : **15th October.**

Typing of S.aureus

00:26:18



used in epidemiological studies by phenotypic and genotypic methods.

Phenotypic methods :

- **Serotyping** (based on antigens).

- **Biotyping** (based on biochemical reactions).
- **Phage typing** (based on susceptibility to a standard set of bacteriophages) : Bacteria are inoculated on a culture plate to get a lawn culture and then inoculated with a standard set of bacteriophages over the predetermined sites on the culture plate using micropipettes.

After overnight incubation, zones of lysis are created if the strain is susceptible to a particular standard phage.

There are **23 standard bacteriophages** that infect *S. aureus*.

S. aureus is divided into 5 phage types/groups.

For example, in MRSA : Phage type is **Phage type III**.



Genotypic methods :

- **Pulsed-field gel electrophoresis (PFGE)**.
- **multi-locus sequence typing**.

Biochemical reactions of *S. aureus*

00:30:52

- Free and bound coagulase test positive.

Slide coagulase test	Tube coagulase test
<p>A suspension of <i>S. aureus</i> with a positive and negative control is prepared, and a drop of rabbit plasma is mixed with it and checked for clumping (bound coagulase : Positive).</p>	<p>A few colonies of <i>S. aureus</i> are added into a tube containing plasma and incubated for 4-6 hours. Free coagulase if present, converts fibrinogen to fibrin and coagulum is formed.</p>
<p><i>S. epidermidis</i> negative control <i>S. aureus</i> strain s4 <i>S. aureus</i> strain s5</p>  <p>Slide coagulase test</p>	 <p>Tube coagulase test</p>

Active space

- Oxidase positive.
- Urease positive.
- Phosphatase positive.
- Ferments mannitol.

S. aureus : culture

00:33:19

Simple medium/nutrient agar prasanth7@gmail.com

Produces a golden-yellow pigment (Staphyloxanthine).



Staphyloxanthine

Blood agar : Pin head colonies surrounded by a zone of β -hemolysis.

 β hemolysis on blood agar

macConkey agar : Form tiny lactose fermenting colonies (bright pink).

Staphylococcus and enterococcus are the only gram-positive cocci which can grow on macConkey agar.

Selective medium :

- Salt agar.
- Salt milk agar.
- Baird parker medium.
- Ludlam's medium.

Selective and differential medium :

mannitol salt agar :

Pale colonies of CONS (non-mannitol fermenters).

Yellow colonies of S. aureus

(mannitol ferments and produces acid which changes the color of phenol red indicator to yellow).



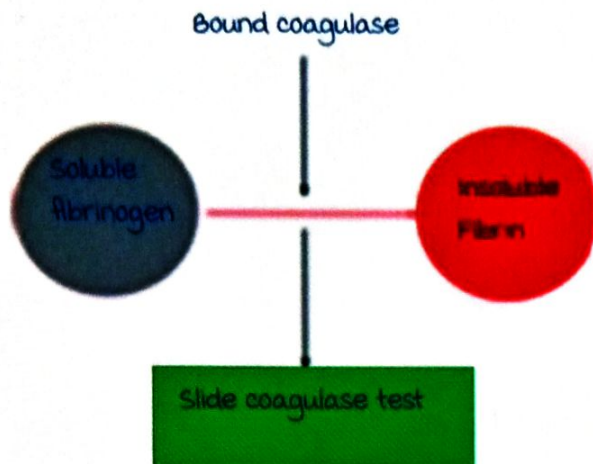
mannitol salt agar

Virulence factors of *S. aureus*

00:37:35

Broadly divided into :

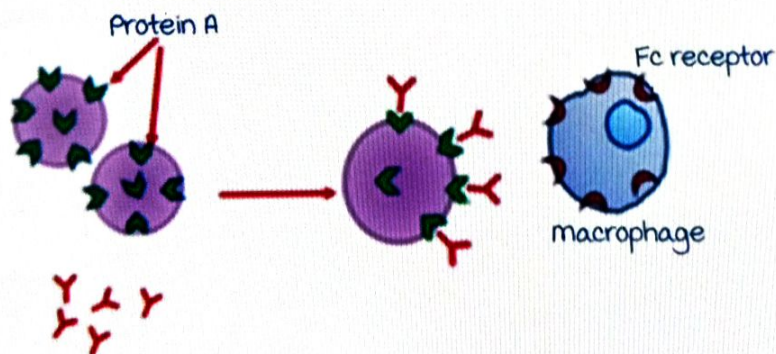
- Cell wall associated.
- Secreted outside.



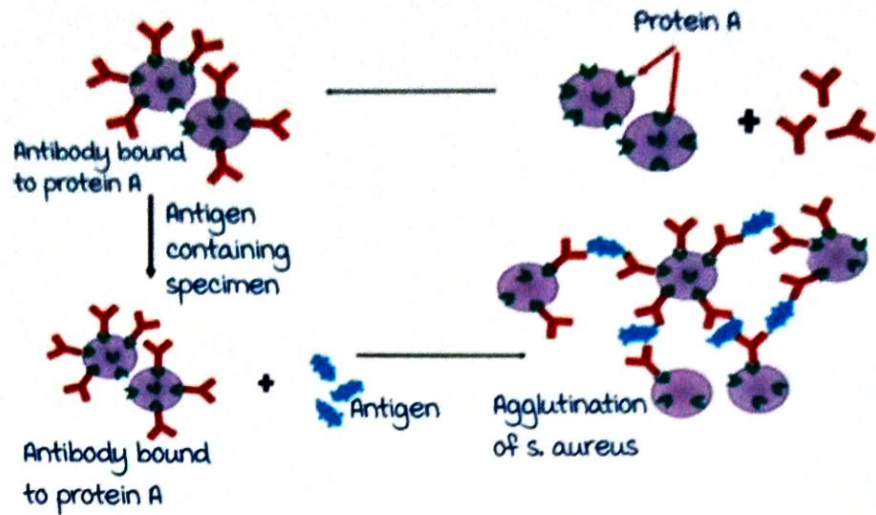
Cell wall associated virulence factors :

- **Bound coagulase** : It converts soluble fibrinogen to insoluble fibrin, which deposits on *S. aureus* and forms an antiphagocytic barrier around itself. Absent in 12% strains.
- **Protein A** : Binds to the Fc part of IgG antibody, making it antiphagocytic.

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Protein A, due to its ability to bind to the Fc portion of antibodies, is used in **co-agglutination tests (COA)** for detection of soluble antigens in clinical specimens.

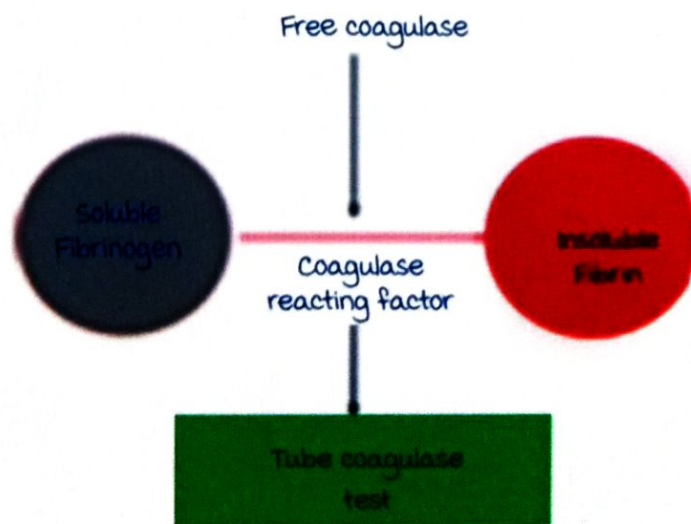


For example, *Salmonella typhi* antigens detected in the urine of a patient with enteric fever are **small soluble antigens** which are infected with a laboratory animal to produce antibodies.

These **antibodies** are then coated onto the *S. aureus* via the **protein A**.

The patient's urine sample is then added to the antibody-bound Protein A, and if these antigens are present in the urine, they will combine with the antibodies and cause **agglutination** of *S. aureus*.

The strain of *S. aureus* rich in Protein A and used in coagulase test : **Cowan I strain**.



Active space

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Secreted virulence factors :

- **Free coagulase** : It converts soluble fibrinogen to insoluble fibrin to form an antiphagocytic barrier. It activates prothrombin in the presence of **coagulase reacting factor (CRF)**.
Present in 100 % of *S. aureus* strains.
CRF is absent in guinea pig plasma, due to which its plasma should not be used in tube coagulation tests.
- **Staphylokinase.**
- **DNAse.**
- **Hyaluronidase.**
- **CHIPS** (Chemotaxis Inhibitor Protein of *S. aureus*).
- **Hemolysins** : Alpha, beta, gamma delta
- 1. **Alpha Hemolysin** shows **paradoxical reactivation** between 80-100°C (it gets inactivated at 60°C and finally gets denatured at 100°C).
- 2. **β Hemolysin / Sphingomyelinase** : Shows the **hot-cold hemolysis** (also seen in alpha-toxin of *Clostridium perfringens*) i.e., when β -hemolysin is mixed with a suspension of RBCs, the hemolysis begins at 37°C and completes at 4°C.
- 3. **Gamma Hemolysin** : It is a **bicomponent toxin** (two separate components secreted extracellularly, combine to form this). It is a membrane damaging toxin. (**Synergohymenotropic toxin**).
- **Panton-valentine leucocidin / PVL toxin** :
Synergohymenotropic toxin.
Dermonecrotic and leucocidal in nature.
It is secreted only by 5% of the strains, but >95% of community-acquired MRSA are positive for PVL toxin.

Enterotoxins

00:51:04

There are more than **15 antigenic types** which are chromosomally encoded.

Responsible for **staphylococcal food poisoning.**

Mechanism of action : By stimulation of the **vagus** and the **vomiting center.**

Active space

Typical history : milk products, meat, pastries, mayonnaise salad, potato salad contaminated by *S. aureus* carrier and the enterotoxins (heat stable, can resist 100°C for 10-40 minutes and are preformed toxins) are secreted.

Staphylococcal food poisoning :

Incubation period : < 6 hours (up to 8 hours).

Clinical features : Nausea, vomiting, watery diarrhoea +/-, no fever.

Self-limited in 10-12 hours.

Diagnosis : Detection of Staph enterotoxin (SE) in the vomitus/stool by ELISA.

Treatment : Symptomatic.

Antibiotics are not needed because it is toxin-mediated.

Exfoliatins / epidermolytic toxins

00:59:00

Antigenic types : EF-A (chromosomal) and EF-B (plasmid mediated).

These are serine proteases.

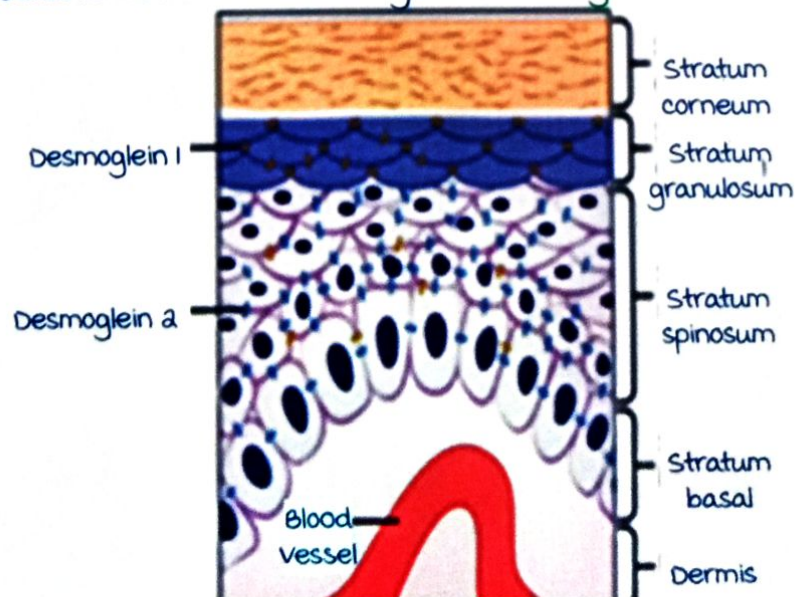
Responsible for :

- Staphylococcal Scalded Skin Syndrome (SSSS)/ Ritter's disease.
- Bullous impetigo.

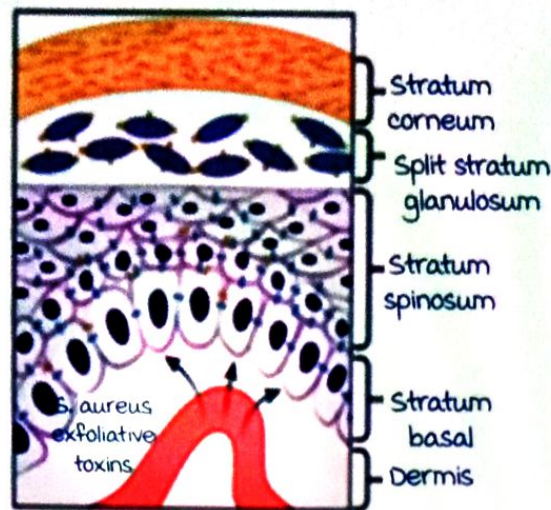
Seen in neonates and young infants.

Site of action : Stratum granulosum of epidermis.

Mechanism of action : Proteolysis of desmoglein-1.



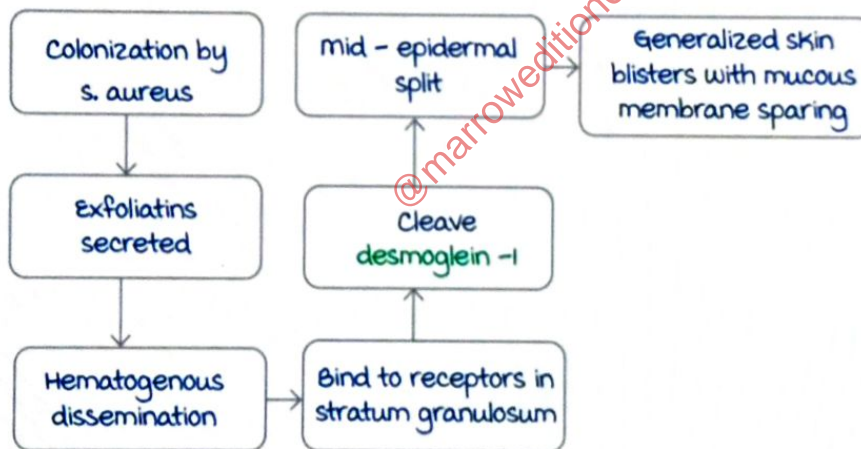
Normal skin



Staphylococcal scalded skin syndrome
with cleavage of desmoglein I

Age group : SSSS : Neonates and young infants.
Bullous impetigo (localized SSSS) : Young children.
Adults do not have the receptors for these toxins.

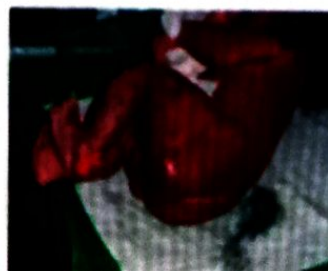
Pathogenesis of SSSS :



Diagnosis : ELISA on the blood for detection of EF-A or EF-B.

Treatment :

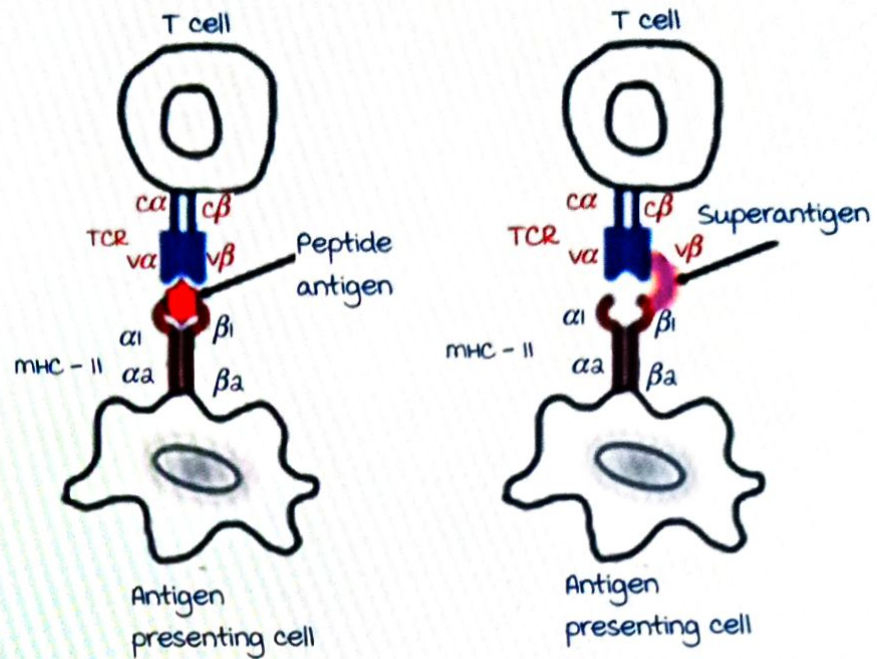
- Topical care with cool saline compresses.
- Parenteral antibiotics :
vancomycin (empirically).
After sensitivity report, if
MSSA : Nafcillin / Cloxacillin /
Flucloxacillin.
If MRSA : Continue vancomycin.



SSSS

Toxic shock syndrome toxin-1 /TSST-1

01:06:52



mechanism of Superantigens

Earlier called Enterotoxin F.

It is a **superantigen/ T cell mitogens** :

- Small /medium-sized proteins.
- They do not need processing by the dendritic cells / APCs.
- Directly bind to the MHC-II antigen of the APCs at a site lateral to the usual antigen-presenting groove.
- Need to be recognized by just the $V\beta$ part of the T-cell receptor.
- Normally peptides are recognised by $<0.0001\%$ of T_H cells.
- 5-20% of T_H cells are activated resulting in **massive cytokine** release which is called Toxic Shock Syndrome (TSS).

Types of Toxic Shock Syndrome :

menstrual TSS :

- vaginal colonization by *S. aureus* strains.
- Associated with the use of high absorbency tampons during menstruation.
- more than 90% of cases are associated with **TSST-1**.
- Other toxins responsible : **SE-B, SE-C, Erythrogenic toxins (A, B, C)** of *Streptococcus pyogenes*.

Non-menstrual TSS :

Due to colonization at another site/infection of *S. aureus* or *Streptococcus pyogenes*.

Same toxins as in menstrual TSS are responsible.

CDC criteria for the diagnosis of TSS :

- **Fever** of $> 102^{\circ}\text{C}$.
- **Hypotension** with SBP $< 90\text{mmHg}$.
- **maculo-papular rash** (generalized rash) which desquamates in 10-14 days.
- Involvement of **3 or more organ systems** of the body.
 1. GIT : Nausea, vomiting.
 2. Kidney : S. Creatinine > 2 times the normal.
 3. Coagulopathy : Thrombocytopenia < 1 lakh/ mm^3 .
 4. Liver : SGOT/SGPT > 2 times the normal.
 5. CNS : mental confusion.
 6. muscle : CPK levels > 2 times normal.
 7. **Mucous membranes** : Hyperemic.
- Negative serology for measles, leptospirosis, RMSF.
- Negative blood or CSF cultures for organisms other than *S. aureus*.

Diagnosis and treatment of TSS :

ELISA on the blood for detection of the toxins.

- Symptomatic treatment.
- Parenteral Penicillin + Clindamycin (for erythrogenic toxins of *S. pyogenes*).
- Nafcillin / Cloxacillin / Flucloxacillin + Clindamycin (for MSSA).
- Vancomycin + Clindamycin (for MRSA).

Two-component regulatory systems

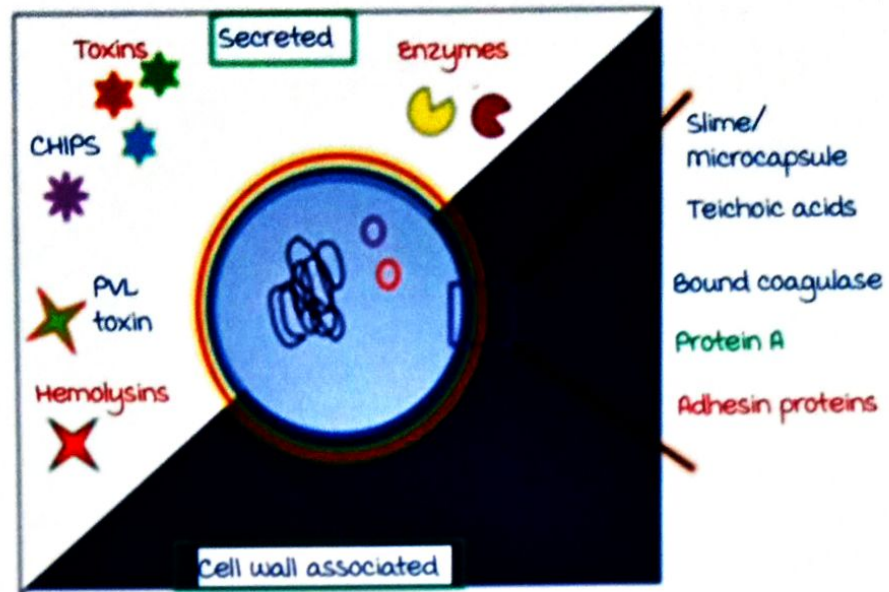
01:22:27

- **Accessory gene regulator (Agr)** : A quorum-sensing system. bnvssprasanth7@gmail.com
- **Staphylococcal accessory regulator (Sar proteins)** : DNA binding proteins which increase the transcription of genes.
- **Regulatory RNAs.**

Active space

Summary of virulence factors :

Cell-wall associated :



- **Slime** : Form biofilms.
- microcapsule.
- **Teichoic acids** : universal factor of all GPs.
- Adhesion proteins - **MSCRAMMs** (microbial Surface Component Recognizing Adhesion matrix molecules) :
 1. Fibronectin binding protein.
 2. Collagen binding protein.
 3. Fibrinogen binding protein.
- Protein A.
- Bound coagulase.

Secreted virulence factors :

- Enzymes :
 1. Free coagulase.
 2. DNase.
 3. Staphylokinase.
 4. Hyaluronidase.
 5. Beta-lactamases.
- CHIPS.
- Hemolysins.
- PVL toxin.
- Toxins : Exfoliatins, enterotoxins, TSST-1.

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STAPHYLOCOCCUS : PART 2

Introduction

00:00:10

Disease and infections caused by Staph. Aureus :

Intoxications :

Toxin mediated disease :

- Food poisoning.
- Scalded Skin Syndrome.
- Toxic Shock Syndrome.

Infections :

most common source of infections due to Staph. Aureus : Skin flora.

Soft tissues and Skin infections :

Staph. Aureus is the most common cause for :

- Stye.
- Carbuncles.
- Folliculitis.
- Furuncle.
- Impetigo.
- Bullous Impetigo.
- Post-operative wound infection.
- mastitis.
- Breast abscess.

Respiratory Tract Infections :

- Sinusitis.
- Otitis media.
- Pharyngitis.
- Pneumonia.
- Lung abscess.

Bone, muscle and joint involvement :

Staph. Aureus is the most common cause for :

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Active space

STAPHYLOCOCCUS : PART 2

Introduction

00:00:10

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- Pneumonia.
- Lung abscess.

Bone, muscle and Joint involvement :

Staph. Aureus is the most common cause for :

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- Osteomyelitis.
- Tropical pyomyositis.
- Septic arthritis.
- Septic bursitis.

Endocarditis :

Staph. Aureus is the most common cause for :

- Acute endocarditis.
- Native valve endocarditis.
- Early prosthetic valve endocarditis (As a group MCC is CONS).
- Late prosthetic valve endocarditis (As a group MCC is VGS : viridans group of streptococci).
- Infective endocarditis in IV drug users.

Nosocomial Infections

Staph. Aureus is the most common cause of :

- Hospital acquired pneumonia.
- Ventilator associated pneumonia.
- Post-op wound infections.
- meningitis following neurosurgery.

Treatment for Staph. Aureus infections :

most effective drugs to treat Staph. Aureus are

Beta-Lactam antibiotics.

Penicillin G is the drug of choice for any non-beta Lactamase producing S. Aureus, but these strains are rarely encountered.

>80% of community strains and >95% of hospital strains are resistant to Beta Lactams.

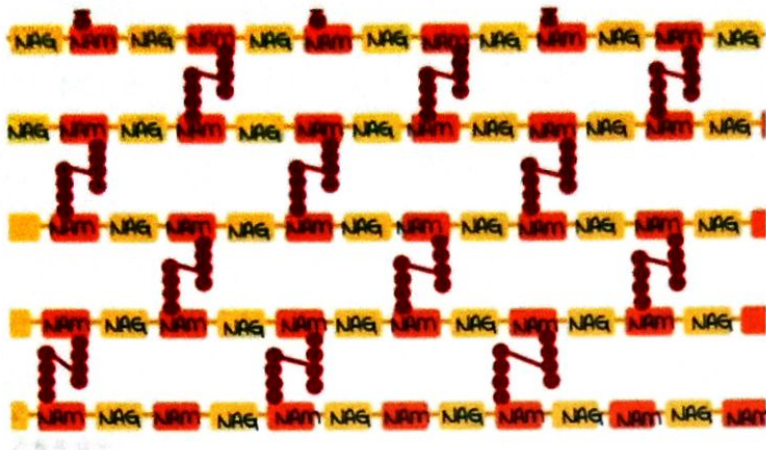
Beta Lactam Mechanism of Action

00:07:10

The bacterial wall has several layers of murein monomers which are cross linked by tetrapeptides and pentapeptide cross bridges.

This cross linking of murein monomers are done by carboxy peptidases and transpeptidases. These are the enzymes

where the sites of action of beta lactam antibiotics are present.

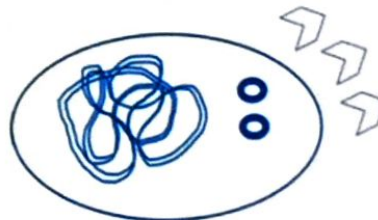


Beta lactams bind to these transpeptidases and prevent the cross linking leading to the lysis of the bacterium. Staph. aureus has 4 transpeptidases (also known as penicillin binding proteins).

Resistance to Beta Lactam Antibiotics in S. Aureus :

By Beta Lactamases/Penicillinases (most common) :

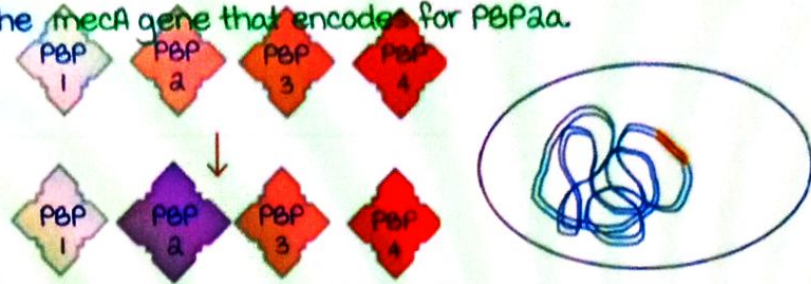
- Plasmid mediated
- Have R-plasmids which hydrolyze the Beta Lactam ring.
- MC mode of resistance to β lactam antibiotics : R plasmid mediated.
- most common mode of spread in S. Aureus is by transduction >> conjugation.
- Treatment is by : Penicillinase resistant penicillin. Example : Nafcillin, Cloxacillin, Flucloxacillin.



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By changing structure of transpeptidases :

- Chromosomally mediated
- methicillin resistant S. Aureus (MRSA).
- The transpeptidase structure is altered and then develops low affinity to beta lactams. Altered Penicillin Binding Protein 2a (PBP2a) is the new restructured binding site and occurs due to a mobile

genetic element on the chromosome known as **SCCmec** which has the **mecA** gene that encodes for PBP2a.



MRSA Diagnosis :

- **Dilution method :** Can be broth or agar dilution. The strain is MRSA : If MIC (minimum inhibitory concentration) of Oxacillin is ≥ 4 mcg/mL or MIC of Cefoxitin ≥ 8 mcg/mL. Cefoxitin and Oxacillin are equally good in producing effective results.
- **Disc diffusion method :** Cefoxitin or Oxacillin disc is used. If a zone of inhibition is found around the disc smaller than recommended, strain is MRSA. Cefoxitin disc is better than oxacillin disc because mecA gene has better expression in cefoxitin.
- **Oxacillin agar screen method :** If a colony grows on mueller Hinton agar with 2-4% salt is used with 6 mcg/mL of Oxacillin, then that strain is MRSA.

mueller Hinton agar with 2-4% salt is used and incubation is done at : $< 35^{\circ}\text{C}$.

Dilution method is superior to disc diffusion method though it takes more time.

- **Latex Agglutination :** Latex particles coated with anti-bodies against the transpeptidases that alter penicillin binding and causes

agglutination if PBP2a is present.

- Immunochromatographic tests
Can be used to detect altered penicillin binding proteins.
- PCR for meca gene :
This is the gold standard procedure for MRSA diagnosis.

methicillin is not used to find MRSA strain because it is an unstable drug and does not give reliable results.

Treatment for MRSA :

Gloves and gowns should be worn and discarded on entry and exit of room. Clean and disinfect all reusable equipment.

- Drug of choice : Vancomycin.
- Alternative drug : Daptomycin.
- Other drugs include : Ciprofloxacin, Septran, streptogramins, Linezolid, Ceftobiprole (5th gen Cephalosporin, new β -Lactams effective against MRSA), Ceftaroline and Razupenem.



STOP

Contact precautions
EVERYONE MUST :



STOP



Clean their hands, including before entering and when leaving the room

PROVIDERS AND STAFF MUST ALSO :



Put on gloves before room entry.
Discard gown before room exit.



Put on gown before room entry.
Discard gown before room exit.



Do not wear the same gown and gloves for the care of more than one person

use dedicated or disposable equipment.
Clean and disinfect reusable equipment before use on another person.



	Community acquired-MRSA	Hospital acquired-MRSA
	No such history in the last one year	Resident of nursing home/hospital ; Recent surgery; Recent dialysis ; Recent contact with health care personnel
SCCmec	SCCmec IV and SCCmec V	SCCmec I, II and III
PVL toxin	>95% of CA-MRSA produce PVL toxin	Not seen
Virulence	+++	+
Anti-Biotic Resistance	Relatively sensitive to other groups of antibiotics	Often MDR

Glycopeptide Resistance in S.Aureus

00:29:19

Vancomycin Intermediate Staphylococcus aureus (VISA) and Vancomycin Resistant Staphylococcus aureus (VRSA)

Vancomycin inhibits cell wall synthesis by binding to cell wall precursors and thereby prevents their addition to the peptidoglycan.

Resistance to vancomycin is always determined by the dilution method.

VISA : MIC of vancomycin : 4-8 mcg/mL.

VRSA : MIC of vancomycin : ≥ 16 mcg/mL.

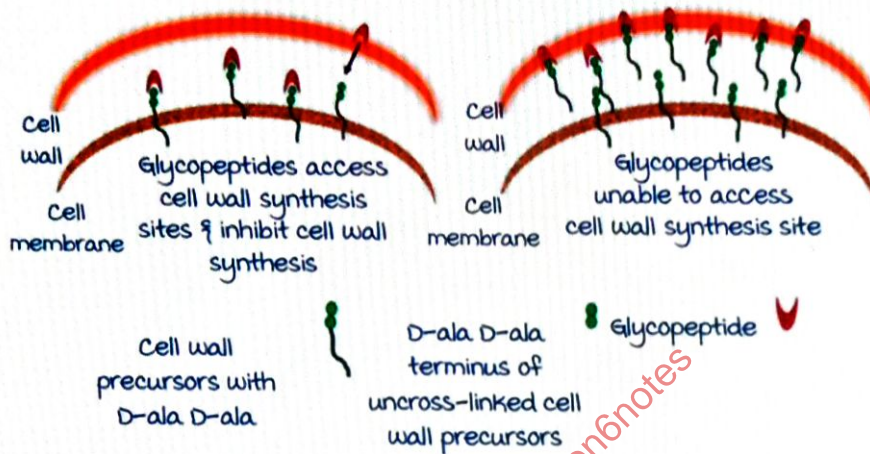
Normally, vancomycin enters the cell wall and binds with the cell wall precursor at the D-Ala D-Ala amino acids and prevents the addition to the growing amino acid chain.

VISA :

Thickened cell wall of the bacteria make it difficult for vancomycin molecules from entering the bacteria and several D-ala molecules are synthesized to trap the vancomycin molecules from binding with cell wall precursors.

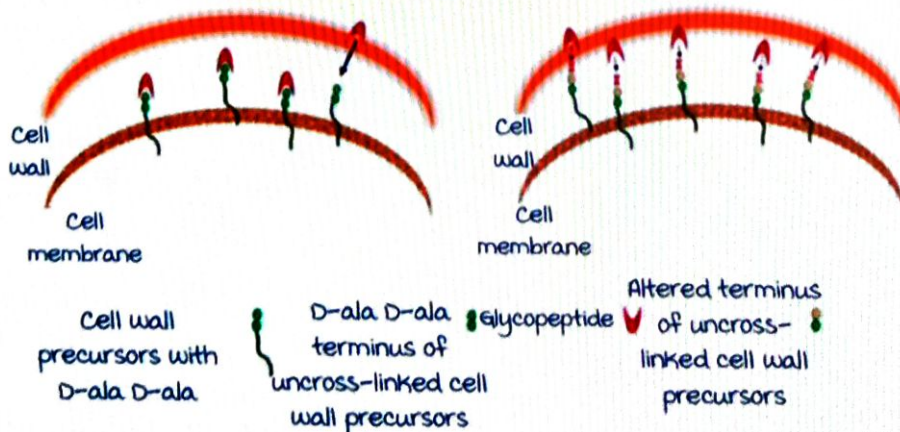
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Increase in dosage of vancomycin can increase treatment effectivity.



VRSA :

Altered cell wall precursors are synthesized rendering vancomycin ineffective in binding with the cell wall precursors. D-ala D-lactate is synthesized from the *van a* gene present either on the plasmid or transposon. Vancomycin plays no role in treatment of VRSA.



Active space

Coagulase Negative Staphylococcus

00:34:07

Staph. epidermis :

- Coagulase negative.
- Non-pigmented.
- Non-hemolytic.
- Does not ferment mannitol.
- Novobiocin sensitive.
- Present on normal skin flora and mucous membranes.
most common Staph. species on the skin.
- Virulence factors :
 1. Produces slime which forms biofilm.
 2. Has adhesive proteins.
 3. Teichoic acids.
 4. Enzymes like lipases, DNases.
- Associated infections :
Amongst the coagulase negative Staph species, Staph. epidermis is the most common cause for :
 1. Healthcare associated blood stream infections.
 2. Early prosthetic valve endocarditis.
 3. Vascular graft infections.
 4. Peritonitis in dialysis patients.
 5. Infections of orthopedic prosthesis.
 6. CSF shunt infections.
- Surgical site infections :
MC cause : S. aureus > E. coli > S. epidermidis.
Staph. epidermis is MDR. Drug of choice : Vancomycin.

Staph. Lugdunensis :

- Bound coagulase positive but free coagulase negative.
- Non-pigmented.
- Non-hemolytic.
- Does not ferment mannitol.
- Novobiocin sensitive.
- Often present as normal skin flora.
- As virulent as Staph. aureus.
- Can cause :
 1. Native valve endocarditis.

Active space

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2. Prosthetic valve endocarditis.
 3. Skin and soft tissue infections.
 4. urinary Tract Infections and bacteremias.
 5. Osteomyelitis.
- It is not an MDR and it is sensitive to most antibiotics including beta lactams.

MCQs

Q. Which of the following gram-positive organism is the most common cause of UTI in sexually active women;

- A. S. aureus.
- B. S. epidermidis.
- C. S. saprophyticus.
- D. E. coli. (E. coli is gram negative)

Q. A 54-year-old woman develops a right shoulder abscess with a strain of S. aureus that is resistant to Nafcillin. She was treated with a 2-week course of intravenous Vancomycin and improved. Three weeks later (week 5), the infection recurred, and she was given 2 more weeks of intravenous Vancomycin and again improved. Four weeks later (week 11), the infection recurred and the patient was again started on intravenous Vancomycin. The MICs for Vancomycin for the S. aureus isolates were as follows : initial isolate (day 1), 1 mcg/mL; week 5, 2 mcg/mL; and week 11, 8 mcg/mL. The patient failed to improve with the third course of Vancomycin, and alternative therapy was used. The mechanism that best explains the relative resistance of the patient's strain of S. aureus to Vancomycin is

- A. Acquisition of the vanA gene from another microorganism.
- B. Active transport of vancomycin out of the S. aureus cell.
- C. Action of β -lactamase.
- D. Increased cell wall synthesis and alterations in the cell wall structure.
- E. Phosphorylation and resultant inactivation of the vancomycin.

Explanation : Case of VISA.

Q. Over a period of 3 weeks, a total of five newborns in the hospital nursery developed *S. aureus* infections with *S. aureus* bacteremia. The isolates all had the same colony morphology and hemolytic properties and identical antimicrobial susceptibility patterns, suggesting that they were the same. (Later molecular methods showed the isolates were identical) which of the following should be done now?

- A. Prophylactic treatment of all newborns with intravenous vancomycin.
- B. Protective isolation of all newborns.
- C. Closing the nursery and referring pregnant women to another hospital.
- D. Hiring all new staff for the hospital nursery.
- E. Culture using mannitol salt agar of the anterior nares of the physicians, nurses, and others who cared for the infected babies.

Q. All of the following are important infection control strategies in containing spread of MRSA in hospitals except

- A. Aggressive hand hygiene.
- B. Routine surveillance for nasal colonization among high-risk individuals.
- C. Contact isolation for patients who are colonized or infected with MRSA.
- D. Routine antimicrobial prophylaxis for all patients hospitalized for more than 48 hours.
- E. Aseptic management of skin lesions.

Q. A 65-year-old man develops an abscess on the back of his neck. Culture yields *S. aureus*. The isolate is tested and found to be positive for the *mecA* gene, which means that

- A. The isolate is susceptible to vancomycin.
- B. The isolate is resistant to vancomycin.
- C. The isolate is susceptible to Nafcillin.
- D. The isolate is resistant to Nafcillin.

Explanation : Nafcillin is a β lactam.

STREPTOCOCCUS : BETA HEMOLYTIC

Types of cocci

00:00:38

Gram negative cocci	Gram positive cocci
Veillonella. Neisseria. Moraxella. mnemonic : VENOM .	Micrococcus. Enterococcus. Staphylococcus. Streptococcus. mnemonic : MESS .

Gram negative spread **VENOM**, Gram positive clean up the **MESS**.

Streptococcus :

Family : streptococcaceae.

Differentiation between streptococcaceae and staphylococcaceae.

streptococcaceae	Staphylococcaceae
Occur in chains .	Occurs in clusters .
Catalase negative.	Catalase positive.

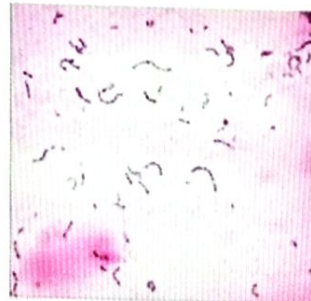
Gram staining : Gram positive cocci in chains/ pairs.

Motility : Non motile.

Gas requirements : **facultative anaerobes**.

Capsule : **Polysaccharide capsule** seen in.

- Streptococcus pneumoniae.
- Group A, B, C, D of beta hemolytic streptococci.



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Active space

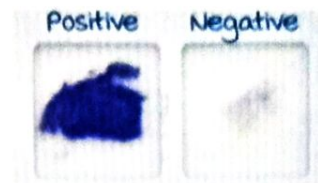
Biochemical reaction :

- Catalase test : Negative



Catalase positive test

- Oxidase test : Negative.



- Sugar utilization :

Ferment sugars both aerobically and anaerobically.

Cultivation :

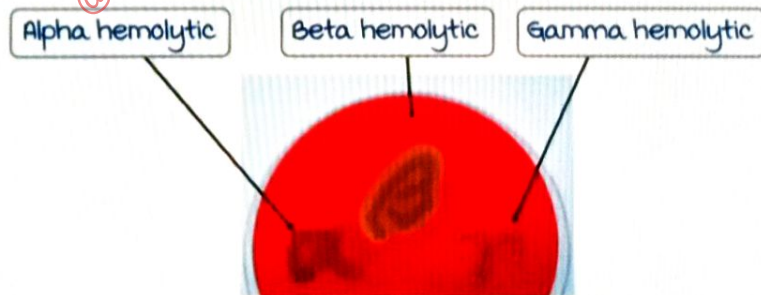
Simple medium : Fastidious organism and need nutrient medium to grow.

Enriched medium : **Blood agar** (5% of sheep blood agar).

Classification of Streptococcus

00:05:18

Also called **Brown's classification**.



Alpha hemolytic	Beta hemolytic	Gamma hemolytic.
Greening around colonies. (Partial hemolysis).	Clearing around colonies. (Complete hemolysis).	No change around colonies.

Alpha hemolytic streptococci :

	viridans group	S. pneumoniae
Arrangement	Chains	Pairs
Colonies	Dome	Carrom coin
Bile solubility	Insoluble	Soluble
Optochin sensitivity	Insensitive	Sensitive
Fermentation of inulin	No	Yes

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Gamma hemolytic streptococci :

Some group D streptococci.

	Enterococcus	Non enterococcal Group D
Bile resistance	Yes	Yes
Aesculin hydrolysis	Yes	Yes
Growth in 6.5% NaCl	Yes	No
PYR test	Yes	No

Beta hemolytic streptococci :

Lancefield sero grouping.

C carbohydrate antigen in cell wall of streptococci.

Classified in 20 sero groups (A to V without I/J) :

- Group A = streptococcus pyogenes.
- Group B = S. agalactiae.
- Group C.
- Group D to Group V.

Beta hemolytic streptococci

00:15:23

Extraction of C carbohydrate from cell wall.

Lancefield used hydrochloric acid for extraction.

Once extracted, C antigen was mixed with antisera prepared in rabbits.

Original Lancefield serogroup was done using ring precipitation test.

Not done nowadays.

Newer methods include :

- Latex agglutination test.
- Agar gel diffusion.

Group A beta hemolytic streptococci:

Also called as Lancefield serogroup A.

Organism : *S. pyogenes*.

Capsule :

- made up of hyaluronic acid.
- Only one capsular serotype.
- Epidemiological studies can't be performed on basis of capsular antigen.

Typing :

- m protein in cell wall.
- >120 m types : Further subtyping done on basis of T and R protein.
- Done by Griffith.

Newer methods : 'emm gene' sequencing.

m1, m3, m6 : more often cause respiratory tract infection.

m49, m57, m61 : more often cause skin and soft tissue infection.

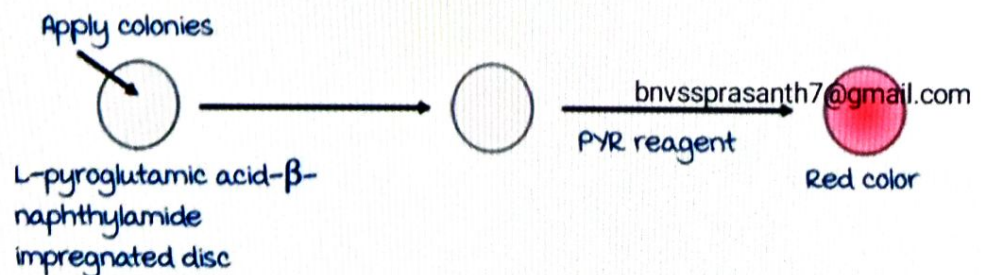
Biochemical reaction :

- Maxted's observation : Sensitivity to bacitracin.
- Ribose fermentation : Negative.



- PYR test : Positive.

Detecting activity of enzyme : Pyrrolidonyl amidase.

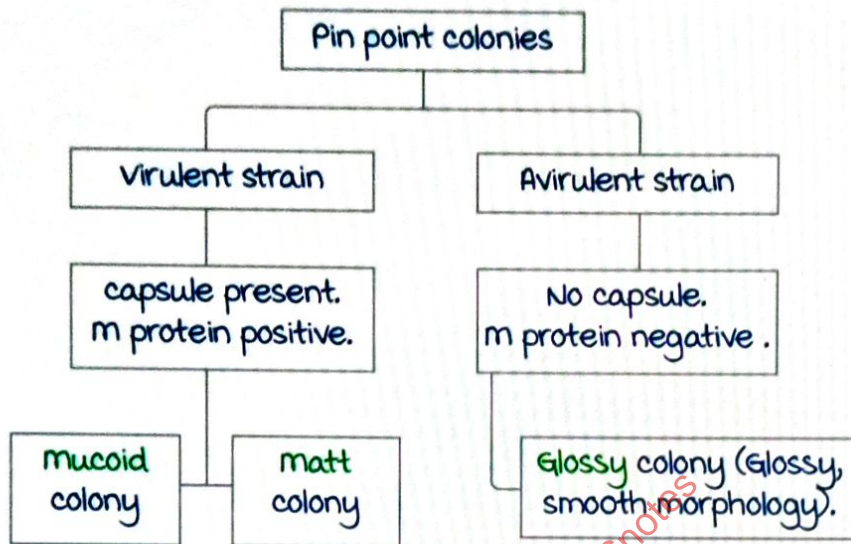
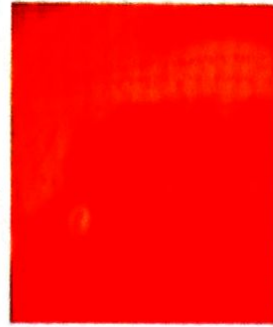


Culture :

Blood agar :

Wide zone of beta hemolysis.

Tiny colonies called **Pinpoint colonies**.



Transport media : **Pike's media**.

Selective media : Crystal violet blood agar.

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Virulence factors of *S.pyogenes*

00:26:16

1. Cell wall :
- Capsule : Hyaluronic acid.
- Adhesive proteins : Fibronectin binding protein.
- **m protein** :

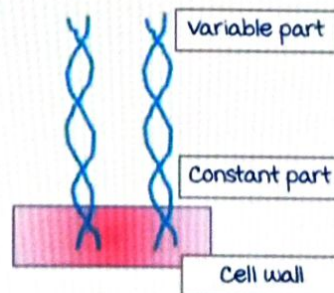
most important virulence factor.

Filamentous dimeric protein.

Anti-phagocytic and anti-complementary.

Two parts :

- Constant part : Carboxy terminal.
- Variable part : Amino terminal, extending outside.



Amino terminal used in Griffith typing.

Highly antigenic and will induce type specific protective antibodies.

Active space

Loss of M protein : Bacteria become avirulent.

a. Secreted by bacteria :

- Streptolysin

Streptolysin O	Streptolysin S
Oxygen labile.	Oxygen stable.
Active only in reduced state.	Active in reduced/oxidized state.
Lysis of RBC's seen only in subsurface colonies : Four plate colonies.	Hemolysis around surface colonies.
Highly antigenic : Induces anti streptolysin O antibodies (ASO). ASO titer used in retrospective diagnosis in acute rheumatic fever.	Non antigenic.
Can also be secreted by group C and group G streptococci.	

- Streptokinase :

Activates plasminogen which helps in lysis of fibrin. Responsible for the **spread of infection** (along with hyaluronidase).

Only of 1 antigenic type.

Also secreted by group C and group K streptococci.

use : fibrinolysis (MI, TE episodes).

S. equisimilis (group C) is used therapeutically for fibrinolysis.

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- Streptodornase/ DNase :

Depolymerizes DNA.

Protects bacterium from **neutrophil extracellular trap**.

Also responsible for the **serous character of the exudates.**

Four distinct antigenic types : A-D.

DNase B : most antigenic.

Anti-DNase B titer used in retrospective diagnosis of streptococcus infection in glomerulonephritis

- Erythrogenic toxin/pyrogenic toxin/scarlatiniiform toxin/ Dick toxins.

3 antigenic types :

A & C : Phage mediated.

B : Chromosomal.

Responsible for :

Streptococcus toxic shock
syndrome (TSS).

Scarlet fever.

Super antigen :

Small to medium sized proteins produced by certain bacteria
don't need processing by antigen presenting cells (APC).

Directly bind to MHC class II antigen.

Recognized by $\nu\beta$ part of T cell receptor at a site lateral to
antigen presenting groove.

Stimulate 5-20% of T cells leading to massive cytokine
response.

Cytokine storm responsible for TSS.

- Hyaluronidase

Also called as spreading factor.

Anti-hyaluronidase antibody titer : Retrospective diagnosis of
streptococcal infection.

- NADase/ DPNase.
- C5a peptidase.

MNEMONIC for virulence factor : SMASHED.

Streptolysins.

M protein.

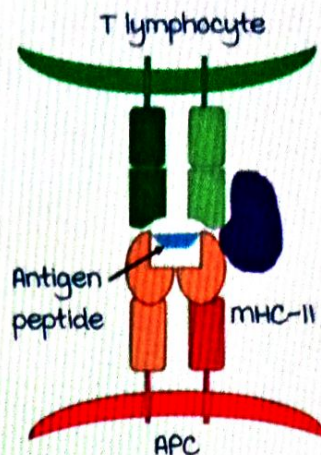
Anti-C5a peptidase.

Streptokinase.

Hyaluronidase.

Erythrogenic toxin.

DNAse.



Infections of *S. pyogenes*

00:46:08

1. Localized infections :

Pharyngitis.

Skin and soft tissue infections :

- Cellulitis.
- Necrotizing fasciitis.
- Erysipelas.
- Lymphangitis.
- Impetigo.



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2. Bacteremia :

Endocarditis.

Meningitis.

Pneumonia.

3. Toxin mediated infections

TSS.

Scarlet fever.

4. Non suppurative :

- Rheumatic fever.

Follows sore throat.

Rheumatogenic M type like M1, M5, M6 etc.

Jones criteria used for diagnosis for rheumatic fever.

ASO titer used.

- Glomerulonephritis :

Follows either sore throat or skin and soft tissue infection.

Nephritogenic M type like M49, M57, M61 etc.

Anti-DNase B used.

molecular mimicry

Structural component of <i>S. pyogenes</i>	Human tissue with which it cross-reacts
Hyaluronic acid	Synovial fluid
Cell wall M protein	Myocardium
Group A carbohydrate	Cardiac valves
Cytoplasmic membrane antigens	Vascular intima
Peptidoglycan	Skin antigens

Treatment : **Penicillin**. No resistance reported.

Pharyngitis

00:52:08

most common infection caused by *S. pyogenes*.

most common bacterial cause of pharyngitis : *S. pyogenes*.

Incubation period : 1-4 days.

Symptoms :

- Fever.
- Sore throat.
- Pain in throat.
- Exudate on tonsil.
- Tender cervical lymphadenopathy.
- Absence of cough.

Pathogenesis of strep pharyngitis :

- mucosal attachment using m protein, Lipo teichoic acids (LTA), Protein F (Fibronectin binding protein).
- Hyaluronic acid, m protein prevent phagocytosis by host macrophages.
- Bacterial streptokinase, DNase, protease, and hyaluronidase helps in invasion.

Complications :

Local :

- Peritonsillar abscess (Quinsy).
- Retro pharyngeal abscess.
- Sinusitis.
- mastoiditis.

Systemic :

Spread through cribriform plate leading to meningitis or brain abscess.

Bacteremia :

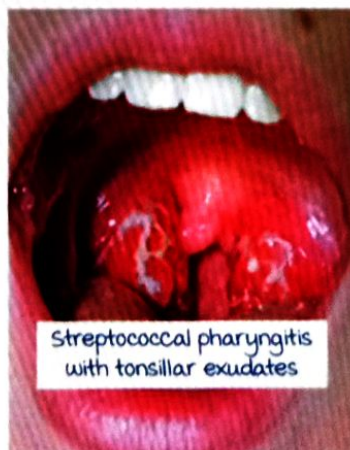
- meningitis.
- Pneumonia.
- Endocarditis.

Non suppurative complications :

- Rheumatic fever.
- Glomerulonephritis.

Diagnosis : Throat swabs.

Transport : Pike's media.



Streptococcal pharyngitis
with tonsillar exudates

Active space

Tests involved :

- Culture :

Done on crystal violet blood agar.

Gold standard method for diagnosis.

most specific method.

- Rapid test :

Immune chromatographic test.

ELISA test.

- PCR test :

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most sensitive method.

Treatment :

Penicillin V for 10 days/ **Benzathine penicillin** 1m 1.2 single dose
1.2 mu (6 L.U in children less than 27 kg).

Allergic to penicillin : **Erythromycin**.

Erysipelas

00:59:14

Acquired by minor trauma, bites, or eczema.

most common cause : **S. pyogenes**.

Can also be caused by Staphylococcus aureus, Group B streptococcus.

Superficial infection of the skin restricted to dermis along with lymphangitis.

Typical appearance :

Raised, salmon pink, well defined, painful lesion.

Symptoms :

Fever along with constitutional symptoms like chills and myalgia.

most common : **Lower extremities**.

Diagnosis : Skin biopsy culture.

Treatment : Penicillin.



Cellulitis :

Skin and subcutaneous infection.

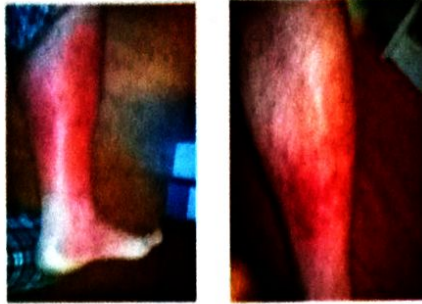
Acquired by **surgical incision or trauma**.

Appearance : **Pinkish** lesions, **not raised**, painful lesion.

Demarcation between involved and non involved area is not distinct.

Causes : *S. pyogenes*, *S. aureus*, Group B, C, & streptococci

Treatment : Penicillinase resistant penicillins (dicloxacillin/
cloxacillin).



Scarlet fever :

Toxin mediated infection : erythrogenic toxin A, B, C.

Follows pharyngitis.

most common : 5-15 years.

Presents with fever.

Appearance :

- Rash :

Sandpapery, red erythematous rash.

Blanches on pressure.

Originates from chest, spreads to neck and
to extremities.

No involvement of palms and soles.

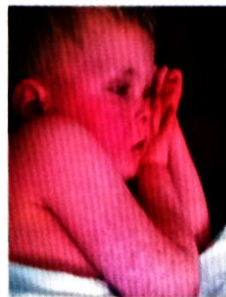
- Rash on face.
- Circum oral pallor.
- Pastia lines :

Deep red lines between the skin folds.

mainly seen in elbow axilla, perineal area, gluteal
folds.

- Strawberry tongue (beefy red tongue).

Treatment : Oral penicillin V for 10 days / Benzathine penicillin
IM single dose.



Group B beta hemolytic streptococcus

01:08:08

S. agalactiae :

Capsulated :

10 capsular serotypes (Ia, Ib, II upto IX).

Biochemical :

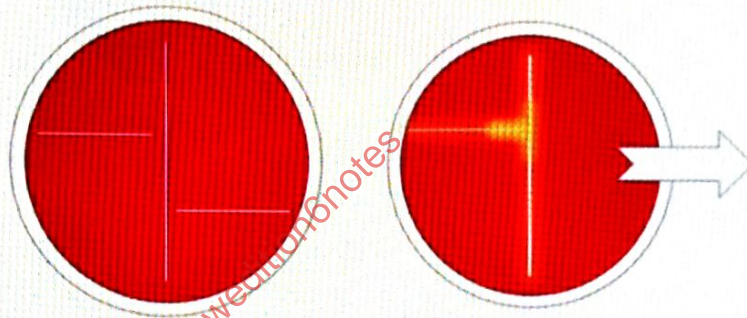
- Bacitracin resistant.
- CAMP test :

Christie Atkins Munch Peterson test.

In blood agar plate :

Streak inoculate colonies of *S. aureus*.

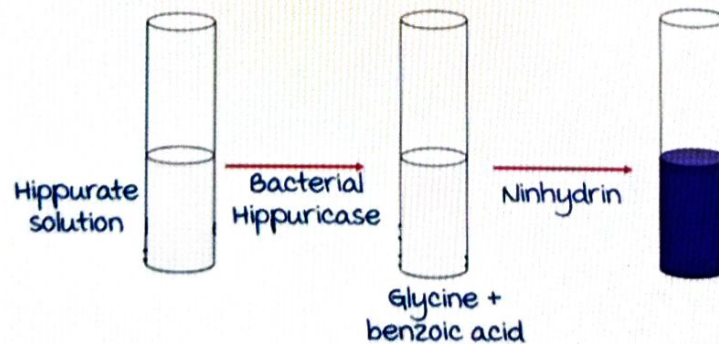
Streak inoculate the colonies of group B beta hemolytic streptococcus at right angles.



Ideally these colonies should be surrounded by respective zones of beta hemolysis.

But hemolysin synergize with each other producing enhanced zones of hemolysis (arrowhead hemolysis).

- Hippurate hydrolysis test : Positive.



Culture :

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Beta hemolytic.

Islam's medium/ Granada's medium : orange colored pigment.

Clinical importance :

Normal flora in lower GIT and vagina in 10-40% of individuals.

Infections :

1. Neonatal diseases :

- Early onset :

Within **first week** of delivery.

Due to vertical (perinatal) transmission during delivery.
most caused by capsular serotypes (Ia, III, V).

manifests with septicemia, pneumonia and meningitis.

Can be prevented by taking **prophylaxis** :

35-37 weeks of pregnancy : rectal and lower vaginal swabs culture.

If positive : **Intra partum penicillin prophylaxis** 4 hourly till delivery.

Prophylaxis therapy also given to pregnant women with a history of delivering a child with similar infection.

- Late onset :

Horizontal transmission.

Generally, a community acquired infection :

Nosocomial transmission (nursery personnel or other colonized neonates).

Community transmission.

Occurs **1 week - 3 months** after delivery.

Commonly caused by **capsular serotype III**.

Generally present as septicemia / meningitis.

2. Puerperal sepsis.

3. **Diabetic, HIV and elderly** :

Skin and soft tissue infections.

Pneumonia.

Bacteremia.

Treatment for group B beta hemolytic streptococci : **Beta lactam antibiotics**.

Group C beta hemolytic streptococci

01:17:22

Includes *S. equi*, *S. equisimilis*, *S. zooepidermicus*, *S. dysgalactiae*.
mainly **animal pathogens**.

Human infections; bnvssprasanth7@gmail.com

Respiratory tract infections.

Skin and soft tissue infections.

Endocarditis.

Clinical questions :

Q. 4 day old infant girl now showing signs of sepsis is brought to the emergency department. She was preterm (33 weeks) and born at home to her 19-year-old mom after 22 hours of labour following the rupture of the membranes. A friend helped the mother deliver the baby. What is the best description for the agent most likely causing the sepsis if it was acquired during labour? All organisms in the answer choices are Gram-positive, catalase negative cocci.

- A. Nonhemolytic organisms found as part of the normal faecal flora; resistant to bile and optochin; carries a high level of drug resistance.
- B. Alpha-hemolytic diplococci sensitive to both bile and optochin.
- C. Beta-hemolytic cocci in chains and carrying Lancefield's group B antigen.
- D. Alpha-hemolytic cocci in chains; resistant to bile and optochin.

Q. A child develops pustule on front of his leg. A swab culture isolates a beta hemolytic bacterium which on staining is gram positive coccus in chains and on biochemical testing is catalase negative and bacitracin sensitive. A similar strain is found by school doctor in students causing pharyngitis. Which test can differentiate between two strains?

- A. M. Protein.
- B. mec A gene.
- C. Capsule.
- D. C carbohydrate antigen.

STREPTOCOCCUS ALPHA AND GAMMA HEMOLYTIC

Gram positive cocci : Seen in chains/pairs.

They are non-motile, facultative anaerobes.

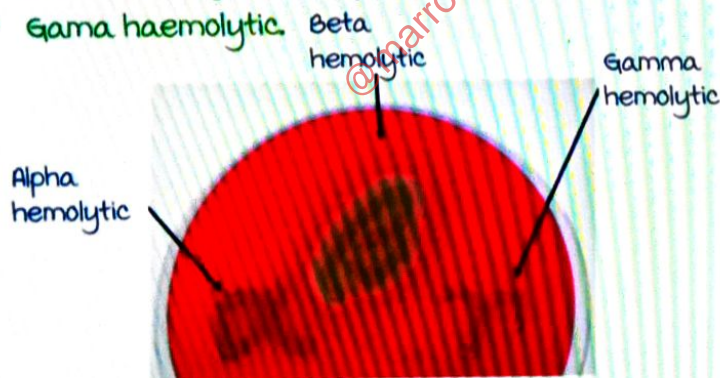
Capsule may/may not be present.

They are catalase negative, oxidase negative & ferment sugars (both aerobic and anaerobically).

They would only grow in an enriched medium (fastidious organisms).

All the streptococci have been classified on the basis of their haemolysis pattern :

- Alpha haemolytic : Produce greening along the colonies due to a green coloured compound (partial haemolysis). They include Streptococci viridans & Streptococci pneumoniae.
- Beta haemolytic.
- Gamma haemolytic.



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Viridans streptococci (VGS)

00:02:08

viridis : Latin origin (means green).

They are alpha haemolytic.

Species :

- S. mutans.
- S. mitis.
- S. sanguis.
- S. salivarius.
- S. anginosus.

Active space

Sites of normal flora :

- Oral cavity → **m/c** site.
- Upper respiratory tract.
- Skin.
- Genitourinary tract.

They cause opportunistic infections as they are organisms of low virulence.

m/c cause of dental caries → **S. mutans** form biofilms around the teeth.

m/c cause of sub-acute bacterial endocarditis/SABE (affecting prosthetic valves) → **S. sanguis**.

m/c cause of late prosthetic valve endocarditis (endocarditis after one year of surgery) → **S. sanguis**.

Relatively sensitive to beta-lactams.

Streptococci pneumoniae

00:05:47

Also called as **pneumococcus**.

Capsulated bacteria with **polysaccharide capsule** (most virulent factor) present.

Non capsulated bacteria become avirulent.

There are > **97 serotypes** on the basis of their capsular antigen.

> **75%** adult infection → By types 1-8.

> **75%** children infections → By types 6, 14, 19, 23.

most virulent strain → **Type 3** (very thick capsule difficult to phagocytose).

On gram staining :

Gram positive diplococci.

Appear as paired/short chains.

Characteristic shape → **Lanceolate/flame shaped**.

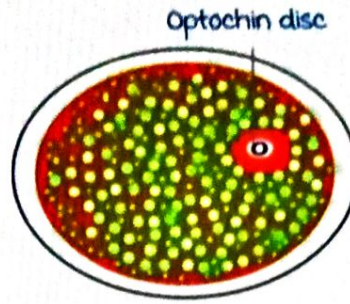
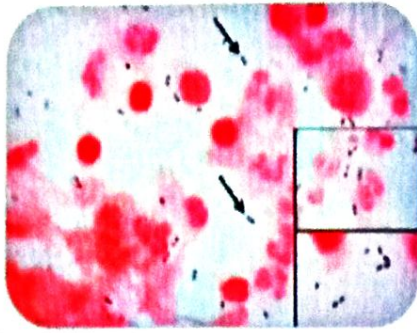
They are **non-motile facultative anaerobes**.

Capnophiles (likes to grow in presence of **5%** carbon dioxide).



Lanceolate / flame shaped

It is sensitive to **optochin (ethyl hydrocuprein)** sensitivity test (disc diffusion test) → After overnight incubation, streptococci pneumoniae will be missing around the disc.

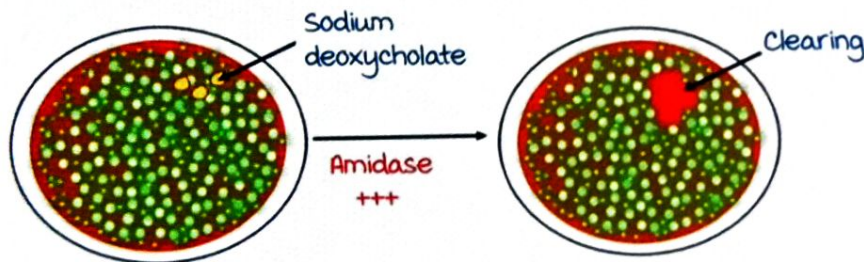


Bile solubility test : *Streptococcus pneumoniae* produces an autolytic enzyme (**amidase**) under normal circumstances.

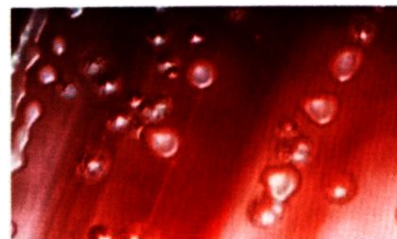
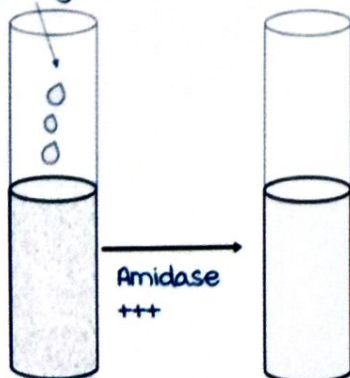
After overnight incubation, streptococcus forms its colonies
 → Place petridish back into the incubator for **2 to 3 days** →
 The colonies autolyse themselves → Clearing patches are left.

If **sodium deoxycholate (bile salts)** is added to the petridish, the automatic enzyme activity increases and clearing occurs in few hours compared to days.

Can be done in a petridish or a test tube (sodium deoxycholate can be added to the test tube) → Turbid colonies disappear .



Sodium deoxycholate



Carron coin/Draughtsman colonies of pneumococcus on blood agar

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Active space

Culture : when grown on blood agar, the colonies are alpha haemolytic with zones of greening.

Carrom coin/draughtsman colonies are seen with central depression (autolysis).

Virulence factors :

- Peptidoglycan & teichoic acid.
- Polysaccharide capsule/specific soluble substance (antiphagocytic).
- Adhesive proteins.
- Pneumolysin.
- Amidase.
- **IgA-1 protease.**

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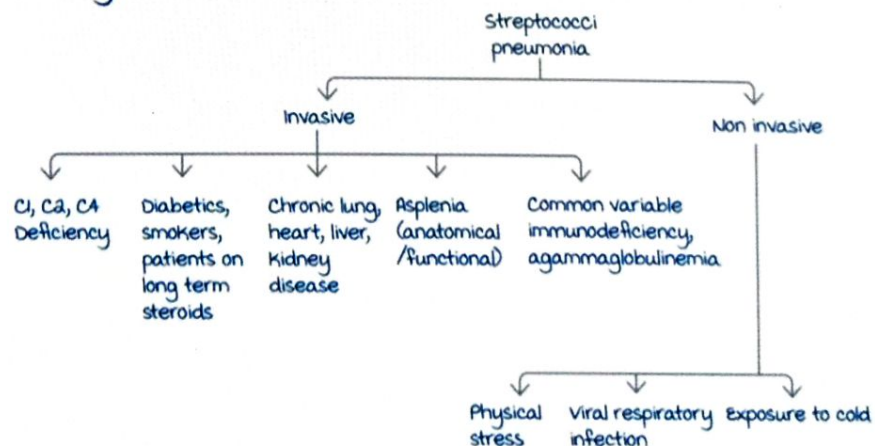
Other bacteria producing Ig A 1 protease : mnemonic - SHIN.

1. **S**treptococcus pneumonia.
2. **H**aemophilus influenzae.
3. **N**eisseria species.

CRP reacts with **C carbohydrate antigen** of streptococcus pneumonia, whenever inflammation present in the body.

Risk factors for diseases caused by streptococcus pneumonia :

Streptococcus pneumoniae is normal URT flora in **50 %** healthy individuals.



Non-invasive (contiguous spread from respiratory tract colonization site) :

- otitis media
 - Sinusitis
- } **m/c** bacterial course.
- Acute exacerbation of COPD : Caused by

1. Rhinovirus.
 2. Coronavirus.
 3. Non typeable haemophilus influenza.
 4. Pneumococcus.
 5. Moraxella catarrhalis.
- Non bacteremic pneumonia.

Invasive (bacteremia):

- meningitis: **m/c** cause is *S. pneumoniae* in all ages except →
 1. Neonates: where common causal organisms are listeria, *E. coli* & group B streptococcus.
 2. 15 to 25 year olds: meningococcus.
 3. HIV: Cryptococcus.
 4. Post neurosurgery: *Staphylococcus aureus*.
- Community acquired pneumonia: **m/c** cause.
- Other diseases: Endocarditis, osteomyelitis, septic arthritis.

Treatment:

most effective drug: **Beta Lactams.**

Resistance → **m/c** mode of resistance to beta lactam:

Altered penicillin binding proteins (transpeptidases) →

mode of resistance is via bacterial transformation (uptaking soluble DNA fragments from the environment and incorporating into its genome).

Associated with resistance to several other antibiotics like macrolides and tetracyclines.

Otitis media → **Amoxicillin ± clavulanic acid.**

Sinusitis → **Amoxicillin ± clavulanic acid.**

Acute exacerbation of COPD → **Amoxicillin ± clavulanic acid.**

meningitis → **IV 3rd generation cephalosporins** (ceftriaxone/cefotaxime).

Pneumonia → **IV Ceftriaxone.**

vaccine: Sub unit capsule vaccine providing type-specific immunity.

- **Pneumovax** : A polysaccharide vaccine containing **23 capsular antigen** serotypes.
Recommended for all individuals who are at risk of invasive pneumococcal disease + elderly.
Single dose provides protective immunity for 5 years.
- **Prevnar 13** : Conjugate vaccine (**capsular antigen + carrier protein, CRM 197**) for children < 2 years.
CRM 197 is a non-toxic toxin of a mutant strain of *Corynebacterium diphtheria*.

Children (< 2 years) are not able to mount an immune response to polysaccharides due to their decreased immunity. Hence conjugation of polysaccharide with carrier protein is a must.

Schedule for children : 2 months, 4 months, 6 months and a booster after 12-15 months.

	<i>S. pneumoniae</i>	Viridans group
Arrangement	Pairs	Chains
Colonies	Carrom coin	Dome
Bile solubility	Yes	No
Optochin sensitivity	Yes	No
Fermentation of inulin	Yes	No

Gamma hemolytic streptococci

00:32:43

Gamma haemolysis : No colour seen around the colonies.

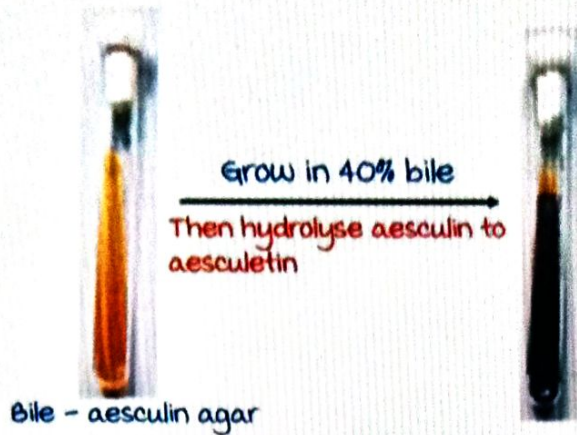
Non enterococcal Group D streptococci containing Lance field **C carbohydrate antigen** which are gamma hemolytic on blood agar.

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Species : *S. equinus* & *S. gallolyticus* (*S. bovis* earlier).

Identifying features :

- They are bile resistant (able to grow in **40% bile medium**).
- They are able to hydrolyse aesculin.
Bile-aesculin test is positive → Black coloured aesculetin produced.



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They can be present as normal gastrointestinal flora in humans.

S. gallolyticus can cause septicaemia and endocarditis in patients of colorectal cancer and rectal polyps.

All gamma hemolytic streptococci are relatively sensitive to betalactams.

Gamma hemolytic streptococci : Enterococcus 00:36:28

Seen as gram positive cocci in pairs or short chains.

Identifying features :

- They are bile resistant.
- Aesculin hydrolysis positive.
- Grow in 6.5% NaCl.
- PYR test (pyrrolidinylamidase test) positive.
- Can grow at 5 - 9.6 pH.
- Grow at 10°C - 45°C.
- Can survive 60°C for 30 minutes.
- They are resistant to sodium azide (toxic).



Bile-aesculin agar

Cultural characteristics :

- On blood agar, gamma haemolysis is produced (some exceptions produce α & β haemolysis).
- MacConkey agar : Tiny lactose fermenting/LF pink coloured colonies are formed.
MacConkey agar is selective for gram negative organisms, but *Staphylococcus* and *Enterococcus* are

Active space

exceptions as they can grow in this agar.

- **Sodium azide agar** is the selective agar.
- **Bile-Aesculin test is positive** → Black coloured aesculetin production.

Enterococcus are part of normal GI flora.

Diseases caused :

- Nosocomial infections :
 1. Catheter associated urinary tract infection.
 2. Surgical site infection.
 3. Intra abdominal abscesses.
- Endocarditis.

m/c clinical isolate → *E. faecalis* (80-90%), *E. faecium* (5-15%).

Treatment :

They are inherently resistant to penicillin (except ampicillin) and first and second generation cephalosporins.

They have inherent low level resistance to aminoglycosides as they cannot cross the cell membrane.

Resistance can be overcome by adding penicillin to aminoglycosides).

Ampicillin + Gentamicin can be given.

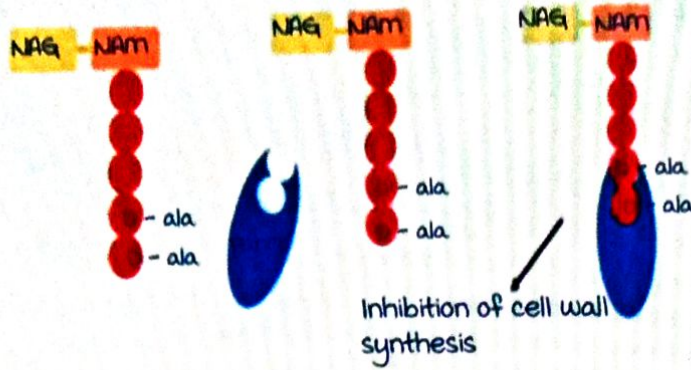
Some strains have high level resistance to aminoglycosides (target ribosomes are altered) → Ampicillin + Gentamicin cannot be given → Drug of choice : **Vancomycin**.

Reports of vancomycin resistance have increased.

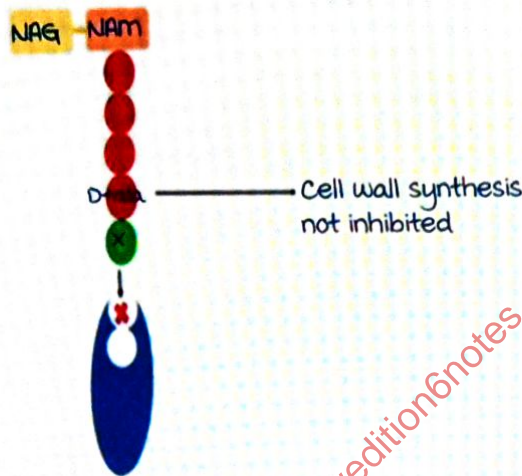
Mutation in Vancomycin Resistant Enterococcus (VRE) → **van A/van B /van c genes** → Start to synthesize altered cell wall precursors.

Alteration : **D-ala - D-lactate, D-ala - D-serine**.

For **vancomycin resistance** : **Daptomycin and linezolid**.



Vancomycin acts by binding irreversibly to the terminal D - alanyl - D alanine of bacterial cell wall precursors, inhibiting cell wall production.



	Enterococcus	Non-enterococcal Group D
Bile resistance	Yes	Yes
Aesculin hydrolysis	Yes	Yes
Growth in 6.5% NaCl	Yes	No
PYR test	Yes	No
Lancefield group D carbohydrate antigen	Yes	Yes

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Clinical scenarios

00:50:12

Q. An elderly man develops low-grade fever and signs of endocarditis over a period of 2 weeks. Following blood culture report from the microbiology department, his doctor also becomes concerned about possible colon cancer. Which of the following is the likely

Active space

cause of the endocarditis?

- A. *Staphylococcus aureus*.
- B. *Enterococcus faecalis*.
- C. *Streptococcus gallolyticus*.
- D. *Staphylococcus epidermidis*.

Q. Blood culture from afebrile patient has growth of gram positive cocci. Further testing revealed that the isolate possessed the group D antigen in cell wall, was not beta-lactamase positive, but was resistant to vancomycin. Which of the following is the most likely identification of this isolate?

- A. *Enterococcus casseliflavus*.
- B. *Enterococcus durans*.
- C. *Enterococcus faecalis*.
- D. *Enterococcus faecium*.

E. *faecium* has 70-80% vancomycin resistant enterococci.

E. *faecalis* has only 1-7% VRE.

Q. A 16 year old female presents with fever severe left ear pain and decreased hearing for the past 3 days. She has had at least 4 similar episodes in the past. Chart review shows that she had been diagnosed with acute myringitis at 10 years of age and acute otitis media on at least 3 occasions. Physical examination demonstrate a thickened erythematous tympanic membrane with apparent separation of some parts of pars tensa. Tympanocentesis confirms middle ear effusion and samples are sent for culture. Given the likely diagnosis, what is the most commonly identified bacterium in this patient's condition?

- A. *Streptococcus pneumoniae*.
- B. *Mycoplasma pneumoniae*.
- C. *Moraxella catarrhalis*.
- D. Non typeable *Haemophilus influenzae*.

m/c cause of otitis media is *streptococcus pneumoniae*.

BACILLUS

Genus : Bacillus

00:01:26

Spores of the members of genus Bacillus, the non pathogenic are present everywhere in the environment and are used as a biological control in an autoclave, hot air oven, etc.

most common contaminants of culture media.

Gram stain : **Gram positive bacilli/rods.**

motility : motile by **peritrichous flagella** (except for Bacillus anthracis; non motile).

Oxygen : Aerobes (except for Bacillus anthracis; facultative anaerobe).

Capsule : Non capsulated (except for Bacillus anthracis; capsulated).

General characters :

Spores :

- Highly heat & desiccation resistant.
- Round / oval.
- Central / subterminal.
- **Non bulging.**
- Spores **never form in the human body**, only form either in soil or culture.

Bacillus anthracis

00:06:16

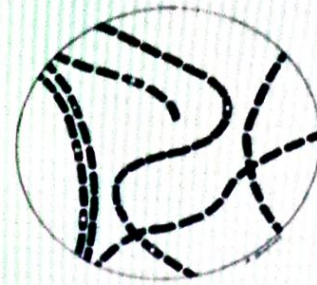
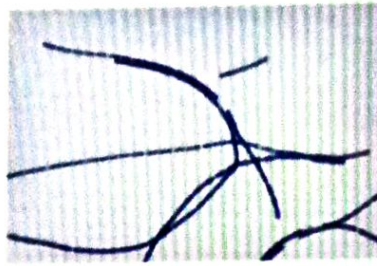
1st pathogenic bacterium to be;
seen under the microscope,
isolated in pure culture,
demonstrate **Koch postulates** by Robert Koch,
made as 1st **live attenuated vaccine** by Louis Pasteur.
largest pathogenic bacterium (sometimes as long as
microns).

A gram positive rod that grows in a long chain (**bamboo stick appearance**).

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Microbiology • v4.0 • Marrow 6.0 • 2022

Page 1/9



Bamboo stick appearance under microscope

Properties :

Non motile.

Facultative anaerobe.

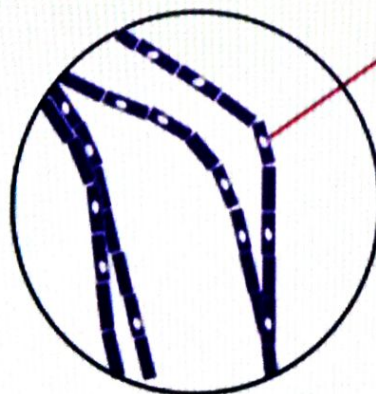
Capsulated ; polypeptide capsule (polymer of D glutamic acid).

Demonstrated by **McFadyean's reaction**, by staining with **polychromemethylene blue**, bacteria take up blue, and around bacteria, the pinkish amorphous material is seen which is the capsule.



Pink amorphous material of the capsule around the blue staining bacteria

Spores do not take up gram stain, which appears as empty spaces inside the bacterium.



Gram staining shows unstained spores

Active space

Spores are demonstrated using **Schaeffer Fulton stain** and **Ashby stain** (modification of the Schaeffer Fulton stain), bacteria take up the red color of the **safranin dye** and the spores take up green from the **malachite green dye**.



Schaeffer fulton stain

Biochemical : Catalase & oxidase positive.

Special properties :

- In **gelatin medium**, stab inoculation of colonies at an incubation of 23 - 24°C produces **inverted fir tree liquefaction** (slow liquefaction).



Inverted fir tree



Zone of lysis; gamma phage

- Susceptibility to **gamma phage**.
- **String of pearl appearance**; seen in penicillin containing medium, bacteria become rounded.

Bacillus anthracis; Culture

00:14:22

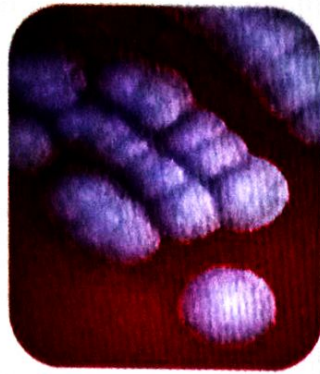
Culture :

Non fastidious ; grows on **nutrient agar**.

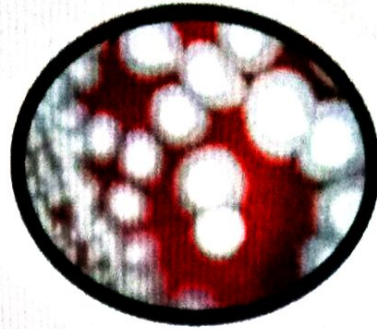
Only species of Bacillus which is **non hemolytic** in blood agar.

Colonies appear as **frosted glass appearance**/ **medusa head appearance**/ **comet tail appearance**/ **beaten egg white appearance**.

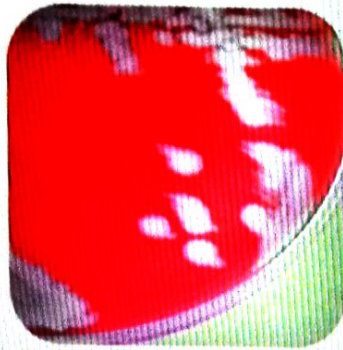
Active space



Frosted glass appearance



medusa head appearance



Comet tail appearance



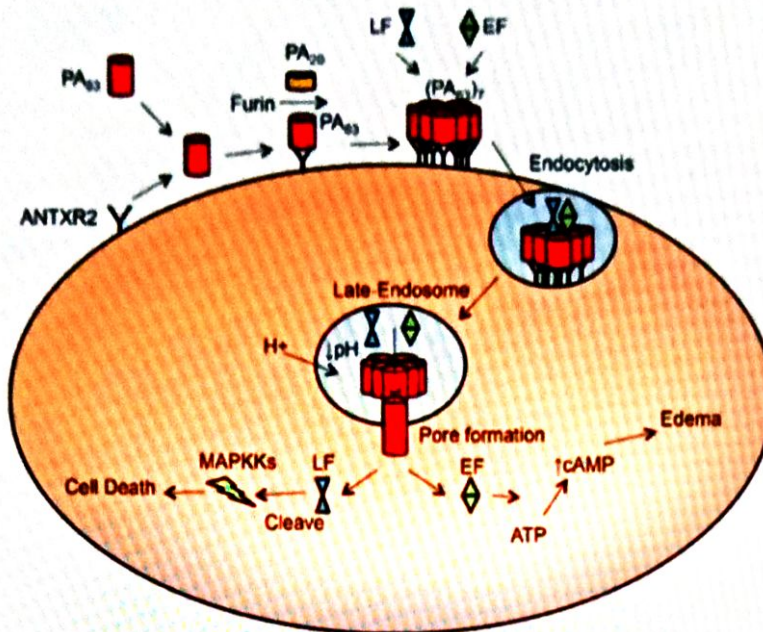
Beaten egg white appearance

Selective medium : Polymyxin Lysozyme EDTA Thallous acetate (PLET medium).

Virulence factors :

- Capsule; anti phagocytic polypeptide capsule, mediated by **px02 plasmid**.
- Anthrax toxin : **Tripartite toxin** (3 components secreted separately : **Protective antigen**, **Edema factor**, **Lethal factor**) encoded by the **px01 plasmid**.

Protective antigen → binds to receptors on host cell → forms a heptamer → endocytosis → Lethal/ Edema factor forms Lethal/Edema toxin by binding with heptamer → brings effects.



effects :

Edema factor + protective antigen heptamer → Edema toxin.
Edema toxin increases cAMP in the host cell by calmodulin mediated mechanisms → Cell edema.

Lethal factor + protective antigen heptamer → Lethal toxin.
Lethal toxin → inhibits enzymes (mitogen activated protein kinase kinases; MAPK's) → Cell death.

Anthrax

00:23:50

Common zoonosis, causes high morbidity and mortality in domestic animals.

mode of infection : Spore, infectious dose ($1D_{50}$: 10 spores).



Cutaneous anthrax/ malignant pustule/ Hide porter's disease

Active space

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3 Types :

	Cutaneous (> 95%)	Inhalation	Gastro intestinal
General	most common Also called as malignant pustule / hide potters' disease.	Also called as wool sorter's disease.	Ingestion of spore / vegetative bacilli contaminated meat.
Presentation & course	Papule → vesicle ulcerates (painless) → black eschar (ANTHRAX: black coal like scar). 90 - 95% resolve spontaneously. In 5 - 10% → bacteraemia, complicated with meningitis.	Primarily a mediastinitis. X ray chest shows widened mediastinum with bilateral pleural effusion. 80 - 90% of untreated patients → bacteraemia.	Pharyngeal disease → pharyngeal ulcer covered with exudates. Intestinal disease → haemorrhagic enteritis. 25 - 60% patients → bacteraemia.
Treatment	Oral Doxycycline / Ciprofloxacin per orally for 7 - 10 days.	Ciprofloxacin / moxifloxacin / Levofloxacin + Meropenem + Linezolid / Clindamycin / Rifampicin. Duration : 60 days Adjunctive : Anthrax vaccine adsorbed (AVA) : 3 doses, 0,2,4 weeks. In severe disease monoclonal antibodies (Raxibacumab).	Same as inhalation anthrax.

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Diagnosis :

Specimen : Swabs, sputum, bronchoalveolar lavage, lung biopsy, stool.

microscopy : Gram positive rods. No spores.

Cultivation under biosafety level III (bacteria forms spores in the culture medium) : Gold standard.

Identification tests : Gelatin liquefaction, McFadyen's reaction; positive, a string of pearl appearance.

NAATs : most sensitive.

Serology : ELISA, Western blot. Antibodies appear by the end of the first week.

Anthrax vaccine & exposure prophylaxis

00:37:02

Anthrax vaccine adsorbed :

- Derived from the filtrate of supernatant of a broth culture of *Bacillus Anthracis* (non capsulated strain), modified by deleting pX02 plasmid.
- A **subunit vaccine**.



Post exposure prophylaxis :

After accidental exposure **vaccine** (dose; 0, 2 & 4 weeks)

+
Antibiotics (Doxycycline / Ciprofloxacin for 60 days).

Pre exposure prophylaxis :

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For individuals at high risk of exposure.

vaccine (5 doses) : 0, 1 month, 6 months, 12 months, 18 months;

Followed by a **booster** every 12 months.

MCQs :

Q. All true about *Bacillus anthrax*, except :

- Non motile.
- Arranged in chains.
- Central/ subterminal bulging spores.
- Capsulated.

Q. False about anthrax :

- Caused by gram positive bacilli.
- Soil is the reservoir.
- Spore formation occurs in the body.
- more common in herbivores.

Q. A tourist visits a cattle ranch and 5 days later develops fever, malaise, a dry cough, and pressure in his chest. These symptoms resolve after a few days. He then develops high fever, severe shortness of breath, chest pain, cyanosis, and

Active space

diaphoresis, and is rushed to the emergency department, where work up reveals hemorrhagic mediastinitis and bloody pleural effusions. Within a few hours, the patient develops septic shock and dies. Which of the following organisms is most likely responsible for this patient's symptoms?

- A. *Bacillus anthracis*.
- B. *Brucella abortus*.
- C. *Legionella pneumophila*.
- D. *Nocardia asteroides*.

Q. A 55 year old government employee of the Army Research Institute for Infectious disease presents with sudden onset of fever, cough, dyspnea, and myalgias. He reports that he was working in the laboratory when a small explosion occurred in one of the isolation rooms containing various specimens. He appears unwell and in moderate respiratory distress. His chest xray demonstrates a widened mediastinum. Which of the following is the most appropriate initial therapy?

- A. Aztreonam.
- B. *Ciprofloxacin*.
- C. Ceftriaxone.
- D. Tobramycin.

Bacillus cereus

00:44:46

Anthracoids.

motile.

Non capsulated.

Aerobe.

Blood agar : Beta hemolytic.

Selective medium : **MYPA** (mannitol, egg yolk, phenol red, polymyxin agar).

Spores are widely distributed in the environment, causes food poisoning.

a types :

	bnvsspraseenth@penta.com poisoning	Cereus diarrheal type Food poisoning
Implicated Food	Chinese rice/ fried rice.	meat contaminated with spores.
Toxin	Contains preformed toxin → Cereulide (plasmid mediated toxin).	The toxin is formed in GI tract → NHE toxin (chromosomal mediated toxin).
mechanism of action :	Similar to <i>S. aureus</i> enterotoxins (vagal stimulation).	similar to <i>Clostridium</i> <i>perfringens</i> enterotoxin (via pore formation in enterocytes).
Incubation period	Less than 6 hours.	8 - 16 hours.
Presenting symptoms	Self limiting illness of nausea, vomiting, abdominal pain.	Self limiting watery diarrhoea and abdominal cramps.

Category A/ Tier 1 bio terrorism agents

00:52:14

Properties :

- Can be easily disseminated or transmitted from person to person.
- Result in high mortality rates.
- Have potential to cause public health panic and social disruption.
- Require special preparedness from public health services.

Mnemonic : **F**rancis is **P**laying with **B**ottles that **A**re **F**illed with **S**mall **A**nts.

1. *Bacillus anthracis* → Anthrax.
2. *Francisella tularensis* → Tularemia.
3. *Yersinia pestis* → Plague.
4. *Botulinum toxin*
5. *Variola major* → Smallpox.
6. Viral hemorrhagic fevers (with high mortality) :
 - Filoviruses** (Ebola & Marburg);
 - Arenaviruses** (Lassa fever, South American hemorrhagic fever).

CORYNEBACTERIUM, LISTERIA AND ERYSIPELOTHRIX

Gram positive rods

00:00:19

(CORY & ERY KNOCKED BACK MY ACTOR LIST in CLOSET).

- Corynebacterium.
- Erysipelothrix.
- Nocardia.
- Bacillus.
- Mycobacterium.
- Actinomyces.
- Listeria.
- Clostridium.

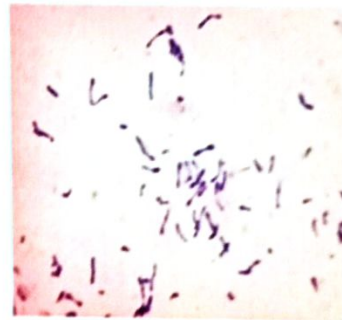
Corynebacterium diphtheriae :

(Korune : Club.

Diphtherite : Leather)

Also called **Kleb Loeffler bacterium**.

morphology : Non capsulated club shaped gram positive rods arranged in **cuneiform/chinese letter pattern**.



Daughter cells split apart after binary fission (chinese letter/cuneiform).

They are **facultative anaerobes** and are non motile.

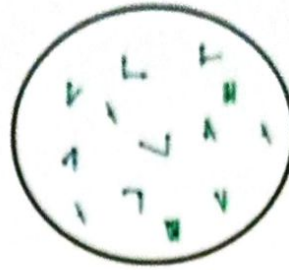
In the cytoplasm, **metachromatic/ volutin/ Babes Ernst granules/ polar bodies** are seen.

metachromasia : when stained with toluidine blue, bacteria appear purplish.

Granules : made of **polymetaphosphate** (acts as energy stores).

Stains :

- Albert A & B stains (poured onto glass slide one after the other).



Cytoplasm : malachite green.
Granules : Stained with toluidine blue.

Albert A stain :

- Toluidine blue 0.15 g.
- malachite green 0.2 g.
- Glacial acetic acid 1 ml.
- Alcohol (95% ethanol) 2ml.

Albert B stain :

- Iodine 2 g.
- Potassium iodide 3 g.
- Neisser's stain.
- Ponder's stain.

Biochemical reactions :

- Catalase : Positive.
- Oxidase : Negative.
- Ferments glucose and maltose.

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Positive



Negative

- Cultivation of *C. diphtheriae* :
- Cannot grow on a simple medium (fastidious organism).
- Grows only on enriched medium (Loeffler's serum slope).
- Forms colonies in 4 - 8 hours.



Loeffler's serum slope

Selective media are potassium tellurite blood/chocolate agar.

Contains 0.03 - 0.04% potassium tellurite.

Selective medium is required to inhibit the growth of commensals in the throat swab.

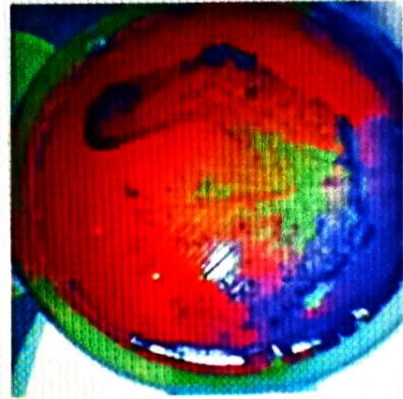
Active space

All bacteria of corynebacteriae family grows on this medium. Converts potassium tellurite to **metallic tellurium** (gives black color).

Forms colonies in 24 - 48 hours.

Potassium tellurite containing media :

1. Macleod medium.
2. Hoyle's medium.
3. Downie's medium.
4. Tinsdale/cysteine tellurite agar.



KTBA

Rapid diagnosis of diphtheriae :
 Loeffler's serum slope.

Best medium : KTCA/KTBA.

Biotypes of C. diphtheriae

00:10:17

	Gravis	Intermedius	mitis	Belfanti
Colony morphology	Pointed colony	Frog egg colony	Poached egg colonies	Nitrate reduction test negative variant of mitis
Fermentation	Starch and glycerol	-	-	
Diphtheriae toxin production	100%	95 - 99%	80 - 85%	
Blood agar	Variable hemolysis	Non hemolytic	Always hemolytic	

Virulence factor of C. diphtheriae :

Diphtheria toxin :

- Cause both local effects and systemic complications.
- All exotoxins are proteins (heat labile).
- Highly potent (minimal lethal dose is small).
- Prophage (beta phage) mediated gene encoding of toxin by tox gene.

Active space

A-B subunit toxin (A : Active part, B : Binding).

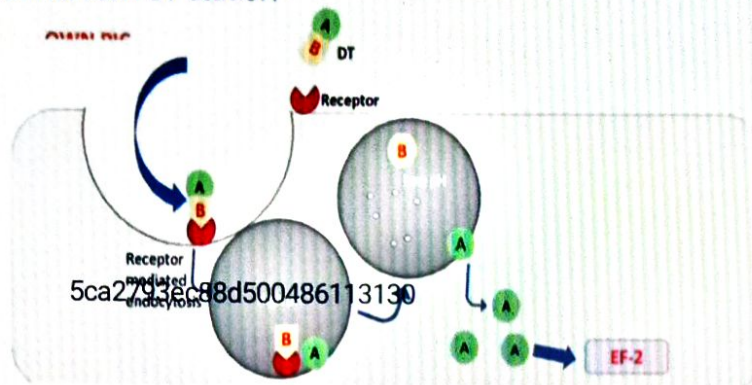
m.w. of A subunit ~ 20kDa; B subunit ~ 40kDa.

Receptor for the toxin is Epidermal Growth Factor (EGF).

All cells are susceptible to effects of toxin **except RBCs**.

Special affinity for myocardium, adrenal and nerve endings.

mechanism of action :



Toxin binds to receptor.

Receptor mediated endocytosis.

A subunit separates (acidic pH in endosome).

A subunit enters bacterial cytoplasm.

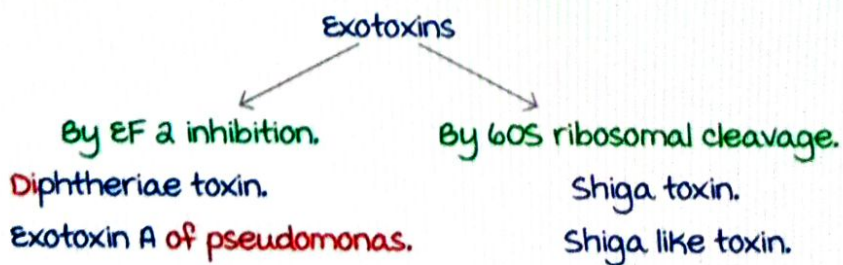
ADP ribosylates Elongation Factor 2 (EF 2).

Inhibition of EF 2.

Blocks protein synthesis.

Exotoxins inhibiting protein synthesis

00:19:25



Preparation of diphtheriae toxoid :

1st preparation by **Ramon**.

Active space

Toxoid : A toxin which has lost its virulence, but maintains its antigenicity.

Grow Park William 8 strain of *C. diphtheriae* in broth medium.



Filter the diphtheriae toxin and incubate filtrate at 37°C.



Add formaldehyde.



Formal toxoid formed after few weeks.



Adsorbed onto aluminium phosphate (adjuvant).



Added to Tdap/DTaP vaccine.

Diphtheria :

Reservoir : Only humans (case/carrier).

Mode of infection : Inhalation of droplets of respiratory secretion/ulcer exudates (cutaneous diphtheria).

Incubation period : 3 - 4 days.

Most common type is faucial diphtheria.



Pseudomembrane

Patient presents with fever, sore throat, cervical lymphadenitis and greyish white pseudomembrane around the tonsils.



Bull neck

Pseudomembrane contains fibrinous exudates with WBCs, RBCs and entangled bacteria. No epithelium.

Other types of diphtheria :

Laryngeal (most dangerous), nasal, otitic, conjunctival, vaginal and cutaneous diphtheria.

Cutaneous diphtheria is generally associated with a shallow ulcer covered with a pseudo membrane.

Complications of diphtheria :

1. **most common is myocarditis.**

Develop upto 10 days after symptom onset.

Can cause arrhythmias, heart block and dilatation of ventricles. Circulatory failure leading to death.

most common cause of death in diphtheria.

2. Neurological :

Due to demyelination.

Onset can be upto 90 days after infection.

manifests as palatine/extraocular muscle/ciliary paralysis.

Paresis of limbs also seen.

Slow, spontaneous and complete recovery. bnvssprasanth7@gmail.com

3. Asphyxia :

Due to pseudomembranes and bull neck.

Differential diagnoses :

Streptococcus pharyngitis.

Infectious mononucleosis.

Vincent's angina.

Oropharyngeal candidiasis.

Acute retroviral syndrome.

Retropharyngeal space infection.

Diagnosis of diphtheria

00:28:43

Specimens : Throat swab with pseudomembrane.

Gram stain/ Albert stain used.

Culture on Loeffler's serum slope/ KTCA/ KTBA.

Toxicity tests to prove presence of tox gene in beta phage.

In vivo on **guinea pigs** by subcutaneous/intradermal injections.

In vitro by **Elek's gel precipitation test** (immunodiffusion).

Also known as **in vitro toxin neutralization test.**

Elek's gel precipitation test :

Serum medium.



Place a filter paper with anti - DT before medium solidifies.



As medium solidifies, filter paper settles deep into it.



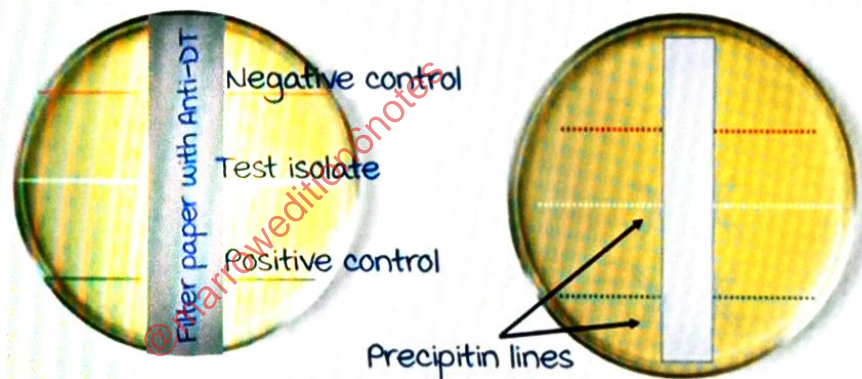
Streak test isolate, negative control (non toxic strain) and positive control (toxic strain) at right angles.



Overnight incubation.



Precipitates formed at the junction where anti - DT and toxin from positive control meets.



Other in vitro toxigenicity tests :

Tissue culture assay, ELISA and PCR (tox gene).

Treatment :

- Droplet precaution using masks till two 24 hours consecutive culture becomes negative.
- Airway management.
- Treat with anti diptheric toxin (blocks toxins unbound to receptors).

Dose depends on disease duration and type.

20,000 - 40,000 units IM/IV in pharyngeal/ laryngeal disease < 3 days.

40,000 - 60,000 units IM/IV in nasopharyngeal disease.

80,000 - 1,20,000 units half IM/ half IV in extensive disease
on presentation/ > 3 days.

+

Penicillin/ erythromycin for 10 - 14 days.

- Age appropriate vaccine.
 - ≤ 7 years : 3 doses at 0, 4, 8 weeks.
 - > 7 years : 3 doses at 0, 6 weeks and 6 months
(1st dose DTap, 2nd & 3rd doses Tdap).

Children's dose of DT : 15 - 25 Lf units.

> 7 years/ adults dose of dT : 1 - 1.5 Lf units.

Post exposure prophylaxis (PEP)

00:37:55

PEP is indicated if :

- most recent vaccine dose < 5 yrs back : Single dose of IM Benzathine Penicillin/ erythromycin 7 - 10 days.
- most recent vaccine dose > 5 yrs back : Antibiotics + DT/dT booster.
- unknown/ incomplete vaccine history : Age appropriate vaccine + antibiotics.

PEP to be stopped if throat swab of contacts becomes negative.

Schick test : In vivo toxin neutralization test.

To evaluate susceptibility/immunity to diphtheria.

Inactivated DT on one arm and small dose of DT (active) on other arm is given.

monitored for reactions.

Erythema + induration : Positive schick test (susceptible).

Negative schick test : Immunity.

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Diphtheroids/Coryneform species :

All other species of Corynebacterium apart from C. diphtheriae.

<i>C. ulcerans</i>	Can produce DT.
<i>C. pseudotuberculosis</i>	Can produce DT.
<i>C. jejuni</i>	Axillary flora, causes septicemia and endocarditis in immunocompromised.
<i>C. urealyticum</i>	UTI and forms struvite stones .

Listeria monocytogenes

00:47:42

Both saprophyte and commensal.

Gram positive rod.

motile at room temperature (peritrichous flagella).

Non motile at 37°C.

This is called **differential motility**.

Also shows **tumbling/ end on end motility**.

Facultative, non capsulated aerobe.

Grows at wide temperatures from 4 - 45°C.

It is a **psychrotroph**, cold enrichment can be used to isolate listeria.

Biochemical reactions :

Catalase positive.

CAMP positive.

Anton's test positive (like sereny test).

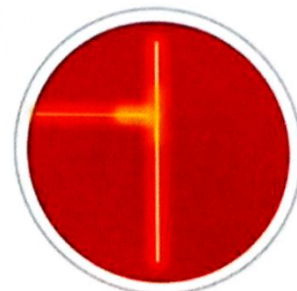
Keratoconjunctivitis in guinea pig :

Positive anton.

Culture :

On blood agar, narrow zone of beta hemolysis.

Selective medium : **PALCAM agar**.



CAMP test

On a blood agar,
streak vertical line :
Staphylococcus aureus.
Horizontal line : Listeria.
Enhanced zones of hemolysis
seen (arrow head) at the
junction.

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Active space

Pathogenicity

00:52:57

Facultative intracellular bacteria (mnemonic: **Some Nasty Bugs may Live Facultatively**).

- **S**almonella.
- **N**eisseria.
- **B**rucella.
- **M**ycobacterium tuberculosis.
- **L**egionella.
- **F**rancisella.
- **L**isteria.
- **Y**ersinia.

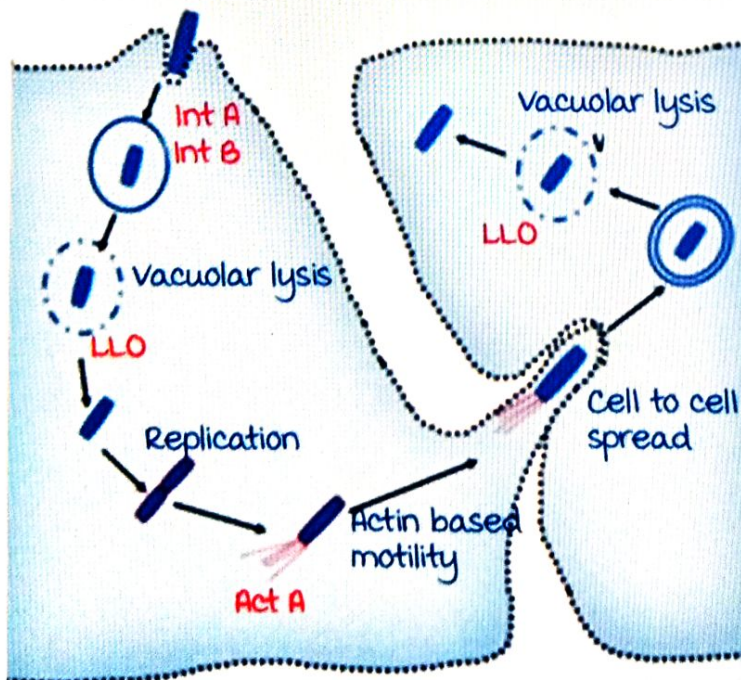
Virulence factors :

Internalins (outer membrane proteins)

↓
Inside endosomes

↓
Listeriolysin - O helps break away from endosome

↓
Act A helps in cell to cell spread



bnvssprasanth7@gmail.com

Active space

Epidemiology :

Saprophyte : In soil, sewage, water.

Animal carriage : Cows, sheep in their GIT.

Humans : 0.5 - 1% as GI commensal.

mode of infection : Ingestion of raw milk, soft cheese, deli meats and refrigerated foods.

m/c serotype causing human GI disease is 1/2a and 1/2b.

m/c serotype causing invasive disease is 4b.

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Listeriosis :

In healthy individuals, febrile gastroenteritis (incubation period 2 days).

In elderly/immunocompromised : meningitis/meningoencephalitis.

Pregnant women have 17 - fold risk of bacteremia leading to chorioamnionitis, spontaneous abortions/still births.

Transplacental infection causes granulomatous infantile septica (skin pustules + multiple visceral abscesses in neonates).

Perinatal transmission from infected birth canal causes neonatal septicemia and neonatal meningitis.

Treatment :

Drug of choice is Ampicillin.

Invasive disease : Ampicillin + Gentamycin.

Allergic to penicillin : Cotrimoxazole.

Erysipelothrix rhusiopathiae

01:02:35

Non motile gram positive rod.

Filamentous forms also seen.

Saprophyte in soil and sea water (fisherman at risk).

Commensal in fish and pathogen in pigs (erysipelas).

At risk occupations :

Fishermen.

Butcher/slaughter house worker.

mode of infection : Traumatic implantation.

Presents with sharply demarcated purplish swellings with itching, burning sensation/throbbing pain ± regional lymphadenopathy.

Non suppurative swelling.

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whale/ seal finger/
pork finger/ fish handler's
disease.

Treatment :

Spontaneous resolution after
3 - 4 weeks.

If required, Penicillin is the drug
of choice.

Other diseases caused :

Erysipelothricosis : Diffuse cutaneous lesions spreading
proximally.

Bacteremia leading to endocarditis and septic arthritis.

Penicillin is the drug of choice.

Active space

ACTINOMYCES AND NOCARDIA

Important gram positive bacilli :

Cory & **Ery** knocked **Back** my **Actor** **List** in **Closet**

Corynebacterium.

Erysipelothrix.

Nocardia.

Bacillus.

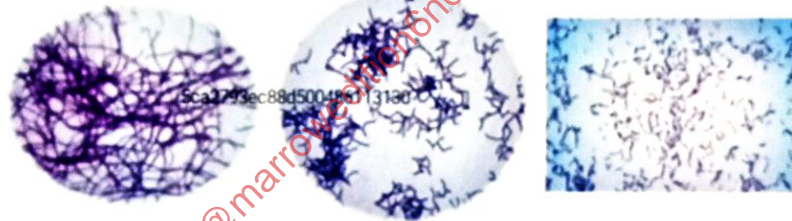
Mycobacterium.

Actinomyces.

Listeria.

Clostridium

Gram positive slender/ filamentous bacteria which typically show branching.



Motility : Non motile.

Capsule : Non capsulated.

Gas requirements : Anaerobes or aerobes.

Anaerobes :

1. Actinomyces.
2. Bifidobacterium.
3. Eubacterium.

Aerobes :

1. Nocardia.
2. Rhodococcus.
3. Actinomadura.
4. Streptomyces.

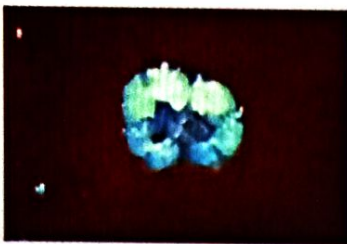
Actinomyces

00:05:31

Anaerobes/ some are microaerophilic & causes actinomycosis.
Acid fast stain : Non acid fast.



Culture : Simple media like
nutrient agar and blood agar
(molar tooth colonies).



Normal habitat : Oral cavity, GI tract & female genital tract.
(Never been isolated in the environment).

most common species : *Actinomyces israelii*.

Actinomycosis

00:08:09

Break in the mucosal barrier → entry into sterile tissue →
provoke inflammatory response → malignancy like hard
nodular lesions associated with sulfur granules → open into
body surfaces or neighboring organs.

It is an unique infection, requires prolonged antibiotic therapy.

Companion organisms usually present in the lesions are
Eikenella, *Aggregatibacter*, *Staphylococci*, *Streptococci*, *E. coli*

Types :

1. Cervico facial :

About 60%, history of dental manipulation/ oral surgery/
head & neck radiotherapy / oral trauma, also associated
with bisphosphonate therapy.

Also called lumpy jaw.

2. Thoracic :

History of aspiration of oral contents (alcoholic/seizures).

Active space



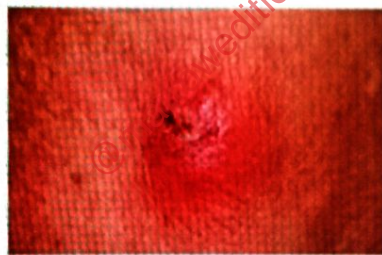
Presents with mass lesions or pneumonia with or without pleural involvement.

3. Abdominal :

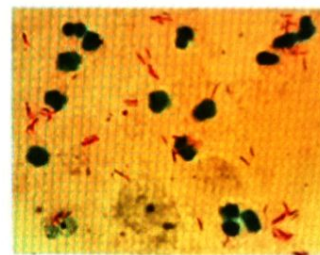
History of appendicitis/ diverticulitis / laparoscopic cholecystectomy. Presents with vague abdominal pain, fever, altered bowel habits.

4. Pelvic :

H/O IUCD, oocyst retrieval, endocervical curettage, etc. Presents with fever, abdominal pain, vaginal discharge.



Nodule



Sinus formed

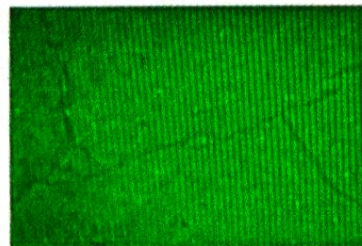
Diagnosis :

Specimen : Tissue biopsy.

Microscopy : Tissue gram stain shows gram positive bacteria with branching.

Culture : most specific.

Nucleic Acid Amplification Test (NAATs) : most sensitive.



Sulfur granules



Histologic appearance of sulfur granules with blue filamentous actinomycetes

Active space

Treatment :

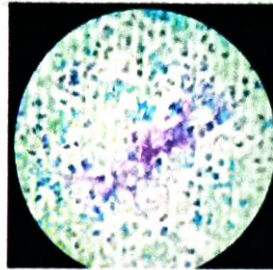
Drug of choice : Penicillin.

Duration 6 - 12 months. Initially IV, f/b oral.

Nocardia

00:19:44

m/c species causing nocardiosis is
Nocardia asteroides complex members.
m/c species causing actinomycetoma
is *Nocardia brasiliensis*.



Partially acid fast filamentous
bacteria showing branching

- Strict aerobe.
- Belong to CMN group
(Corynebacterium, Mycobacterium & Nocardia),
a special group of bacteria that contain
mycolic acids in cell wall.
- Nocardia have 40 - 60 carbon mycolic acids.
- Acid fast stain : Partially acid fast (with 0.5 - 1% H_2SO_4).

Culture :

- Simple medium : Nutrient agar.
- Lowenstein Jensen (LJ) medium.
- Sabouraud Dextrose Agar (SDA).
- Blood agar.
- Thayer martin medium.



Colonies of nocardia

Special property : Uses paraffin as a sole source of carbon
(used in paraffin bait technique to isolate Nocardia).

Epidemiology : Soil saprophyte; Soil, organic matter, sewage
sample (never a commensal).

Nocardiosis

00:25:52

An exogenous infection, seen in both healthy and
immunocompromised individuals

Types :

Cutaneous nocardiosis :

Active space

Acquired by traumatic implantation from an environmental

sample
bnvssprashant@gmail.com

- Seen as cellulitis/ abscess.
- **Sporotrichoid/lymphocutaneous nocardiosis** or also called nodular lymphangitis.

Other causes of sporotrichoid lesions : Sporotrichosis, swimming pool granuloma, cutaneous leishmaniasis, tularaemia

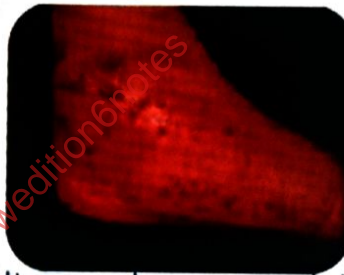


Cellulitis



Typical lymphatic spread

- **mycetoma** : Actinomycetoma.



Actinomycetoma presentation with sinuses and granules

Pleuropulmonary nocardiosis :

Only seen in immune deficient individuals, following inhalation from the environment.

Risk factors :

- Long term corticosteroid therapy.
- HIV patients.
- Haematological malignancies.
- Chronic alcoholism.
- Transplant : Solid organ or hematopoietic stem cell recipients.

Patient presents with pneumonia/lung abscess / cavities formation / pleural effusion or empyema.

In severe immunosuppression, it can disseminate most commonly to the brain, also to eyes, bones, joints & skin.

Diagnosis :

Specimen : Tissue biopsy, sputum, bronchoalveolar lavage.

microscopy : Tissue gram stain shows beaded gram positive filamentous rods.

Cultivation : most specific.

NAATs : most sensitive.

Treatment :

Drug of choice : Cotrimoxazole.

Immunocompetent : 1-6 months sometimes upto 12 months.

immunosuppressed : Cotrimoxazole + Amikacin +

Carbapenem/ 3rd generation Cephalosporin for 6 - 12 months or more if required.

Family Actinomycetes :	
Gram positive, slender bacilli, filamentous, branching.	
Non motile, non capsulated.	
Nocardia	Actinomyces
Aerobe.	Anaerobe.
mycolic acids in cell wall.	Absent.
Partially acid fast.	Non acid fast.
Utilize paraffin as sole carbon source.	Cannot.
Never a commensal.	Commensal in mouth, GIT, vagina.
Soil saprophyte.	Not found in soil.
Nocardiosis is exogenous.	Actinomycosis is endogenous.
DOC : Sulfonamides.	DOC : Penicillin.

MCQs :

Q1 : A homeless, malnourished chronic alcoholic presents with severe headache & dyspnea. Physical examination reveals a disheveled male with poor hygiene. His temperature is 39°C, BP is 110/78 mm Hg, and his pulse is 96/min and regular.

Auscultation of chest reveals absence of breath sounds over the left middle lung fields. A chest X ray confirms left lobar

Active space

pneumonia. Sputum stain reveals partially acid fast bacilli with branching. Which of the following is false about the most likely cause of infection in this patient?

- a) Ubiquitous saprophytes in soil.
- b) Strict aerobe.
- c) It can cause infections in both healthy and immunodeficient.
- d) Infection is endogenous.
- e) Treatment is best done with sulfonamides.

Answer : d.

Infection is exogenous with nocardia.

@marroweditionsnotes

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Active space

CLOSTRIDIUM PERFRINGES AND C. DIFFICILE

General properties

00:01:25

Widely distributed in soil as well as in animal and human GIT.

Normal GI flora : *C. tetani*, *C. perfringens*.

Gram stain :

- Gram positive pleomorphic rods.

Motility :

- motile : Peritrichous flagella, except *C. perfringens*, *C. tetani* VI.

Capsule :

- Non capsulated, except : *C. perfringens* 9 & *C. butyricum*.

Gas requirements :

- Strict anaerobes, except *C. histolyticum* which is the aerotolerant species.

Spores :

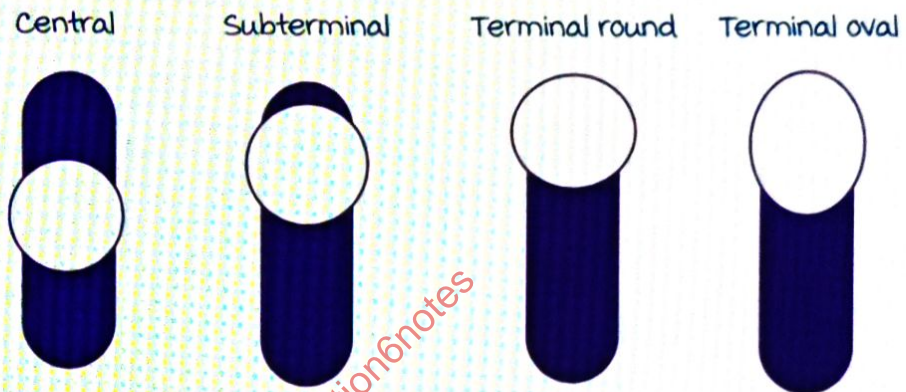
- Bulging spores.
- Formed in soil, culture and in human body, except *C. perfringens*, which doesn't form spores in human body.

Position of spores :

- Central spore : *C. bifermentans*.
- Subterminal spores :
C. perfringens.
C. botulinum.
C. novyi.
C. septicum.

- Terminal and round spores (**drumstick appearance**):
C. tetani.
C. tetanomorphum.
C. sphenoides.
- Terminal and oval spores (**tennis racket appearance**):
C. difficile.
C. tertium.
C. cochlearum.

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Cultivation of clostridium

00:07:08

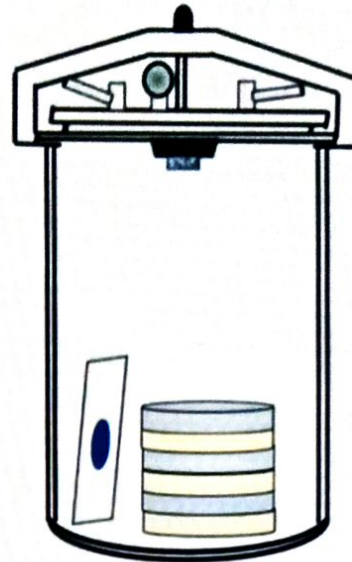
1. **Low redox potential** in the medium (oxygen deficient):

This is achieved by chemicals such as:

- Thioglycolate.
- Glutathione.
- Cysteine.
- Vitamin C.
- Unsaturated fatty acids.
- metallic iron filing.

2. **Strict anaerobic condition**:

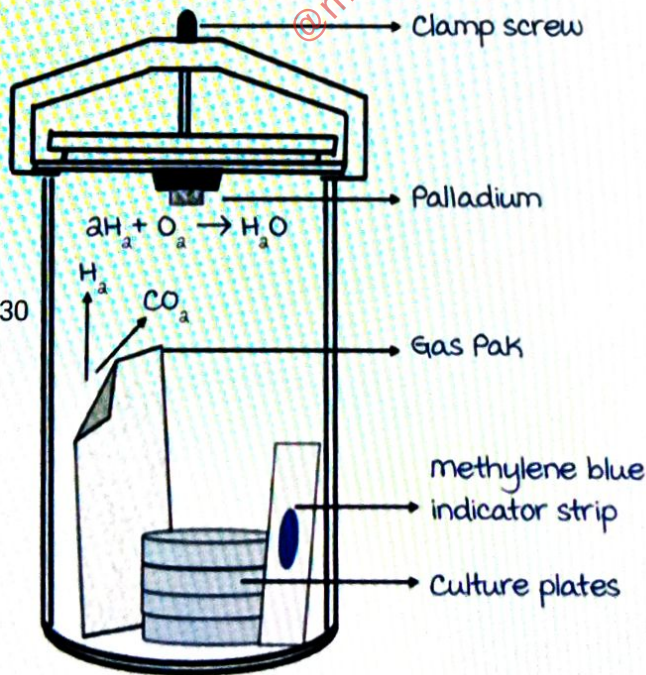
- mcIntosh Fildes' / anerobic jar:
methylene blue indicator inside jar, which on anaerobic condition turns colorless.



- **Anerobic gas pack system :**
Sachet containing chemicals added to it, which forms hydrogen and carbon dioxide on adding water.
The generated H_2 reacts with remaining oxygen to form water droplets.

Palladium : Acts as catalyst in the reaction.

Indicator : **methylene blue.**



- **Sterile petroleum jelly:**
Pour **petroleum jelly** on top of medium.
It prevents diffusion of oxygen.

Active space



medium used for cultivation :

1. Robertson's cooked meat broth :

- mildly proteolytic (black) :
C. tetani.
- uninoculated
- Predominantly saccharolytic (Pink) :
C. perfringens
C. novyi.
C. septicum.
C. difficile.

Black

Uninoculated

Pink



Active space

2. Nutrient agar.
3. Blood agar.
4. Selective media : Blood agar or nutrient agar with polymyxin / neomycin.
5. Pre reduced anaerobically sterile (PRAS) media

Clostridium perfringens

00:14:25

Previously known as *C. welchii*.

Widely distributed in feces, soil, air, and water.

Non motile, capsulated.

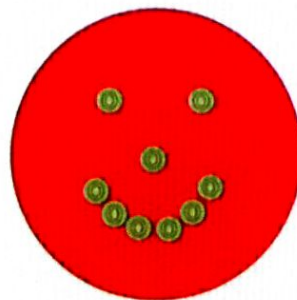
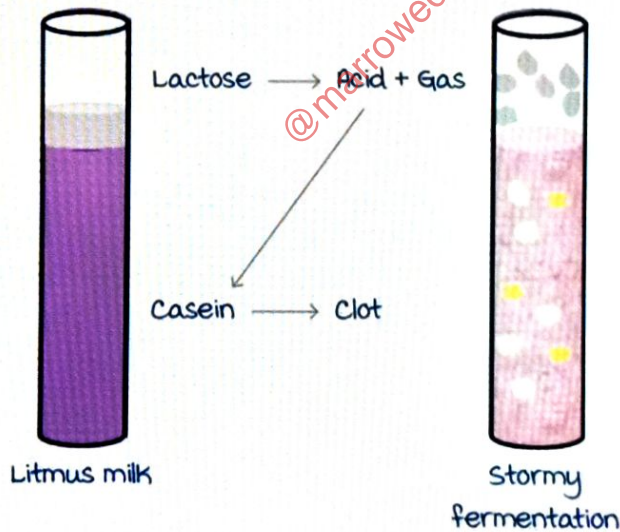
Spores:

- Has sub terminal spores.
- Formed in soil, culture (*Eliker's medium*).
- No spores in human body.

1. Only species which grows at 45°C.

2. Litmus milk: *Stormy fermentation*.

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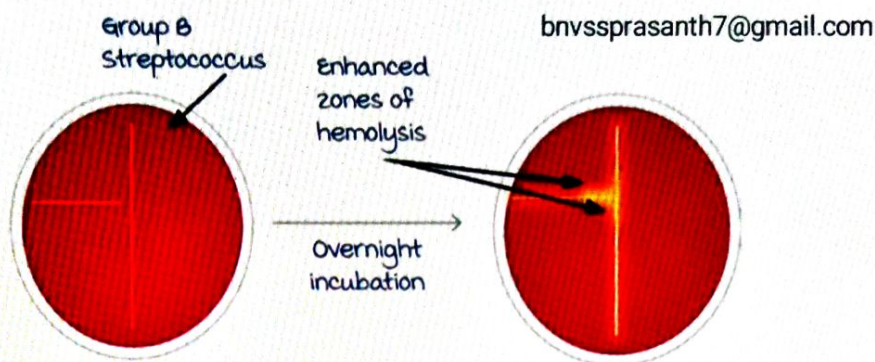
Target hemolysis on blood agar

Active space

3. Blood agar: Target hemolysis.

Narrow zone of complete (θ toxin) hemolysis surrounded by wide zone of incomplete hemolysis (α toxin).

4. Reverse camp test positive.



Streak group B streptococcus (*S. agalactiae*), and at right angle streak clostridium perfringens.

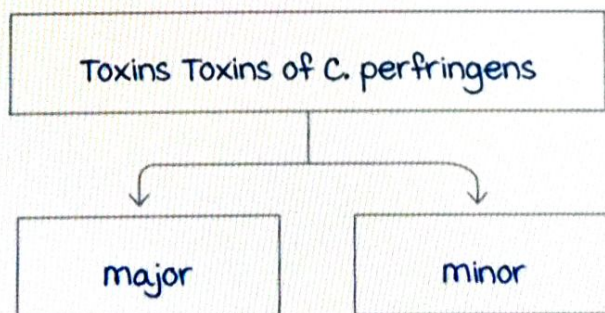
Hemolysis synergizes to produce enhance zone of hemolysis.

Also known as arrowhead hemolysis.

Mnemonic: Clostridium perfringens targeted 45 storms on the reversing CAMP.

Toxins of *C. perfringens* :

Produces at least 16 histolytic toxins.



- major toxin :

Includes $\alpha, \beta, \epsilon, i$

All strains produce α hemolysin.

The other 3 major toxins, β, ϵ, i are lethal and dermonecrotic.

Depending upon the spectrum of other 3 major toxins, *C. perfringens* has been divided into 5 types : A to E.

- minor toxins:

Includes η , μ , θ and so on.

Has **variable action**.

Some are collagenase, DNase, hyaluronidase, neuraminidase.

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Alpha toxin

00:21:20

most important toxin produced (especially in gas gangrene).

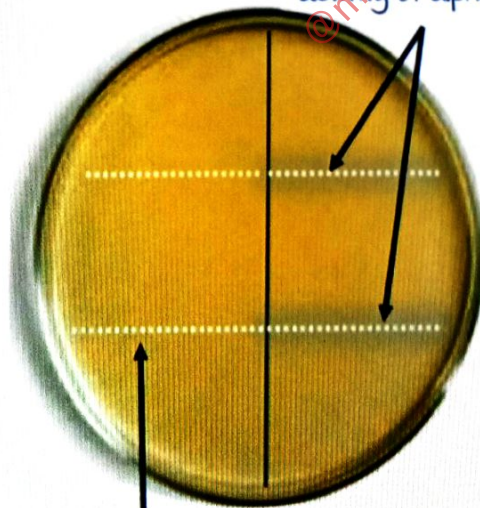
Properties include:

- Lethal.
- **Dermonecrotic**.
- Hemolytic.

Hot cold phenomenon of alpha toxin:

- Also seen in beta hemolysin of *S. aureus*.
- Alpha hemolysin mixed with a suspension of RBC.
- Hemolysis begins at 37°C, but gets completed at 4°C.

Zone of opacification around the colonies due to lecithinase activity of alpha-toxin



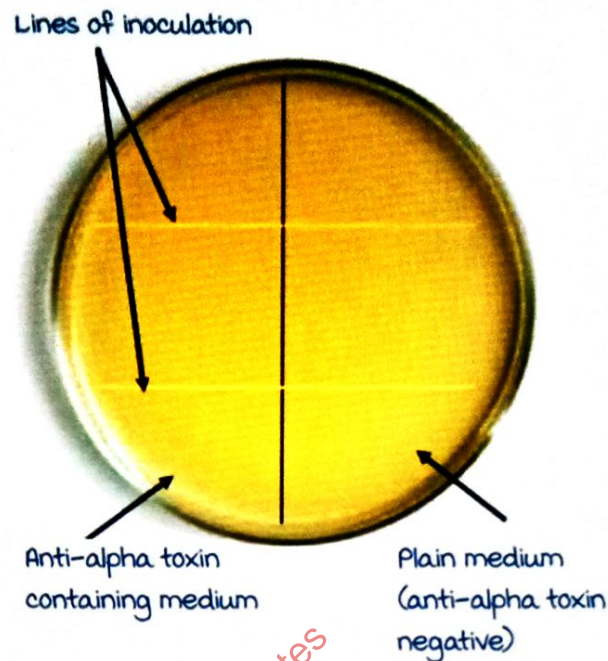
Alpha toxin
neutralized

Lecithinase activity of alpha toxin :

- Tested by **Naegler reaction**.
- Naegler reaction : **In vitro toxin neutralization test**.
- *C. perfringens* produce **lecithinase**.

Active space

- Creates opalescence in the egg yolk media.
- This opalescence gets neutralized, where alpha anti toxin is added.



Alpha toxin has phospholipase activity.
Induces platelet aggregation in humans.

Theta toxin :
Also called as **perfringiolysin**.
Has hemolytic activity.

Gas gangrene

00:29:10

Also called as **anaerobic myonecrosis**.
Life threatening condition with High mortality (50%).
Rapidly progressive infection of **skeletal muscles** caused by clostridia species.
Disseminated toxins from the site of production will cause multi system failure and disseminated intra vascular coagulation.

Causative organism

- **Clostridium perfringens** (80-95% of cases).
- **Clostridium novyi**.
- **Clostridium septicum**.

- Also caused by *C. sordellii*, *C. fallax*, *C. histolyticum* (never by tetani & botulinum). bnvssprasanth7@gmail.com
- most common species causing gas gangrene :
***C. perfringens* type A.**

Pathogenesis :

- entry of spores via **trauma** of any kind (RTA, war wounds, surgery, septic abortion).
- Local vessels get destroyed leading to **ischemia**.
- This will result in formation of **devitalized tissues** at the area.
- The area act as an anaerobic environment for the multiplication of clostridia species.
- In case of **mixed infection** (presence of *E. coli*, *Klebsiella*, facultative anaerobes along with clostridia), these will also utilize the oxygen available, further reducing oxygen levels creating an anaerobic environment.
- In anaerobiosis → multiplication of Clostridia species → Release of **histolytic toxins** → Tissue necrosis.
- Incubation period : 1-3 days.



Discoloration of involved tissue



Hemorrhagic bullae with discolored and edematous skin.

Clinical presentation :

- Severe pain.
- Edema.
- Discoloration of overlying skin (initially **red** → **brown, purple**).

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- **Crepitus** of muscle.
- Foul smelling exudates oozing out from the edges of wound.
- **Toxemia** → Disseminated intravascular coagulation, shock and multi organ failure.

Alpha toxin :

- Intravascular hemolysis (also by theta toxin).
- Aggregation of platelets.

Spontaneous gas gangrene

00:35:10

Also known as **non-traumatic gas gangrene**.

Lack of an obvious portal of entry.

Hematogenous seeding of normal muscle.

GIT portal of entry → **ileitis, diverticulitis, colonic carcinoma**.

Diagnosis :

Specimen collected :

- Tissue bits from the depth of wound.
- Foul smelling exudate oozing out.

Gram stain smear:

- Gram positive rods with **'box car'** appearance.
- No presence of spores.

Culture under anaerobic condition :

Colonies put up for **identification test**.

Treatment :

Surgical exploration and wide debridement.

Parenteral antibiotics:

- IV Penicillin + clindamycin for **2 weeks**.
- Clindamycin : Protein synthesis inhibitor.
Reduce toxin production.
- Penicillin : **kill organism**.

Hyperbaric oxygen :

- Increase oxygen supply to tissues.

- Helps surgeons in marking of exact demarcating lines between normal and gangrenous tissues.

Granulocyte colony stimulating factor :

- Enhance activity of bone marrow.

Anti-gas gangrene serum :

- used earlier.
- Rarely available now.

Clostridial food poisoning

00:40:18

Etiology :

C. perfringens type A : most common cause.

Produce heat resistant spores.

Can survive up to 100°C for 2-3 hours.

Toxin :

Cpe (Clostridium perfringens enterotoxin).

Produced in GIT.

Cause :

Consumption of meat products contaminated with spores of type A.

mode of action :

Pore formation in enterocytes leading to outpouring of water and electrolytes.

Clinical features :

Incubation period : **8-24 hours**.

Presents with

- Abdominal cramps.
- Watery diarrhea.
- Self-limiting (usually in **24 hours**).

Diagnosis :

1. Detection of Cpe toxin in stool :

- ELISA.

Active space

- Latex agglutination test.
- Tissue culture assay.

2. PCR for Cpe gene in stool.

3. Quantitative culture of stool/ food : Diagnosis is made if

- Stool : $\geq 10^6$ /g of stool.
- Food : $\geq 10^5$ /g of food.

Treatment :

- Symptomatic.
- No antibiotics needed.

Necrotizing enteritis / **enteritis necroticans** :

Also called as **pigbel**.

Etiology :

- **Ingestion of pork** contaminated with spores of *C. perfringens* Type C.
- **Heat resistant spores.**

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Toxin:

- Beta toxin formed in GIT \rightarrow Inflammation and necrosis of mucosa.

Clinical features :

- Fever.
- Abdominal cramps.
- Bloody diarrhea.

Diagnosis :

- **Beta toxin** in stool (ELISA).

Treatment :

- **IV Penicillin + metronidazole.**
- Symptomatic treatment : Fluid and electrolytes.

Clostridioides difficile

00:49:28

Leading cause of hospital acquired gastrointestinal

infections.

Spores :

Present in large numbers in **hospital** environment.

GI carriage in community :

- Healthy individual : 1-3%.
- Hospitalized : 20%.

Gram stain :

Gram positive rod with subterminal spores in soil culture and in human body.

motility :

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motile.

Capsule :

Non capsulated.

Toxins of C. difficile :

6-8% strains : Toxigenic.

Causes **Antibiotic associated colitis (AAC)** and severe form, **pseudomembranous colitis (PMC)**.

- Toxin A : **Enterotoxin.**
- Toxin B : **Cytotoxin.**

Action of toxins :

They are **Glucosyl transferases.**

Glucosylate Rho proteins (GTP binding proteins).

Results in the disruption of activation of actin cytoskeleton.

This will lead to death and provokes mucosal inflammation.

Apart from cytoskeletal derangement leading to apoptosis and necrosis of colonic epithelial cells, toxins induce secretion of **IL-8** (chemo attractant).

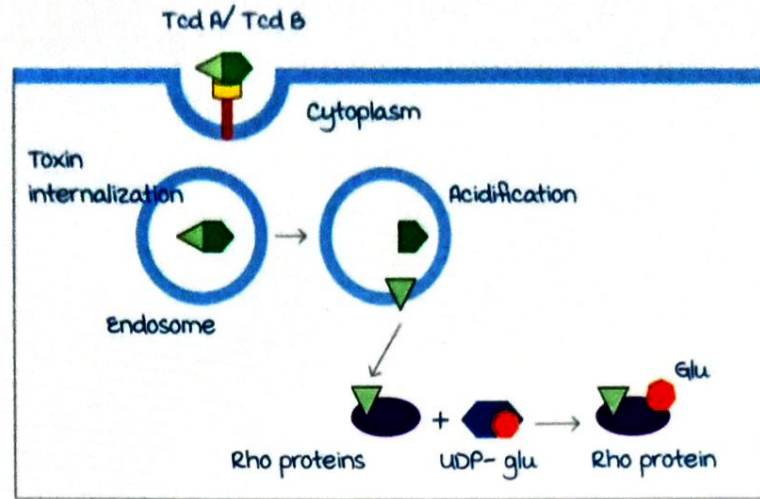
Rho proteins:

Function : Actin polymerization and maintenance of cytoskeletal architecture.

Types :

- Rho A, B, C.
- Rac 1, 2, 3.

- Cdc42.

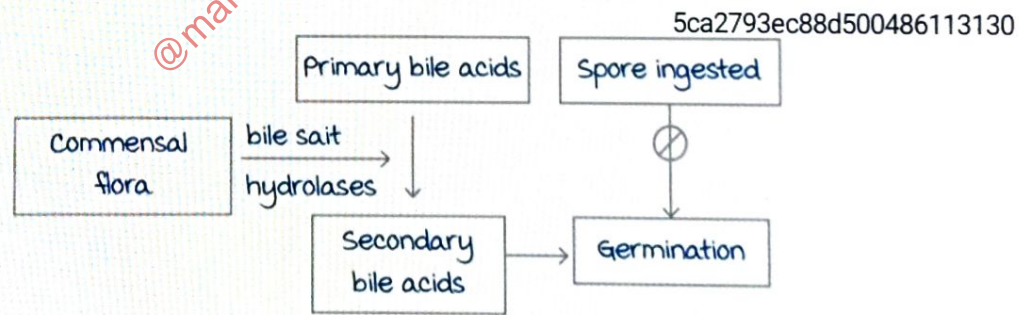


Cytopathic effects :

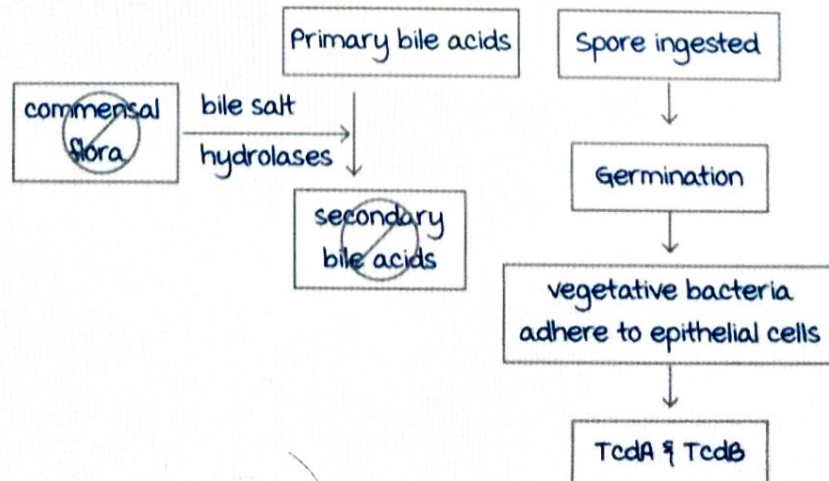
- Breakdown of actin cytoskeleton.
- Loss of cell-to-cell contact.
- Increased epithelial permeability.

Cytotoxic effects :

- Increased ROS.
- Induction of apoptosis.



On Antibiotic therapy



Active space

Normal GI flora has :

- Immunity in GI tract.
- Prevent spread of pathogenic micro-organisms in GI tract.
- Helps in digestion of dietary complex polysaccharides.

Risk factors of C. difficile infection

00:58:54

1. Antibiotics :

- Clindamycin.
- 2° and 3° cephalosporins.
- Ampicillin and amoxicillin.
- Fluoroquinolones.
- Aminoglycosides.
- Carbapenems.

2. Proton pump inhibitors/ H₂ blockers.

3. Elderly.

4. Immuno deficient individuals.

Clinical features :

- Soft to watery diarrhea.
- Fever (25%).
- Abdominal cramps (25%).
- Leucocytosis (25-50%).

Complications :

- Adynamic ileus.
- Toxic megacolon.
- Perforation → Sepsis.
- If these complications occur, it's called **fulminant C. difficile** infection.

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Diagnosis

01:02:22

Diarrhea:

- ≥ 3 unformed stools/24 hour for ≥ 2 days with no other recognized cause.
- Detection of toxin A or B in the stool.

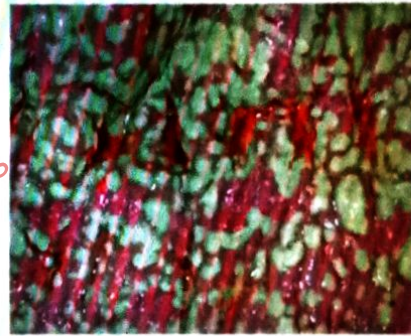
Active space

- Detection of toxin producing *C. difficile* in stool.
- Visualization of **pseudo membranes** in the colon.
100% specific and 50% sensitive also is Expensive.

Detection of toxin in stool :

1. Tissue culture assay / cytotoxic assay :

- **Gold standard** for toxin detection.
- Patient's **stool supernatant** is dropped over a cell line and incubated for **24-48 hours**.
- If stool contains toxin, cytotoxic effects of toxin is visible.
- Test can only be carried out in specialized research labs.
- Time consuming test.



Pseudomembrane

2. ELISA for Toxin A/B on stool :

- Rapid method.
- **Low sensitivity** than tissue culture assay.

3. PCR for genes of Toxin A/B.

Detection of organism in stool :

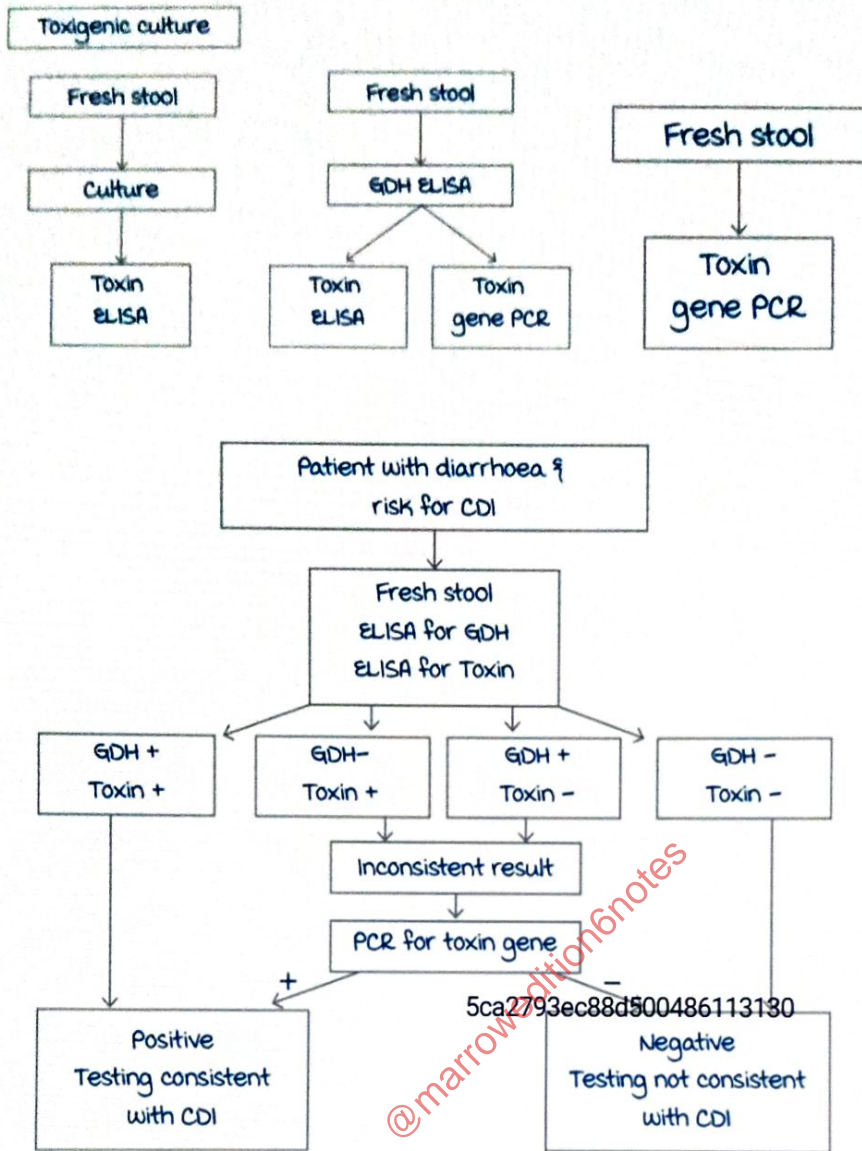
1. Culture of stool on CCFA medium :

- **CCFA medium** : Cycloserine Cefoxitin Fructose Agar.
- **Gold standard** for detecting organism in stool.

2. ELISA for *C. difficile* specific glutamate dehydrogenase in stool.

Recommended method :

Toxicogenic culture.



Treatment of C. difficile infection

01:12:22

Always wear gloves.
 Put on gown before room entry.
 Discard gloves and gowns during room exit.
 Hand hygiene.



1. mild/moderate CDI infection :

- Drug of choice : **Fidaxomicin**.
- Alternative : **Vancomycin** > **metronidazole** for 10 days.

2. Severe CDI infection :

Active space

- Leucocytosis : $> 15000/\text{mm}^3$.
- Creatinine : 1.5 times normal.
- Hypoalbuminemia : $< 2.5 \text{ mg/dl}$.

Drug of choice : oral Fidaxomicin.

Alternative : Oral vancomycin.

3. Fulminant CDI :

- Paralytic ileus.
- Toxic megacolon.
- Septicaemia.

Drug of choice : vancomycin (oral) + IV metronidazole/
tigecycline for 10-14 days.

Severe paralytic ileus : Add vancomycin as rectal retention enema.

Questions

01:18:49

Q. Which organism would most likely cause infection after a disinfection procedure that killed vegetative cells, but did not kill spores?

- Chlamydia.
- Clostridium.
- Escherichia.
- Pseudomonas.

Answer : B.

Q. A middle-aged woman with type 2 diabetes presents to the emergency with a very painful right lower leg. She has a wound on that extremity and stated that she stumbled on a fallen tree limb in her yard. You observe that her lower leg is discoloured and swollen with several areas of crepitus. The abscess fluid was sent to the microbiology laboratory for aerobic and anaerobic culture. The organism grew best on anaerobic cultures both at 37°C and 45°C . Gram stain of the abscess fluid showed gram positive rods. What is the most likely organism?

- Fusobacterium nucleatum.

- B. Bacteroides fragilis.
- C. Clostridium perfringens.
- D. Clostridium tetani.

Answer : C.

Q. True about gas gangrene?

- A. Alpha toxin is the main cause of toxæmia associated with gas gangrene.
- B. Caused by C. intestinale.
- C. Low oxygen tension in tissue is important precondition.
- D. Devitalized tissue predisposes to gas gangrene.
- E. Does not occur if dead tissues is not present.

Answer : A, C and D.

Q. Correct about Clostridium perfringens, except

- A. It produces an enterotoxin.
- B. It produces a double zone of haemolysis on blood agar.
- C. It is the most common cause of antibiotic associated diarrhoea.
- D. It can cause intravascular haemolysis.

Answer : C.

CLOSTRIDIUM BOTULINUM AND C. TETANI

C. botulinum and C. tetani

00:00:37

Gram positive rods.

Characteristic features : Produce spores (very resistant and bulging).

Strictly anaerobic (lacks catalase, peroxidase and SOD).

C. botulinum :

Gram positive rods with sub terminal bulging spores (resists boiling for several hours).

motile (peritrichous flagella).

Non capsulated.

Strict anaerobes.

Botulinum toxin (BoNT) : Category A bioterrorism agent.



Bioterrorism agents : Category A

Mnemonic : Francis is Playing with Bottles that Are Filled with Small Ants.

Francisella tularensis, Plague, Botulinum, Arena viruses, Filoviruses, Smallpox, Anthrax.

- most potent toxin (minimum lethal dose is very small - 1-2 nanogram).
- Exotoxin released only on lysis of bacterium.
- Heat labile.
- 8 antigenic types of neurotoxins: A, B, C, D, E, F, G, F/A hybrid.
- C_a : Enterotoxin, not neurotoxin.
- Human botulism : A, B, E rarely F and F/A Hybrid.
- Chromosomally mediated except C & D → Phage mediated.

most severe disease : Type A.

C. baratii.

C. butyricum.

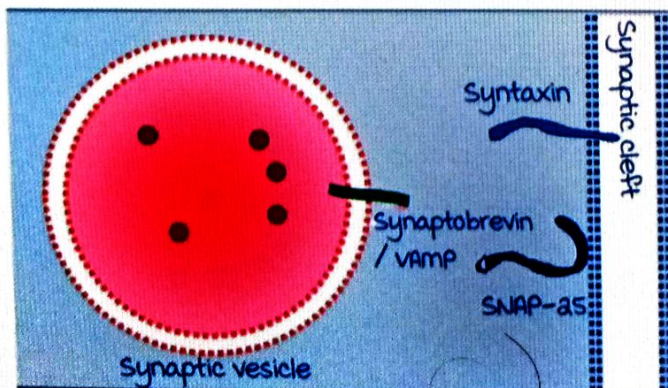
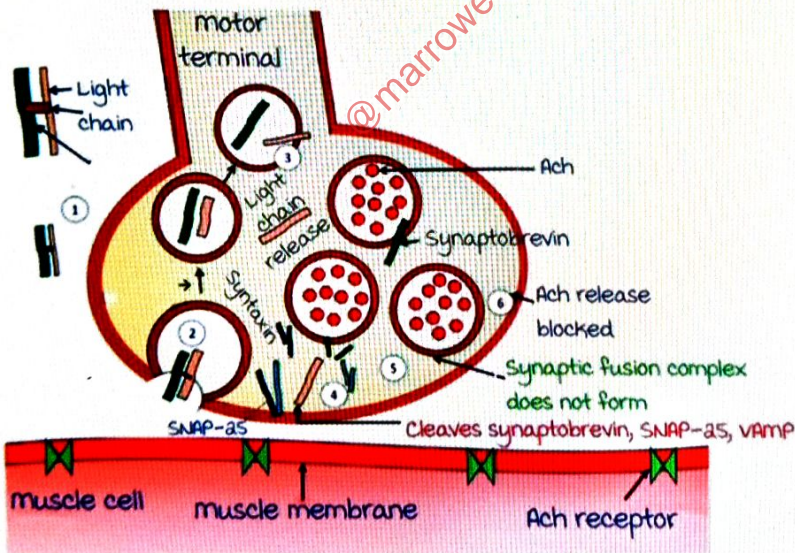
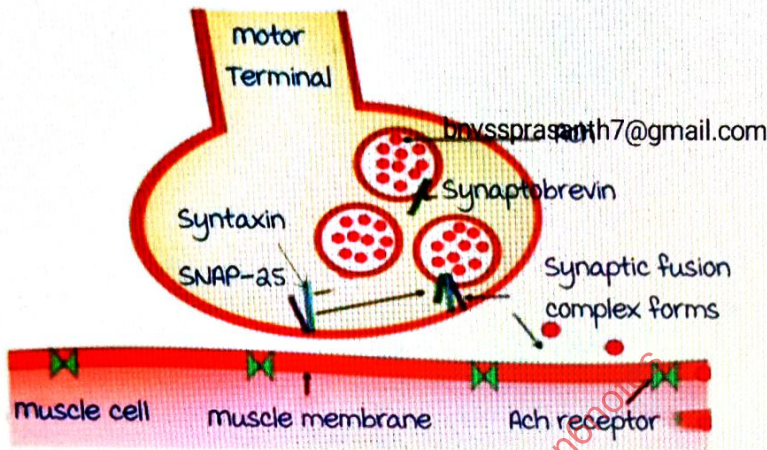
C. argentinense.

Also produce BoNT

Mode of action

00:09:24

Site of action : All peripheral cholinergic synapses.
 Snare proteins : Responsible for Ach release at synapses.
 BoNT proteolyzes snare proteins.
 1,50,000 Dalton pro toxins → Proteolysis → Heavy chain linked by disulfide bond to light chain → Enter blood and lymph nodes → Presynaptic inhibition of ach release.



Synaptobrevin/VAMP : BONT B, D, F, G.

Syntaxin : C1.

SNAP 25 : A, E, F/A hybrid.

Site of action :

- NMJ.
- Autonomic ganglia.
- Parasympathetic post ganglionic terminals.
- Sympathetic post ganglionic terminals (which release Ach).

Clinical features of botulism

00:15:07

Acute onset b/l cranial nerve Palsies.	ANS symptoms :
Dry mouth.	Postural hypotension.
Double Vision.	Urinary retention.
Drooping lids.	Constipation.
Dysarthria.	No sensory deficits
Dysphagia.	No fever.
Dilated pupils.	DTR : Normal
Descending b/l symmetric flaccid paralysis.	mentation : Normal

Cause of death : **Respiratory paralysis.**

mortality rates : **25-70 %** in untreated patients, timely treatment can reduce it to **< 10 %**.

Food-borne botulism

00:19:00

Home preserved foods >>> Industrialized can foods.

Preformed botulinum toxin (heat labile).

Incubation period : **12-36 hours.**

Prodromal symptoms : Nausea, vomiting, diarrhea leading to B/L cranial nerve palsies. Resulting in Descending Symmetric Flaccid Paralysis (DSFP).

Wound botulism :

Wound contaminated with spores.

Skin popping → Intradermal black tar heroin.

Incubation period : **1 week.**

No GI prodromal symptoms.

Infant botulism :

Floppy baby syndrome.

most common type.

H/O ingestion of honey, baby powders or milk powders with spores.

Leading to BONT formed in GIT.

1st symptom : Constipation.

Poor feeding.

Weak cry.

Drooping of eyelids.

Drooling.

Floppiness.



Floppy baby syndrome

Diagnosis of botulism

00:26:19

Screening tool :

- Fever negative.
- Presence of any 1 of these symptoms : Diplopia, dysphagia, dysarthria, change in voice, thick tongue.
- Presence of any 1 of these signs : Ptosis, extraocular muscles palsy, facial palsy, dilated/fixated pupil, DSFP.

Toxin assays :

Specimen : Serum gastric secretion, stool/food

- a) mouse bio assay.
- b) ELISA.
- c) PCR.
- d) mass spectroscopy.

Culture : Anaerobic conditions → Low sensitivity.

Management of botulism

00:30:23

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Supportive care and antitoxin.

untreated → Death in 1-7 days in 25-70%.

Good supportive care → Decreases mortality < 10%.

- Airway management : monitor vital capacity.
- Nutritional support.
- Antitoxin (neutralize toxin not bound to receptors).

In adults : H - BAT (Heptavalent equine botulinum antitoxin).

F/A hybrid → Neutralized by A antitoxin.

Infantile : HBIG : Human botulinum Immunoglobulin.

Wound botulism : Debridement and antibiotics (penicillin G / metronidazole).

Recovery : Slow over several months (max recovery : First 3 months).

Sprouting of new axons → Formation of new synapses.

Therapeutic uses of botulinum toxin :

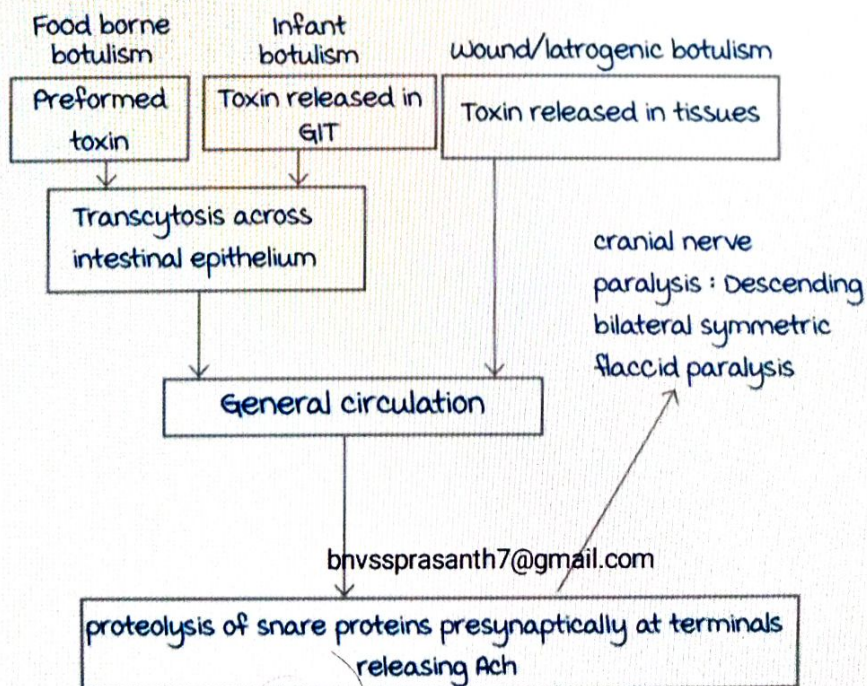
- Blepharospasm.
- Achalasia.
- Strabismus.
- myoclonus. effect starts in 1 week
- Tics. and lasts for 10-12 weeks
- Dystonia.
- Reduces frowning and wrinkles (cosmetic purpose).

Rare types of botulism :

Iatrogenic : Excess dose.

Inhalational : Intranasal use of contaminated cocaine.

Adult botulism of unknown etiology : Inhibit commensal flora.



Active space

Differential Diagnosis

00:39:21

Differential diagnosis	manifestation
Guillain-Barré syndrome	Sensory deficits and areflexia. Rarely begins with cranial nerve dysfunction. Slower onset, ascending paralysis.
miller fisher variant of GBS	Begins with oculomotor dysfunction. Prominent ataxia (not seen in botulism).
Poliomyelitis	Fever + Asymmetric paralysis
myasthenia gravis	Not a fulminant onset, absence of autonomic features
Tick paralysis	Engorged tick. Acute onset of ascending paralysis.

Preformed toxin related diseases :

- Food-borne botulism,
- Staph food poisoning,
- Bacillus cereus emetic food poisoning.

Clostridium tetani

00:43:56

Drumstick morphology of gram positive rods.

Except type II.

10 antigenic types of tetani (type 6 is non motile).

Non capsulated.

Special property :

Swarming best seen in blood agar.

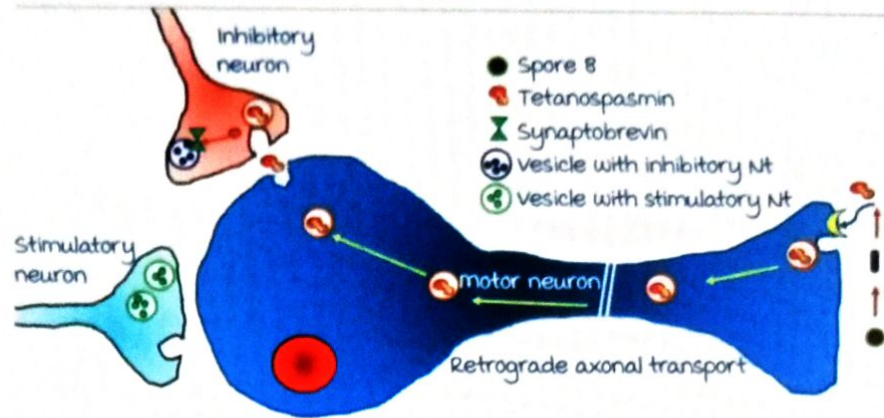
Filde's technique : In subculture can recover pure cultures of

C. tetani.

Gelatin stab culture : Fir tree liquefaction.



Active space



Toxins :

Plasmid mediated : Tetanolysin and tetanospasmins

Tetanolysin : Hemolysin (no role in tetanus).

Tetanospasmin (Tent) : Sole virulence factor of tetani.

1,50,000 dalton pro toxins → Proteolysis by bacterial protease
→ Heavy chain linked by disulfide bond to light chain.

Site of action : Spinal cord and brain stem.

Presynaptic inhibition of release of inhibitory neurotransmitters.

Stimulatory neuron : Ach.

Inhibitory neuron : GABA & glycine.

Uncontrolled stimulation of motor neurons → Responsible for rigidity and spasm.

Tetanus, spores in soil, sewage and animal & human GIT.

Wounds → Spores → Germinates → Secrete Tent.

Incubation period : 3-21 days.

Trismus/lock jaw. }
Risus sardonicus. } → 1st symptoms

Descending symmetric paralysis.

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Trismus : Spasm of
orbicularis oris



Active space

Opisthotonos : Spasm of extensor of neck, trunk and lower limbs.



ANS Symptoms : Hypertension, tachycardia and pyrexia
mnemonic for general tetanus : ROAST.

- Risus sardonius.
- Opisthotonos.
- ANS symptoms.
- Spasm.
- Trismus.

Death (cause of death : Respiratory paralysis)
mortality rates : 15 - 50%.

Generalised tetanus : most common, Generalized with cranial nerve palsies.

In rare cases :

Localized tetanus : At the wound site.

Cephalic tetanus : Chronic otitis media/head trauma. Cranial nerve palsies are present → Generalised tetanus

Neonatal tetanus : unimmunized mother → umbilical stump contamination.

mortality : 80 - 90%.

A.K.A 8th day disease.

Diagnosis

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00:57:20

mainly clinical.

Culture under anaerobic condition : mouse bio assay of toxin.

Management :

Supportive care + Antitoxin + Wound care + Antibiotics + Toxoid.

Supportive care : Quiet room, sedate patient, muscle relaxant, ventilatory and nutritional support.

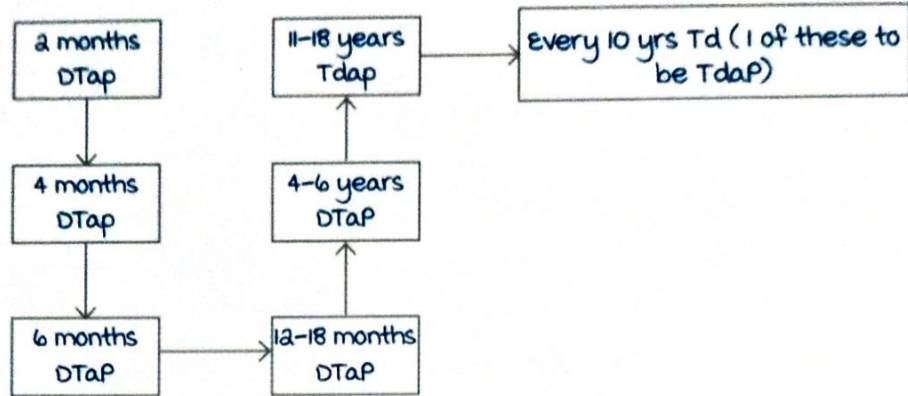
Antitoxin : Tetanus **IG IM/IV 500-3000 units.**

Antibiotics : metronidazole IV X 7 days/penicillin IV X 7 days.

Toxoid : Start 1st dose of TT at separate site from IG

< 7 years : **0, 4, 8 weeks** and > 7 years : **0, 4 and 6 months.**

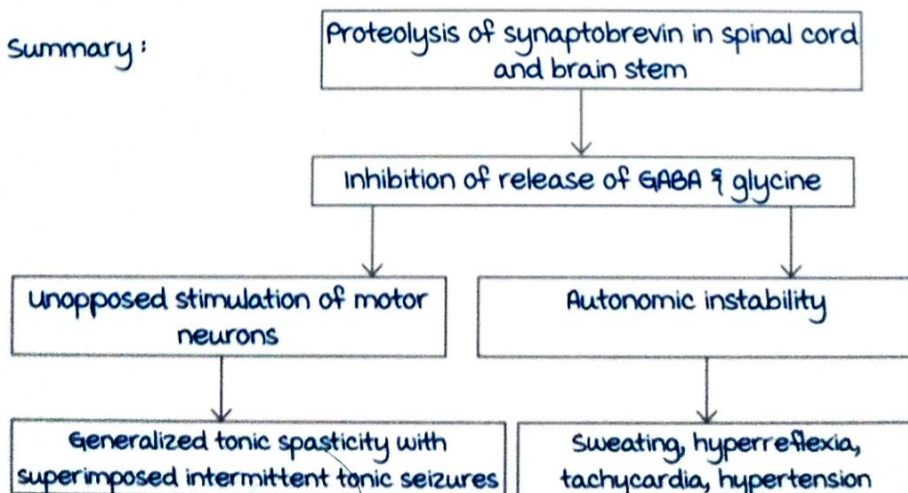
Pre-exposure tetanus prophylaxis



Post exposure prophylaxis :

Status	Clean, non penetrating minor wounds	All other wounds
Complete course of TT/ booster in last 5 yrs	Nothing except wound toilet	Nothing except wound toilet
Complete course of TT/ booster > 5 yrs ago but < 10 yrs ago	Toxoid - 1 dose	Toxoid - 1 dose
Complete course of TT/ booster > 10 years ago	Toxoid - 1 dose	Toxoid - 1 dose + HTIG
Not completed TT course or immunity status unknown	Toxoid complete course	Toxoid complete course + HTIG

Summary :



Active space

Cases :

A 19-year old woman is brought to the emergency department by her boyfriend because she has had worsening dry mouth, double vision, and slurred vision over the past hour. Her boyfriend says that she had abrupt onset of nausea and vomiting, abdominal cramping, and diarrhoea 3 hours ago. 24 hours ago, the patient and her friends attended a country fair where she ate many different types of food; she says that several of her friends have similar symptoms. Botulism is suspected, and the patient is admitted to the hospital. When closely monitoring this patient, serial measurement of which of the following is the most important because of the progressive paralysis that accompanies this conditions ?

- A. Blood pressure.
- B. Sensation.
- C. Vital capacity.
- D. Temperature.

How are the clinical pictures of foodborne and of wound botulism different ?

- A. Paralysis.
- B. Urinary retention.
- C. Cranial nerve involvement.
- D. Gastrointestinal symptoms.

A 45 year old female executive goes to a cosmetic surgeon with the complaint of frown lines on her forehead which she feels are negatively affecting her appearance.

Rather than undergoing surgery, she opts to try the botox injection. What is the mechanism of action of this toxin

- A. It ribosylates Gs.
- B. it inhibits glycine and GABA.
- C. it is a lecithinase.
- D. It blocks release of acetylcholine.
- E. It ribosylates eukaryotic elongation factor-2.
- F. It is a super antigen.

MYCOBACTERIUM

General characteristics

00:00:10

Criteria :

mycolic acid present in cell wall.

Acid fast.

High guanine & cytosine content in the genome.

Characteristics :

Gram positive bacilli.

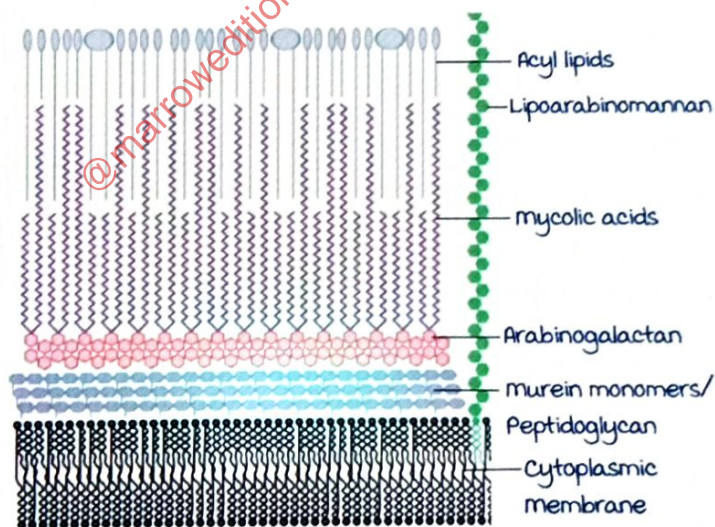
Non motile.

Non sporing.

Non capsulated.

Strict aerobes except mycobacterium bovis which is micro aerophilic.

Cell wall of mycobacterium :



Acid fast stain : ZN Stain.

Introduced by Paul ehrlich.

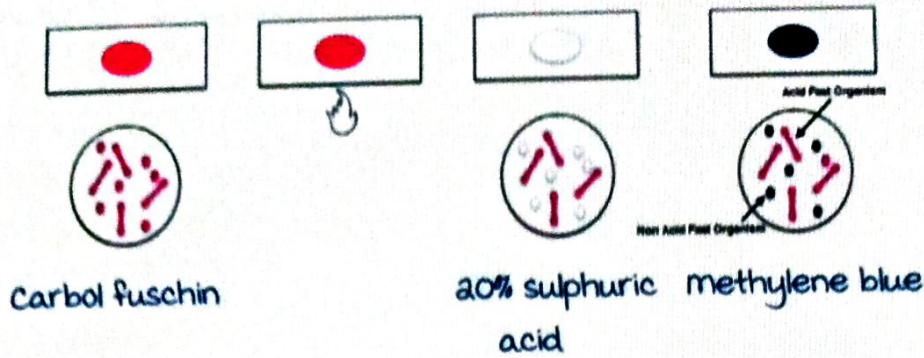
modified by ziehl neelsen.

Active space

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Microbiology • v4.0 • Marrow 6.0 • 2022

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make the smear of sample culture on a clean microscopic slide and heat fix it.

Pour the smear with **carbol fuchsin**.

Heat gently for 3 to 5 minutes.

Allow to stand for 5 minutes & wash it off gently under running tap water.

Add **20 % sulphuric acid** & leave for 1 to 2 minutes (for decolorisation).

wash under running water.

Add **methylene blue dye**, leave it for 2 to 3 minutes & then wash under water.

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Air dry & examine under oil immersion lens.

Acid fast bacteria : **Reddish pink in colour.**

Non acid fast : Blue in colour.

Atypical Mycobacteria

00:05:02

Also known as **tubercloid mycobacteria/non tubercle mycobacteria/mycobacteria other than tubercle bacillus (MOTT).**

Also known as **environmental mycobacteria** (soil & water saprophytes).

No human to human transmission.

mainly causes diseases in individuals with **deficient cell mediated immunity** (HIV patients with CD4 less than 50, malignancy, transplant recipients).

Low grade tuberculin reaction.

False positive sputum smears as they are acid fast with 20 % sulphuric acid same as mycobacterium tuberculosis.

When **3 % acid alcohol** (3 % HCl in 95% alcohol) is used,

mycobacterium tuberculosis retains the color as they are both acid & alcohol fast whereas atypical mycobacteria are only acid fast.

Biochemical reactions :

	Atypical	MTBC
Catalase test	+++ bnvssprasanth7@gmail.com	+
Peroxidase test	-	+
Arylsulfatase test	+	-
Niacin test	-	+

Cultivation

00:11:54

LJ medium :

Forms colonies within 3 to 8 weeks, except rapid growers (5 to 7 days).
Orange red pigment +.



Classified based on Runyon's classification :

Based on rate of growth & pigment production.

Classified into 4 types :

1. **Photochromogens :**
Produces pigment only on exposure to light.
Examples : *M. marinum*, *M. asiaticum*, *M. simiae*, *M. kansasii*.
2. **Scotochromogens :**
Produces pigment in both light & dark.
Examples : *M. szulgai*, *M. scrofulaceum*, *M. goodii*.
3. **Non photochromogens :**
Do not produce pigments.
Examples : *M. abscessus*, *M. fortuitum*, *M. mucogenicum*, *M. chelonae*, *M. smegmatis* & *M. phlei*.
4. **Rapid growers :**
May or may not produce pigments but they form colonies in 5 to 7 days.
Examples : *M. abscessus*, *M. fortuitum*, *M. mucogenicum*, *M. chelonae*, *M. smegmatis* & *M. phlei*.

Pathogenicity

00:17:29

1. Granulomatous disease of the lung :
Resembles tuberculosis.
most common cause : **MAC**.
2nd most common cause : **m. kansasii**.
2. Disseminated granulomatous disease :
most common cause : **MAC**.
2nd most common cause : **m. kansasii**.
3. Skin & soft tissue infections :
 - A. Post surgical/injection/traumatic abscess :
most common cause : **Rapid growers**.
 - B. Swimming pool granuloma :
Also called as **fish tank granuloma**.
Caused by fish tubercle bacillus (**m. marinum**).
Nodule → ulcer (few acid fast bacilli).
Ulcerative lesion show **sporotrichoid spread**.
 - C. Buruli ulcer :
Also called as **Bairdsdale ulcer**.
Caused by **m. ulcerans**.
Trauma → Indurated nodule (after few weeks).
↓
Indolent ulcer rich in AF bacilli.
Immunoreactive phase
↓
Heal with disfiguring scars.
4. Lymphadenitis :
most common cause : **MAC**.
2nd most common cause : **m. scrofulaceum**.



Treatment :

Resistant to anti tubercular drugs.

m. kansasii/MAC : Treated with combination of INH +
Rifampicin + Ethambutol (HRE).

Antibiotic sensitivity done first.

Drugs which are effective include **rifabutin, clofazimine, newer macrolides** (given in combination).

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Species with immunomodulating properties : *m. vaccae*,
m. w (renamed as *m. indicus pranii* : used as vaccine for
prophylaxis of leprosy in India).

Only species producing exotoxin mycolactone : *m. ulcerans*.

Species which is scotochromogen at 37°C & photo chromogen
at 28°C : *m. szulgai*.

Tap water scotochromogen : *m. gordonae*.

most common rapid grower causing pulmonary disease :
m. abscessus.

Mycobacterium leprae

00:27:08

Also called as Hansen's bacillus (Hansen's disease).

Acid fast : 5% sulphuric acid.

Live *m. leprae* : Uniform staining.

Dead *m. leprae* : Irregular staining.

Cultivation :

Non cultivable on cell free media.

Obligate intra cellular.

Grows in :

Foot pad of mice (30°C).

9 banded armadilloes (*Dasypus novemcinctus*) :

Better experimental animal.

Unlimited replication.

40% of them develop leprosy (similar to humans).

Present only in south America.

Other animals used :

Hairy armadilloes.

Indian pangolin.

Slender loris.

Chimpanzees.

m. tuberculosis complex (MTBC) members :

members	Reservoir	
<i>m. tuberculosis</i>	Humans	Antigenic
<i>m. africanum</i>		TB
<i>m. canetti</i>		

m. bovis	Cattle	Zoonotic TB
m. caprae		
m. microti	Voles	
m. pinnipedi	Seals & Sea lions	
m. mungi	Banded mongoose	
m. orygis	Oxyxes	
m. surricatae	meer cats	

Diagnostics in TB

00:33:40

I. microscopy :

Sensitivity : $\geq 10^4$ bacteria/ml.

Stains used :

ZN Stain.

Kinyoun stain :

Also known as **cold stain/gabbet stain.**

No step of heating.

The phenol concentration in carbol fuschin (basic fuschin in phenol) is increased to avoid the step of heating.

Acid fast : **20 % sulphuric acid.**

Advantages of microscopy :

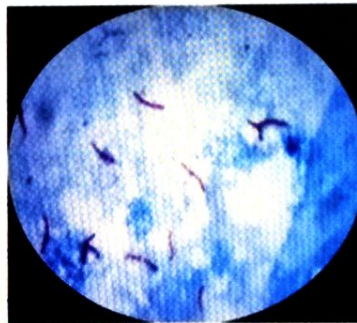
Rapid method (< 30 minutes).

Cheap method.

Disadvantages :

Poor sensitivity.

No differentiation of live from dead bacteria.



Sensitivity can be increased by **fluorescent dyes.**

Dyes include :

Auramine O.

Rhodamine B.

Both can bind to **mycolic acid.**

Advantages :

Increased sensitivity than ZN stain.

One fifth of the time is required in fluorescent microscopy when compared to other staining methods.

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Disadvantages : Expensive.

Homogenization & decontamination :

Petroff's method :

WHO recommended method

4% of sodium hydroxide is used

Equal amounts of sputum & chemical is taken.

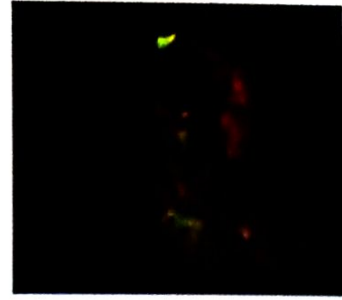
manually shake test tube for 15 to 20 minutes.

Centrifuge the tube at 3000 rpm for 15 to 20 minutes.

Discard the supernatant in phenol & take the lower 1 ml of the sediment.

The sediment is neutralized by $N/10$ HCl & then used for culture.

Instead of 4% NaOH, 2% NaOH + N acetyl L cysteine (NALC) can be used.



Cultivation

00:41:47

a. Cultivation :

Diagnostic gold standard.

Sensitivity : 10 to 100 bacteria per ml of specimen.

Generation time of *M. tuberculosis* : 14 to 15 hours.

Strictly aerobic condition needed.

0.5 % of glycerol & sodium pyruvate, enhance the growth of *M. tuberculosis*. bnvssprasanth7@gmail.com

0.5 % glycerol inhibits *M. bovis* (micro aerophilic).

Solid culture media :

medium	Examples
Egg containing	LJ medium, Dorset egg medium & petragni medium
Blood containing	Tharshi's medium
Serum containing	Loeffler's medium
Potato containing	Pawlowsky medium
Agar containing	middle brook 7H 10/ middle brook 7H 11 medium

LJ medium :

Lowenstein jensen medium.

Contains whole eggs, mineral salt solution, asparagine, glycerol and malachite green.

Dorset egg medium :

LJ medium without glycerol.

m. tuberculosis :

Take about 3 to 8 weeks to grow.

Eugonic growth.

Rough, tough & buff colonies.

m. bovis :

Smooth, shiny & white colonies.

Liquid culture media :

Rapid growth is present (10 to 20 days).

Types :

Middle brook 7H 9.

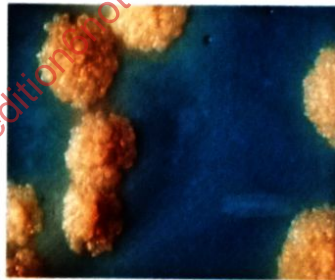
Middle brook 7H 12.

Duo's medium.

Sula's medium.

Souton's medium.

Proskauer & Becks medium.



On liquid medium, they form a **surface pellicle**.

In case of virulent strains : **Serpentine chords of growth** are seen (due to virulence factor : **Cord factor**).

Cord factor is useful in intracellular survival.

Cord factor is one of the mycolic acid present in the cell wall of MTB.

Automated methods : ^{bnyssprasanth7@gmail.com}

Turn over time : **8 to 14 days**.

medium used : middle brook 7H 9/ 7H 12.

method	Principle
Bactec 460 TB	Automated radiometric method. Obsolete method.
Bactec MGIT	Automated fluorometric method.
BACT ALERT	Automated colorimetric method.

VersaTrek	monitor pressure changes in the medium with gas consumption/gas liberation
-----------	--

Advantages :

Detection of live bacteria.

Disadvantages :

Expensive.

Molecular methods

00:57:40

Takes < 1 day.

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Types :

Hybridization probe : Line probe assay.

NAAT's :

Can detect 1 bacterium/ml of specimen.

Examples include :

LCR.

TMA.

SDA.

Real time PCR.

Target amplified : IS6110 (specific for MTB).

Another sequence is IS1081.

Advantages :
Highly sensitive.

Disadvantages :

Cannot differentiate between live or dead bacteria.

Expensive.

mycobacteriophage based tests :

- Also called as fast plaque assay.
- Takes around 5 to 7 days.
- Specimen → Phages added.

Virucidal solution added



Virucidal solution neutralized.

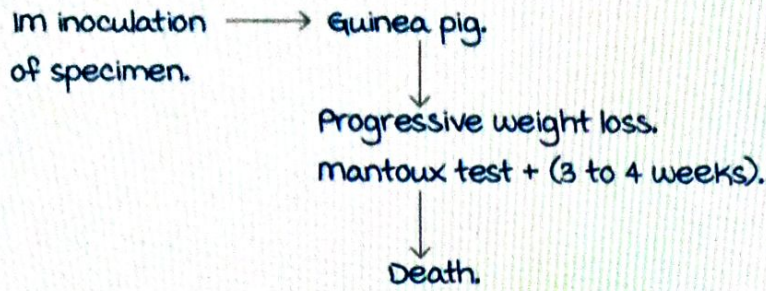


Plated on a lawn culture of *m. smegmatis* (sensor cells).



Plaques on lawn culture.

3. Animal pathogenicity tests :



Antibiotic sensitivity tests

01:04:26

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4. Antibiotic sensitivity tests :

Disc diffusion method is not used for sensitivity testing of MTB.

Conventional/solid medium based methods :

A. Absolute concentration method :

- LJ medium with serial concentration of ATD's is added with standard inoculum of test.
- Resistance is determined by the lowest concentration of the drug that inhibits growth.

B. Resistance ratio method :

- 2 sets of LJ medium, one with standard inoculum of test bacterium & the other with standard inoculum of lab strain (H37 RV).
- Determine the MIC of both & the ratio between them will tell about the resistance.

C. Proportion method :

WHO recommended :

- 2 sets of LJ medium, one is drug free & the other is having fixed drug concentration.
- Serial dilutions of standard inoculum are added, and the number of colonies formed is counted to determine the resistance.

Liquid medium based methods :

A. MODS :

microscopic observation of drug susceptibility.

uses a liquid culture medium.

Report obtained in 5 to 10 days.

B. Automated methods :

MGIT, BACT ALERT.

molecular methods :

A. NAAT : To amplify the specified gene mutation.

B. Real time PCR based :

Results within 90 to 120 minutes.

Detects MTB.

Simultaneously detects rifampicin resistance.

95 % of rifampicin resistance is due to mutation in RRDR region of rpoB gene. 5ca2793ec88d500486113130

• Xpert MTB/RIF assay/CBNAAT :

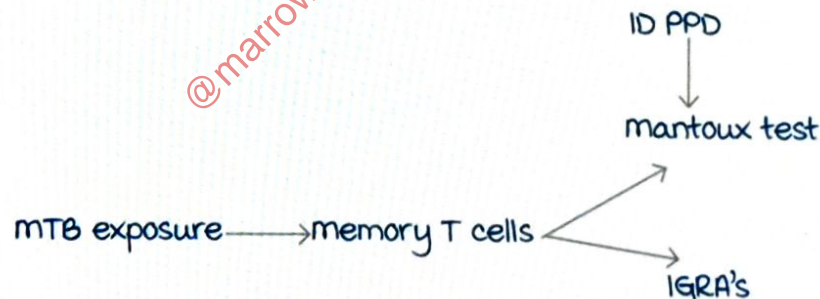
Target sequences amplified : IS16110 & RRDR of rpoB.

• Xpert MTB/RIF Ultra assay :

Target sequence : IS16110, RRDR of rpoB & IS1081.

Screening tests for infection with MTB

01:13:44



Mantoux test :

- Also known as tuberculin/pirquet test.
- 5 tuberculin units of purified protein derivative is injected intradermally in the forearm .
- After 48 to 72 hours, measure the diameter of induration perpendicular to the long axis of forearm.

Disadvantages :

Positive in BCG vaccinated individuals.

No differentiation of latent TB infection from active TB.

5. Interferon gamma release assay :

In vitro test.

Whole blood of the patient is mixed with antigens specific to mTB.

Antigens like **ESAT 6**, **CFP 10** or **TB 7.7** are added and left overnight.

Amount of IFN gamma released from the memory T cells is measured.

Types :

- Quantiferon gold in tube.
- T spot TB.

Advantages :

Negative in BCG vaccinated.

Disadvantages :

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No differentiation of latent infection from active TB.

6. **MPB64 skin/transdermal patch test :**

Diagnosis of active TB.

Torii patches dipped in **MPB 64** antigen is applied to the forearm of the patient.

After **48 to 72 hours**, patch is examined to look for erythema and induration.

7. **Lipoarabinomannan assay**

These are **lateral flow assay**/immunochromatographic test.

Lipoarabinomannan antigen released into blood and excreted through urine in active TB.

Advantages :

Good sensitivity in HIV patients with active TB.

Disadvantages :

Poor sensitivity in healthy individuals with active TB.

Typing of MTB

01:21:30

useful in :

Tracing transmission.

Distinguishing exogenous reinfection from endogenous reactivation.

Types :

Phenotypic methods :

Phage typing.

Active space

Serotyping.

Bacteriocin typing.

Genotypic methods :

Spoligotyping.

MIRU VNTR (mycobacterial interspersed repeat units variable number of tandem repeats).

Nucleic acid sequencing (best method).

MCQ's :

Q. XDR TB is defined as :

- A. Resistant to two most effective first line therapeutic drugs, isoniazid and rifampin irrespective of resistance to any other drug.
- B. MDR with resistance to fluoroquinolones & at least 1 of the three injectable second-line drugs (amikacin, kanamycin, or capreomycin).
- C. MDR TB that is resistant to either one of the injectable second line drugs or second line therapeutic fluoroquinolones.
- D. Strain resistant to more than one drug (excluding co resistance to INH and rif).

Q. Gene/s amplified in Xpert MTB Rif ultra assay :

- 1. KatG gene.
- 2. IS 6110.
- 3. IS 1081.
- 4. oxyR gene.
- 5. RRDR of rpoB gene.

- A. 1 and 2.
- B. 2 and 3.
- C. 2, 3 and 4.
- D. 2, 3 and 5.

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Q. Pyrazinamide resistance in mycobacterium tuberculosis is due to mutation in which of the following genes?

- A. rpoB.
- B. pncA.
- C. gyrA.
- D. etnB.

Drug resistance	Gene mutation
H	Kat G (most common). inhA, ahpc, oxyR & kasA
R	rpoB gene (RRDR).
Z	pnc A
E	Emb B

Q. Which of the following statements about the PPD and the tuberculin skin test is most correct?

A. A positive PPD skin test result implies that a person is immune to active tuberculosis.

B. Persons immunized with BCG rarely, if ever, convert to positive PPD skin test results.

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C. The intradermal skin test is usually read 4 hours after being applied.

D. A positive tuberculin test result indicates that an individual has been infected with *M. tuberculosis* in the past and may continue to carry viable mycobacteria.

Q. Select the odd one out:

A. *M. abscessus*.

B. *M. ulcerans*.

C. *M. fortuitum*.

D. *M. chelonae*.

Q. Absence of which locus in the vaccine strain *M. bovis* Bacille Calmette Guerin (BCG) has been shown to be a key attenuating mutation?

A. RD1.

B. sigC and sigH.

C. leuCD.

D. panCD.

Q. Which of the following anti tubercular testing method uses a broth medium? (multiple correct options.)

A. MODS.

B. Resistance ratio.

C. Proportion method.

D. MGIT.

E. Line probe assays.

Active space

NEISSERIA AND MORAXELLA

Galen in 180 AD introduced the word gonorrhoea (flow of seeds). He used it to describe urethral discharge.

Neisseria meningitidis can be a normal flora in the nasopharynx. It renders immunity being a commensal for months.

But the invasive form can cause meningitis & meningococemia.

Moraxella had multiple other names earlier like micrococcus, neisseria, branhamella.

Gram negative cocci

00:01:46

Mnemonic : VENM (Venom)

Veillonella (Normal flora, gram negative diplococcus).

Neisseria.

Moraxella.

Neisseria :

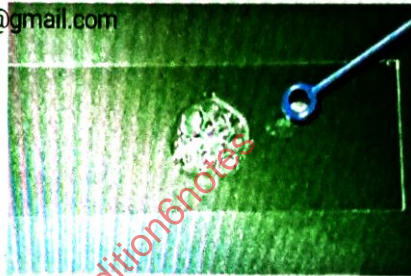
	Pathogenic	Non-pathogenic
Species	<i>N. meningitidis</i> , <i>N. gonorrhoeae</i>	<i>N. flava</i> , <i>N. subflava</i> , <i>N. sicca</i> , <i>N. lactamica</i> mainly seen in oral cavity and upper respiratory tract.
Growth temperature	35 to 37°C	22 to 37°C
Growth on simple media	Do not grow (Fastidious)	Growth seen
Growth on selective medium	Growth seen	Do not grow
CO ₂ requirement	Capnophiles (need CO ₂)	Not required

General characteristics of pathogenic Neisseria :

- Gram stain : Gram negative diplococci (seen in pairs).
N. meningitidis : Half-moon/lens shaped
N. gonorrhoeae : Kidney/bean shaped } cannot be seen
- Motility : Non-flagellated, twitching motility (type 4 pili).
- Gas requirement :
N. meningitidis : Strict aerobe.
N. gonorrhoeae : Facultative anaerobe.
 Both are **capnophiles**.
- Capsule : *N. meningitidis* : Present (13 capsular antigens).
N. gonorrhoeae : Absent.

Biochemical tests privssprasanth7@gmail.com

- Catalase : Positive.
- Oxidase : Positive.
- Sugar utilization :
 Glucose by all species.
N. meningitidis : also use **maltose**.
N. gonorrhoeae : Only **glucose**.



Positive



Negative

Oxidase test :

Oxidase reagent is put on a filter paper and bacterial colonies are streaked with a wooden stick.

Purple color : Positive.

No color : Negative.

Catalase test :

H_2O_2 is added to the colony suspension.

If bubbles are seen, it is

catalase positive.

Cultivation :

Simple medium : No growth.

Transport media : **Stuart's medium**, **Amie's medium**.

Amie's medium is black in color because of added charcoal to absorb toxic metabolites.



Stuart's

Amies

Enriched medium : Blood agar, chocolate agar.

Selective medium : used when swab is from nasopharynx/ genital swab.

To inhibit commensal growth.

Thayer martin medium : Chocolate agar with 3 antibiotics, which are Vancomycin (inhibits gram positive), Colistin (inhibits gram negative) and Nystatin (inhibits fungal growth).

modified Thayer martin medium :

Chocolate agar with antibiotics.

(mnemonic : Very Typically Cultures Neisseria).

Vancomycin.

Trimethoprim.

Colistin.

Nystatin.

New York city medium : modified thayer martin + translucent medium instead of chocolate agar.

N.meningitidis

00:12:48

Typing (for epidemiological studies) :

Capsular serogroups : 13.

Predominantly only 6 capsular serogroups are responsible for human diseases.

A : mainly epidemic (most common in India).

B : Epidemic and outbreaks.

C : Outbreaks.

W, X, Y : Sporadic illness.

Pathogenicity factors :

- Capsular antigen (anti phagocytic).
- Lipooligosaccharides (stunted o antigen).
- **Type 4 pili** (helps in adhesion).
- Outer membrane proteins (por A & B) for adhesion.
- Siderophores (acquire iron from transferrin/lactoferrin).
- IgA₁ protease (protects from mucosal IgA).

Epidemiology :

Reservoir : Strict human pathogens. Asymptomatic nasopharyngeal carriers.

Transmission : **Respiratory droplets** (From an asymptomatic nasopharyngeal carrier).

Symptomatic disease : < 1% of infected individuals.

Spread : **mostly hematogenous spread** or along the olfactory nerve (via cribriform plate) to reach meninges.

Risk factors for invasion :

mnemonic : Recently, Hypo despite his Age, Asplenia and Terminal HIV Travelled to Africa.

Recent viral RTI/m. pneumoniae infection.

Hypogammaglobulinemia.

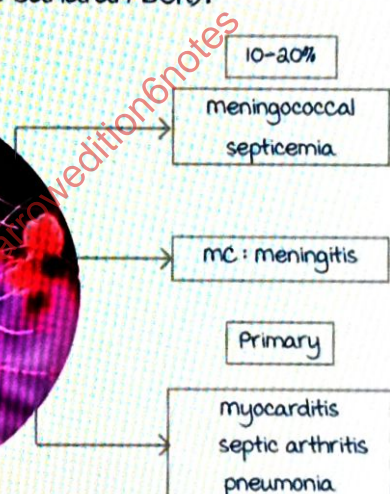
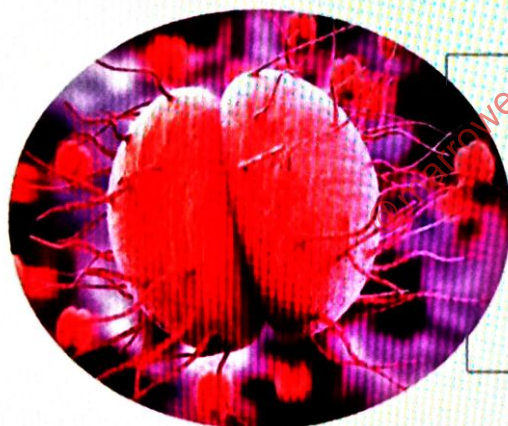
Elderly Age.

Functional/anatomical Asplenia.

Terminal complement component deficiency.

HIV : Advanced AIDS.

Travelled to **Africa** (endemic sub saharan belt).



meningococcal meningitis :

mostly as **acute meningitis**.

Presents with fever, nausea, nuchal rigidity, headache, photophobia. Signs of meningeal irritation may also be seen.

Petechiae/purpura/ecchymosis can be seen (endotoxemia).

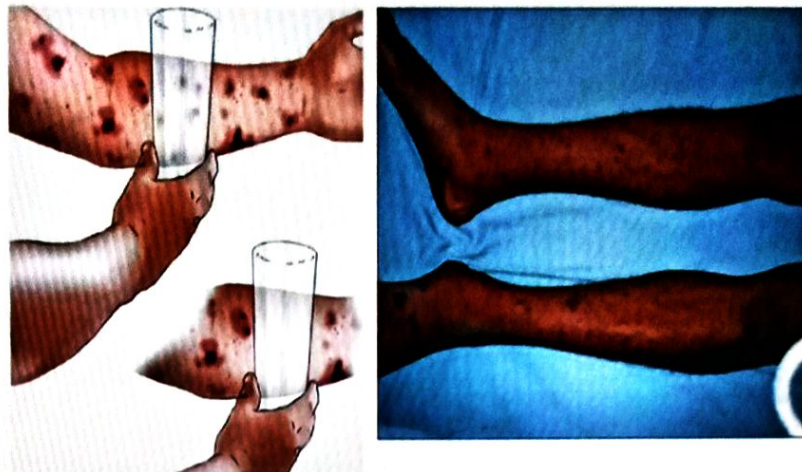
Typical clinical case scenario :

A 22 year old college student who is confused and lethargic is brought to the emergency room. His roommates say he was complaining of a severe headache and neck stiffness last night. Vital signs are remarkable for a temperature of 104.3° F, blood pressure of 80/60 and heart

Active space

rate of 120/min. Physical exam reveals a purpuric rash on the patient's chest and back. Lumbar puncture reveals a CSF WBC count with high neutrophil count, glucose levels of 35 mg/dL and protein concentrations 60 mg/dL.

Non blanching rash :



Meningococcal septicemia

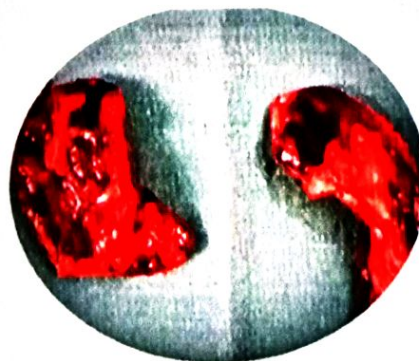
00:24:31

Seen in 10 to 20% patients who developed invasive disease. Due to **endotoxemia**.

High grade fever, typical rash, severe hypotension, DIC, shock with multi-organ failure.

Complications of meningococcal invasive disease :

1. Immune complex mediated : Aseptic arthritis, iritis, pleuritis.
2. Neurological deficits : Hemiparesis, cranial nerve palsies.
3. **Waterhouse-Friderichsen syndrome** : Due to severe adrenal hemorrhage.
4. Gangrene of toes and fingers.



Adrenal gland hemorrhage

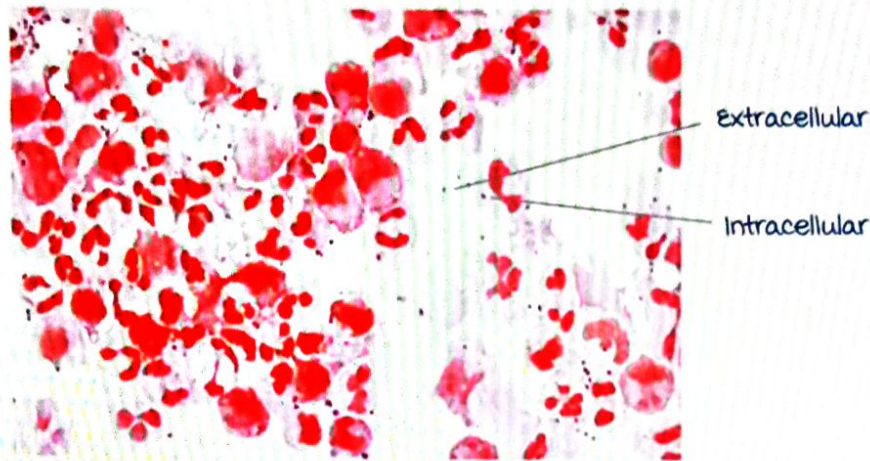


Gangrene of fingers

Diagnosis :

Specimen : CSF, blood, synovial & pleural fluid.

Gram stain (after centrifuging CSF and staining the sediments) : Intra and extracellular diplococci seen.



Latex agglutination : Capsular antigen detection in CSF and blood.

Culture : most specific (selective medium : Carriers. Blood/ chocolate agar : Sterile specimen like blood, CSF)

NAAT like PCR : most sensitive.

Treatment :

Empirical : Ceftriaxone/cefotaxime with vancomycin.

If *N. meningitidis* is identified : Continue with only ceftriaxone/cefotaxime.

Duration : 1 to 2 weeks.

Allergy to penicillin and cephalosporins : Chloramphenicol, moxifloxacin.

Post exposure prophylaxis :

Adults : Ciprofloxacin 500 mg single dose orally.

Ceftriaxone 250 mg IM single dose.

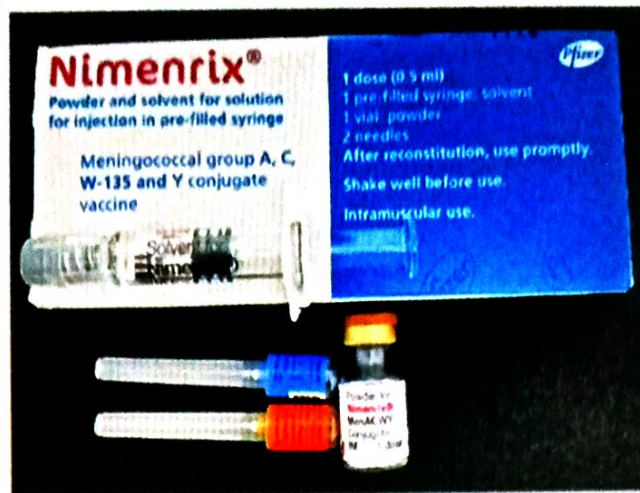
Rifampicin BD for 2 days orally.

Children : Ciprofloxacin is not used. use other two.

Pregnant : Ceftriaxone 250 mg IM single dose.

Pre exposure prophylaxis vaccine (high risk people) :

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meningococcal conjugate vaccine 4 (mCV-4) :

Contains 4 capsular antigens (A, C, W, Y).

Conjugated with protein (diphtheroid toxoid/tetanus toxoid/
non-toxic toxin of *C. diphtheriae* CRM 197).

CDC recommendation :

All adolescents 11 to 12 years of age : First dose.

Booster of same dose at 16 to 18 years.

Also, for both adults & children at risk.

men B vaccine :

Capsular B antigen is like neural
cells of fetus.

B : **Non immunogenic.**

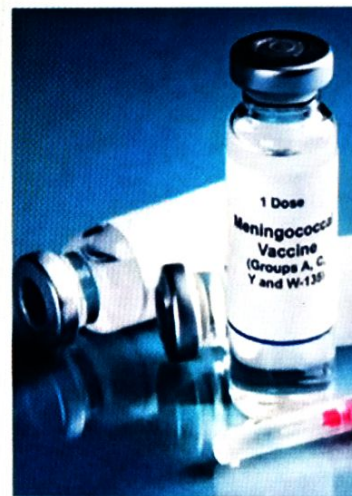
Component : Outer membrane
proteins of capsular serotype B.

1/2 doses schedule : 0, 6 or

0, 2, 6 months.

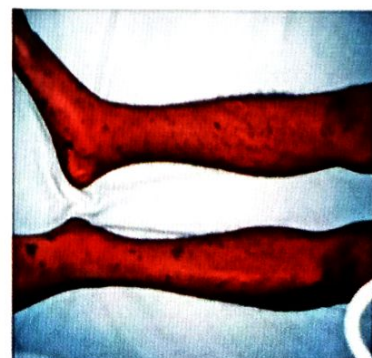
CDC recommendation : At 16-18

years of age. At risk individuals > 10 years.



mCQs :

Q. A 23 year old presents with
fever, headache and altered
sensorium for the last two days
with a rash shown in the figure.
Her BP is 80/50 mm Hg. O/E she
has neck rigidity. An LP is
performed. The CSF is cloudy,



PMN's are increased, proteins are elevated and glucose levels are reduced. Which of the following correctly describes the organism responsible?

- A. Gram Positive diplococci (kidney shaped), oxidase positive.
- B. Gram negative diplococci, utilizes glucose and maltose.
- C. Gram positive cocci, catalase negative, bacitracin sensitive (Streptococcus pyogenes)
- D. Gram positive lanceolate shaped diplococci, catalase negative, optochin sensitive (Strep. pneumoniae)

Streptococcus pneumoniae is m/c cause of meningitis.

2. A 20 year old woman is brought to the emergency by her college roommate due to 1 day of fever, neck pain and altered mental status. On physical examination neck stiffness is elicited and a non-blanching erythematous rash is noted on her extremities. Upon evaluation, meningitis is suspected, and lumbar puncture is performed. CSF gram stain reveals gram negative diplococcus. The roommate is further questioned who mentions that patient often volunteers as teacher assistant in a 3rd grade classroom, however, she has not been to work for past week since she just returned 2 days ago from a vacation trip overseas. Which of the following control measures is most appropriate after confirming the diagnosis?

- A. Rifampicin prophylaxis for the pregnant nurse who performed initial vital signs measurement.
- B. Ciprofloxacin prophylaxis for patient's roommate even if she has completed a full set of meningococcal vaccines.
- C. Rifampicin prophylaxis for all the 3rd grade students in the classroom she volunteers at.
- D. All of the above.

N. gonorrhoeae

00:41:50

GN diplococci, bean shaped.
 Facultative anaerobe.
 Non capsulated.
 utilizes only glucose.

Typing :

Serotyping :

PorB is outer membrane protein. Earlier called as protein-1.

Divided into : PorB IA : Disseminated gonococcal disease.

PorB IB : Localized disease.

Auxo typing : On basis of utilization of special substrates like arginine, hypoxanthine, uracil etc.

AHU auxotype : Associated with disseminated gonococcal disease.

Colonies :

Primary isolates : Colonies are small, dome shaped and brown in color.

Auto agglutinable (automatic clumping on suspension)

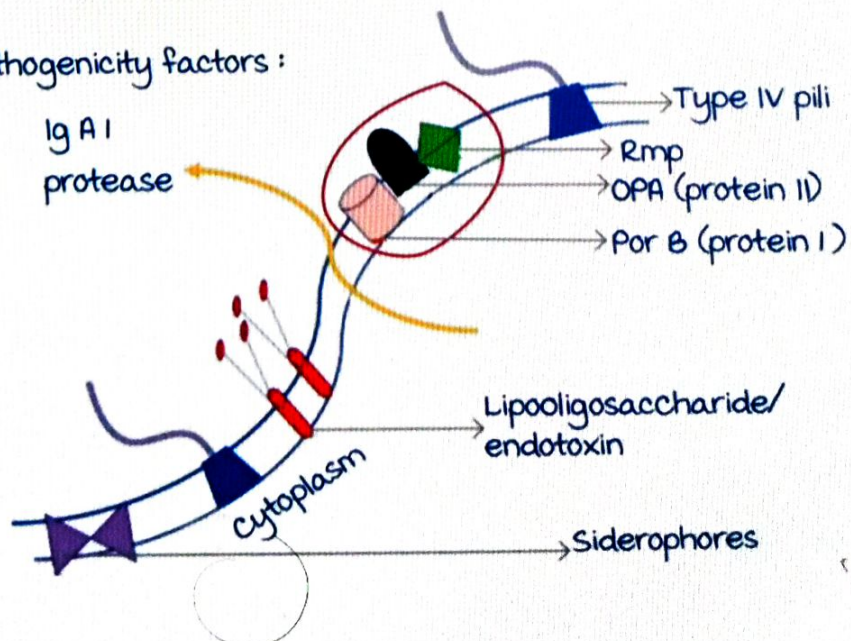
Lots of pili are present (P^+/P^{++}). Responsible for auto agglutination.

Serial subculture isolates : Flat white colonies, forms smooth suspension.

They are P^- (non-agglutinable).

Pathogenicity factors :

Ig A 1 protease



Type 4 pili : twitching motility.

Outer membrane proteins :

- Por B : Forms channel.
- OPA : Helps in adhesion.
- Reduction modifiable protein (RMP).
- Siderophores : For iron trapping.

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> 96% gonococcus have **cryptic plasmid** (unclear function).

Gonorrhoea :

It means flow of seed.

Reservoir : Strictly human.

mode of infection : Sexual transmission from asymptomatic carrier females.

Incubation period : 2 to 5 days.

Clinical manifestations :

male : Symptomatic.

urethritis (m/c) : mucoid discharge followed by purulent discharge & dysuria.

Chronic urethritis (urethral strictures because of fibrosis).

Complications : Prostatitis, epididymitis, seminal vesiculitis.

Females : Asymptomatic.

m/c site : **Cervix**. mucoid discharge followed by purulent cervical discharge.

Complications : Endometritis, salpingitis, **Fitz Hugh Curtis syndrome** (perihepatitis), PID (infertility).

Vaginitis : Prepubertal females and postmenopausal females.

Urethritis : Causing discharge + dysuria.

Chronic urethritis : Strictures.

Spread to perineal tissues : multiple abscess (**watercan perineum**) leading to multiple sinuses in both men & women.

Anal sex : can cause Proctitis.

Oral sex : can cause exudative pharyngitis.

Ocular gonorrhoea : Conjunctivitis (auto inoculation via contaminated fingers), perinatal (ophthalmia neonatorum).



Active space

Disseminated gonococemia

Risk factors : **C**AMPA (mnemonic)
 Terminal **C**omplement component
 deficiency.
AHU auxo type.
Menstruation.
Por **B** IA.

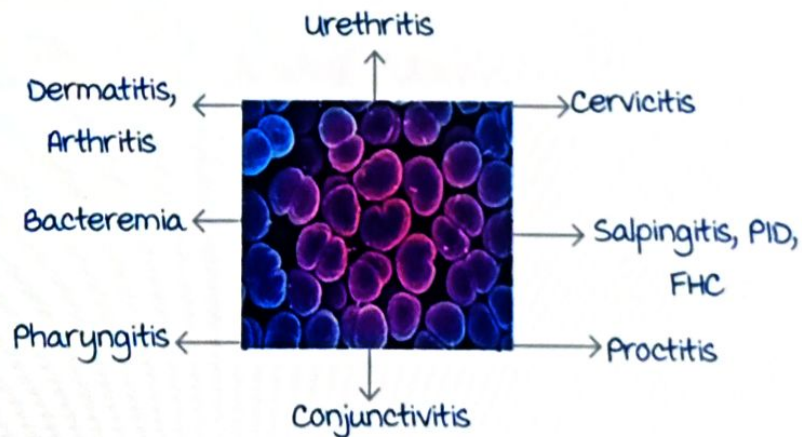
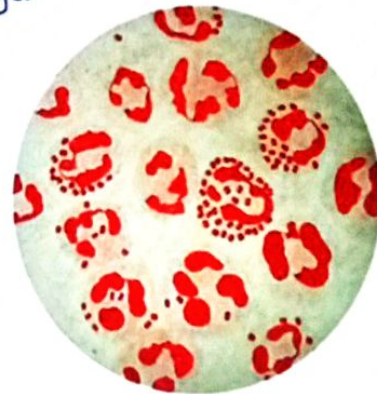


Complications of gonococcaemia :
 mnemonic : **D**ATeME

Dermatitis (papular/pustular), **A**rthritis, **T**enosynovitis,
Meningitis, and **E**ndocarditis.

Diagnosis :

Specimen : urethral swab/discharge.
 Cervical swab/discharge.
 Transported by Stuart's/
 Amie's medium.
 Gram stain : **GN** diplococci both
 intra and extracellular.
 Culture : Selective medium.
 NAAT: Rapid and sensitive.



Treatment :

Condition	Treatment
Uncomplicated cervicitis, urethritis, proctitis, pharyngitis	Ceftriaxone 500 mg once Im + Azithromycin 1g (single dose)
Conjunctivitis	Ceftriaxone once

Active space

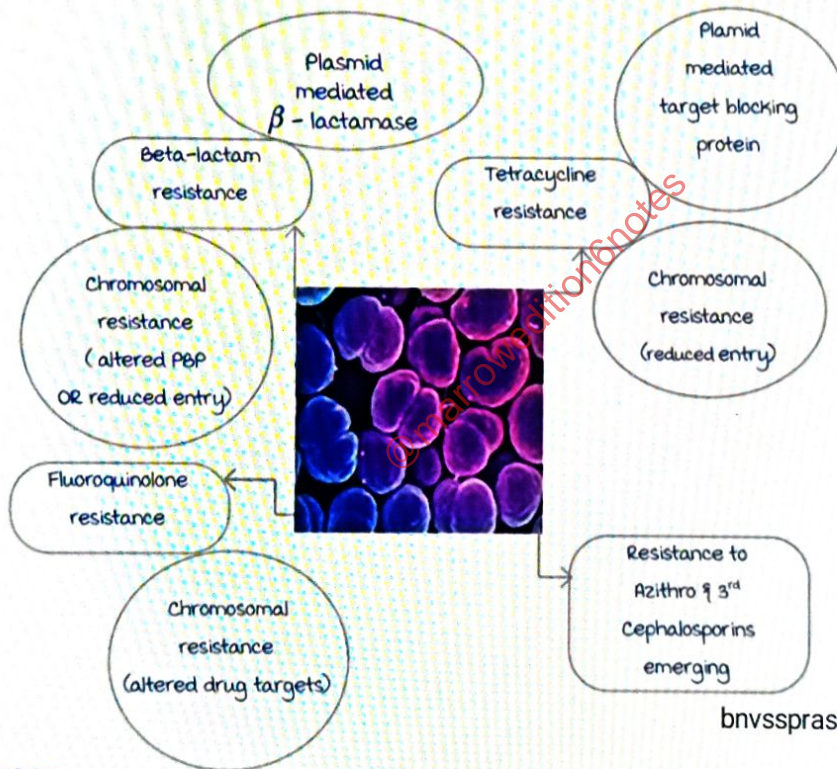
Ophthalmia neonatorum	Ceftriaxone once
DOI : Arthritis, tenosynovitis, skin lesions	Ceftriaxone IM for 2 days followed by Cefepime PO for next 5 days
meningitis	Ceftriaxone IM for 2 weeks
Endocarditis	Ceftriaxone IM for 4 weeks

Ceftriaxone is the **DOC**.

To treat concomittant infection with Chlamydia trachomatis d-k serovars, Azithromycin is given.

Resistance

01:03:33



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MCQs :

Q. A 23 year old man presents to your office with a five day history of unremitting fever and right wrist pain. He denies a history of illicit drug use. He is sexually active, has been with his recent girlfriend for about 1 month. Vitals are as follows: HR 118, BP 135/90, RR 20, T 101.9F. The patient is noticeably anxious. On physical exam, his right wrist and left elbow are both painful to palpation. No needle marks are visible. Small papular, non-pruritic lesions are noted on his back. Gram negative cocci inside macrophages are

Active space

seen on microscopy of synovial fluid, which of the following is the likely event that introduced the pathogen for this patient's presentation?

- A. Airborne droplets.
- B. Unprotected sexual contact.
- C. Needle stick injury.
- D. Feco-oral.

Q. A 26 year old woman comes to the office due to fever, malaise, and joint pain. A week ago, the patient had pain in the small joints of her right hand, which improved spontaneously but was quickly followed by pain in right ankle and left wrist. She has no prior medical problems and taken no medication. Temperature is 100.4 F, blood pressure is 124/82 mm Hg, and pulse is 90/min. The extremity joints are not erythematous or swollen, but tenderness is present along the tendons of the left wrist & right ankle. Several small, non-tender pustules are seen on the extremities. There is no other rash. Blood culture in a selective growth medium consisting of chocolate agar and multiple antibiotics yields that causative pathogen. Which of the following strategies would most like have prevented this patient's current condition?

- A. Avoiding bites from ticks.
- B. Obtaining appropriate vaccine.
- C. Using condoms consistently.
- D. Cooking meat products throughly.

Differences between meningococcus & gonococcus :

	meningococcus	Gonococcus
Shape	Lens shaped	Bean shaped
Oxygen requirement	Strict aerobe	Facultative anaerobe
Capsule	Present	Absent
Sugar utilization	Glucose + maltose	Glucose

Moraxella catarrhalis

01:09:22

Gram negative diplococci (mimics neisseria on Gram stain).

Non motile.

Catalase and oxidase positive.

Asaccharolytic (do not break down sugar)

Produces DNAase.

Upper Respiratory Tract (URT) flora in young children.

Causes **otitis media, sinusitis** in children (3rd m/c cause after streptococcus pneumoniae, H. influenzae).

In adults: **Acute exacerbation** of COPD and pneumonia.

Produce **beta lactamases**.

Treatment: Oral Amoxiclav. Others like Fluoroquinolones, macrolides can also be used.

Differentiating points between moraxella & Neisseria:

	Neisseria	moraxella
Sugar utilization	Yes	No
DNAase	No	Yes
Tributylin hydrolysis : Butyrate esterase test	Negative	Positive

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E.COLI AND SHIGELLA : PART 1

more than 40 genera present.

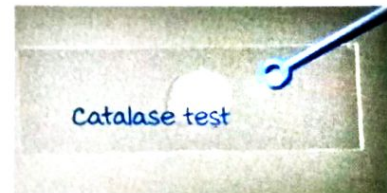
morphologically : Gram negative bacilli and non sporing.

motility : motile by peritrichous flagella except :

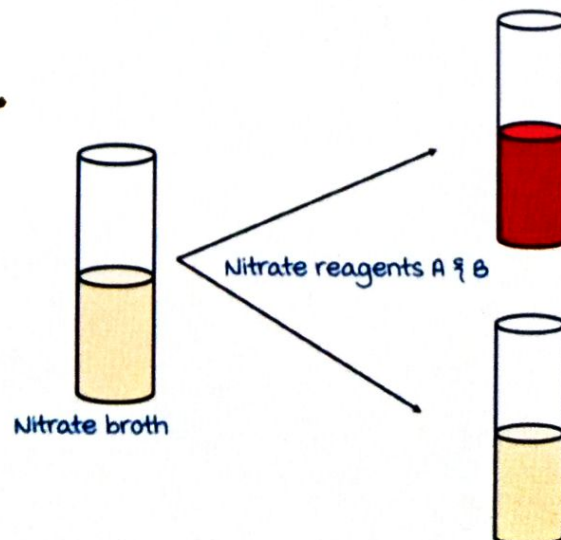
- Klebsiella.
- Shigella
- Atypical E.coli.
- Yersinia Pestis.
- Salmonella gallinarum and pullorum.

These are non-motile organisms.

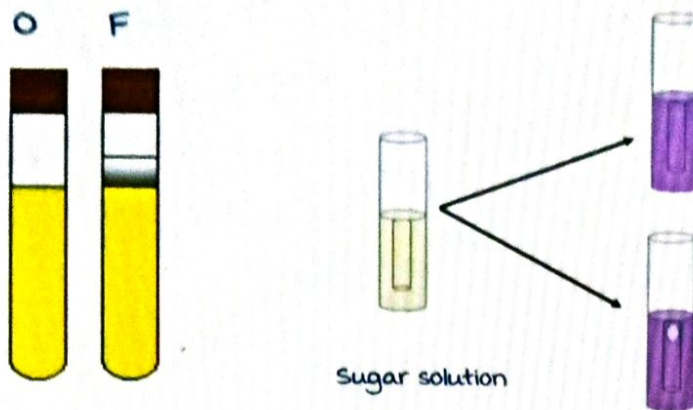
- All Enterobacteriaceae are facultative anaerobes.
- All of them are catalase test positive except Shigella Dysenteriae Type 1, which is catalase negative.
- All of them are oxidase test negative.



- All of them are Nitrate reduction test positive. i.e. in a nitrate broth, if they are incubated overnight and with the addition of nitrate reagents A and B, red color is seen. Nitrates have been converted in to nitrites. (NO_3^- to NO_2^-).



They all ferment sugars with production of acid only (anerogenic) or with acid and carbon dioxide (aerogenic).



Hugh Leifson's oxidative fermentative medium is used to check fermentation of sugars under aerobic or anaerobic conditions.

- Acid is produced both aerobically and anaerobically as they ferment sugars in the medium. To check whether they produce CO_2 along with the acid or not, liquid sugar solutions are used (glucose solution or lactose solution).
- A pH indicator along with few colonies of the bacterium are added into the tubes after which the tubes are placed overnight with a Durham's tube in it.

In case the organism is aerogenic : Bubbles (CO_2 gas) are seen inside the Durham's tube along with change in pH.

In case the organism is anaerogenic : Only pH change is seen.

Cultivation of enterobacteriaceae :

They easily grow on simple/basal media (non-fastidious in their requirement).

They grow on selective medium for gram negative rods. On MacConkey medium, they either produce lactose fermenting or non lactose fermenting colonies.

MacConkey medium consists of :

mnemonic PLANT

Peptone : Provides nutrition acts as a nitrogen source.

Lactose : Sugar which gets broken down.

Agar : Serves as a solidifying agent.

Neutral red : pH indicator.

Sodium Taurocholate (bile salt) : Selective agent which inhibits growth of gram positives.

Lactose fermenting colonies :

When the bacteria breaks down lactose, acid is produced and neutral red becomes bright pink in color.

These include :

- Escherichia coli.
- Klebsiella.
- Enterobacter.



Non-lactose fermenting colonies :

Pale colored colonies are seen, the neutral red remains pale in the absence of acid.

These include :

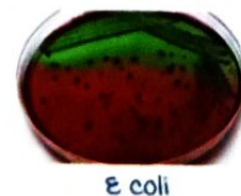
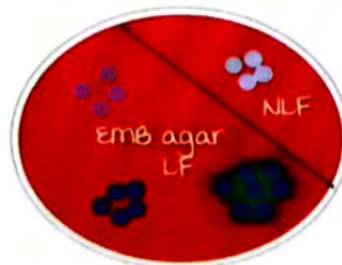
mnemonic : SPM SPY.

- Shigella.
- Proteus
- Morganella.
- Salmonella.
- Providencia.
- Yersinia.



Epson methylene Blue (EMB) Agar :

Another mildly selective medium for gram negative rods. Helps in differentiating lactose fermenting from non-lactose fermenting.



E coli

If lactose is fermented :

- Pink colored colonies or
- Bluish purple-colored colonies or
- Purple colored colonies with green metallic sheen surrounding them (typical of E.coli).

If **lactose is not fermented**, pale colored colonies are seen.

MacConkey and EMB media are mildly selective for gram negative, therefore some gram positive bacteria can also grow in these media.

Staphylococci and Enterococci can both grow on EMB and MacConkey agar as lactose fermenting colonies resisting the effects of sodium taurocholate.

Drug Resistance :

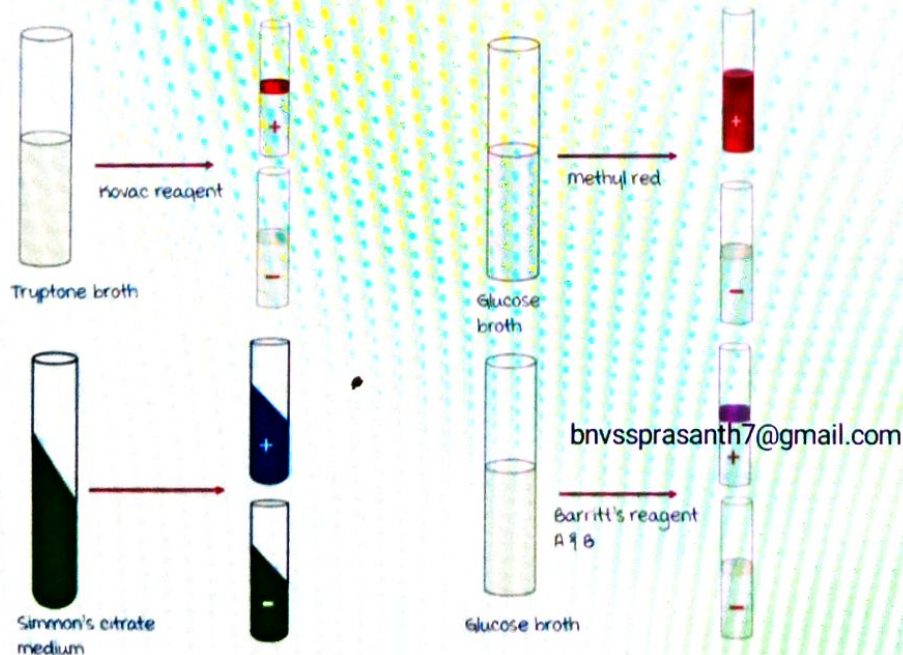
Resistance to several antibiotics is common in all members of Enterobacteriaceae, especially Klebsiella, Enterobacter which is resistant to a wide variety of drugs.

members of Enterobacteriaceae :

- Escherichia.
- Shigella.
- Klebsiella.
- Enterobacter.
- Citrobacter.
- Edwardsiella.
- Serratia.
- Salmonella.
- Proteus.
- Morganella.
- Providencia.
- Yersinia.

IMViC Tests

00:13:41



Biochemical tests used for the detection of members of Enterobacteriaceae.

I : Indole Test.

m : methyl red test.

Vi : Voges Proskauer Test.

C : Citrate Utilization Test.

Indole test :

Ability of a bacteria to **convert tryptophan to indole.**

- Tryptone broth is used.
- Overnight incubation in the tryptone broth.
- A Kovac's reagent is added.
- If indole is formed a **red colored ring** is seen.

methyl Red test :

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This checks whether the bacteria can break **glucose via the mixed acid pathways.**

- Colonies are incubated overnight in a **glucose broth.**
- After adding methyl red, the tube stays **red for hours** due to the **acid present** in them (pH remains < 4 for a very long time).

Voges Proskauer test :

When the organism utilizing glucose **does not use the mixed acid pathway**, it produces **acetoin**. **Bright pink colored ring** is seen when Barrit's reagents A & B are added into colonies which were incubated in glucose broth overnight.

A bacterium can either be voges test positive or methyl red positive.

Citrate utilization test :

Ability to utilize citrates as the **sole source of carbon**. If the medium turns **blue** after overnight inoculation, it means the bacterium has grown and utilized **citrates as the sole source of carbon**.

Otherwise the medium remains **without changes** after overnight inoculation.

Urease test :

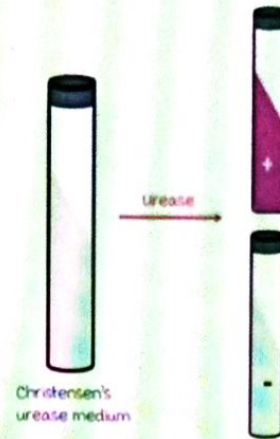
Christensen's urease medium is used here and contains **urea** and **pH indicator**.

If the bacteria produce **urease**, it will breakdown the medium and **ammonia is generated**. Ammonia will turn the medium alkaline and in the presence of the pH indicator **turn bright red** in color.

Urease positive organism include :

PUNCH MSKB

- Proteus.
- Urea Plasma.
- Nocardia.
- Cryptococcus neoformans.
- Helicobacter pylori.
- Morganella.
- Staphylococcus aureus and saprophyticus.
- Klebsiella.
- Brucella.



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Shigella

00:18:30

Non-motile and non-capsulated. On the basis of its somatic "O" antigen (part of the LPS molecule), it is divided into 4 groups/species.

	Group A	Group B	Group C	Group D
Species	Shigella dysenteriae	Shigella flexneri	Shigella boydii	Shigella sonnei
Serotype	>15	19	20	1
Lactose ferment	NLF (non lactose fermenting)	NLF	NLF	Late LF
Mannitol ferment	NMF	MF	MF	MF

Active space

Other	<p>Shigella dysenteriae type 1 is called as Shiga bacillus.</p> <p>Only serotype of shigella which produces shigella toxin. Shigella dysenteriae is the cause for most severe dysentery/shigellosis</p>	<p>most common species reported in developing countries (including India).</p> <p>Reactive Arthritis associated with Shigella Flexneri.</p>	<p>Least common species in the world</p>	<p>Antigenically it is the most homogenous. All Shigella sonnei will have the same antigen</p> <p>Causes the mildest disease. most common species in the west and in the world. The most resistant to chemical disinfection.</p>
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NLF : Non-lactose fermenting.

MG : mannitol fermenting.

Biochemical tests :

Sugar breakdown : Anaerogenic (except few S.flexneri, few S.boydii which are aerobic)

H₂S : Does not produce H₂S.

ImVIC : - + - -.

Urease : Negative.

Cultivation of shigella

macConkey medium : NLF.

Transport medium : Sachs buffered glycerol saline and Cary Blair medium.

Enrichment medium : Selenite F broth.

Selective medium : Deoxycholate Citrate Agar (DCA), Xylose

Lysine Deoxycholate (XLD) medium, Hektoen Enteric agar,

Salmonella Shigella agar.

Pathogenesis of Shigella :

virulence factors of Shigella.

Overall pathogenic mechanism : Invasive diarrhea.

Site : Colon and rectum is the site of invasion.

Shigella has a virulence plasmid which encodes 2 important virulence factors :

- Type 3 secretion system by which it pumps in special proteins into the cell.
- Multiple translocated proteins are secreted, these include ipa A to D (detectable in stool samples).

Site of invasion is not apical but basolateral invasion of the mucosal cells of the intestine.

- Shigella invades the large intestine by entering the macrophages (at MALT) via the m cells (microfold cells).
- Shigella induces the apoptosis of macrophage and then translocate proteins (via type 3 secretion system) into the mucosal cells of the large intestine from the basolateral aspect.
- Actin polymerization in the cells occurs followed by formation of microspikes and shigella is taken up intracellularly.



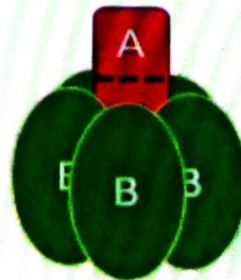
Shigella then spreads from one cell to another and causes apoptosis of the cell it leaves.

This followed by infiltration of inflammatory cells like neutrophils. The infection is controlled within 5-7 days. The diarrhea that occurs is self limiting.

Shiga toxin :

This toxin is secreted only by *Shigella dysenteriae* Type 1.

It is a **AB5 subunit toxin**, 5 B's in the form of a ring with A subunit in the center.



Receptor : **Gb3** is the receptor for shiga toxin.

Site of action : Endothelial cells of the small blood vessels in GI tract.

mode of action :

Once the B subunit binds with Gb3 receptor.

- The toxin undergoes receptor mediated endocytosis and, reaches the endoplasmic reticulum, where the A subunit is broken down to A1 and A2.

A1 : Enters cytoplasm and cleaves the 28S ribosomal RNA of the 60S ribosomal subunit. This leads to inhibition of protein synthesis.

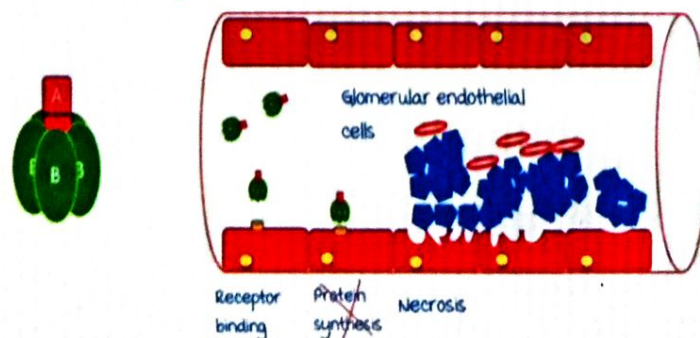
Therefore, shiga toxin causes inhibition of protein synthesis. This causes necrosis of endothelial cells, activates coagulation pathways and causes local ischemia.

4 toxins that act by inhibiting protein synthesis.

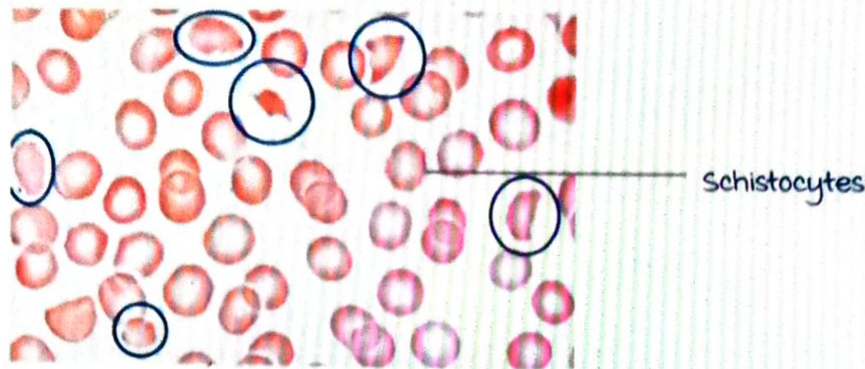
- Those which cleave 60S ribosomal subunit are :
 1. Shiga toxin.
 2. Shiga like toxin.
- Those which act by inhibiting elongation factor-2 :
 1. Diphtheria toxin.
 2. Pseudomonas toxin.

Generally, shiga toxin is restricted to the GI. In a small percentage of people, the shiga toxin disseminates via blood and reaches the glomerular capillaries where its receptors are present on the endothelial cells.

- This causes necrosis of the endothelial cells.
- Platelets are activated and causes binding of platelets at the site of necrosis and compromises renal vasculature.
- This results in acute renal failure and consumptive thrombocytopenia.



microangiopathic hemolytic anemia is also seen due to RBCs passing through the partially clogged blood vessels.



Peripheral blood smear in HUS

Hemolytic uremic Syndrome (HUS) :

- Acute renal failure.
- Consumptive thrombocytopenia.
- microangiopathic hemolytic anemia.

most common cause of hemolytic uremic syndrome in India : shigella dysentery Type I.

On peripheral blood smear fragmented RBC / Schistocytes.

Bacillary dysentery / shigellosis

00:39:18

Bacillary dysentery is caused by both shigella and entero-invasive E. coli (EIEC).

Reservoir for Shigella : Humans only.

Infective dose : < 100 bacilli (therefore easily transmitted by contaminated fomites/fingers/food).

most communicable bacterial diarrhea.

Incubation Period : 2 days (12hrs to 5 days).

Clinical features :

- Fever.
- Abdominal cramps.
- Initially watery diarrhea.
- Bloody diarrhea with tenesmus and urgency.

Spontaneous resolution occurs in 4-5 days.

Complications :

- Fluid and electrolyte imbalance.
- Toxic megacolon.
- Intussusception.

- Reactive arthritis : Only seen with *S. flexneri*.
- HUS (seen with *Shigella dysenteriae* Type 1)

Diagnosis :

Specimen used : Fresh stool >> Rectal swab.

Microscopy : Sheets of WBCs seen.

Cultivation :

Transport medium : Cary Blair medium.

Enrichment medium (liquid medium) used first where they replicate rapidly, then after 6 hours, the colonies are subcultured on selective medium.

Selective medium : XLD or DCA

ELISA :

Directly on the stool sample for detection of ipa proteins (also known as virulence marker antigens)

PCR can also be done for the shiga toxin gene on stool sample.

Tests for invasiveness :

- Sereny test :

Suspension of colonies of shigella is dropped into the eye of a rabbit and keratoconjunctivitis occurs if shigella is invasive.

Only of historical importance.

- Invasion of Hela/Hep-2 Cell lines :

After overnight incubation of colonies on the cell line, under an inverted microscope, all shigella become intracellular if invasive.

Treatment for bacillary dysentery :

Self-limiting condition. Therefore only treatment required is maintenance of hydration to normal physiological levels.

In neonates, elderly, immunodeficient or in cases of severe disease : 3rd generation cephalosporins given.

Q. Which other pathogens have a low Infective Dose (ID50) ?

- Shigella
- Enterohemorrhagic E. coli (EHEC).
- Entamoeba histolytica
- Giardia
- Cryptosporidium.

E.COLI AND SHIGELLA : PART 2

E. coli

00:00:08

Serotyped on the basis of 3 antigens.

- **O antigen :**
Somatic antigen.
> 170 'O' serotypes.
- **K antigen :**
Capsular antigen.
> 75 'K' serotypes.
- **H antigen :**
Flagellar antigen.
> 100 'H' serotypes.

General characters :

All are motile except the atypical group of E. coli.

80% are capsulated.

Biochemical tests and culture:

Sugars broken down : Aerogenic

Does not produce H₂S.

IMVIC reactions ++--.

urease -ve.

Lactose fermenting members of enterobacteriaceae :

1. Escherichia coli
2. Klebsiella
3. Enterobacter

MacConkey agar : Bright pink lactose fermenting colonies.

EMB agar : Dark purple colonies with green sheen around them.

Blood agar : Beta hemolytic.

GI commensal/flora/microbiota :

Strict anaerobes : Facultative anaerobes = 1000 : 1

Anaerobes	Facultative anaerobes
Bacteroides : MC in human colon.	E. coli : most common.
Clostridium.	Klebsiella.
Actinomycetes.	Enterobacter.
Lactobacillus.	Citrobacter.
Prevotella.	Proteus.
Porphyromonas.	Enterococcus.
Pepto streptococci.	Group B Streptococci.

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Diarrheagenic *E. coli* :

According to their **pathogenicity**, they are classified as:

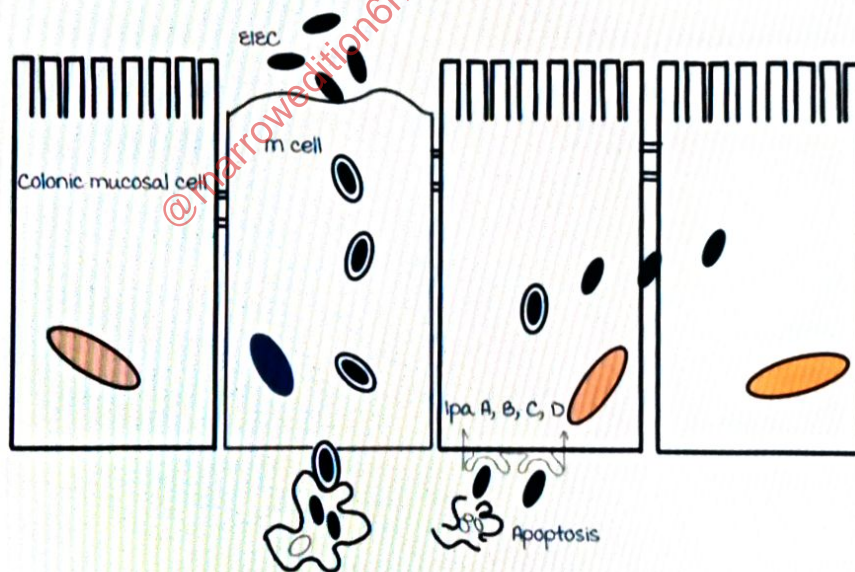
- Enteroinvasive *Escherichia coli* (EIEC).
- Enterotoxigenic *Escherichia coli* (ETEC).
- Enteropathogenic *Escherichia coli* (EPEC)
- Enteroaggregative *Escherichia coli* (EAEC).
- Shiga toxin producing *Escherichia coli* (STEC).
- Diffusely adherent *Escherichia coli* (DAEC).

Never present as normal GI flora.

Enteroinvasive *Escherichia coli* (EIEC) :

Causes disease similar to that caused by *Shigella*.

EIEC is taken up by the **microfold cells** (m cells) → Induces **apoptosis** of the neighboring colonic mucosal cells from the basolateral end → Releases the **virulence marker antigens** → Spreads from cell to cell.



Infective dose is **100 times** more.

No strain produces **Shiga toxin**.

Causes slightly **milder disease** compared to *Shigella*.

Belong to **atypical *E. coli*** (resembles *Shigella*) :

- Non-motile.
- Anaerogenic.
- Non-lactose fermenting.

Enterotoxigenic E. coli (ETEC)

00:08:13

most common cause of **traveler's diarrhea**.

most common cause of **bacterial diarrhea** in children.

Causes diarrhea in all age group.

Large infective dose : $> 10^9$.

- No transmission by fomites, person to person or transmission by flies.
- Transmitted only by human feces contaminated food or water.

Virulence factors : **Plasmid mediated**.

- **Colonization factor antigens** → Adhesion to small intestinal mucosa.

25 adhesive molecules identified.

E.g: CFA- I, CFA- III, CSI, CSA.

- **Plasmid mediated heat labile toxin (LT)** → A&B subunit toxin. Similar to cholera toxin.
- **Plasmid mediated heat stable toxin (ST)**.

LT & ST are responsible for diarrhea that the patient has.

Pathogenesis :

ETEC adheres to the small intestinal mucosal cell.

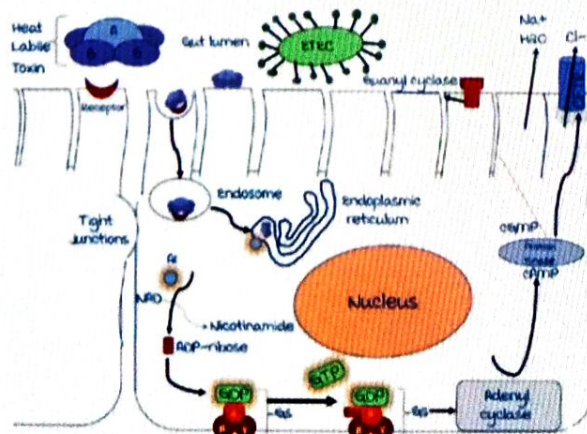
Releases the **heat labile toxin**, which is an A&B-subunit toxin.

Toxin is taken into the cell via receptor mediated **endocytosis**.

In the **endoplasmic reticulum**, the A and B subunits separates, and A splits into A1 and A2.

A1 takes an ADP-ribose moiety from **NAD** and transfers it to the subunit of the **Gs protein** → Persistent activation of **adenyl cyclase** → Increase in cAMP.

cAMP continuously activate protein kinases → Phosphorylates **CFTR** (cystic fibrosis transmembrane regulator) → Watery diarrhea.



Active space

Heat stable (ST) toxin binds with **guanylyl cyclase** at the apical end of the mucosal cell.

Increases cGMP → Activates protein kinases.

Contributes to the formation of **watery diarrhea**.

Mnemonic :

Labile in the **Air** : **A**denyl cyclase.

Stable on the **Ground** : **G**uanylyl cyclase.

Labile toxin	Stable toxin
AB5 subunit toxin similar to cholera toxin in structure. Less potent.	19 AA polypeptide (small)
Highly antigenic.	Low antigenicity.
Increases cAMP.	Increases cGMP.

IP ~ 2 days.

Presents with **watery diarrhea ± vomiting**.

Self-resolving in 3-5 days.

Diagnosis :

Stool sample taken.

Gram stain : No pus cells or RBCs (as diarrhea is non invasive/non inflammatory).

Culture : macConkey and blood agar → **Lactose fermenting colonies** in macConkey agar.

To prove it is ETEC : Toxigenicity tests.

• **Labile toxin** :

Y-1 adrenal tumor cell assay.

Chinese hamster ovary assay.

ELISA and PCR.

Cell lines

• **Stable toxin** :

ELISA.

PCR.

Treatment :

Fluids and electrolytes.

Severe diarrhoea : **Ciprofloxacin** or **Rifaximin**.

Antimotility drugs like **loperamide** (not used in invasive/bloody diarrheas).

Enteropathogenic E. coli (EPEC)

00:20:36

most common cause **bacterial infantile diarrhea**.
Rarely cause diarrhea in other age groups.

2 virulence plasmids :

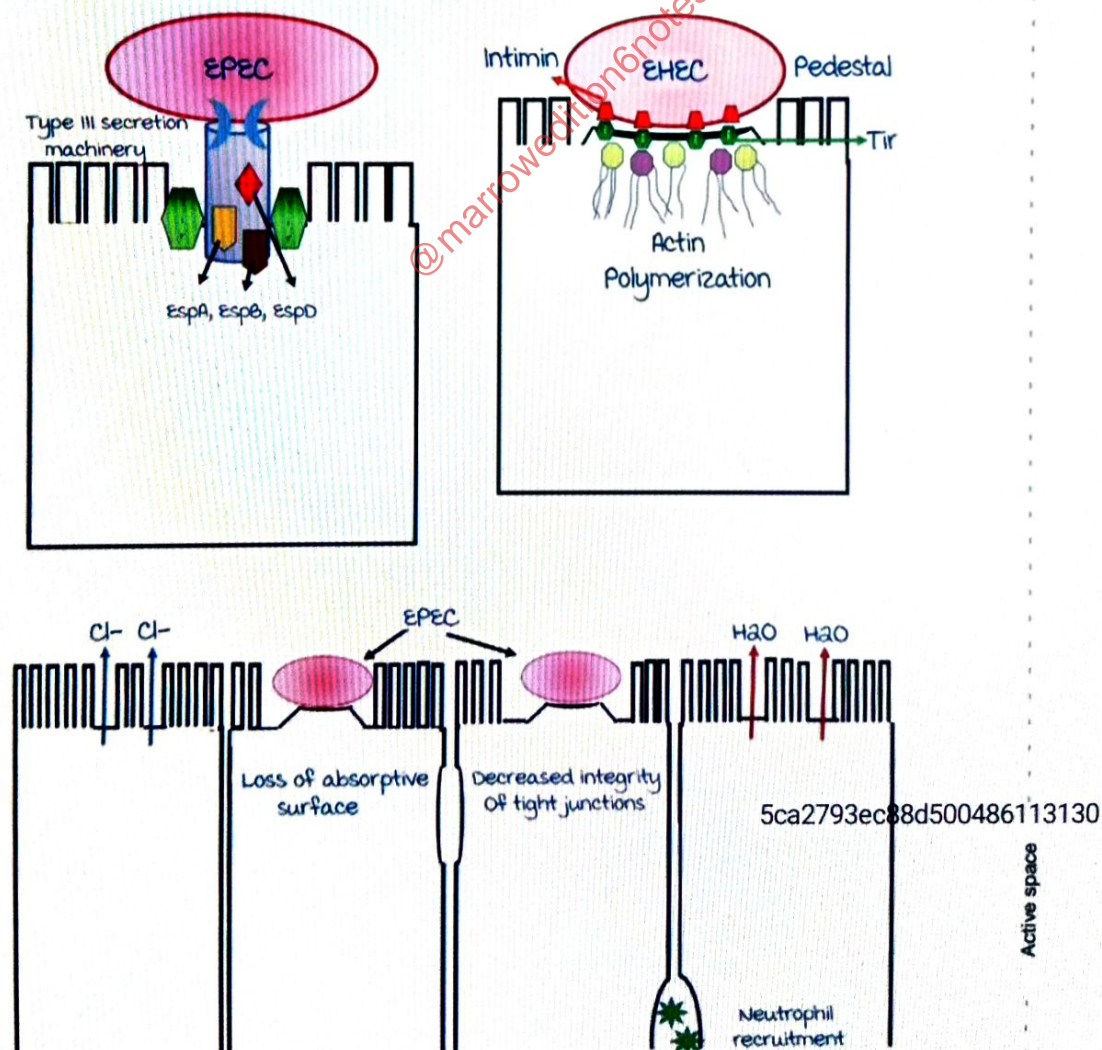
- **EPEC adherence factor plasmid**.
encodes Bundle forming pilus (BFP).
Adhesion to small intestinal mucosa.
- **Locus of enterocyte effacement plasmid**.
Type III secretion system.
multiple translocated proteins like Tir, Intimin, EspF.

Pathogenesis :

Cause attachment-effacement effect (A- ϵ effect).

Attachment to cup like **pedestals** \rightarrow Effacement of villi.

Disrupt the tight junctions between the enterocytes.



The baby presents with watery diarrhea.

Diagnosis :

Stool collected.

Culture : **macConkey and blood agar** → **Lactose fermenting colonies in macConkey agar.**

EPEC is proven with :

Localized adherence on the **Hep-2 adherence assay.**

Adhering in small bunches over the cell line.

Rapid test : PCR for gene encoding the BFP.

Treatment :

Fluid and electrolytes.

Antibiotics according sensitivity testing.

Enterohemorrhagic E. coli (EHEC)

00:27:34

Also called **verotoxigenic E. coli (VTEC).**

Animal reservoir : Present as normal GI flora of cows (zoonotic infection).

modes of infection : Small infective dose (< 1000 bacteria).

- Contaminated **poorly cooked beef or hamburgers.**
- Contaminated unwashed salads.
- Contaminated water.
- Fomites and person to person.

most common cause of **HUS** (hemolytic uremic syndrome) in the world.

virulence factors :

1) Locus of **enterocyte effacement plasmid.**

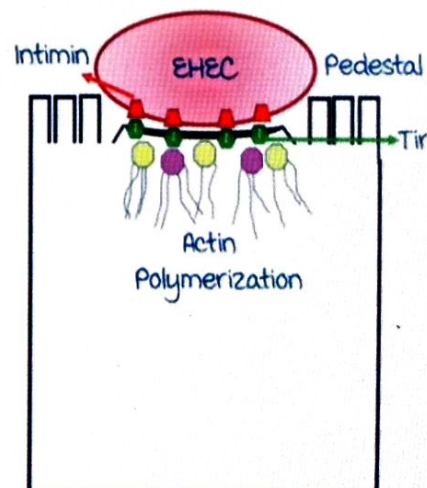
→ Attachment-effacement effect.

2) Phage mediated Shiga-like toxin/verocytotoxin.

→ Phage encoded toxin.

Acts by inhibition of protein synthesis.

Site of action : Endothelial cells.



Causes HUS when it disseminates to glomerulus.

Virulence factors causing bloody diarrhea is unknown.

IP: 3-8 days.

Presents with abdominal cramps, initially diarrhea → Later dysentery.

Fever is absent.

Self limiting in 5 days.

Some develop the complication of HUS.

Most severe disease and occurrence of HUS are commonly seen with *E. coli* O157:H7.

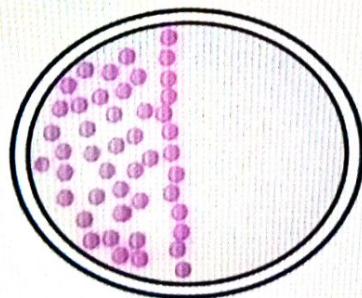
Diagnosis :

Stool sample.

MacConkey and blood agar → Lactose fermenting colonies in MacConkey agar.

EHEC is proven by :

- To identify *E. coli* O157:H7 → Sorbitol MacConkey test.
Sorbitol instead of lactate.
O157:H7 does not ferment sorbitol and produced pale colonies.
All other *E. coli* are sorbitol fermenting.
- Rest of EHEC (non O157:H7).
Identified by cytotoxicity assay/tissue culture assay.
uses vero cell lines.
PCR for the gene of Shiga like toxin/verotoxin.



Sorbitol MacConkey test

Treatment :

Symptomatic treatment.

Fluid and electrolytes.

No antibiotics should be used → worsens the disease.

No antimotility drugs.

Phage mediated toxins :

The gene for the toxin is present on a phage DNA which is integrated by the bacterial genetic material → **Lysogenic conversion.**

Mnemonic : ABDCE.

ShigA like toxin.

Botulinum toxin C and D.

Cholera toxin.

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Diphtheria toxin.

Erythrogenic toxins → Pyrogenic toxins A and C of Streptococcus pyogenes.

Enteraggregative E. coli (EAEC)

00:39:06

Causes traveler's diarrhea.

Most common cause of **persistent diarrhea** (> 14 days).

Leads to **malnourishment** in children.

Virulence factors : Plasmid mediated.

Aggregative Adherence Fimbriae (AAF) → Adhere to small intestinal mucosa.

SPATE proteins → Increase the mucus production →

Formation of mucus biofilm around them.

EAST-1 (Enteraggregative Stable Toxin) → Increases cGMP.

Presents with watery or mucoid diarrhea.

Diagnosis :

Lactose fermenting colonies on MacConkey agar.

Proved as EAEC → **Stacked brick adherence** pattern on Hep-2 adherence assay.

Treatment :

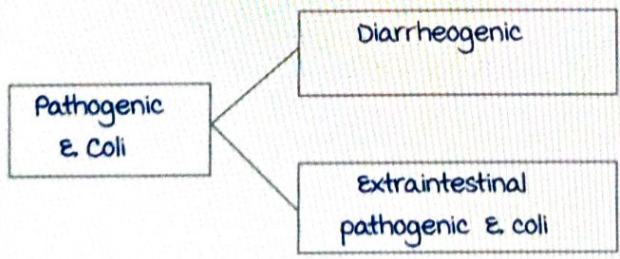
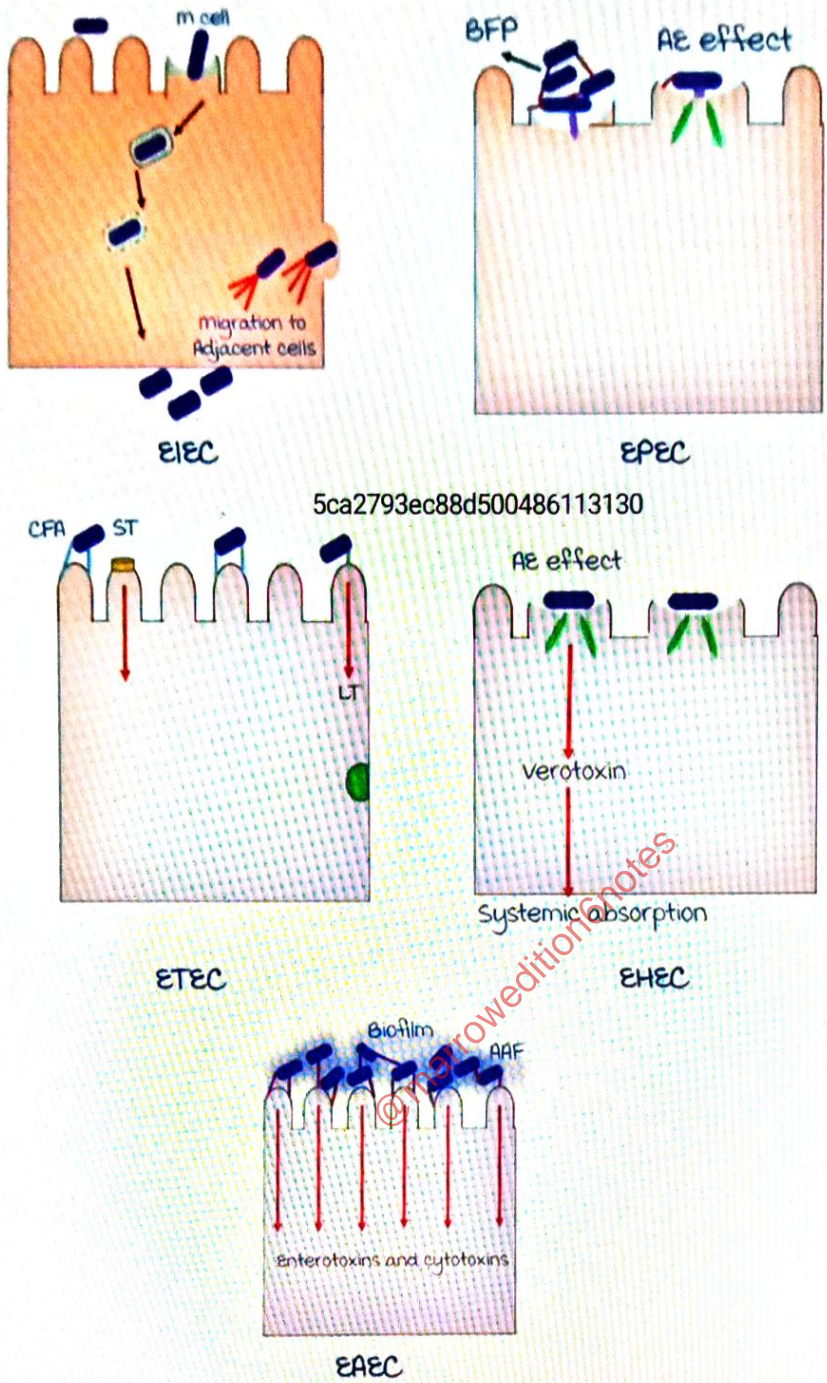
Fluid and electrolytes.

Antibiotics according to sensitivity testing.

Diffusely Adherent Escherichia coli (DAEC) :

Causes diarrhea in children of 1-5 years.

Shows diffuse adherence on Hep-2 adherence assay.



Extraintestinal pathogenic E. coli (ExPEC) 00:47:07

Normal gastrointestinal flora which has acquired special virulence factors and has entered a normally sterile

Active space

extra-intestinal site.

Causes community acquired and nosocomial infections.

Uropathogenic Escherichia coli (UPEC) :

most common cause of UTI, including :

Community acquired UTI.

Nosocomial infection.

In any age or sex.

Source : vaginal or fecal microbiota.

Special virulence factor : Helps to adhere to urothelial mucosa.

- Type I fimbriae which bind to uroplakin.
- P pili : Bind to P blood group antigen.

E. coli are most common cause of neonatal meningitis (India and world).

K-1 capsular serotype is the most common.

E. coli is the most common cause of intra-abdominal abscess and peritonitis.

MCQs

00:52:40

Q. A 20-yr old boy ate pani-puri from a roadside vendor. Three days later he developed abdominal cramps with mild fever followed by increased frequency of stools with blood and mucus. Stool microscopy showed many pus cells. Which of the following is the likely etiology of his symptoms?

- ETEC.
- Vibrio cholerae.
- Shigella dysenteriae.
- Shigella flexneri.

The question mentions pani-puri roadside vendor, which indicates that the location is India. S. flexneri is the most common cause of invasive diarrhea in India.

Q. A 2-week-old infant who was born 2 weeks preterm, is brought to the OPD with the history of vomiting, fever, poor feeding and crying more than usual for the last 24 hours. On examination, the infant has a temperature is 102.4 degree

Celsius, has bulging anterior fontanelles, increased respiratory and heart rates.

Lab investigation results show peripheral WBC count of 15,400/cumm.

The child starts to have focal seizures in front of the consultant. CSF examination shows 3900 WBCs/cumm (92% neutrophils), protein levels of 350 mg/dl and glucose level of 2 mg/dl. The gram stain picture of the sediment shows gram negative bacilli.

Which other property is correct about the causative bacterium?

- A. Oxidase positive.
- B. Urease positive.
- C. Strict aerobe.
- D. Lactose fermenting.

Ans : This is a case of neonatal meningitis which is caused by gram negative bacillus.

Common causes are :

E.coli : Gram negative bacillus.

Group B streptococcus : Gram positive cocci.

Listeria : Gram positive rod.

All enterobacteriaceae are oxidase negative and are facultative anaerobes.

Q. An HIV positive from UK recently travelled to India for a 2 week vacation. During the 2nd week, he developed acute watery diarrhea and abdominal pain without fever. 3 weeks later, he is having persistent symptoms and beginning to lose weight. You suspect :

- A. EPEC.
- B. ETEC.
- C. EAEC.
- D. EHEC.

Ans: Case of traveller's diarrhea + persistent diarrhea.

Q. Three days after eating in a fast-food hamburger restaurant, a 12 year-old boy develops abdominal pain and bloody diarrhea. He is admitted to the hospital. His stool

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smear shows many leukocytes, a gram stain shows many gram-negative rods. The organism forms pink colonies on macConkey agar. The responsible organism is thought to be associated with development of which of the following conditions?

- A. Fever, migratory polyarthrititis, and carditis.
- B. Fever, new murmur, small erythematous lesions on the palms, and splinter hemorrhages on the nail beds.
- C. Petechial rash and bilateral hemorrhage into the adrenal gland.
- D. Thrombocytopenia, anemia, and uremia.

Ans : Causative organism is EHEC associated with hemolytic uremic syndrome.

member	motility	LF/NLF	Gas (CO ₂) H ₂ S	Imvic	urease
E. coli	m	LF	Aerogenic -	++--	-
Shigella	Nm	NLF	Anaerogenic -	-+--	-
Klebsiella	Nm	LF	Aerogenic -	--++	+
Enterobacter	m	LF	Aerogenic -	--++	-
Citrobacter	m	LF/NLF	Aerogenic -	-++ Cf ++-CK	-
Salmonella	m	NLF	Aerogenic -	-++	-
Proteus	m	NLF	Variable +	Variable	+
Yersinia	Nm	NLF	Anaerogenic -	-+--	-

m : motile.

Nm : Non-motile.

LF : Lactose fermenting.

NLF : Non-lactose fermenting.

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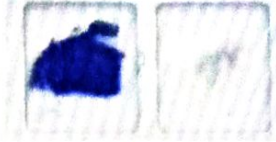
Gram negative rods.
 Non sporing facultative anaerobes.
 motility by **peritrichous flagella** (except klebsiella, shigella, Yersinia pestis, atypical E. coli, Salmonella gallinarum pullorum).

members include :

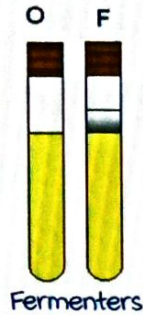
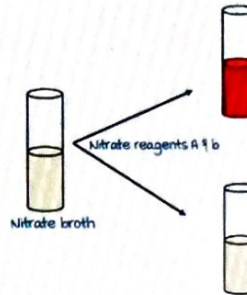
- Klebsiella.
- Enterobacter.
- Serratia.
- Proteus.
- Yersinia, etc,



Catalase +



Oxidase -



Features :

- Catalase positive.
- Oxidase negative.
- Reduce nitrates to nitrites.
- All ferment sugars (can utilize sugars aerobically and non-aerobically).
- Can easily grow on simple and basal media.
- On macConkey's : Lactose fermenting or non-fermenting.

Klebsiella

Also called : Friedlander's bacillus.

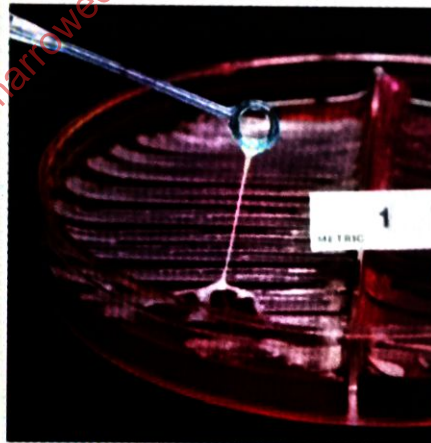
Family : Enterobacteriaceae.

motility	Non-motile.
Capsule	Capsulated (> 80 capsular serotypes : K1, K2 most virulent).
Sugar fermentation	When sugars fermented → Produce both acid + gas (aerogenic).

Indole	Negative
Methyl red	Negative
Wok's Proskauer (WP) test	Positive.
Citrates	utilized
H ₂ S	Do not produce H ₂ S on sugar

Active space

Indole	Negative
methyl red	Negative
Wok's Proskauer (WP) test	Positive.
Citrates	Utilized.
H ₂ S	Do not produce H ₂ S on sugar fermentation.
urease	Positive.
macConkey's medium	Lactose fermenting.
Blood agar	Highly mucoid colonies (thick capsules : Hyperviscous) : Hypervirulent Klebsiella (HVKP).
Species	<ol style="list-style-type: none"> 1. K. pneumoniae (3 subspecies : Pneumoniae, ozaena, rhinoscleromatis). 2. K. oxytoca. 3. K. granulomatis
MC cause of community acquired liver abscess (previously, E.coli).	



Hypermucoviscous *Klebsiella pneumoniae* or Hypervirulent *Klebsiella pneumoniae* (HVKP)

Subspecies of *Klebsiella pneumoniae* :

1. ***Klebsiella pneumoniae pneumoniae*** :
 - Also called **Friedlander's bacillus**.^{5ca2793ec88d500486113130}
 - Normal GI flora.
 - many virulence factors : Capsule, pili, lipopolysaccharides, siderophores (iron).
 - Diseases caused : Community + Nosocomial infections (UTIs, intrabdominal abscesses, wound or burn infections,

surgical site infections, bed sore infections, pneumonias (nosocomial post tracheostomy), meningitis post neuro surgery, neonatal septicemia.

Friedlander's pneumonia : Community acquired pneumonia.

- Risk factors : Alcohol, diabetes, COPD, advanced age.
- Has a propensity for upper lobes of lungs & abscess formation.

CXR : Bulging fissure sign.

Hemoptysis + and patient expectorates a

blood tinged sputum called currant jelly sputum.

Also causes community acquired liver abscess (mainly seen in people with pre-existing hepato-biliary disease).

- manifests as single unilocular pus collection.
- MC cause of this liver abscess : K1 type (hyper mucoviscous-hypervirulent).
- metastatic spread very common (brain, eye).
- Eyes : Ophthalmitis.
- Brain : meningitis, abscess.

Treatment :

multidrug resistant organism.

Chromosomal resistance & R-plasmid mediated → Beta lactamases.

Drugs :

- Extended spectrum beta lactamases.
- Carbapenemases (metallo beta lactamases).

Klebsiella pneumoniae rhinoscleromatis

00:12:30

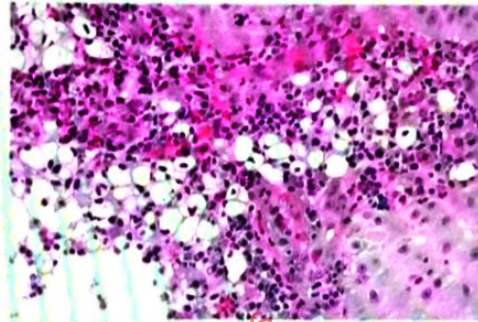
- Causes rhinoscleroma/respiratory scleroma.
- Chronic granulomatous infection of nasal cavity.

Associated with formation of nodules/masses in the nasal passage → erosions of nasal cavity → Destruction of bone and cartilage → Hebra nose.

mostly seen in poor people, in Africa, Asia, South America.



Preoperative views of the patient, showing the swelling both internally and externally



mixed inflammatory infiltrate with plasma cells and vacuolated macrophages (Mikulicz cells)

Biopsy : Mikulicz cells (foamy macrophages filled with Klebsiella).

Requires prolonged treatment.

Sensitive to most antibiotics (fluoroquinolones/tetracyclines).

Klebsiella pneumoniae ozaena :

- Ozaenae : Stench (bad smell).
- Normal nasal flora.
- Sometimes, leads to atrophic rhinitis associated with foul smelling nasal discharge (ozaenae).
- Easily treatable.
- Treatment : Tetracyclines/Fluoroquinolones.

Klebsiella oxytoca

00:16:26

Indole positive (IMVIC : +-++).

Nosocomial pathogen.

Associated with antibiotic associated colitis.

Causes surgical site infections, meningitis post neurosurgery.

MDR bacterium : Treated after sensitivity testing.

Klebsiella pneumoniae pneumoniae and Klebsiella oxytoca :
MDR bacteria.

Klebsiella granulomatis

00:17:40

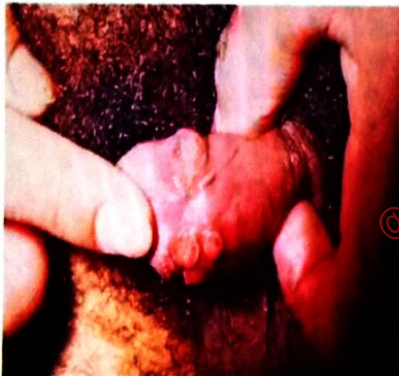
Earlier called Calymmatobacterium granulomatis.

Difficult to culture.

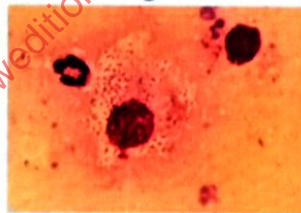
Causes **granuloma inguinale/donovanosis** (sexually transmitted disease).

- Painless genital ulcer disease.
- Incubation period : 1-6 weeks.
- Painless nodule/papule → Beefy red ulcer with rolled edges → Bleeds on touch.
- Can auto inoculate → multiple ulcers → Coalesce → Big ulcer → Destruction of the underlying tissues.

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Klebsiella granulomatis



Donovan bodies seen in large, mononuclear (Pond) cells as intracytoplasmic cysts

Diagnosis :

Difficult of culture.

Scraping from edge of the ulcer or **punch biopsy** → Stain with giemsa → **Pond cells** (large mononuclear cells with intracellular bacteria (**donovan bodies**)).

Treatment :

- **DOC** : Azithromycin for 3 weeks.
- **Alternative** : Doxycycline.

Enterobacter

00:22:36

Closely related to Klebsiella (belongs to enterobacteriaceae).

Motility	motile
Capsule	Capsulated
Sugar fermentation	Aerogenic
Wok's Proskauer (WP) test	Positive
Citrates	utilized
Urease	Negative
MacConkey's medium	Lactose fermenting
Blood agar	Mucoid colonies (thick capsule).
Species	Common species associated with human beings : 1. <i>Enterobacter cloacae</i> . 2. <i>Enterobacter aerogenes</i> .
Resistance	Chromosomal and R-plasmid mediated resistance. Encodes lots of extended spectrum beta lactamases and carbapenemases.
	Normal gastrointestinal flora. Nosocomial infections, like UTI, pneumonia, Surgical site infections

WHO priority escape agents :

- Nosocomial infections.
- Highly virulent.
- MDR pathogens.

Include : ESKAPE :

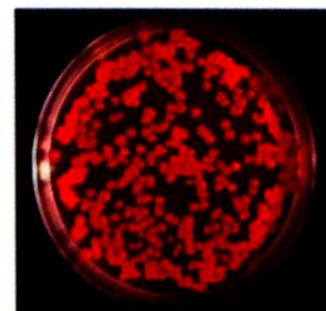
- Enterococcus faecium*.
- Staphylococcus aureus*.
- Klebsiella pneumoniae*.
- Acinetobacter baumannii*.
- Pseudomonas aeruginosa*.
- Enterobacter* species.

Serratia marcescens

00:25:17

Produces red color pigment called **prodigiosin**.

Acts as an efficacy tester for pore size of standard bacterial filters.
motile.



Causes :

1. Nosocomial pneumonias : Pseudo hemoptysis (red pigment).
2. Contact lens associated keratitis (pink hypopyon).
3. Infections in Chronic Granulomatous Diseases (CGD).
MDR bacterium.

Treatment based on sensitivity.

Pathogens causing infections in CGD :

1. Staphylococcus aureus.
2. Burkholderia cepacia.
3. Nocardia.
4. Actinomyces.
5. Aspergillus.
6. Serratia marcesans.

Proteus

00:28:13

Belongs to tribe **Proteae**.

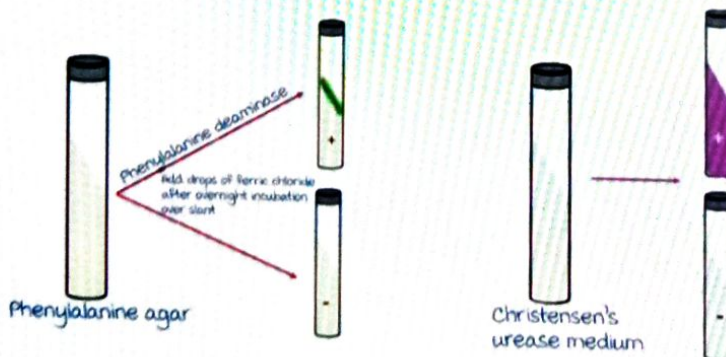
Others include :

1. Proteus.
2. Morganella.
3. Providencia.

bnvssprasanth7@gmail.com

Family : Enterobacteriaceae.

PPA test positive (phenyl pyruvic acid test : Ability to convert phenylalanine to phenyl pyruvic acid).



Active space

motility	motile
Capsule	Non-capsulated
H ₂ S	When sugars are utilized → They produce H ₂ S.
Urease	Positive
MacConkey's medium	Non-lactose producing
Blood agar	Swarming
Species	Proteus vulgaris, Proteus mirabilis

Swarming used in **Diene's phenomenon** (use in epidemiological typing of Proteus strains).

On a sterile blood agar plate, spot inoculate 2 strains and incubate them overnight, both will swarm over the plate.

If same strain → merge together.
If different strains → They form a zone or a line separating them.



Diene's phenomenon

Clinical importance:

Normal gastrointestinal flora.

Associated with:

Nosocomial infections.

1. Catheter associated UTIs.
2. Pneumonias.
3. Intra-abdominal abscesses.
4. Peritonitis.

Community acquired UTIs (recurrent UTIs with proteus

→ Persistent alkalization of urine because of urease → **Struvite stones** in kidney).

- Sensitive to most antibiotics.
- Resistant to tetracycline, nitrofurantoin (P. vulgaris > P. mirabilis).

H₂S producing organisms:

1. Salmonella.
2. Cyclobacter.
3. Proteus.

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Bacteria showing **swarming** :

Gram positive : *Bacillus cereus* & *Clostridium tetani*

Gram negative :

1. *Vibrio alginolyticus*
2. *Vibrio parahaemolyticus*
3. *Proteus*

Swarming can be **inhibited by** :

1. Increasing agar concentration (5-6%).
2. Using MacConkey's medium or CLED medium.
3. Add chemical agents (alcohol, boric acid, chloral hydrate, sulfonamides, sodium azides or surface acting agents).

Yersinia

00:40:56

member of enterobacteriaceae.

Species:

1. *Y. pestis*.
2. *Y. enterocolitica*.
3. *Y. pseudotuberculosis*.

Yersinia pestis :

Category A bioterrorism agent.

Highly pleomorphic (different sizes) gram negative rods.

bnvssprasanth7@gmail.com	
Motility	Non motile.
Capsule	Capsulated (1 capsular serotype : Protein capsule).
Temperature	Can grow at wide temperature ranges (2-45°C). Optimum temperature of growth : 27°C. Cold enrichment can be used for isolation.
pH	Can grow at wide pH ranges (5-9.6).
Sugar utilization	Anaerogenic.
H ₂ S	Does not produce H ₂ S.
IMVIC	-+--
Urease	Negative.
MacConkey's medium	Non-lactose producing.

Active space

Blood agar	Brown colored colonies (hammered copper colonies) due to deposition of hemin.
Selective media	CIN (Cefsulodin-Irgasan-Novobiocin) agar.

Facultative Intracellular bacteria.

Virulent factors :

1. Anti-phagocytic capsule (F1/fraction I antigen).
2. Lipopolysaccharides (gram negative : Endotoxins).
3. V and W proteins : Intracellular survival.
4. Protease (coagulase activity at 37°C and fibrinolytic activity at 25-28°C).

Epidemiology :

- Reservoir : Wild rodents.
- Vector : Rat flea (Xenopsylla cheopis).
- Enzootic in : Africa, South America, Southeast Asia (India). In India : Uttaranchal, Rhoru etc.
- Epizootic in : moist/humid seasons.

Human plague :

	Bubonic plague	Pneumonic plague	Septicemic plague
Comment	most common	Highly infectious form	Secondary to bubonic/pneumonic plague. 10-25 % individuals → Primary manifestation.
Transmission	Bite of flea	Inhalation of aerosols	Bite of flea or aerosols
Clinical features	Incubation period (2-7 days) → Painful regional lymphadenopathy (buboes). Some may have sentinel papule/pustule → Resolve spontaneously in 50% → Or septicemic plague if untreated.	Incubation period (1-2 days) → Symptoms of hemorrhagic pneumonia (fever, productive cough, hemoptysis, dyspnea, prominent cyanosis etc.) If left untreated → Septicemia (85-90%).	Severe endotoxemia : Fever +++ with low BP with typical endotoxic manifestations. DIC : Leading to gangrene of toes and fingers + multi organ failure.

Diagnosis :

Specimen : 5ca2793ec88d500486113130

- Bubo aspirate.
- Sputum/broncho-alveolar lavage.
- Blood specimen.
- CSF (if meningitis suspected).

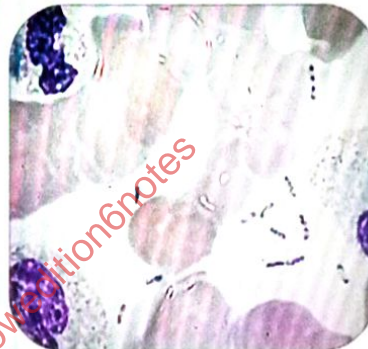
Specimen handling in **BSL 2 lab.**Giemsa staining or methylene blue staining : Typical **safety pin appearance** (bipolar stain).

Gram stain : Gram negative pleomorphic rods.

DFAT (Direct Fluorescent Antibody Testing) to detect **FI antigen.**

Culture : most specific.

- Done in **BSL 3 Lab.**
- Contaminate (sputum/BAL) : use CIN agar.
- Sterile specimen (bubo aspirate/CSF) : macConkey's/ blood agar.



Giemsa stain

Nucleic acid Amplification tests : PCR (most sensitive).

Serology :

- Antibodies increase at the end of **1st week.**
- used for **retrospective diagnosis.**

Safety pin appearance seen in :

1. Yersinia pestis.
2. Hemophilus ducreyi.
3. Klebsiella granulomatis.
4. Burkholderia mallei
5. Burkholderia pseudomallei.

Treatment of *Yersinia pestis*

01:00:46

TOC : Streptomycin BD for 7-10 days.

Pregnancy and children : Gentamicin for 7-10 days.

meningitis/pleuritis/myocarditis : Fluoroquinolones or chloramphenicol.

Droplet precautions for at least 2 days after starting treatment in case of pneumonic plague.

Safety pin appearance

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Post exposure prophylaxis :

Doxycycline or ciprofloxacin or levofloxacin for 7 days.

High risk individual : Killed vaccine (0, 1, 6 months dose + boosters every 6 months).

Sokhey's modification of Haffkine vaccine is the killed vaccine .

Yersinia enterocolitica and *Yersinia pseudotuberculosis* : Disease caused by these are called yersiniosis.

motility	Differential motility : <ul style="list-style-type: none"> • motile at room temperature. • Non motile at 37°C.
Temperature	Can grow at lower temperature → Cold enrichment.
macConkey's medium	Non-lactose producing.
CIN agar	Colonies with red centers and pale periphery (bull's eye colonies).
Reservoirs	Several birds/animals (domestic/wild).
mode of transmission	Ingestion of unpasteurized milk, poorly cooked meat, unwashed vegetables or contaminated milk.

Clinical aspects :

<i>Y. enterocolitica</i>	<i>Y. pseudotuberculosis</i>
unpasteurized milk ingestion → Invasive diarrhea (fever with abdominal pain with bloody stools). Self resolving.	

mesenteric adenitis (pain resembles appendicitis : Pseudo appendicitis).

People with iron overload states & liver disease → **Septicemia with visceral abscesses.**

Treatment :

Wait for **self-resolution.**

Persistent diarrhea/severe mesenteric adenitis →

Ciprofloxacin or septran.

Septicemia → Gentamicin + 3rd gen cephalosporin.

They can be differentiated from Yersinia pestis by motility and urease positivity.

Differential motility seen in :

1. Yersinia pestis.
2. Listeria.

MCQs

01:10:20

Q. Which of the following statements is NOT correct about the characteristics of Yersinia pestis?

A. The bacteria can cause pneumonia in humans through airborne droplets.

B. The suitable bacterial growth temperature is room temperature using MacConkey agar.

C. The bacteria is lactose positive.

D. The common source of transmission to humans is through rats.

Q. Which of the following is the best selective media used for the isolation of the pathogen Yersinia enterocolitica from a stool specimen?

A. Regan-Lowe medium.

B. CellSulodin-irgasan-novobiocin agar.

C. medium

D. Lowenstein-Jensen media.

Important properties of Enterobacteriaceae members :

member	motility	LF/NF	Gas(CO ₂) H ₂ S	IMVIC	Urease
Escherichia	m	LF	Aerogenic -	++--	-
Shigella	Nm	NLF	Anaerogenic -	-+--	-
Klebsiella	Nm	LF	Aerogenic -	--++	+
Enterobacter	m	LF	Aerogenic -	--++	-
Citrobacter	m	LF/NLF	Aerogenic	-++ ⁺ Cf ++- ⁺ CK	-
Salmonella	m	NLF	Aerogenic +	-++	-
Proteus	m	NLF	Variable +	Variable	+
Yersinia	Nm	NLF	Anaerogenic -	-+--	-

@marroweditionsnotes

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Active space

SALMONELLA

Gram negative rods.

Non sporing facultative anaerobes.

Catalase positive, Oxidase negative.

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Reduce nitrates to nitrites.

Can utilize sugars both aerobically or anaerobically :

Fermenting sugars.

Easily grow on simple or basal media.

macConkey Agar : Lactose or non-lactose fermenting colonies.

Salmonella

00:01:38

motile by peritrichous flagella, except salmonella galinarum pullorum.

Non capsulated except typhi, Paratyphi C and Dublin are capsulated.

2 species : Salmonella enterica and salmonella bongori.

Predominantly disease is caused by Salmonella enterica.

Salmonella enterica is divided into six subspecies :

Enterica, indica, arizona etc.

These 6 subspecies are subdivided into > 3000 serotypes.

Serotyping is done under Kaufmann and white scheme.

In India, reference centre for serotyping : CRI Kasauli (all serotypes are available).

Kaufmann and white used 3 antigens.

- O antigen : Somatic antigen (LPS).
- H antigen : Flagellar antigen.
- Vi antigen : Capsular antigen (typhi, Paratyphi c and Dublin).

Salmonella typhi : Salmonella enterica subspecies enterica serotype typhi - complete name.

- Identified by 9, 12 Ag in cell wall and flagellar Ag d type and vi.

Salmonella paratyphi A : Salmonella enterica subspecies enterica serotype Para typhi A.

- Expresses a) 1,2,12 antigen in cell wall.
- b) Flagellar antigen A type.
- c) Flagellar variations of 1 and 4 is called as phase variations.

Salmonella paratyphi B : Salmonella enterica subspecies enterica serotype Para typhi B

- Expresses a) 1,4,5,12 O antigen,
- b) B type flagellar antigen at phase I,
- c) 1 and 2 types of flagellar Ag at phase II.

All 3 salmonella serotypes causing typhoid in India share O-12 antigen in their cell wall.

In Widal test, we detect O and H antibodies in the serum. For detection of O antibodies, only O antigen of typhi needs to be added, as the same antigen can be used for typhi, paratyphi A & B detection. low sensitivity & specificity for enteric fever.

Paratyphi C is common in middle eastern countries, not India, hence not tested in Widal test.

Antigens of salmonella

00:11:50

H Antigen	O Antigen	Vi antigen
Flagellar antigen : Protein in nature.	Somatic antigen of salmonella (boivin antigen). Polysaccharide in nature.	Capsular antigen. Polysaccharide in nature.

Active space

<p>Highly antigenic and shows phase variations.</p> <p>Heat and alcohol labile.</p>	<p>Lower antigenicity.</p> <p>Heat and alcohol stable.</p>	<p>Least antigenic.</p> <p>Heat labile and alcohol stable</p> <p>Vi antigen covers O antigen and hence it prevents agglutination with O anti serum.</p> <p>Same Vi antigen may be shared by some E coli, Citrobacter strains.</p>
<p>H antibodies appears earliest after infection.</p> <p>They persist for several months.</p> <p>Presence of H abs in absence of O antibodies indicates remote infection.</p>	<p>O antibodies appear after H antibodies.</p> <p>O antibodies disappear after few weeks.</p> <p>Presence of O abs indicates recent infection.</p>	<p>Vi antibodies appear only in the stage of convalescence</p> <p>Absence of Vi antibodies indicate poor prognosis.</p> <p>Persistence of Vi antibodies indicate carrier state.</p>

Because of the presence of Vi antigen, agglutination of O antigen will not occur in Widal's test, showing false negative results. In order to precipitate O antigen, the sample is first heated to destroy Vi antigen (heat labile antigen).

Phage typing

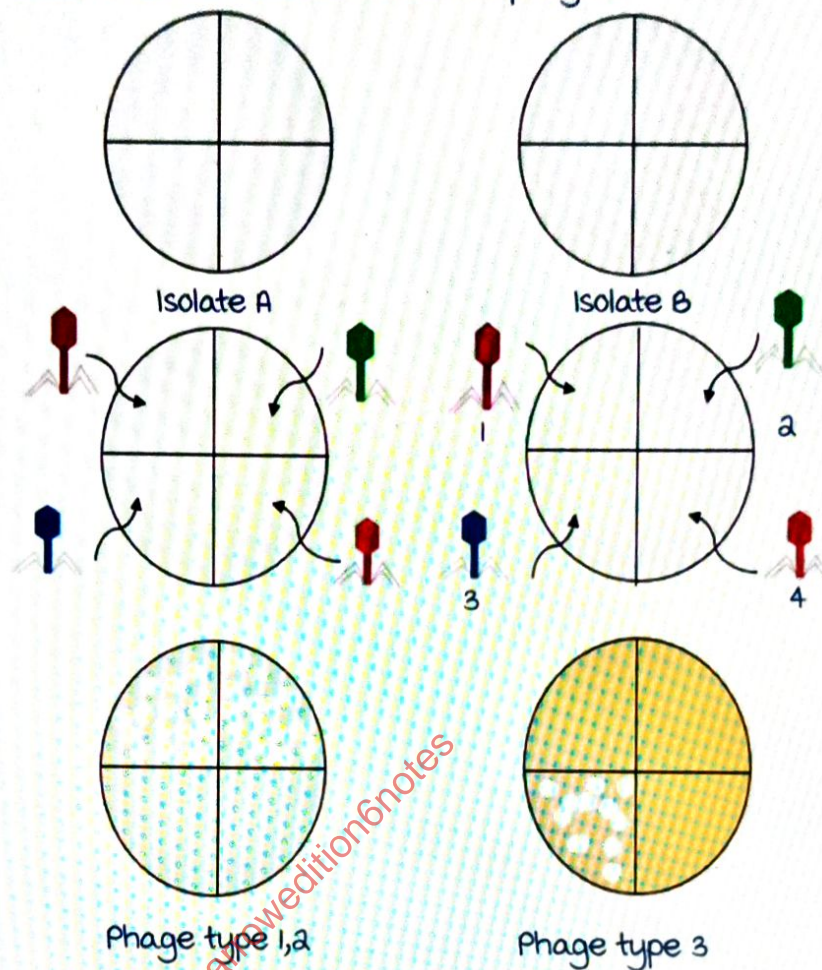
00:23:48

Typing of bacteria based on susceptibility to invasion by bacteriophages is phage typing.

Typing is useful in epidemiological study, virulence study and transmission study.

S. typhi phage type common in India is A and EI.

Similar strains are detected by specific lysing patterns on media to various standard bacteriophages.



Phage typing is also seen in :

- Salmonella.
- *Staphylococcus aureus*.
- *Vibrio cholerae*.
- *Corynebacterium diphtheriae*.
- *Mycobacterium tuberculosis*.

Salmonella can also be typed on the basis of superior genotypic methods :

- Restriction Fragment Length Polymorphism (RFLP).
- Pulse Field Gel electrophoresis (PFGE).
- Multi Locus Sequence Typing (MLST).

Biochemical reactions :

While utilizing sugars : Aerogenic.

Produce both acid with gas.

Except: *Salmonella typhi* - Anaerogenic, only acid no CO_2 .

H_2S : Positive for all except para typhi A, typhi suis and Cholera suis.

ImViC: - + - +. Except for *S. Typhi*: - + - -.

Does not produce urease.

Cultivation:

MacConkey agar: Non lactose fermenting.

Transport agar: Cary Blair medium.

Enrichment medium: Selenite F broth and tetrathionate broth.

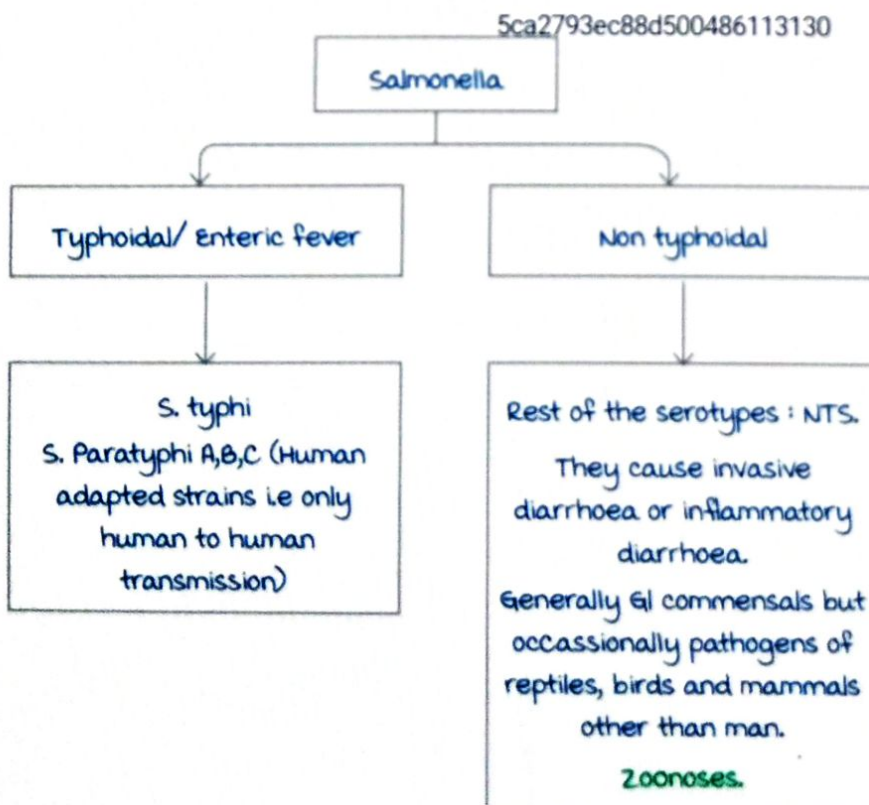
Selective medium (black colonies due to H_2S):

- Deoxycholate citrate agar.
- Xylose lysine deoxycholate.
- Hoektoen enteric agar.
- *Salmonella shigella* agar.

Wilson and Blair bismuth sulphite medium: most selective medium.

Clinical classification of salmonella

00:31:12



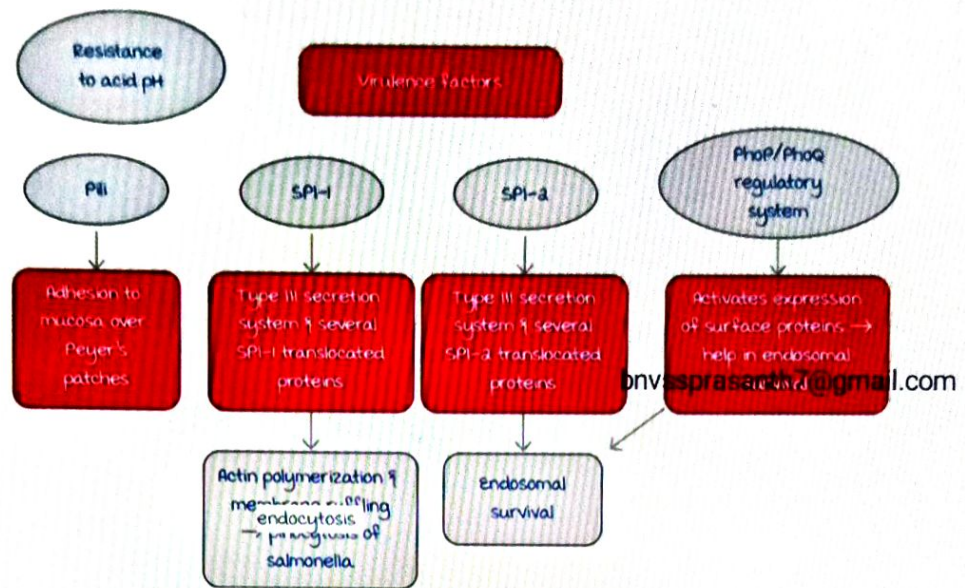
Active space

enteric fever :

S. typhi : Typhoid.

S. paratyphi A,B,C : Paratyphoid.

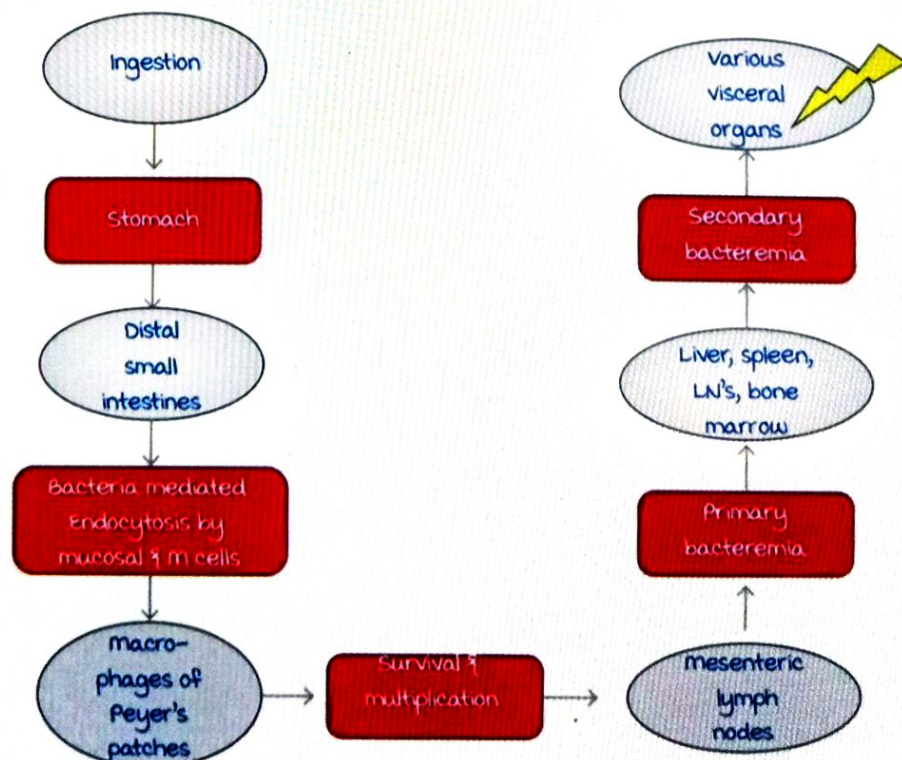
Virulence factors :



Salmonella survives & multiplies inside macrophages

Quorum sensing system : Bacterial communication among each other after reaching a particular intensity via PhoP and PhoQ regulatory system.

Pathogenesis :



Active space

Clinical features

00:40:21

mode of infection :

Ingestion of food or water contaminated with human feces.

Infective dose : 10^3 - 10^6 .

Incubation period : 1-2 weeks (3-21 days).

Constitutional symptoms :

Step ladder pyrexia (Rise of temperature → remain stable → further rise → remains stable etc), malaise, anorexia.

Physical examination :

- Bradycardia (**Faget's sign**) – Sphygmothermic dissociation.
- Coated tongue.
- Rose spots (erythematous macules).
- Hepatomegaly, splenomegaly.

Course of 3-4 weeks, followed by resolution in most.

Complications :

- **Intestinal hemorrhage** (m/c) : Seen in 9-10% of population when secondary bacteraemia occurs, bacteria reappear in Peyer's patches causing massive inflammation.
- Intestinal perforation : Seen in 1-3% of population (m/cly seen in 3rd week).
- Psychosis, meningitis, deafness, arthritis, nephritis, cholecystitis, and visceral abscesses.

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Diagnosis of enteric fever

00:44:31

Gold standard : Culture.

Specimen : Blood, bone marrow aspirate, stool, urine sample and duodenal aspirate.

Blood becomes **positive in first week** of clinical features.

Stool, urine and duodenal aspirate become positive in the later part of illness (end of 2nd week).

Rates of positivity of blood culture :

1st week : 90% .

2nd week : 75% .

3rd week : 60% .

4th week : 25% .

Advantage of bone marrow aspirate than blood culture :

- more sensitive than blood culture at all stages of illness.
- Remains positive for 5 days after antibiotics.

Disadvantage : very painful.

medium : Brain heart infusion agar/broth.

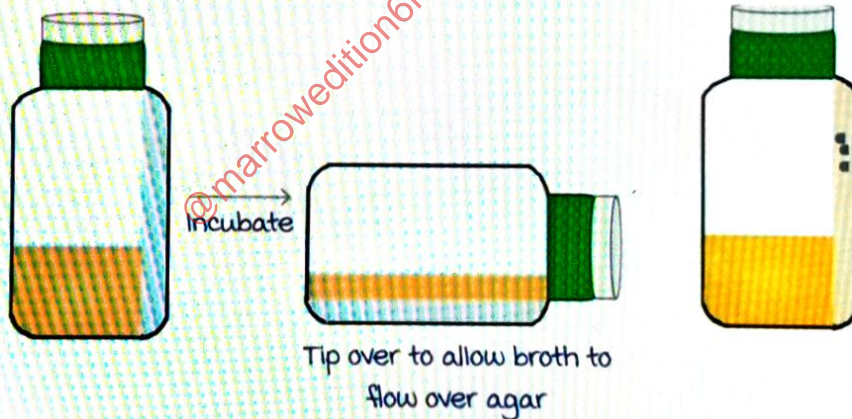
10 ml of blood inoculated in the broth aseptically and incubate at 37°C.

Subcultures daily on both macConkey and blood agar for 10 days.

Increased chance of contamination.

To avoid the risk of contamination : Castaneda/ Biphasic medium is used.

Incubate → Tip over to allow broth to flow over agar.



Stool and duodenal aspirate : Positive at the end of 2nd week in 40-50% of patients.

Cultured on selective medium and simultaneously on enrichment broth (Selenite F broth).

From the enrichment broth after 6 hours of incubation, colonies are subcultured in selective medium, then biochemical tests are done.

Urine culture : Positive at end of 3rd week in 30-50% of patients.

Serology

00:53:23

Presence of antibodies in serum is detected.

Neither sensitive nor specific as antibodies from previous subclinical infections maybe present, and also due to polyclonal activation of B cells causing secretion of antibodies following an unrelated infection.

WIDAL test : Type of tube agglutination test.

Presence of H antibodies and O antibodies in patient's serum.

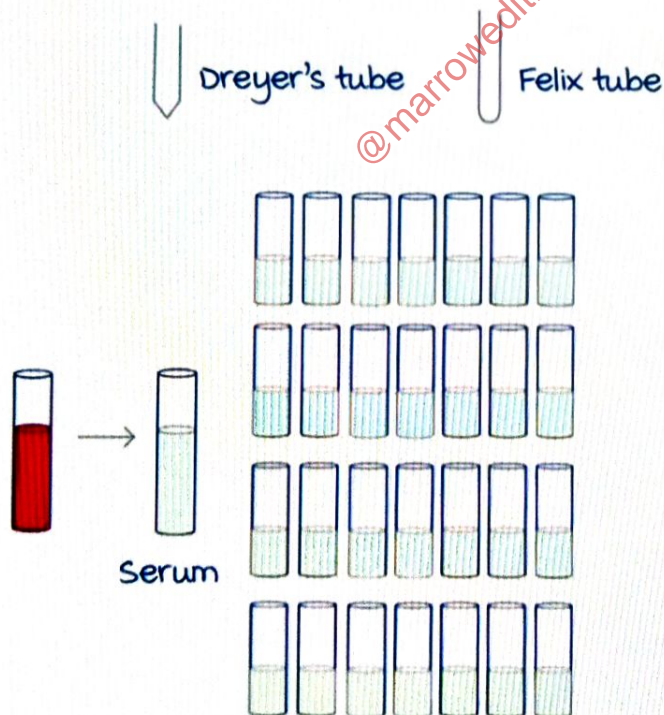
Serum H Abs + H Ag \rightarrow Fluffy or woolly agglutination (seen best in **Dreyer's tubes**).

Serum O Abs + O Ag \rightarrow Granular/chalky agglutination (seen best in **Felix tubes**).

m/c cause of typhoid in India : **Salmonella typhi** > Paratyphi A.

Patient's serum is taken and serial dilutions are made in 4 sets of test tubes.

Dilutions - 1 : 10, 1 : 20, 1 : 40, 1 : 80, 1 : 160, 1 : 320, 1 : 640.



1st set O antigen of *S. typhi* is added.

2nd set H antigen of *S. typhi*.

3rd set H antigen of Paratyphi A.

4th set H antigen of Paratyphi B.

Active space

Incubate overnight in water bath at 37°C. Look for fluffy agglutination in 'O' set and granular agglutination in other 3 sets.

Interpretation : Highest dilution of patient's serum which shows agglutination is taken.

In the image, titre of O antibody - 1 : 160.

Significant titer of O abs in India is > 1 : 80 (recent infection).

Here, highest dilution of patient serum of H agglutination - 1 : 320.

Significant titer of H antigen > 1 : 160.

Paratyphi A H antigen - 1 : 20 which is insignificant.

Paratyphi B H antigen - 1 : 10 which is insignificant.

Diagnosis : Acute or recent infection of salmonella typhi.

Example 2 :

O Abs - 1 : 160.

H Abs - 1 : 20.

Paratyphi A H antigen - 1 : 320 (significant).

Paratyphi B H antigen - 1 : 10.

Diagnosis : Recent infection due to paratyphi A.

Example 3 :

O Abs - 1 : 160.

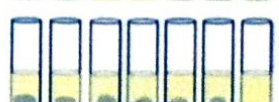
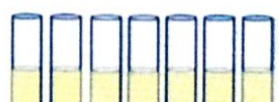
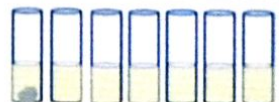
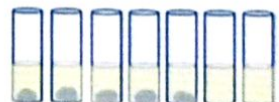
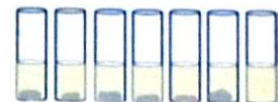
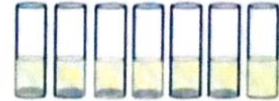
H Abs - 1 : 10.

H Abs of Paratyphi A : Nil.

H Abs of Paratyphi B - 1 : 320.

Diagnosis : Recent infection due to para typhi B.

Widal test



Active space

Widal test : Important points

01:03:44

- Antibodies start to appear at the **end of 1st week of illness**.
- A significant titer of O antibodies in India is $\rightarrow \geq 80$
 \rightarrow Indicates recent infection.
- Significant titers of H antibodies indicate serotype responsible for infection.
- Significant H antibodies titer in India $\rightarrow \geq 160$.
- Rise in titer of antibodies should be demonstrated.
- A single high titre may be an **anamnestic response**, due to activation of memory B cells from previous exposure to salmonella and unrelated illnesses, causing sudden spike in antibody levels which will come down in 2-3 days. Repeating the test after 4-5 days is recommended.

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Enteric fever diagnosis - Other tests

01:08:08

Typhi dot : Immunochromatography test (lateral flow assays).

Separately detect IgM and IgG antibodies.

IgG positive : Past infection.

IgM positive : Early acute infection.

Both IgG and IgM positive :
Acute enteric fever.

Disadvantage of typhidot : Nonquantitative.

Antigen detection :

Latex agglutination, ELISA or Co-agglutination.

Good sensitivity only in the 1st week of illness.

Nucleic acid amplification test (e.g. PCR) :

most sensitive.

Limited availability.

most specific test : Culture.



Active space

Empirical treatment :

Ceftriaxone or Cefotaxime 1m/IV x 7-14 days.

Azithromycin PO x 7 days.

Fully drug susceptible :

Ciprofloxacin or Ofloxacin (1st line) PO/IV x 5-7 days.

Amoxicillin (2nd line) PO/IV x 14 days.

Chloramphenicol PO/IV/1m x 14-21 days.

Cotrimoxazole PO/IV x 14 days.

MDR infection :

Resistant to Ampicillin, Cotrimoxazole or Chloramphenicol.

Ceftriaxone 1m/IV x 7-14 days.

Azithromycin PO x 7 days.

Ciprofloxacin PO/IV x 5-7 days.

Fluoroquinolone (FQ) resistant :

Ceftriaxone 1m/IV x 7-14 days.

Azithromycin PO x 7 days.

Carriers

01:15:00

m/c site of carriage : Gall bladder → Fecal carriers.

2nd site : Kidney → urinary carriers.

Carriers classification :

Convalescent carrier : upto 3 months after clinical cure.

Temporary carrier : 3 months - 12 months after clinical cure.

Chronic carrier : > 12 months after clinical cure.

Diagnosis of carrier state :

1. Persistence of Vi antibodies : Vi agglutination test (screening test).
2. Stool or urine culture (confirmatory test).

Treatment of carrier state :

Depending upon susceptibility report.

Ciprofloxacin → 4 weeks.

Amoxicillin/ Ampicillin/ Cotrimoxazole → 3 months.

Vaccines

01:19:48

Type specific immunity for *S. typhi*.

Typhoral : **Oral LAV** → mutant strain Ty 21a - enteric coated capsule.

≥ 6 years of age.

4 doses on alternate days → 5 years protection → Full dose repeated after 5 years.

Typhim vi : Injectable subunit vaccine (SC or IM).

≥ 2 years of age → Single dose every 2 years.

Non typhoid salmonella (NTS) :

Reservoir : GI tract of many mammals, birds and reptiles.

modes of infection :

Ingestion of unpasteurized milk or milk products.

Ingestion of poorly cooked poultry.

Ingestion of raw or runny eggs.

Exotic pets like turtle/tortoise/lizard/snakes.

They cause **invasive diarrhoea**.

D/O of invasive diarrhoea :

- Enteroinvasive *E. coli*.
- Enterohemorrhagic *E. coli*.
- Shigella.
- Campylobacter.
- *Yersinia enterocolitica*.
- *Vibrio paralyticus*.

Incubation period varies about : 8-24 hours (upto 48 hours).

C/F : Fever with abdominal cramps

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Diarrhoea (non bloody).

Self resolving (1-2 days).

Diagnosis : Stool culture.

m/c serotype of NTS : ***S. typhi* murium**.

Active space

NTS bacteraemia :

In neonates, elderly and immunodeficient and patients with chronic hemolytic anemia (sickle cell anemia and thalassemia) → Invasive enteritis → Septicemia → meningitis, osteomyelitis, arthritis, nephritis, etc.

Diagnosis : **Blood culture.**

m/c serotypes : Cholera suis, Dublin, enteritidis.

Treatment : **Symptomatic treatment.**

Antibiotics not required as it is self resolving.

In case of neonates and severe disease : 3rd generation cephalosporin (in risk of septicemia also).

Q. A 23 year-old man comes to the emergency department complaining of bloody diarrhea and a fever for the last 1 day. On questioning, he says that his symptoms started with just loose stools with mild abdominal pain. On examination he is febrile and blood pressure is 110/65 and pulse is 84/min. Laboratory tests of blood and stool cultures show an oxidase-negative, motile, gram negative bacillus that grows as clear colonies on macConkey agar. Which of the following is the most likely causative organism?

- A. Escherichia coli.
- B. Pseudomonas aeruginosa.
- C. **Salmonella species.**
- D. Shigella species.
- E. Vibrio cholerae.

Q. A 3 year-old boy presents with a 1-day history of loose stools, fever, abdominal cramping, headache, and myalgia. He has no blood in the stool. A careful history reveals that he has several pet turtles. Which of the following is most likely the causative agent of his diarrhea?

- A. Chlamydia psittaci.
- B. Entamoeba histolytica.
- C. **Salmonella spp.**
- D. Shigella.

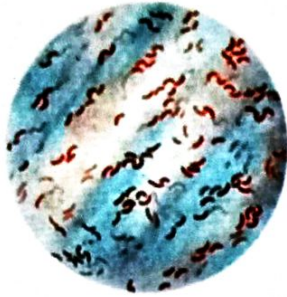
VIBRIO

Morphology

00:01:30

Gram negative, curved rods.

Robert Koch gave the name 'Komma bacillus' for vibrio cholerae and cultured it for the first time.



Reservoir for all vibrios : Sea water and brackish estuaries.
Vibrios cannot survive in contaminated waters due to vibrio phages.

Have 2 chromosomes : Large and small.

motile by monotrichous flagellum.

motility is described as darting/shooting star.

Some are capsulated : v. parahaemolyticus, v. vulnificus and v. cholerae O139.

Facultative anaerobes except v. cholerae which shows poor growth anaerobically.

pH requirement : Optimum pH is 8.2-8.4 (alkaliphiles).

Salt requirement :

- 0.5-1% salt required (Halophilic).

~~Scal 279 cholerae and v. vulnificus~~
v. cholerae and v. vulnificus are non halophilic.

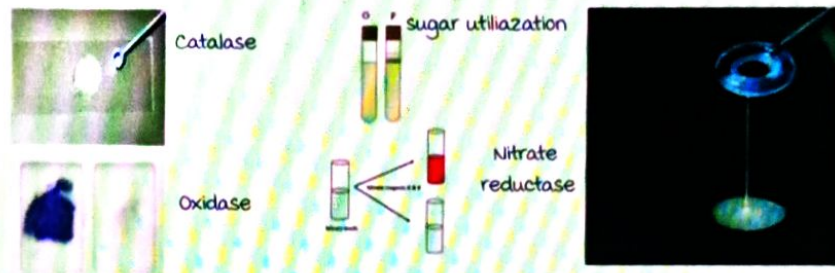
- v. parahaemolyticus requires 2-4% of salt.

- Above 6% of salt concentration → No growth except v. parahaemolyticus (can tolerate up to 8%) and v. alginolyticus (up to 10%).

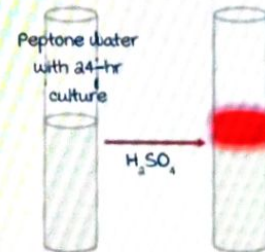
Active space

Biochemical reactions :

- Catalase test positive.
- Oxidase test positive.
- All utilize sugars : **Fermentative**.
- All reduce nitrates to nitrites.
- **String test** is positive : A drop of bile salt (0.5% sodium deoxycholate) is taken on a slide. With a loop a few colonies of vibrios are added. On mixing, bile salt appears milky. It comes out with a string attached when the loop is taken out.



- **Cholera red reaction** is positive only for *V. cholerae*. It checks ability to reduce nitrates to nitrites and ability to convert tryptophan into indole. Few colonies of *V. cholerae* are added to peptone water (contains salt) and incubated. Nitrites and indole are produced that combine to form nitrosoindole. On adding few drops of sulphuric acid,



bnvssprasanth7@gmail.com a red colored ring develops.

String test is positive for hypermucoviscous strains of *Klebsiella pneumoniae*. Touch the colonies of *K. pneumoniae* on a culture medium using a loop. It comes out with a string attached when the loop is taken out.

Vibrio cultivation

00:12:42

Simple media : Grows on simple media (Non fastidious).

Blood agar : **Hemodigestion** → Vibrio growth on blood agar plate produces a chemical compound that diffuses around colonies causing lysis of RBCs - Greening around cluster of colonies but not around individual colonies.



Transport medium :

- Venkatraman Ramakrishnan medium.
- Cary Blair medium.
- Autoclaved sea water.

Enrichment medium :

- Alkaline peptone water.
- Monsur's taurocholate tellurite peptone water.

They can also be used as transport media.

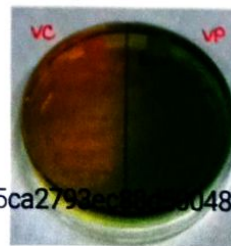
Selective medium :

- Bile salt agar.
- Monsur's gelatin trypticase taurocholate tellurite agar.

Selective and differential medium :

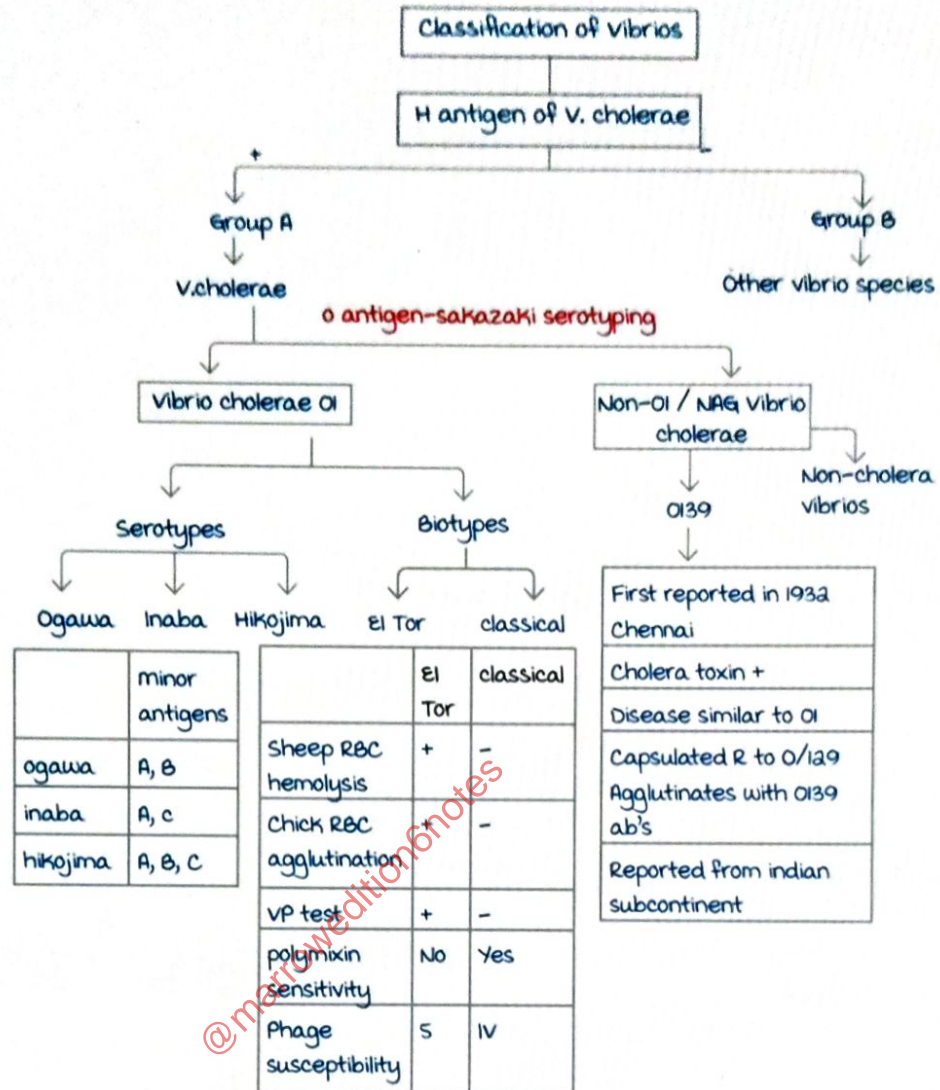
TCBS - Thiosulphate citrate bile salt sucrose agar -

- Helps to differentiate sucrose fermenting vibrios from non-sucrose fermenting vibrios.
- **Sucrose fermentation** produces acid and pH indicator (bromothymol blue) changes color : **Bright yellow**.
- Colonies of **sucrose fermenters** are yellow colored (v. cholerae). Colonies of **non-sucrose fermenters** are **green colored** (v. parahaemolyticus).



Classification of vibrios

00:17:20



Vibrios were classified by Gardner and Venkatraman on the basis of flagellar antigen: H. antigen of *V. cholerae*.

- **Positive H antigen**: Group A vibrios. *V. cholerae*.
- **No H antigen (own flagellar antigen)**: Group B vibrios. All other species except *V. cholerae*.

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Sakazaki serogrouping:

- *V. cholerae* serogrouping on the basis of **O antigen**.
- O1 - O200 serogroups.
- Only **O1 serogroup** produces cholera toxin.
- Cholera is gastroenteritis caused by toxin of O1 serogroup. Other serogroups cause gastroenteritis, but they do not secrete cholera toxin.
- Other vibrios are called as **non-cholera vibrios/ non O1 vibrios/non-agglutinable vibrios/NAG vibrios** (no agglutination with O1 antiserum).

In 1992, a new strain was isolated in Madras. Though a non-cholera vibrio, it produced cholera toxin but there was no agglutination with O1 antiserum → Identified as **O139 V. cholerae**.

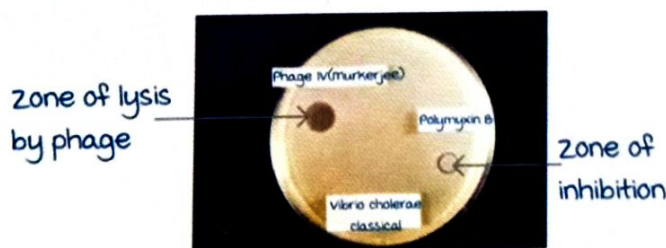
V. cholerae O1 is further biotyped into **El Tor** and **Classical**. In addition, it is serotyped based on minor antigens (A, B and C) into **Ogawa**, **Inaba** and **Hikojima**.

- Ogawa has **A and B** minor antigens.
- Inaba has **A and C** minor antigens.
- Hikojima has **A, B and C** minor antigens.

Basis of bio typing V. cholerae into El Tor and Classical :

	El Tor	Classical
Sheep RBC hemolysis	+	-
Chick RBC agglutination	+	-
VP (Voges-Proskauer) test	+	-
Polymyxin sensitivity	Resistant	Sensitive
Phage susceptibility	Susceptible to 5	Susceptible to 4

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Lawn culture of V. cholerae

Epidemiology :

	El Tor	Classical
Hardiness in sea water	more (survives longer)	Less
Subclinical cases	more	Less
Severity of disease	Less	more
mortality	Less	more
Secondary attack rate	Less	more
Carriers	more	Less

Active space

O139 V. cholerae :

- First isolated in 1992 in Chennai.
- Produces cholera toxin but does not agglutinate with O1 antiserum.
- It spread to Bangladesh in 1992 killing more than 50 thousand people. Hence it is also called as Bengal strain.
- It causes disease similar to cholera. Unlike V. cholerae O1, it is capsulated and agglutinates with O139 antiserum.
- It is resistant to vibriostatic compound : O/129.
- Presently it is reported in some parts of India and Bangladesh.

Pandemics of cholera

00:33:16

Over the last 200 years, there have been 7 pandemics.

First 6 pandemics → Originated in Indian subcontinent. Due to V. cholerae O1 classical biotype.

7th pandemic → Originated in Indonesia (1961). Due to V. cholerae O1 El Tor biotype.

Cholera virulence factors :

Flagellum :

Sheathed flagellum → Helps to cross mucous layer covering the small intestinal mucosa.

Toxin coregulated pili (Tcp) → Helps in adhesion to small intestinal mucosa. Role in formation of microcolonies on small intestinal mucosa.

Cholera toxin/cholera toxin :

Prophage (CTX ϕ) mediated. ABS subunit toxin. B subunit binds to GM1 ganglioside receptor.

Site of action → Small intestinal mucosa.

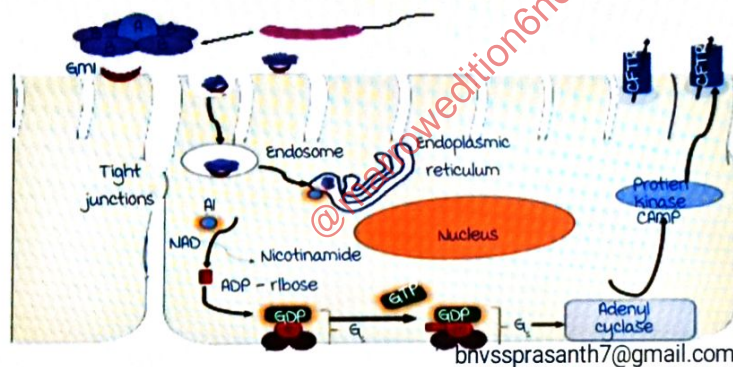
Mechanism of action → Upon binding to receptor, toxin undergoes receptor mediated endocytosis.

Toxin is transported to endoplasmic reticulum → The A subunit separates from B subunit, and it is further proteolyzed into (A1 + A2) parts.

A1 part is active enzymatic part → It transfers ADP ribose moiety from NAD to alpha subunit of G_s stimulatory protein. ADP ribosylation of alpha subunit causes persistent stimulation of adenylyl cyclase and massive increase in cAMP.

Increase in cAMP causes continued activation of protein kinase and phosphorylation of cystic fibrosis transmembrane regulator (chloride ion channels).

This leads to pumping out of chloride ions from crypt cells into the lumen of small intestine and reduced absorption of sodium chloride from villous tip cells.



Toxins acting by cAMP :

- ETEC : Labile toxin.
- Anthrax toxin.
- Bordetella pertussis : Pertussis toxin, adenylyl cyclase toxin.
- Cholera toxin.

[Mnemonic : Exotoxins Acting By cAMP].

Rabbit ileal loop assay, Y1 adrenal tumor cell line assay, Chinese hamster ovary cell line assay are toxigenic tests for cholera toxin. Not used nowadays due to easier tests like ELISA, PCR.

minor toxins :

- Zot (Zonula occludens toxin) : Disrupts tight junctions between enterocytes.

- Ace (Accessory cholera enterotoxin) : Contributes to pumping out of ions into intestinal lumen.

Enzymes :

- Neuraminidase → Generates receptors for cholera toxin.
- Collagenases, proteinases, elastases, mucinases etc. contribute to virulence.

Quorum sensing :

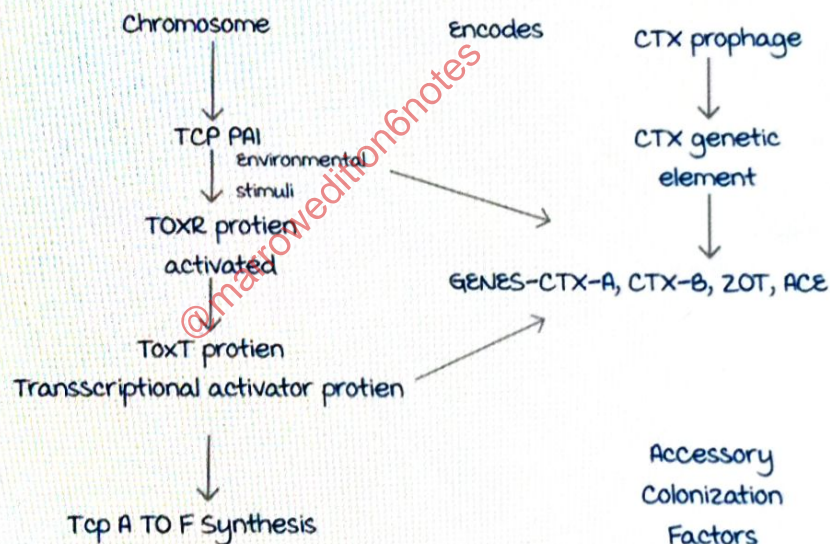
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Group of bacteria communicating with each other for the co-ordinated expression of virulence factors.

Lipopolysaccharide :

No role in the disease cholera.

Encoding of virulence factors :



TCP PAI → Toxin co-regulated pilus : Pathogenicity island.

Cholera

00:49:08

Reservoir : Humans (carriers), sea water.

Epidemics : All ages are affected.

Endemic areas : Only children are affected.

Infective dose (ID50) : With water $>10^6$ bacilli. With food - 10^3

bacilli. vibrios are susceptible to acidity of stomach. Food raises the overall gastric pH and hence infective dose reduces.

Symptomatic infection: Only seen in 2-5% of infected individuals.

Risk factors for symptomatic disease :

- gastrectomy, achlorhydria, hypochlorhydria, antacids, proton pump inhibitors, H_2 blockers.
- "O" blood group people and people with retinol deficiency develop more severe disease.
- AB blood group individuals develop less severe disease.

Incubation period : 1 day to 5 days.

Clinical features :

- effortless vomiting.
- Severe watery diarrhea (up to 25 L/day).
- Hypovolemia and metabolic acidosis.
- Rice water stools.

Diagnosis :

Specimen : Fresh stool/ rectal swab.

Wet mount → Fish in stream appearance. Under dark ground microscopy/phase contrast microscopy → motility can be demonstrated which is inhibited by adding a drop of O1/O139 antiserum.

Culture of stool sample :

- **macConkey** : mildly selective → shows non lactose fermenting colonies (late lactose fermenting as it turns pink after 1 day)
- **TCBS medium** : Highly selective, it shows yellow color due to sucrose fermentation.
- Biochemical tests such as oxidase test, string test, cholera red reaction are used for identification.
- Serogrouping, biotyping and serotyping done after identification using respective antisera/biochemical tests.

Cholera treatment :

Assess hydration status.

	< 5 % fluid deficit	(5-10) % fluid deficit	> 10 % deficit
Mentation	Alert	Restless, irritable	Lethargy or unconscious
Eyes	Normal	Sunken	Sunken

Skin turgor	Normal	Slow recoil	Very slow recoil
Pulse	Normal	Rapid, low volume	Weak/absent
Thirst	Normal	Drinks eagerly	Poor/unable

< 5 % fluid deficit : Give ORS.

> 5 % fluid deficit : Parenteral rehydration with ringer lactate.

Antibiotics are required only in moderate to severe dehydration.

They reduce the duration and severity of diarrhea.

Azithromycin 1g single dose or Doxycycline 300 mg single dose can be used.

For children < 5 years of age, zinc supplementation is given.

Patient develops short lasting (6-12 months) protective immunity following recovery.

Cholera vaccine

01:00:49

Oral live attenuated vaccine :

- Contains genetically modified strain of *V. cholerae* O1. In vitro gene deletion of CTX A gene is done.
- Single dose is given.
- Provides immunity for 6 months to 2 years.
- It is 60-100% efficacious.

Group B vibrios :

V. parahaemolyticus :

- Requires 2-4% of salt concentration. Can tolerate up to 8% of salt.
- Capsule is present.
- Flagella : Peritrichous on solid media and monotrichous on liquid media.
- Shows swarming on blood agar.
- Pale/green colored colonies on TCBS agar (non-sucrose fermenting colonies).
- Wagatsuma agar : Human blood agar with 2% salt. Shows Kanagawa phenomenon : Pathogenic strains are hemolytic and non-pathogenic strains are non-hemolytic.

- Disease is acquired by ingestion of contaminated and poorly cooked sea food → **Shell fish and oysters**. Causes invasive diarrhea. After incubation period of **1-3 days** → Watery diarrhea.
- Bloody diarrhea in 15%. → (Self-limiting). If it persists for >5 days, **Doxycycline/ fluoroquinolones** can be administered.

V. vulnificus :

- Also called as **L+Vibrio** as it is lactose fermenting after overnight incubation.
- Ingestion of contaminated sea food causes toxin mediated mild/self-limited diarrhea. bnvssprasanth7@gmail.com
- Other illness :
In **cirrhosis/ iron overload states** and in people on **Deferoxamine therapy**, ingestion of contaminated sea food causes septicemia → High grade fever with fulminant myonecrosis and cellulitis.
Treatment : **Parenteral fluoroquinolones or tetracyclines**.
It causes cellulitis in wounds exposed to sea water.

V. alginolyticus :

- Shows **swarming** on blood agar.
- Can tolerate **up to 10%** of salt concentration.
- Causes **eye and ear infections** in sea swimmers.
- Causes cellulitis following exposure of abraded skin to sea water.

PSEUDOMONAS AND BURKHOLDERIA

Pseudomonas aeruginosa

00:00:34

Gram negative Bacillus/rods.

motile, monotrichous flagella.

Strict aerobe.

No capsule, instead produces **slime** (helps in formation of **biofilms**).

Special characteristic : Ability to **grow at 42°C**.

Absent in other Pseudomonas species.

Biochemical tests :

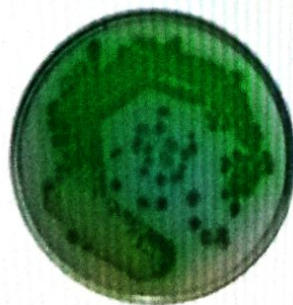
Catalase test : Positive.

Oxidase test : Positive.

Sugar utilization : Oxidative utilization of sugar.

Culture characteristics :

- Simple medium : Nutrient agar, Non fastidious.
- Colonies : **mucoid colonies**.
- Pigments : **Pyocyanin (blue green)**,
pyoverdinin (yellow), pyorubin (red),
pyomelanin (brown).
- Blood agar : Produces β hemolytic colonies.
- MacConkey medium : Pale colour colonies.
- Selective medium : **Cetrimide agar**, have **fruity or grape like smell**.



Pyocyanin (blue green)



β hemolytic colonies on blood agar



Pyoverdinin (yellow),

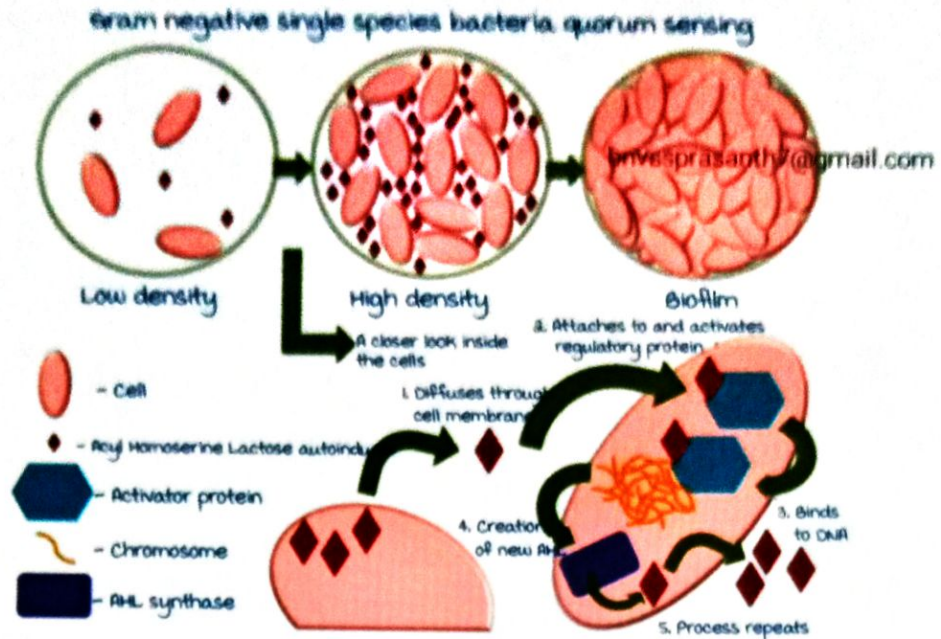
Pale colonies on
MacConkey medium**Virulence factors :**

- Slime → Biofilms → Adhesion, antiphagocytic & reduce entry of antibiotics.
- Fimbriae/pili : Type 4 pili, twitching motility (flagella independent).
- Endotoxin/ Lipopolysaccharide.
- Secretion system : Type 1, 2, 3.
- Flagellum : Helps in adhesion.

Extracellular virulence factors :

- Proteases : Elastases (Las A, B), Alkaline protease.
- Toxins : Exotoxin A (MOA like Diphtheria toxin, ADP ribosylation of elongation factor 2 → inhibiting protein synthesis).
Exoenzyme S, T, U.
- Haemolysins : Phospholipase, rhamnolipid.
- Siderophores : Help bacteria acquire iron, pyoverdinin, pyocyanin (eliminates toxic oxygen radicals).

Quorum sensing : Cell to cell communication via auto inducers for the coordinated release of virulence factor.



Epidemiology :

Normal soil saprophytes.

Sometimes normal commensals in humans present in moist areas (ears, axillae, perineal area).

Environment : Large numbers.

- Community : Soil and natural water bodies, artificial water bodies (jacuzzi, swimming pools, home humidifiers).
- Hospitals : Sinks, mops, respiratory therapy equipment's, endoscopes etc.

Infections caused by Pseudomonas

00:15:15

Community infections :

1. Corneal ulcers in contact lens users.
2. Jacuzzi syndrome/ hot tub folliculitis.
3. Hot hand and foot syndrome.
4. Infective endocarditis, osteomyelitis, septic arthritis (seen in IV drug users)
5. Swimmer's ear/simple otitis externa.
6. Malignant/necrotizing otitis externa (in diabetics)
7. Paronychia & green nail syndrome (chloronychia).



malignant otitis externa

Nosocomial infections :

1. Burn infections (most common gram -ve).
2. Hospital acquired pneumonia and ventilator acquired pneumonia (most common gram -ve).
3. Catheter associated urinary tract infections.
4. Surgical site infections.
5. Septicaemia with high mortality rate, manifesting characteristically with *Ecthyma gangrenosum* in immune deficient (ICU patients).



Ecthyma gangrenosum

Resistance to antibiotics :

Intrinsic resistance :

- Low outer membrane permeability (lack of outer membrane proteins).
- Chromosomally mediated Beta Lactamases.
- Several efflux pumps.

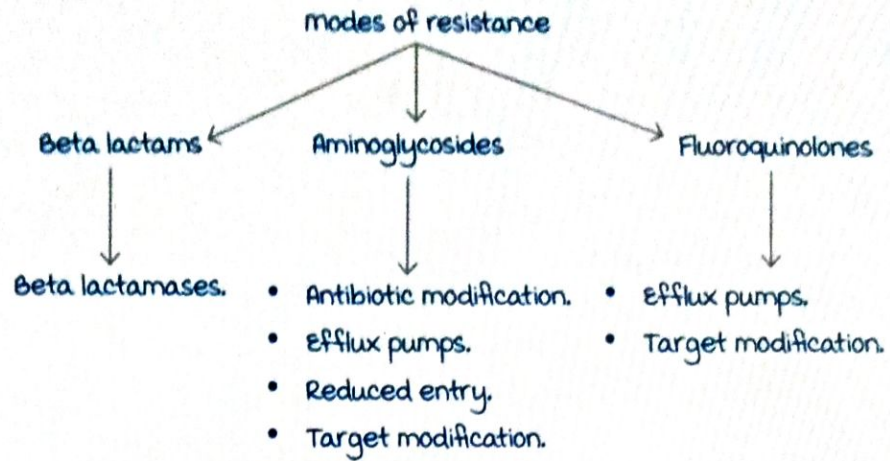
Acquired resistance :

- Horizontal gene transfer.
- mutation driven.

Treatment :

1. Fluoroquinolones.
2. Aminoglycosides.
3. Extended spectrum cephalosporins.
4. Carbapenem.
5. Piperacillin ± Tazobactam.

multidrug resistance : when it is resistant to 3 or more drugs of these antimicrobial classes.



ESKAPE organisms :

WHO list of priority organisms causing nosocomial infections and exhibit multi drug resistance and high virulence.

Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species.

Burkholderia

00:24:57

B. mallei.

B. pseudomallei.

B. cepacia.

Belongs to family Pseudomonadaceae.

Characters similar to Pseudomonas :

- motile.
- Strict aerobe.
- Gram negative rods.
- Oxidase positive.
- Non lactose fermenting.
- Environmental saprophytes.

Burkholderia mallei :

Gram negative rods (safety pin appearance / bipolar staining).

Non motile.

Oxidase negative.

Strict parasitic existence (horses are sole reservoir).

In horses, causes nasal abscesses called Equine glanders.

And causes subcutaneous nodules and abscesses called

Farcy buds & **farcy pipes**.

Biosafety level-3 pathogen.



Primarily an occupational disease :

Seen in Jockey's, Butchers, Veterinarians.

Percutaneous infection → skin nodules → septicemia.

Inhalation → pneumonia → septicaemia.

Resistant to 1st & 2nd generation **Cephalosporins** & **Penicillins**.

Intensive phase : **Ceftazidime/ Imipenem/meropenem** + **Cotrimoxazole** at least 2 weeks.

Eradication phase : **Cotrimoxazole** at least 3 months or more.

Burkholderia pseudomallei

00:30:06

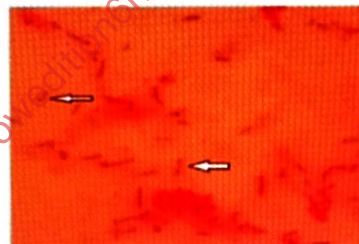
Gram stain **safety pin appearance/bipolar staining**.

Culture : **Ash down's medium**, it forms pink to purple colonies.

BSL-3 pathogen.

Found in soil and water.

Endemic in south east Asian countries, northern Australia.



safety pin appearance/
Bipolar staining

Mechanism of infection :

Inhalation of soil and water : Pneumonia.

Percutaneous : Local ulcers.

Ingestion : GI ulcer, lymphadenopathies.

Causes **melioidosis**, also called as **Whitmore's bacillus**.

Risk factors for septicaemia :

- Alcoholics.
- Diabetics.
- Chronic renal failure.
- Cystic fibrosis.

Can cause **latent infection** & Can reactivate after months or years, hence called **Vietnamese time bomb disease**. (Many American soldiers were infected during Vietnam war).

Treatment :

Intensive phase : **Ceftazidime/ Imipenem/meropenem + Cotrimoxazole** at least 2 weeks.

• Eradication phase : **Cotrimoxazole** for at least 3 months or more.

Safetypin appearance seen in :

Burkholderia mallei

- *Burkholderia pseudomallei*.
- *Hemophilus ducreyi*.
- *Y. pestis* (only with Wayson stain not with gram stain).
- *Klebsiella granulomatis*.

Burkholderia cepacia complex (BCC)

00:36:00

mainly a **plant pathogen**.

Environmental saprophytes.

Apart from environment, It is also found in **nebulisers, humidifiers & respiratory equipment**.

Culture media called **Burkholderia cepacia selective medium**.

It is resistant to common antibiotics (**Polymyxin, Cholestin**), these are added in the medium.

Risk factors :

Cystic fibrosis.

Chronic granulomatous disease.

Cepacia syndrome : Rapidly progressive pneumonia with frequent systemic dissemination.

Treatment :

- Cotrimoxazole.
- meropenem.
- Doxycycline.
- minocycline.

B. cenocepacia & B. multivorans are the most common species causing **infections in humans**.
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Cystic fibrosis - lung diseases

00:41:23

Common organisms :

1. **Mucoid Pseudomonas aeruginosa (more common)** :
Normal flora of cystic fibrosis patients undergoes mutation and produce slime called **Alginate**.
2. **Staphylococcus aureus**.
3. **Burkholderia cepacia**.

Jumping joker in Burkholderia : **Burkholderia mallei**.
(Non motile, Oxidase negative, Strict parasitic existence).

MCQs :

Q. You receive a urine culture report from the lab of a 45 year old patient who is a quadriplegic with an indwelling urinary tract catheter. The urine culture is growing >100,000 per ml oxidase positive gram negative bacilli. The most likely organism is :

- A. E. coli.
- B. Enterococcus.
- C. Klebsiella.
- D. **Pseudomonas aeruginosa**.

Q. All of the following statements about Pseudomonas aeruginosa are true except :

- A. It is oxidase positive.
- B. It does not grow well at 42°C.
- C. **It is a common opportunistic pathogen.**
- D. It contains fimbriae.

Q. True about melioidosis :

- A. It is caused by Pseudomonas pseudotuberculosis.
- B. **It is more common in diabetes.**
- C. It should be treated with cefuroxime.
- D. It is confined to equatorial Africa and South America.

Active space

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Q. A 73 year old woman with a history of diabetes presents with left ear pain and drainage of pus from the ear canal. She has swelling and tenderness over the left mastoid bone. Which of the following microorganisms is the most likely causative agent?

- A. Hemophilus influenzae.
- B. mucor sp.
- C. Pseudomonas aeruginosa.
- D. Streptococcus pyogenes.

Q. A 65 year old man with a history of diabetes presents with severe pain in his right ear. The patient was diagnosed with external otitis. Further tests suggested that the patient suffered bone and nerve damage. Clinical laboratory analysis showed that the isolated microorganism produced a distinct blue pigment. Which of the following is true about likely etiologic agent?

- A. Catalase and oxidase negative.
- B. Ferments glucose producing acid and gas.
- C. Produces an ADP-ribosylating toxin.
- D. All are true.

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HEMOPHILUS AND BORDETELLA

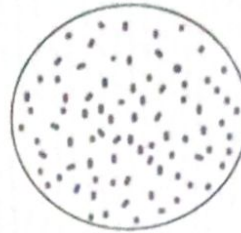
Gram negative coccobacilli.

Hemophilus :

Heme + loving.

X factor : Hemin/any other porphyrin compound.

V factor : NAD/NADP.



Those which require factor X + V are : H. influenzae, H. hemolyticus, H. aegyptius.

Require only V : H. parainfluenzae, H. parahemolyticus.

Require only X : H. ducreyi.

Hemophilus influenzae

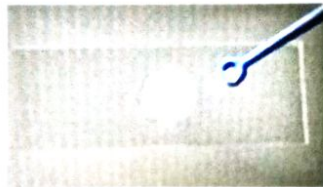
00:02:51

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Gram negative coccobacilli.

Non mobile.

Facultative anaerobes.



Catalase positive

Capsulated strains :

Capsular serotyping : a, b, c, d, e, f.

B serotype (most pathogenic) is made of **polyribosyl ribitol PO₄** (PRP).

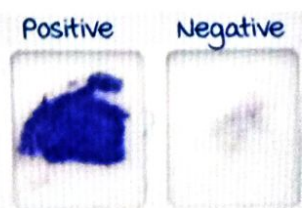
most human infections amongst the capsular serotypes are primarily caused by **Hib**.

Can also present normally in upper respiratory tract flora in <1% of humans.

Non capsulated strains :

Non typeable H. influenzae (NTHI).

Can be present as normal upper respiratory tract flora in 30 - 80% population.



Oxidase positive

Active space

Biochemical reactions :

Catalase positive (tested with H_2O_2).

Oxidase positive.

Sugar : Fermentatively utilized.



Blood agar showing satellitism

Culture :

Blood agar shows poor/no growth.

X factor is freely available.

V factor is trapped in RBC's.

Blood agar with *Staphylococcus aureus* streak shows satellitism.

Chocolate agar.

Other media like Levinthal medium,

Filde's agar.

Filde's agar is considered best medium for primary isolation.



Chocolate agar

Virulence factors :

- Polysaccharide capsule : Hib is most virulent.
- Endotoxin : Lipo oligosaccharide.
- Pili.
- Adhesin protein.
- IgA1 protease (also produced by pneumococcus & meningococcus).

Infections :

Invasive :

H. influenzae is a primary pathogen.

They are associated with concurrent bacteremia.

most common age group affected is infants and young children.

most common serotype : Hib.

- meningitis complicated with subdural effusion.
- Pneumonia.
- Epiglottitis (can cause asphyxia).
- Bacteremia.
- Septic arthritis.

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Active space

Non invasive :

Superimposed on a viral infection.

usually not associated with bacteremia.

most commonly affects adults and older children.

most common serotype : **NTHI**

- Otitis media } Caused most commonly by : Streptococcus
- Sinusitis } pneumoniae > H. influenzae > moraxella catarrhalis
- Acute exacerbation of COPD (most commonly caused by NTHI).
- Conjunctivitis.
- Community acquired pneumonia.

Treatment :

Ampicillin or Amoxicillin should not be used empirically. Only to be used after sensitivity testing as 25% of Hemophilus contains β lactamases.

Invasive : Third generation Cephalosporins.

Non invasive : Amoxicillin + Clavulanic acid.

Chemo prophylaxis of close contacts of Hib is done with Rifampicin.

Prevention :

Vaccine : Hib.

Type : Subunit vaccine.

Protective component : Capsular antigen (PRP).
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Carrier protein : Tetanus toxoid/

Diphtheria toxoid/ outer membrane

protein of meningococcus/ CRM197 of Corynebacterium diphtheria.

Immunity against only Hib serotype.

Schedule : 2, 4, 6 weeks followed by a booster after 12 - 15 months.

Hemophilus aegyptius :

Also known as **Koch weeks bacillus.**

Requires both X and V factor.

Infections :

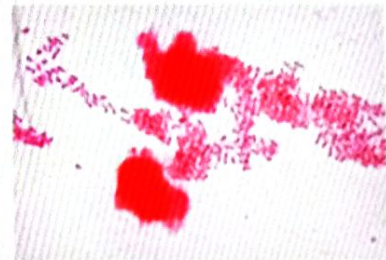
- **Pink eye** : Conjunctivitis.
- **Brazilian purpuric fever** : mainly reported from South America.
- Pink eye followed by **septicemia** (associated with 70% mortality rate).

Treatment : Third generation cephalosporins.

Hemophilus ducreyi

00:21:13

Safety pin appearance/bipolar staining. (Due to increased staining of pole compared to cytoplasm)
Requires only X factor.



Hemophilus ducreyi

Culture :

- **Chocolate agar + isovitalex + fetal calf serum.**
- Chorio allantoic membrane of chick embryo.
- Fresh clotted rabbit blood.

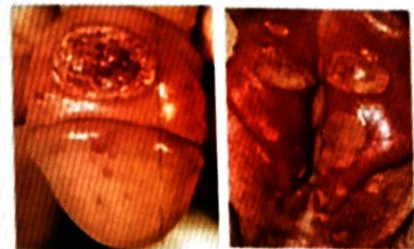
Infections :

mainly transmitted sexually.

Soft sore/chancere/chancroid :

Painful non indurated genital chancre (ragged edges) with inguinal lymphadenopathy called **buboes**.

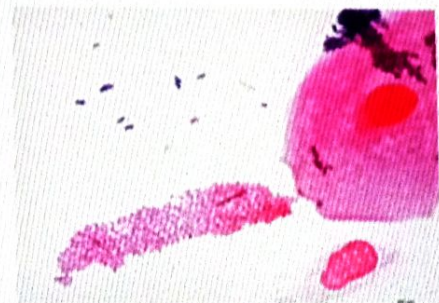
As no protective antibodies develop, patient can be reinfected.



Soft sore/chancere/chancroid

Diagnosis :

- Collect exudate and stain with gram's stain. **School of fish/rail road track appearance** is seen.
- Culture.

School of fish/
rail road track appearance

Treatment :

Single dose of **Ig Azithromycin.**

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Q : False about *Hemophilus ducreyi* :

- A. It is a highly fastidious gram negative coccobacillus.
- B. Is a strict human pathogen.
- C. **Infection confers protective immunity.**
- D. Infection facilitates HIV transmission.
- E. Does not grow on plain chocolate agar.

Q : False about *Hemophilus aegyptius* :

- A. Also called Koch Weeks bacillus.
- B. **Cannot cause invasive disease.**
- C. Is a biotype of *Hemophilus influenzae*.
- D. Requires X and V factors to grow.

Explanation → *H. aegyptius* is considered a biotype 3 of *H. influenzae*.

Q : A 2 year old child has a high fever and is irritable. He has a stiff neck. Gram stain smear of spinal fluid reveals gram negative, small pleomorphic coccobacillary organisms. What is the most appropriate procedure to follow in order to reach an etiological diagnosis?

- A. **Culture the spinal fluid in the chocolate agar and identify the organism by growth factors.**
- B. Culture the spinal fluid in mannitol salt agar.
- C. Perform a catalase test with the isolated organism.
- D. Perform a coagulase test with the isolated organism.

Q : In a child admitted with *H. influenzae* meningitis, cefotaxime was started instead of ampicillin. Which of these is the likely reason for this? (AIIMS)

- A. ***H. influenzae* strains are known to produce beta lactamase.**
- B. *H. influenzae* strains known to have altered penicillin binding proteins.
- C. Easier to administer IV cefotaxime than IV ampicillin.
- D. There is concern that the child will develop penicillin allergy.

Active space

Bordetella

00:32:16

Species :

B. pertussis : 95% cases

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B. parapertussis : Nearly 5% cases.

B. bronchiseptica : Less than 1% cases. mainly a pathogen of dogs, etc.

General characteristics :

- Gram negative coccobacilli.
- Non motile.
- Strict aerobe.
- Non antigenic capsule.

Biochemical reactions :

- Catalase positive.
- Oxidase positive.
- Sugar utilization : Oxidative.



Bordet Gengou agar with *Bordetella pertussis* colonies

Culture :

- On simple medium no growth is seen.

Selective medium :

Bordet Gengou / glycerine potato blood agar.

Regan Lowe's medium (charcoal cephalixin agar).

On these medium, mercury drop/bisected pearl colonies are seen.



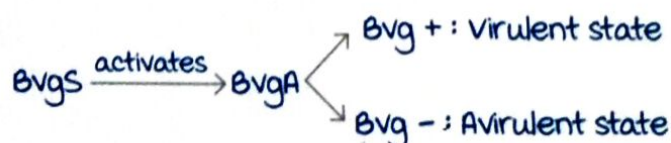
mercury drop colonies

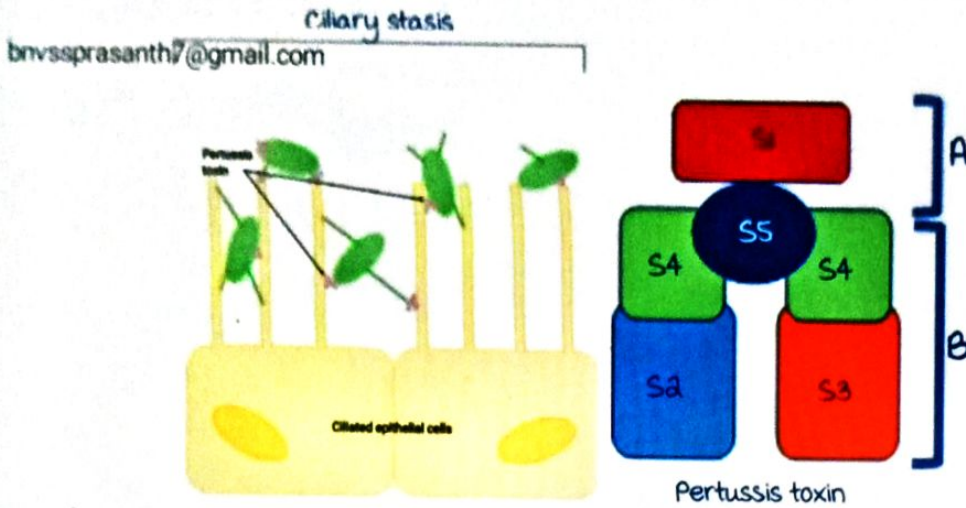
Virulence factors :

many virulence factors are present whose expression is controlled by

BvgAS : A 2 component regulatory system.

BvgS responds to environmental stimuli and activates BvgA (activator gene). This gene further activates the expression of virulence factors.





Filamentous hemagglutinin (FHA) helps in adhesion to the nasopharyngeal mucosa.

Pertactin (outer membrane protein) also helps in adhesion.

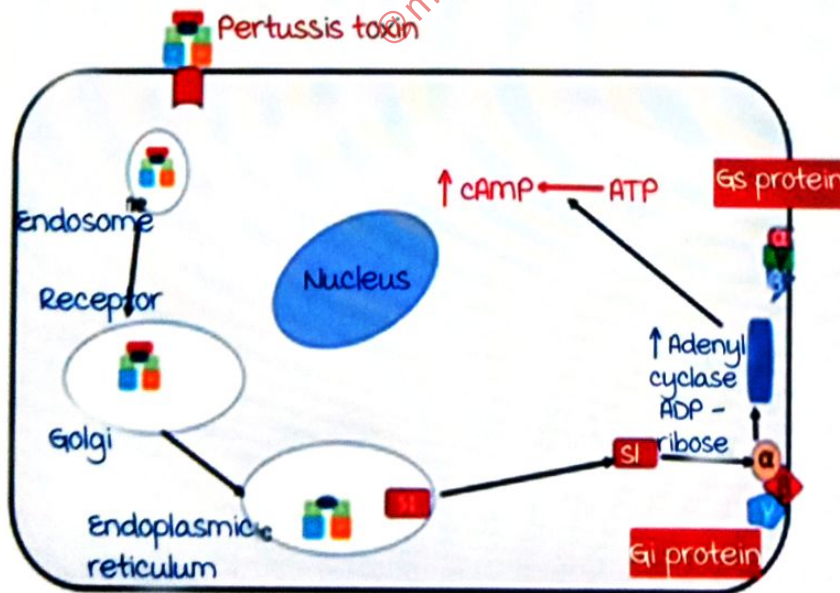
Fimbriae/surface agglutinogens : Helps in adhesion.

Pertussis toxin : Only produced by *B. pertussis* (A - B toxin).

A is the active part(S_2), B is the binding part (S_3-S_4) of toxin.

Functions :

- Helps in adhesion to the cilia of epithelial cells.
- Helps in evasion of innate immune cells. (macrophages and neutrophils)



S1 part of toxin : It ADP ribosylates the α subunit of G_s protein leading to massive increase in cAMP which further inhibits the phagocytic function.

- Systemic effects : Sensitization of islet cells leading to hyperinsulinemia causing hypoglycemia in patients.
- Also induces lymphocytosis.

Active space

Adenyl cyclase toxin : Increases cAMP by calmodulin mediated mechanism.

Tracheal cytotoxin
Dermonecrotxin } Damage ciliated epithelium of upper respiratory tract.

Piracy of adhesins : Virulence factors of *B pertussis* mediate adherence of other bacteria to mammalian respiratory cilia.

Whooping cough

00:46:54

Aka **100 day cough**.

Transmission occurs via droplets of respiratory tract secretions.

Incubation period : 7 - 10 days.

Stages :

Catarrhal stage :

- mild upper respiratory symptoms are present like rhinorrhea, watery eyes, mild fever, slight cough.
- **most infectious stage**.
- Lasting for about 2 weeks.

Paroxysmal stage :

- Repetitive bursts of worsening cough, inspiratory whoop, post tussive vomiting.
- Lasts for about 2 - 8 weeks.

Convalescent stage :

- Gradual resolution of symptoms.

Pertussis vaccine immunity wanes over time. So CDC now recommends booster doses at adolescence and adulthood.

Complications :

Pneumonia : most common complication.

may be due to *B pertussis* or superadded viral infection (RSV). dayssprasad7@gmail.com

Pressure effects of cough may cause :

- Subcutaneous emphysema
- Conjunctival hemorrhage.
- Fracture of ribs.
- Hernia.

Encephalopathy : may be toxin mediated.

Presents as seizures, deafness, aphasia, paralysis.

Diagnosis :

Specimen :

- Nasopharyngeal aspirate (best).
- Nasopharyngeal swab (pernasal).
- Cough plate method.

Swabs :

- Use dacron/rayon/calcium alginate swabs.
- Cotton swabs should not be used as it's toxic to bacterium.

Transport media :

- Modified Stuart medium.
- Liquid Regan Lowe medium

1. Cultivation :

- **Gold standard** : Inoculate the bacteria on culture media and isolate.

2. Gram staining :

- **Thumb print whorl** appearance is seen.

3. Biochemicals :

- **Oxidase positive** (differentiates from *B. parapertussis*).
- **Urease negative** (differentiates from *B. bronchiseptica*).

4. Direct fluorescent antibody test (DFAT) :

- Not sensitive, not specific.

5. Serology (ELISA/ IFAT) :

- Significantly raised antibody titre.
- Four fold rise in antibody titre.

6. NAATs : **most sensitive**.

Optimal timing for diagnostic testing :

- In early stages (0 - 2 weeks) : Culture methods.
- In later stages (0 - 4 weeks) : PCR.
- **After 2 weeks** : Serological tests.

Blood analysis :

- Leukocytosis.
- **Absolute lymphocytosis** (neutrophils in large amount negates the diagnosis).

Treatment :

Antibiotics : Drug of choice is **macrolides**.

- Azithromycin for 5 days or
- Erythromycin for 7 - 14 days or
- Clarithromycin for 7 days.

most effective in resolving the symptoms and decreasing the infectivity in early stages.

In later stages, only decreases the infectivity.

Alternative treatment :

In case of allergy to macrolides, Cotrimoxazole is used.

Precautions : Droplet precautions to be taken.

Vaccine :

Earlier DTP vaccine was used. It contained killed B pertussis.

Now, DTaP vaccine (subunit vaccine) is used.

subunits : Pertussis toxoid, FHA, Pertactin, Fimbrial antigen 1 & 2.

It is less reactogenic.

Also, less effective and immunity wanes off more rapidly.

Chemo prophylaxis :

Close contacts should receive macrolides, whether they have been vaccinated or not.

A 3 month old infant is brought to the pediatric emergency department in severe respiratory distress. The child appears dehydrated, and there is a prominent peripheral lymphocytosis. He is diagnosed with pertussis. The factor responsible for the profound lymphocytosis is :

- A hemagglutinin.
- A polysaccharide capsule.
- An A/B structured toxin.
- A heat labile toxin.
- A neuraminidase.
- F.

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Explanation : Pertussis vaccine is also know as lymphocytosis promoting factor.

Q. A 16 year old female patient has a 10 day history of a severe cough, which is followed by a mild cold. She reports further that the cough comes in the form of intense episodes lasting several minutes, followed by a gasp. She has

vomited 3 to 4 times after the coughing fits. She denies any other symptoms, and her past medical history is unremarkable other than a lack of childhood immunizations. On examination, she is afebrile, and there is no significant finding on the auscultation of her chest. What should be done to manage this patient's illness?

- A. Start her on azithromycin and supportive treatment.
- B. Send for sputum culture and wait for report.
- C. Quarantine and send for the ICU team.
- D. Start supportive treatment only.

Q. A 5 month old boy is admitted to the hospital with a 24 hour history of choking spells. These began with repeated coughing where the child turned red and gasped for breath. In the previous 2 days he had also had 2 episodes of vomiting. The baby was found to have an increased breathing rate, which was labored, but CXR showed no evidence of pneumonia. Blood work showed an abnormally large percentage of lymphocytes (70%). Nasopharyngeal swabs cultured on a selective medium yielded grayish, "mercury like" colonies, and a gram stain revealed minute gram negative coccobacilli. Which of the following is false about diagnosis/etiological agent?

- A. The causative organism is fastidious and requires an enriched medium for growth.
- B. The factor responsible for the profound lymphocytosis is an A - B structured toxin.
- C. An effective vaccine is available to prevent the illness.
- D. It requires X and V factor for growth.

Q. False about pertussis :

- A. Also called 100 day cough.
- B. most infectious stage is catarrhal.
- C. Erythromycin after onset of the paroxysmal phase can drastically alter the clinical course.
- D. PCR and other NAAT methods are the most sensitive methods to diagnose pertussis.

BRUCELLA AND BACTEROIDES

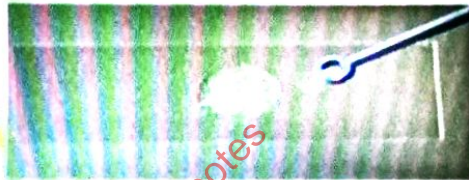
Cultivation of bacteria

00:00:58

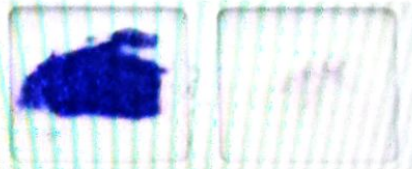
- Gram negative coccobacillus.
- Non-motile.
- Strictly aerobic.
- **B. abortus** : Capnophilic, requires carbon dioxide to grow.
- Non-Capsulated.

Brucella biochemicals :

Catalase test positive.

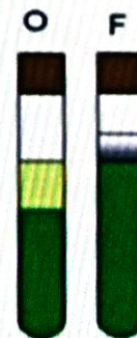


Oxidase test positive : Purple colour when colonies are applied to filter paper strips containing oxidase reagent.



Oxidative utilisation of sugars : Nonfermenters.

- Done in the oxidative fermentation medium.
- Glucose is broken down in the presence of oxygen as in tube O.
- Glucose is not broken down in the absence of oxygen as in tube F.
- In tube F, a layer of petroleum jelly on top prevents the diffusion of oxygen.



Some are H₂S positive.

- If bacterial colonies are incorporated into the solid media containing any sugars like glucose, it turns black in colour.

Some are urease positive.
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Stenson's urease medium will
turn pink.

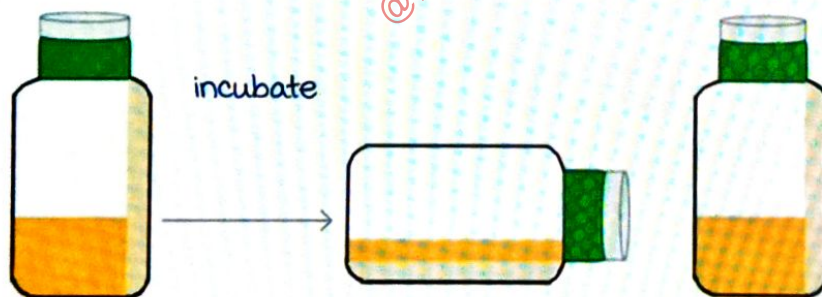


Brucella Cultivation :

Simple medium : Shows poor growth.

Special culture media : Biphasic medium/Castaneda medium.

- Brain Heart infusion agar with broth.
- Tryptose agar with broth.
- Serum dextrose agar with broth.



Tip-over to allow the broth to flow over the agar.

The universal container containing agar medium on the side is inoculated with a blood sample.

It is placed horizontally for half an hour every day after overnight incubation to let the broth flow over the agar slant and it is placed back vertically.

Colonies appear on the agar slant.

The growth of Brucella is very slow and takes several weeks.

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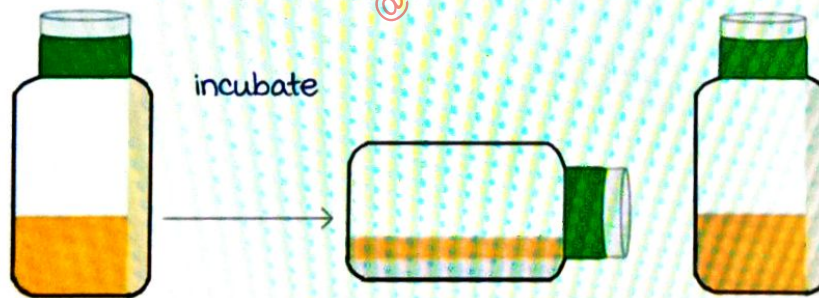


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The growth of Brucella is very slow and takes several weeks.

Brucella : Virulence factors

00:06:16

It is a **facultative intracellular** bacteria. Others are :

mnemonic : **Some Nasty Bugs may Live Facultatively.**

- **S**almonella.
- **N**eisseria.
- **B**rucella.
- **m**ycobacteria.
- **L**isteria.
- **F**rancisella.
- **L**egionella.
- **Y**ersinia.

Lipopolysaccharide (LPS) of brucella has a unique 'O' somatic antigen and a unique core polysaccharide.

LPS of brucella is **antiphagocytic** and protects from serum killing or complement system.

Brucella reservoir :

B. melitensis most common cause of brucellosis most severe	Sheep Goat camels
B. abortus	Buffalo, cattle
B. suis	Pigs, rodents
B. canis : Rarest	Dogs

In these animals, Brucella is present in milk and placenta.

Species identification features :

- CO_2 requirement.
- Urease production.
- H_2S production.
- Susceptibility to Tbilisi phage.

Brucella mode of incubation :

- most common route is the ingestion of **unpasteurized milk**.
- Percutaneous route while handling infected animal products.
- Inhalation of aerosols.
- Human to human via blood transfusion is rarest.

Brucella : Clinical features

Brucellosis is also known as **Mediterranean fever**/malta fever/Gibraltar fever/undulant fever.

Undulant fever because of remitting and relapsing high grade fever.

Occupations at risk :

- Farmers.
- Dairy workers.
- Slaughterhouse/Abattoir workers.
- Veterinarians.

Presentation is varied.

Acute presentation :

most common organism : **B. melitensis**.

Incubation period : 1 to 4 weeks.

Presentation : Waxing and waning high grade fever.

Along with chills, night sweats, malaise, anorexia, sudden loss of weight, and lymphadenopathy.

Chronic presentation :

Low grade fever with fatigue, depression.

O/E : Anemia, leukopenia.

Focal Disease : Disease localized to a particular site.

- Nervous system : Lymphocytic meningitis, encephalitis or polyradiculopathies.
- Heart : Peri/myo/endocarditis.
- Liver : Hepatitis, focal hepatic abscess.
- vertebral column : **Spondylodiscitis**/Osteomyelitis of the lower thoracic or lumbar vertebrae.
- Large joints : Arthritis of hip, knee/Sacroiliac joint.
- Genitourinary tract : Epididymitis, prostatitis, salphingitis, renal abscess, orchitis.
- In Pregnancies : Abortions, intrauterine death, premature delivery.





Diagnosis of Brucellosis

00:18:35

Hemogram shows anemia, leukopenia.

Biopsy of lymph nodes & liver shows **non-caseating granulomas**.

Cultures of blood & bone marrow show growth.

- Handling of Brucella cultures is done in the BSL 3 lab.
- The culture medium used is the biphasic medium/ Castaneda medium.
- Cultures are most specific but are a slow process i.e., it takes several weeks.

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Automated culture methods are more rapid, it detects the organisms in 7 to 10 days.

Nucleic acid amplification test by PCR of specimens (blood & bone marrow) is the most sensitive test.

Serology for specific antibody detection is the most commonly used test.

In Brucellosis :

- IgM and IgG antibodies appear after 1 week after the onset of symptoms.
- Initially, both are raised.
- IgM titers reduce over time.
- IgG antibodies persist or it raises with time

Serological tests :

Standard Agglutination test :

Carried out in test tubes.

Detects IgM antibodies.

Can be negative in **chronic brucellosis**.

Often associated with the **pro-zone phenomenon** :

- Seen because a very high titer of IgM antibodies compared to antigens results in a negative standard agglutination test.
- It is overcome by **serial dilutions** : When the antigen and antibody are in equivalent proportions, results in a positive standard agglutination test.

2-mercaptoethanol test (2-ME) :

- Detects IgG antibodies.
- 2-ME selectively destroys IgM antibodies.
- IgG antibody detection is important for confirmation of Brucellosis.

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Incomplete IgG antibodies are detected by Direct Coomb's test.

ELISA and complement fixation tests, separately detect Igm and IgG antibodies.

The immunochromatographic test by Rose Bengal card test is a rapid test.

Treatment of Brucellosis

00:28:57

monotherapy is not recommended.

Doxycycline orally for 6 weeks + Streptomycin Im for 2 to 3 weeks is the gold standard.

WHO recommended regimen is Doxycycline oral + Rifampicin oral for 6 weeks.

Alternatively, Doxycycline oral for 6 weeks + Gentamycin Im for 1 week.

For Children : Cotrimoxazole oral + Rifampicin oral for 6 weeks.

For neurobrucellosis : Ceftriaxone iv (for 1 month) + Rifampicin oral for 4 to 5 months.

Lab exposure prophylaxis : Doxycycline oral + Rifampicin oral for 3-6 weeks.

Strict Aerobes:

Mnemonic : Nagging Pests must Breathe For Life

Neisseria.

Pseudomonas.

Mycobacterium tuberculosis, Micrococcus.

Brucella, Bordetella.

Francisella.

Legionella.

Gram-negative Anaerobic Rods/Bacillus/

GNARs

00:32:58

most human infections of GNARs are caused due to :

- Bacteroides.
- Prevotella.
- Porphyromonas.
- Fusobacterium.

These GNARs are normal commensals.

Features of GNARS :

- Gram negative.
- Non-motile.
- Strict anaerobes.
 - Lacks enzymes catalase, peroxidase, superoxide dismutase.
 - Cannot eliminate oxygen radicals generated by the metabolism generated in the presence of oxygen.

Earlier all GNARS were included in genus *Bacteroides*, recently they have been split into 3 different genera :

- Pigmented & saccharolytic : *Prevotella* (Brown or black pigment).
- Pigmented & asaccharolytic : *Porphyromonas*.
- Non-pigmented and saccharolytic : *Bacteroides*.

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Prevotella and *Porphyromonas* colonies show brick red fluorescence under ultraviolet light.

They are both normal oral and gastrointestinal flora.

They are implicated in gingivitis, periodontitis and aspiration pneumonia.

***Bacteroides* :**

- most common commensals in the human colon. (30% of colon commensals).
- They play a role in maintaining mucosal immunity : Due to colonization resistance as it antagonizes pathogenic bacteria.
- Help in the digestion of complex dietary polysaccharides.

Bacteroides Fragilis

00:39:23

- It is the most common clinical isolate in Intra-abdominal infections.
- Superoxide dismutase is present, so it is aerotolerant.
- Its capsule is zwitterionic capsule which makes it antiphagocytic and has a role in abscess formation.
- Its lipopolysaccharide has low endotoxic activity.
- It has bile resistance : Can grow in the presence of 20% of bile, helps to differentiate from other anaerobes.

Clinical importance :

- Normal GI flora.
- Commonly causes Intra-abdominal abscesses due to break in GI mucosal barrier.
- Can lead to septicemia. Shock and DIC are rarely seen with septicemia.
- Also associated with cutaneous and brain abscesses.

Treatment :

They inherently secrete Beta-lactamase. Hence resistant to Penicillin and 1st and 2nd generation cephalosporins.

Also, they are inherently resistant to Kanamycin, Vancomycin and colistin.

These features are used to differentiate it from Prevotella and Porphyromonas.

Effective antibiotics : Clindamycin, metronidazole.

Beta lactams with beta-lactamase inhibitors :

- Ampicillin with Sulbactam.
- Piperacillin with Tazobactams.
- Amoxicillin with Clavulanic acid.
- Carbapenems.

Mnemonic (important anaerobes) : **L**osers **C**hoked **B**y **A**ir.

Lactobacillus.

Clostridium.

Bacteroides.

Actinomyces.

Other examples :

- Prevotella.
- Porphyromonas.
- Fusobacterium.
- Cutibacterium (Propionibacterium).
- Bifidobacterium.
- Eubacterium.
- Veillonella.
- Mobiluncus.

RICKETTSIA AND RELATED GENERA

PART 1

Rickettsia and related bacteria

00:00:46

- **Rickettsia** → Family Rickettsiaceae.
- **Orientia** → Family Rickettsiaceae.
- **Coxiella** → Family Coxiellaceae.
- **Bartonella** → Family Bartonellaceae.
- **Ehrlichia** → Family Anaplasmataceae.
- **Anaplasma** → Family Anaplasmataceae.

Common features of rickettsia and related bacteria :

- **Gram negative** coccobacilli.
- **Obligate intracellular** (non-cultivable on cell free media) except Bartonella (cultivable on blood agar and chocolate agar).
- Transmitted by **arthropods** except Coxiella.
- Parasitize **endothelial cells** except for Ehrlichia and Anaplasma (localize in blood cells).
- Clinical presentation → Fever, myalgia, headache, **rash** (never seen in Coxiella infection/Q fever), vasculitis.
- They all form **basophilic inclusion bodies** stained by Giemsa, Gimenez, Castaneda, machiavello stains.
- Cultivation is done on laboratory animals like Guinea pig/mouse, yolk sac of hen's egg, cell lines like HeLa etc

Rickettsia

00:05:36

Diseases caused by Rickettsia :

Typhus group → **CNS** symptoms are dominant.

- Epidemic typhus.
- Endemic typhus.

Spotted fever group → Rash (dominant manifestation):

- RMSF (Rocky mountain Spotted Fever).
- Indian tick typhus.
- Kenyan tick typhus.
- mediterranean tick typhus (Boutteneuse fever).
- Rickettsial pox.

Epidemic typhus/Gaol fever/Jail fever:

- Etiology → *Rickettsia prowazekii*.
- Vector → Human body louse
(*Pediculus humanus corporis*).
- mode of infection → Crushing of the louse against the skin, which leads to faeces inoculation into the skin.
- Reservoir → Only humans but in a small pocket of North America, reservoir is flying squirrel (exception).
- Clinical features → Fever, headache, myalgias, rash which starts from the trunk and then spreads to the extremities but doesn't involve the palms and soles, meningitis, meningoencephalitis (delirium, stupor, coma).
- mortality rate → up to 40%.
- Latency → Reactivates months to years later with the same manifestations → Recrudescence typhus/
Brill Zinsser disease.



Human body louse

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Endemic typhus:

- Etiology → *Rickettsia typhi*
(earlier known as *R. mooseri*).
- Vector → Rat flea (*Xenopsylla cheopis*).
- mode of infection → Bite of the flea/
inoculation of the faeces onto the skin.
- Reservoir → Rodents.
- Clinical features → Slightly milder than
epidemic typhus.
- No latency period is seen.



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mnemonic for reservoir/agent/vector :

epidemic typhus : HUMAN EPIDemics are PROfound and LOUSy.

(Reservoir - Human, Agent - Prowazekii, vector - Louse).

endemic typhus : ENDEMIC disease in RODENTS is TOUGH but FLEETING.

(Reservoir - Rodents, Agent - R. typhi & vector - Rat flea)

Rocky Mountain Spotted Fever (RMSF)

00:13:25

- Spotted fever rickettsiosis (SFR's).
- Endemic in Rocky mountains of North America.
- Etiology → *Rickettsia rickettsia* (mnemonic : RRR).
- vector → Hard tick (example : Dermacentor).
- mode of infection → Bite of the tick.
- Reservoir → Ticks (transovarian transmission) & rodents.
- Clinical features → Fever, headache, myalgias, rash.
- The rash starts from wrists, palms, ankles and soles → Spreads to the other parts of the body.
- Severe cases : Hemorrhagic rashes & meningoencephalitis.

Other spotted fevers

00:15:55

- Common feature → eschar/Tache noire (site of entry).
- This lesion is not present in epidemic typhus, endemic typhus and RMSF.

eschar/Tache
Noire



Hard Ticks



Rat flea



Gamasid mite

Disease	Causative agent	vector/ MOI	Reservoir	Features
Indian tick typhus	R. conori	Hard tick bite	Ticks and rodents	Fever, headache, myalgias, rash, eschar.
Kenyan tick typhus				
Boutteneuss fever				
Rickettsial pox (mildest type)	R. akari	Gamasid mites bite	mouse	

Diagnosis of typhus fevers and SFRs

00:19:52

- Specimen → Blood, rash biopsy, eschar biopsy.
- Giemsa / Castaneda stain → Demonstration of basophilic inclusion bodies inside the endothelial cells.
- Cultivation :
bnvssprasanth7@gmail.com
 1. Laboratory animal → Guinea pig (Neil mooser reaction).
 2. Intraperitoneal inoculation of patients blood sample into the guinea pig.
 3. Scrotal inflammation (enlarged with adhesions and testes cannot be pushed back into the abdomen).
 4. Epidemic typhus → Only fever present.
(NM reaction is negative).
 5. RMSF → Scrotal necrosis is seen.
Others → Scrotal swelling is seen (tunica reaction).
- Serology :
 1. Nonspecific test : Weil-Felix reaction .
(Patient develops antibodies against antigens which are shared with Proteus strains).
This test has very poor specificity and sensitivity.
 2. Specific antibody test by IFAT → Gold standard
(Indirect Fluorescent Antibody test).

3. Nucleic acid amplification tests (NAAT's) →
On blood/ biopsy samples.

most sensitive but have poor availability in most laboratories.

Treatment of typhus fevers and SFRs

00:26:17

- DOC → Doxycycline for (7-10) days.
- Alternatives → macrolides, Fluroquinolones, Chloramphenicol.
 1. Penicillins and aminoglycosides → Ineffective in Rickettsial diseases.
 2. Sulfonamides → Enhance the replication of rickettsia → worsen the disease → Contraindicated.

Epidemic and endemic typhus can be differentiated by :

- Neil mooser reaction.
- Specific antibodies by IFAT tests.
- Latency is seen in epidemic tick typhus.
- most severe SFR → RMSF.
- mildest SFR → Rickettsial pox.
- SFR with no eschar → RMSF.

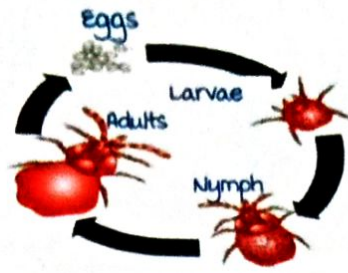
Orientia

00:28:47

- Causes scrub typhus/Chiggerosis.
- most common rickettsial disease reported in India especially from mountain regions.
- Etiology → *Orientia tsutsugamushi* (5 serotypes) :
O. chuto → UAE (latest).
- vector → Chiggers : Larval stages of trombiculid mites (*Leptotrombidium* sp.).
- mode of infection → Bite of chiggers.
- Reservoir → Birds and rodents.
- Transovarial transmission is present.



Chigger mite



- For establishing a microfocuss of transmission, zoonotic tetrad constituents are :

1. Orientia.
 2. Chiggers.
 3. Rodents.
 4. Scrub vegetation.
- Incubation period → 1 week.
 - Clinical features → Eschar, fever, headache, myalgia, rash which starts from the trunk, tender regional/generalised lymphadenopathy.
 - Rare complications → meningitis/meningoencephalitis, respiratory and renal failure, DIC (Disseminated intravascular coagulation).
 - Diagnosis →
Specimen : Blood sample/ Eschar biopsy/LN biopsy/
Rash biopsy.
Giemsa : Demonstration of basophilic inclusion bodies inside the endothelial cells.
 - Cultivation → Guinea pig/cell-lines.
 - Serology :
1. Weil-Felix reaction.
2. Specific anti-orientia antibodies detection by IFAT.
3. NAATs.
 - Treatment → DOC = Doxycycline for (1-2) weeks.
Alternatives : macrolides, Chloramphenicol.
 - Mnemonic for scrub typhus →
ROD SCRUBS for OTs, he CHANGES into MITTENS.
Reservoir → Rodents, Disease → Scrub typhus,
Agent → Orientia Tsutsugamushi, Vector → Chigger mite.

Weil-Felix reaction

00:36:50

- **Heterophile agglutination test** which is done in a tube (tube agglutination test).
- Heterophile antigens → Antigens which are shared over species, genera, classes and kingdoms.
- Heterophile antibodies → Shared among rickettsial & Proteus sp (Proteus vulgaris and Proteus mirabilis).
- O → Somatic antigen.
- X → Non-motile strain.
- Weil-Felix reaction is **positive for all rickettsial diseases** except rickettsial pox & Brill Zinsser disease.

Rickettsial disease	OX-19 (Proteus vulgaris)	OX-2 (P. vulgaris)	OX-K (P. mirabilis)
Epidemic typhus	+++	+	-
Brill Zinsser	-	-	-
Endemic typhus	+++	+/-	-
Spotted fevers (not rickettsial pox)	++	++	-
Scrub typhus	-	-	+++

RICKETTSIA AND RELATED GENERA : PART 2

Rickettsia and related genera

00:00:20

Coxiella.

Bartonella.

Ehrlichia.

All rickettsia are :

Gram negative coccobacilli.

Obligate intracellular except *Bartonella* (cultivable on freshly prepared blood agar and chocolate agar).

Multiply in endothelial cells except *Ehrlichia* and *Anaplasma* (found in blood cells).

Anthropod transmitted except *Coxiella*.

Forms basophilic inclusion bodies.

Stained by Giemsa stain, Gimenez, Castenada or Machiavello stain.

Coxiella burnetii :

Family : Coxiellaceae.

BSL-III pathogens.

Exists in environment in a spore like forms.

Phase variations :

- Fresh isolates : Only phase I antigens.
- Serial subcultures in cell lines : Phase II antigens.

Acute Q fever : Significant rise in antibodies against only phase II antigens.

Chronic cases : Significant rise in antibodies against phase I.

Both phase I & II are expressed.

They both can survive in Holder method of pasteurization.

Reservoir : Domestic animals like sheep, cows and goats.

Present in large numbers in their urine, feces, milk and placenta +++.

Mode of infection : Inhalation of anaerobes (MC), raw milk,

Active space

percutaneous (while handling animal products).

Occupation at risk : veterinarians, abattoir/ slaughter house workers, dairy workers and farmers.

Q fever (Query fever)

00:07:18

Query fever.

most infections are asymptomatic.

Symptomatic individuals develop acute stage of Q fever.

Acute stage : Fever, myalgia, chills, headache.

Resolves spontaneously.

Severe (2-5%) : Atypical pneumonia (resembling Legionnaire's disease).

Small % of population : **Hepatitis**.

Some cases : **meningitis** or meningoencephalitis, characterized by **lymphocytes in CSF**.

Diagnosis : Serology.

IgM $\geq 1 : 50$ and IgG $\geq 1 : 28$ → Significant titre of phase II antigen.

Treatment : Acute infection.

Doxycycline x 2-4 weeks.

Acute Q fever in pregnancy : very dangerous and can cause **spontaneous abortions**.

Risk factors for chronic Q fever :

Immunodeficient & damaged or prosthetic heart valves.

After several months to years, develops **culture negative endocarditis** (as it is an obligate intracellular organism that cannot be isolated in routine culture media and requires blood culture for diagnosis) associated with **nodular vegetations** on heart valves.

Acute Q fever with risk factors : **Follow up (for atleast 2 years)** is recommended → Can end up in culture negative endocarditis.

Diagnosis :

Blood sample : Cell line culture (Bio Safety Level - III) & PCR.

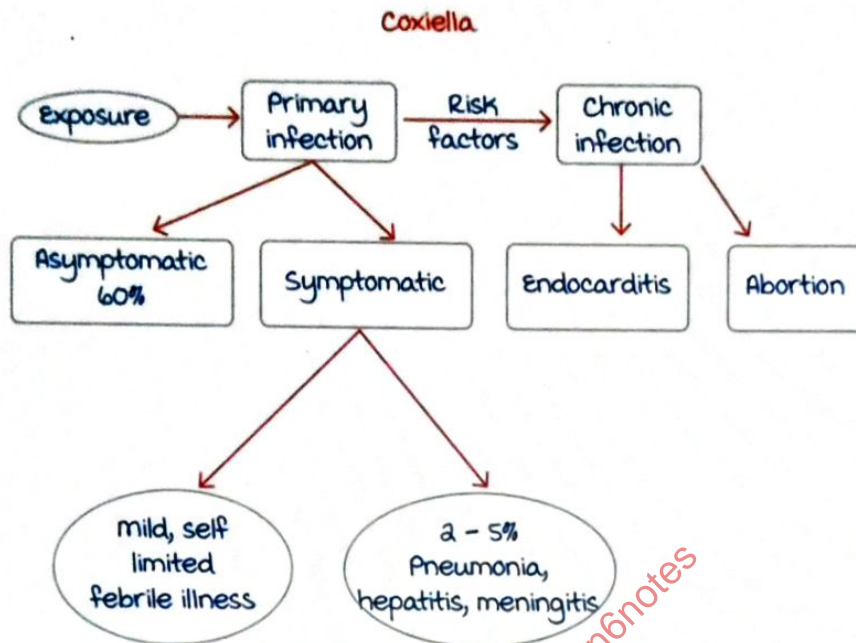
Chronic stage : **Serology** shows significant rise in **IgG** against **phase I antigens ($> 1 : 800$)**.

Treatment : Chronic infection.

Doxycycline + Hydroxychloroquine (best Rx) or

Doxycycline + Rifampicin/ Ciprofloxacin X 18 - 24 months.

In case of pregnancy with chronic infection : Cotrimoxazole continued throughout pregnancy.



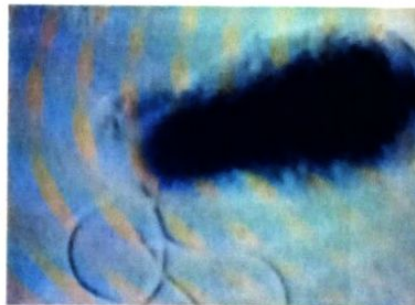
Bartonella

00:16:28

Family : Bartonellaceae.

Special features :

Not an obligate intracellular parasite : Can be grown on blood agar and chocolate agar.
motile.



Types :

Bartonella bacilliformis.

Bartonella quintana.

Bartonella henselae.

Bartonella bacilliformis :

Endemic in South America.

vector : Sandfly (Lutzomyia or Psychodopagus).

Reservoir : Humans.



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Transmission : Bite of sandfly.

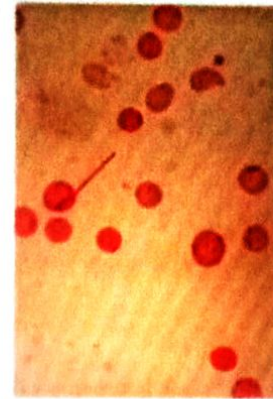
Acute stage : Oroya fever/ Carrion's disease.

Typically associated with hemolytic anemia, hepatomegaly and severe immunosuppression.

45 - 90% mortality rate.

Diagnosis : Peripheral blood smear stained with Giemsa to demonstrate inclusion bodies followed by PCR.

Treatment : Ciprofloxacin + Ceftriaxone.



Giemsa stained blood smear of Oroya fever, showing overwhelming parasitism of erythrocytes

Chronic stage : weeks to months later (if untreated)

aka verruga peruana / Peruvian wart.

Vascular proliferative lesions on the skin (subdermal).

Diagnosis :

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Biopsy : Warthin starry stain shows vascular proliferation + clumps of black coloured bacilli.

Treatment : Azithromycin.



Bartonella quintana

00:22:44

vector : Body louse (Epidemic typhus, Relapsing fever, Trench fever).

H/O homeless, very poor or overcrowding.

Reservoir : Humans only.

mode of infection : Louse feces inoculated through skin.

1. In healthy individuals : Causes **trench fever/ Shin bone fever / 5 days fever.**

Trench fever : Fever (periodic remitting relapsing fever for 5 days), headache, retro orbital pain, typical pain in pretibial area, \pm truncal rash and \pm hepatosplenomegaly.

Diagnosis : Serology.

Treatment : **Doxycycline for 4 weeks combined with Gentamycin for 2 weeks.**

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2. Healthy individuals $\&$ damaged/prosthetic heart valves :
Culture negative endocarditis.

Diagnosis :

Serology : Rise in antibody titre.

Blood culture : Blood agar/ chocolate agar (5 - 7 days).

PCR on blood.

Treatment : **Doxycycline with Gentamycin.**

3. Advanced HIV $\&$ other immunodeficiency states :

Bacillary / Epithelioid Angiomatosis :

Vascular proliferative lesions on skin, subcutaneous tissues and bones.

Never in the liver and spleen.

Diagnosis : Biopsy.

On Biopsy with **Warthin starry silver stain** : Endothelial cell proliferation + clumps of black coloured bacilli.

Resembles Kaposi's sarcoma.

PCR.

Treatment :

DOC : Doxycycline x 3 - 6 months.

Alternative drugs : **Erythromycin/ Azithromycin.**

Bartonella henselae

00:30:56

Reservoir : **Cats.**

vector : **Cat flea** (role is still investigated in human disease).

mode of infection : Cat scratch/bite (? cat flea feces

inoculation into abraded skin).

Active space

1. In healthy individuals : **Cat scratch disease** : m/c human infection reported due to Bartonella.

Papule or **pustule** at the site +
ipsilateral regional lymphadenopathy.
Incubation period : 3-10 days.
Resolves spontaneously in few weeks.

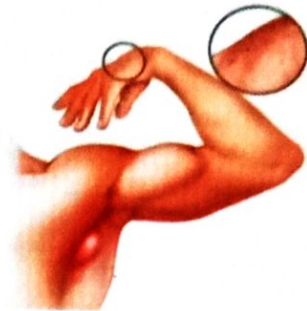
1 - 5% : **Neuroretinitis**.

1 - 7% : **Encephalitis**.

Diagnosis : Biopsy.

Warthin starry stain : **Granulomas** with **stellate necrosis** +
clumps of black coloured bacilli.

PCR.



Atypical forms of cat scratch disease :

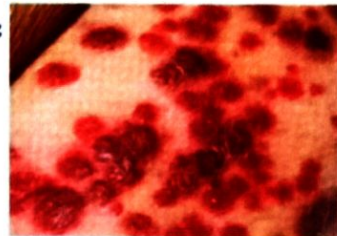
Parinaud's oculo glandular syndrome.

Granulomatous conjunctivitis +
pre auricular lymphadenopathy.

Treatment : Resolves spontaneously.

If extensive lymphadenitis : **Azithromycin** X 5 days.

Neuroretinitis/ Encephalitis : **Doxycycline** + **Rifampicin** X 4 - 6 weeks.



2. Culture negative endocarditis : mainly seen in **damaged/ prosthetic** heart valves.

3. HIV and other immunodeficiency states : **Bacillary/ Epithelioid Angiomatosis**.

m/c site : **Skin** resembling **Kaposi's sarcoma**.

Other sites are difficult to diagnose : **Liver, spleen, bones**.

Diagnosis : Biopsy (similar to *B. quintana*)

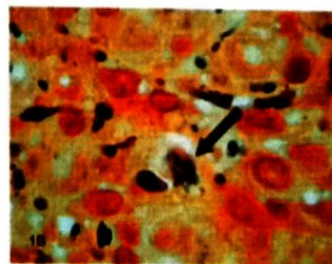
Warthin starry silver stain : **Endothelial cell proliferation** +
clumps of black bacilli.

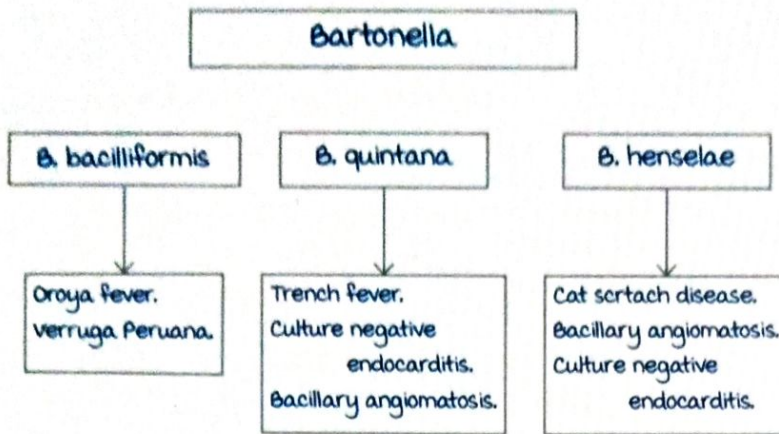
PCR.

Treatment :

DOC : **Doxycycline**.

Alternative : **Azithromycin/ Erythromycin**.





Ehrlichia and anaplasma

00:42:16

Family : Anaplasmataciae.

Special features : Localize in blood cells.

Vector : Hard ticks.

Reservoir : Cats, dogs, etc.

Obigate intracellular gram negative coccobacilli.

Species causing human diseases :

- E. chaffeensis.*
 - E. muris euclariensis.*
 - E. ewingii.*
 - A. phagocytophilum.*
- Human monocytotropic ehrlichiosis.
- Human granulocytotropic anaplasmosis / ehrlichiosis.

C/F : Fever, headache, myalgia, ± rash.

Severe disease : Renal failure, meningitis, DIC.

Diagnosis :

Complete blood count (CBC) :

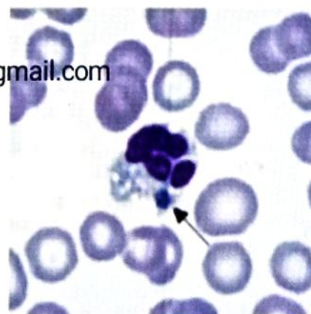
Anemia + thrombocytopenia + leucopenia.

Peripheral blood smear : mulberry shaped inclusions inside granulocytes and monocytes : morula.

PCR on blood.

Indirect fluorescence antibody testing.

Treatment : DOC is Doxycycline.



Active space

Clinical cases :

Q. A 58 year old man presents to the emergency with 1 day of high fever to 39.5°C (103.1°F) along with muscle aches and malaise. During the initial interview, he remembers his name but is not oriented to place or time, and he cannot recall the names of his four children. He has no known medical problems. His wife claims that he is in "perfect health" and reports that he was out playing in the yard with their grandchildren 3 days ago. They have not been out of the country in >2 years and have no animals at home. A peripheral blood smear demonstrates morulae within several monocytes. Laboratory studies show, Platelet count : $84,000/\text{mm}^3$, wbc count : $2500/\text{mm}^3$, hemoglobin : 14.2 gm/dl , serum transaminases : mildly elevated. Which of the following is most likely to have caused this patient's disease ?

- A. Babesia microti.
- B. Borrelia burgdorferi.
- C. Ehrlichia chaffeensis.
- D. Leishmania donovani.
- E. Rickettsia rickettsii.

Q. A 39 year old man presents with sudden, influenza like symptoms. He states that he works in a slaughter house, and several of his co workers have similar symptoms. Early stages of pneumonia are detected on x ray. Which of the following is the most likely etiologic organism ?

- A. Coxiella burnetii.
- B. Rickettsia rickettsiae.
- C. Taenia solium.

D. Taenia saginata. Q. A 16 year old male presents with a 7 day history of fever, malaise, left sided eye pain, and blurry vision. Three dogs and three cats live in the home with the patient and his family. He has no known drug allergies. His BP is $110/70 \text{ mmHg}$, pulse $70/\text{minute}$, temperature 100°F , and respiratory rate $18 \text{ breaths per minute}$. The left eye examination reveals visual acuity of $20/40$ and an afferent

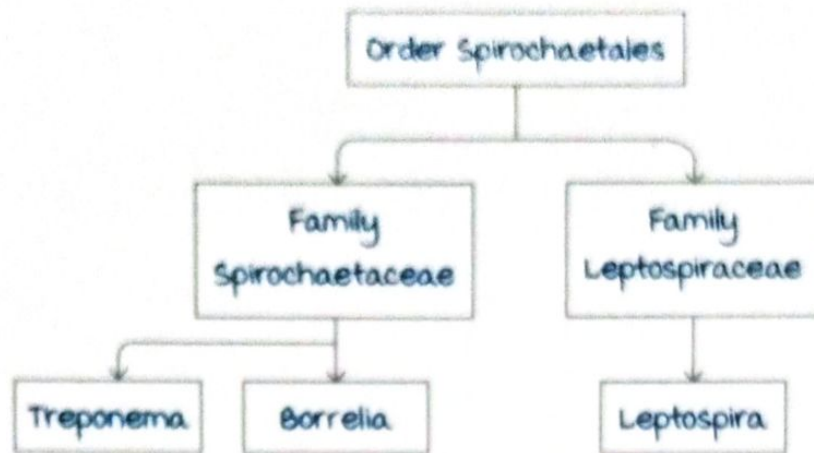
pupillary defect. Fundoscopy reveals cotton wool spots, hemorrhages, and multiple lesions in the deep retina. The rest of the systemic exam is unremarkable, except for a small lymph node in the cervical region. Blood work shows a WBC count of 14,000/ microl, hemoglobin 15 gm/dl, and platelet count 250,000/ microl. Blood cultures are negative. Fluorescein fundal angiography of affected eye reveals disc edema and blocking fluorescence in areas of hard exudates. MRI of the brain is normal. Cervical lymph node biopsy reveals necrotizing granulomas with organisms that stain positive with silver stain. What is the most appropriate treatment for this patient?

- A. Doxycycline plus Rifampin.
- B. Pyrimethamine plus sulfadiazine
- C. INH plus Rifampin.
- D. Ganciclovir plus foscarnet.

Q. A 17 year old female presents with fever, myalgias, cough, and headache. She has just returned home after working at a summer camp. She recalls finding a tick attached to her left thigh, which she removed. Initial lab work reveals WBC count 3,400/ microl, platelets 80,000 microl, AST 80 U/L, and ALT 74 U/L. Given her presentation and history of exposure, there is a high degree of suspicion for anaplasmosis. What should be expected to be seen on the peripheral smear?

- A. Spirochete on peripheral smear.
- B. Presence of morulae in monocytes.
- C. Presence of morulae in granulocytes.
- D. Parasites inside red blood cells.

TREPONEMA



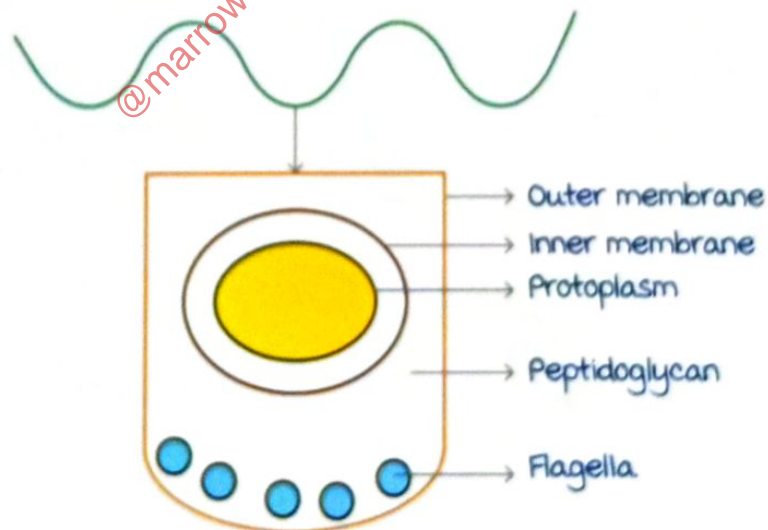
In members of the order **Spirochaetales** → **endoflagellae**.

Also known as **axial filaments**.

Do not extend out to the environment.

Present in the **periplasmic space**.

3-4 polar endoflagellae



Treponema

00:02:02

There are two pathogenic species : **Treponema pallidum**,
Treponema carateum.

Treponema pallidum divided into 3 varieties or subspecies.

Treponema pallidum var pallidum : **Venereal syphilis**.

Treponema pallidum var **endemicum** : **Endemic syphilis** /
Bejel.

- Reported from Africa.

Treponema pallidum var **pertenue** : **yaws**/ **Framboesia**.

- Eradicated from India.

Treponema **carateum** causes **Pinta**.

Reported from South America.

venereal syphilis : **Sexual** transmission.

Others : **Non-venereal** treponematosis.

- Spreads via **contact of skin breaks** or mucus membrane to lesions of the disease.

All the pathogens mentioned above are **morphologically** and **antigenically similar**.

Test for **syphilis** like VDRL/ RPR/ FTA-ABS would be positive in **non-venereal** treponematosis.

Non-pathogenic species :

E.g. : T. microdentium, T. macrodentium, T. denticola, T. phagedenis, T. refringens.

Normal oral and genitourinary tract flora.

T. phagedenis : **Reiter's strain**.

T. refringens : **Noguchi's strain**.

Treponema pallidum subsp. pallidum

00:07:44

General characters :

Gram negative spiral shaped organism.

10 - 14 μ long.

0.1 - 0.18 μ broad.

It is **too slender** to be seen by light microscope.

Seen by **negative staining**/ **dark ground** microscopy.

Endoflagellae present → **Corkscrew motility**.

Non-cultivable on cell free media → obligate intracellular organism.

It can only grow on **rabbit testis** by **serial passage** (live

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cells).

Limited growth can be achieved on Eagle and McCoy cell culture.

1st strain of *T. pallidum* grown on rabbit testis in 1912 was Nichol's strain.



Organisms that are non-cultivable on cell free media: divyprasanth7@gmail.com
 mnemonic: VIRUS infected RICKETTY LEPER Climbed a SPIRAL TREE TRUNK.

All viruses.

Rickettsia and related genera like *Orientia*, *Ehrlichia*,
Anaplasma
Mycobacterium leprae.

Chlamydia

Spirillum minus → rat bite fever.

Treponema pallidum.

Tropheryma whippelii → Whipple's disease.

Venereal syphilis

00:12:58

Earlier called French disease.

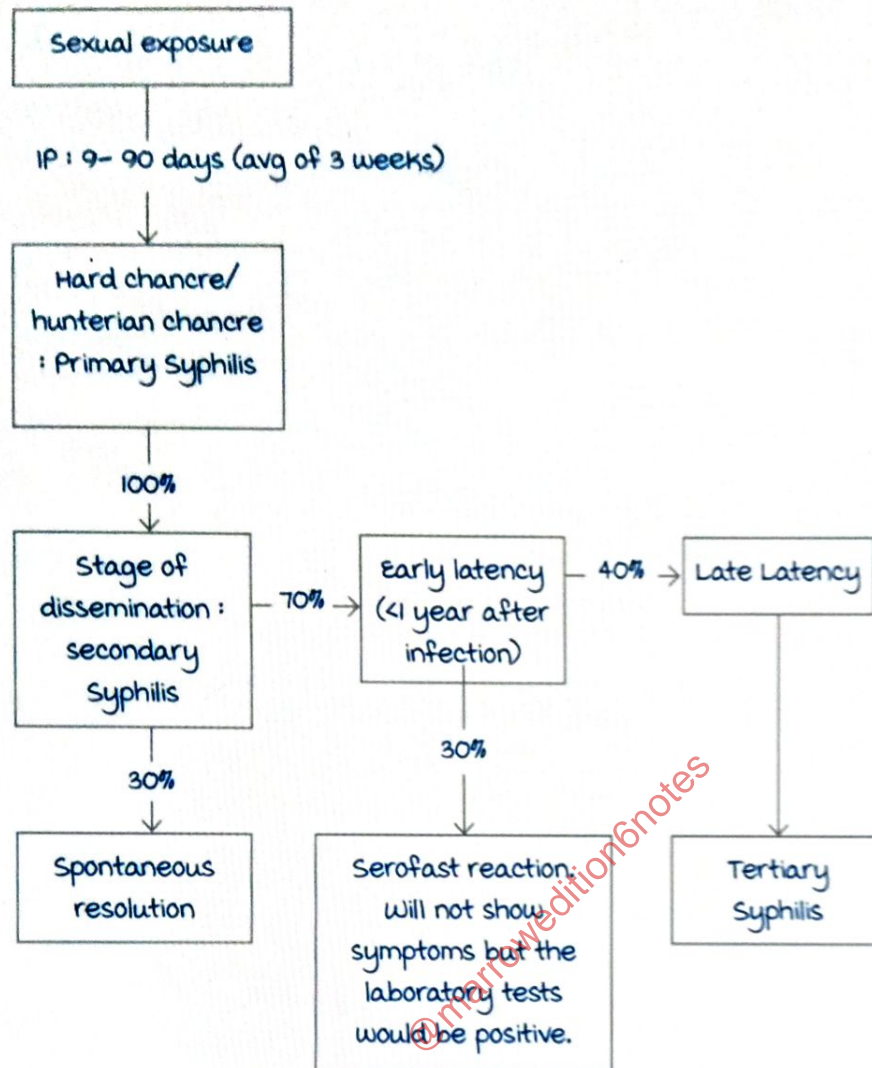
Sexually transmitted disease.

Hard chancre resolves in 2-8 weeks of time if not treated.

The course of syphilis was studied in the infamous

Tuskegee study, where the course was studied on black

individuals without giving them treatment.



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Primary syphilis

00:16:59

IP : 9- 90 days.

Patient presents with **hard/ Hunterian chancre**.

Starts as a **painless papule** → **painless ulcer** with indurated base and raised regular edges.

Usually, **1 or 2** in number.

multiple chancres seen in HIV patients.

Genital ulcer is associated with **non-tender regional lymphadenopathy**.

Highly infectious lesion.

Heals spontaneously in **2 - 8 weeks** time if untreated.

Active space



Secondary syphilis :

100% patients develop 2° syphilis if left untreated → stage of dissemination.

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Varied manifestations :

- Rash : macular / papular / pustular / mixed rash. most often involves the trunk and spreads to limbs. Palms and soles are also affected.
- Generalized non-tender lymphadenopathy.
- **Condyloma lata** : Highly infectious papules / pedunculated lesions in oral cavity or anogenital region.



Papular rash on back



Pustular rash on palms



Genital condyloma acuminata

- **mucous patches** : Silvery grey erosions surrounded by a zone of erythema in the oral mucosa and genital mucosa.

Patient may also develop :
meningitis.

Chorioretinitis, iritis, optic neuritis.

Hepatitis.

Nephrotic syndrome.

Periostitis, arthritis.

Secondary stage was **earlier** called as the **great imitator**.

Latent syphilis :

Lesions of secondary syphilis **resolves** in 1- 6 months time if not treated, and patient goes into latency. bnvssprasanth7@gmail.com

Early latent syphilis : < 1 year after infection.

- Presents with **relapses** of manifestations of 2° stage.

Late latent syphilis : > 1 year after infection.

Early syphilis : 1° stage, 2° stage and **early latent** stage.

- One dose of benzathine penicillin.

Late syphilis : **Late latent** and 3° stage.

- 3 doses of benzathine penicillin.

Tertiary Syphilis

00:23:51

5- 30 years after infection.

Gummas :

most common manifestation.

Granulomas involving **skin**, **bones** (saddle nose), **liver**, **spleen**.

Seen in ~15% of patients.

Seen after 5 years of infection.

Cardiovascular manifestations :

Seen in ~10% of patients.

Seen from 10 years onwards.



Gumma

Active space

End arteritis of the vasa vasorum supplying the ascending aorta > transverse region > descending aorta.

Causes aortitis → aortic regurgitation and aortic aneurysm.

Neurosyphilis :

manifestations are due to :

- End arteritis of cerebral vessels → meningovascular syphilis → symptoms of cerebral infarcts like aphasia, hemiparesis, visual loss.
- Actual destruction of neurons by the treponemes : parenchymatous manifestations.

Tabes dorsalis → affecting the dorsal columns of spinal cord.

Bladder and bowel disturbances.

Foot slap.

Impotence.

Ataxia.

Loss of position and vibration sense.

General paresis of the insane → neurons of cerebral cortex are involved.

Argyll-Robertson pupil.

Illusions, delusions and hallucinations.

Slurring of speech.

Reduced memory.

Diagnosis :

Gold standard : Rabbit infectivity testing → done in research laboratories.

Others include :

microscopic methods.

Antigen detection.

NAATs → most sensitive test.

Serology → useful in all stages of syphilis.

} useful only for early syphilis

microscopy :

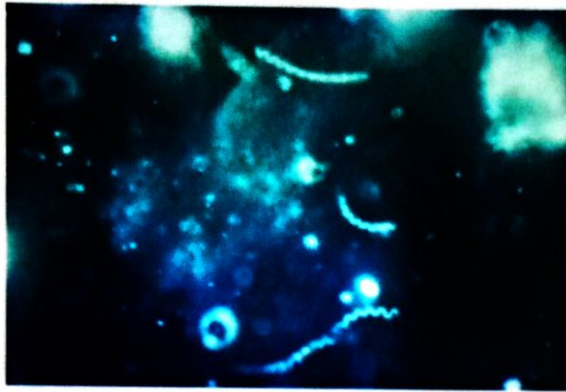
useful only in **early syphilis**.

Specimen collected are :

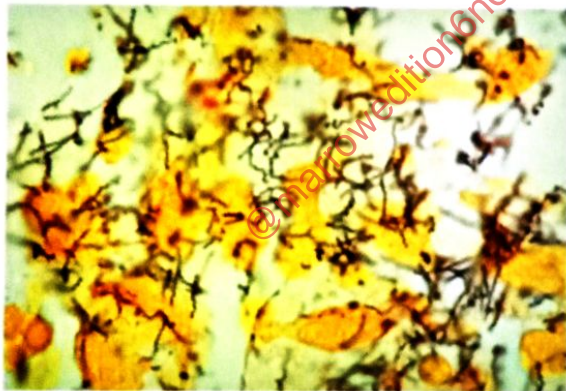
Exudate from hard chancre.

Biopsy from chancre.

Biopsy from lesions of secondary syphilis (mucous patches, condyloma, rash, CSF).



Dark ground microscopy



Levaditi impregnation stain

Fluid specimen : **Dark ground microscopy/ Fontana's silver**
~~stain and the gold stain.~~

Biopsy specimen : **Levaditi silver impregnation stain.**

Antigen detection :

more sensitive than routine microscopy.

Specimen collected are :

Exudate from hard chancre.

Biopsy from chancre.

Biopsy from lesions of secondary syphilis (mucous patches, condyloma, rash, CSF).

Direct Fluorescent Antibody Testing (DFAT) is used.

Smear made on a slide with the specimen.

monoclonal antibodies against *T. pallidum* antigens are conjugated with fluorescent dyes like fluorescein isothiocyanate.

Wash the slide to remove the unbound antibodies and observe under the fluorescent microscope.



Serology :

most commonly used method of diagnosis.

- Non-treponemal tests / standard tests of syphilis.
- Treponemal tests.

Non-treponemal tests:

Antigen used to detect the antibodies → cardiolipin antigen (alcoholic extract of ox heart).

It is conjugated with lecithin and cholesterol (increases the antigenicity of cardiolipin) → lipoidal antigen.

Antibody detected is known as reagin antibody.

Non-specific antibody that appears in the patient's serum, probably in response to the antigens released from the damaged syphilitic tissues.

IgG class of antibody.

Types of non-treponemal tests :

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- Wasserman test : First test used for diagnosis of syphilis.
- Complement fixation test.
- Kahn test : Tube flocculation test (type of precipitation test).
- VDRL test (venereal disease research laboratory) : Slide flocculation test.

One slide for one patient, each containing 12 wells.
 Serial dilution of patient's serum (1:2, 1:4, 1:8 etc).
 Lipoidal antigen added to each well.
 VDRL slide is then placed on the VDRL shaker for 4 mins.
 Flocculation observed under the microscope →
 cannot be used on the field.



VDRL slide



VDRL shaker

- Rapid plasma reagent (RPR) test.

Lipoidal antigen is coated
 onto carbon particles.

The serum is diluted serially
 and the lipoidal antigen
 added.

It is then placed on the
 RPR card.

Agglutination visible with the
 naked eye.

TULIP
RPR card

- TRUS test : Toluidine red unheated serum test.

Biological false positives :

Patient **does not** have syphilis, but one of the **non-treponemal tests** are positive.

Antibody titers are **< 1:8**.

1% of total population

Of two types :

Acute : Lasts for **< 6 months**.

- **Pregnancy.**
- Infectious mononucleosis.
- measles.
- malaria.
- Chicken pox.
- Viral pneumonias.
- Viral hepatitis.
- Recent immunizations.
- Acute HIV infection.
- Relapsing fever.

Chronic : Lasts for **> 6 months**.

- SLE.
- Lepromatous leprosy.
- Collagen disorders.
- Narcotic addictions.
- malignancies like myeloma.

Treponemal tests

00:46:09

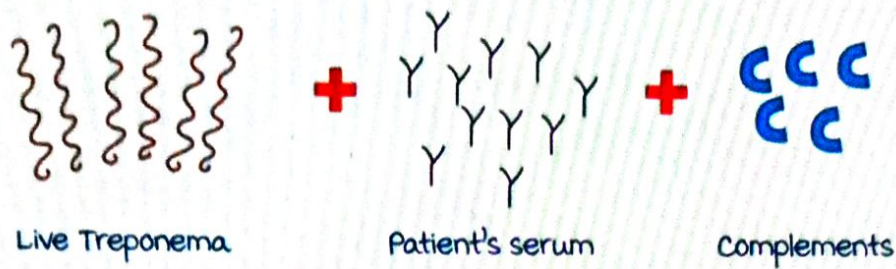
Antigen used is **specific** T. pallidum antigen.

Detects anti-treponemal antibody.

Treponema pallidum immobilisation test (TPD) :

Historical significance.

1st treponemal test used for diagnosis of syphilis.



Live treponema was added to patient's serum and complement (from guinea pigs).

Incubation → immobilization of the treponema by the antibodies in the serum.

most specific test for diagnosis of syphilis.

Fluorescent treponema antibody absorption test (FTA-ABS)

Indirect fluorescent antibody test.

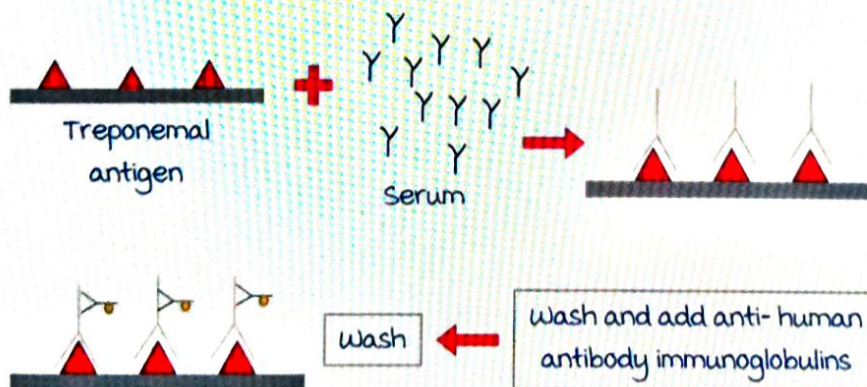
Slides precoated with treponema pallidum antigens.

Add the patient's serum.

Antibodies bind to the antigen.

Slide is washed and anti-human antibody immunoglobulins are added, which are conjugated with the fluorescent dye, FITC.

Wash the slide and observe under the microscope.



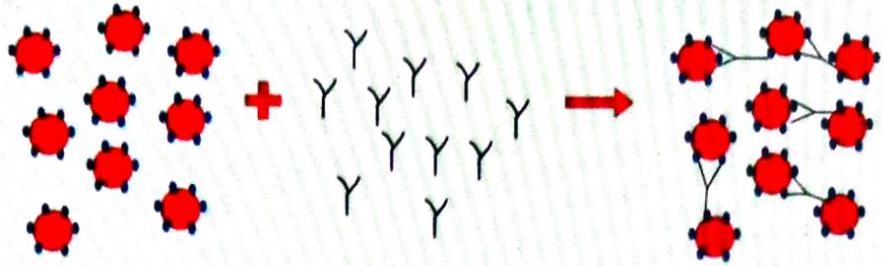
most sensitive test for diagnosis for syphilis.

1st serological test to become positive after infection.

Treponema pallidum hemagglutination test :

bnvssprasanth7@gmail.com

Sheep RBCs coated with antigens mixed with patient's serum.



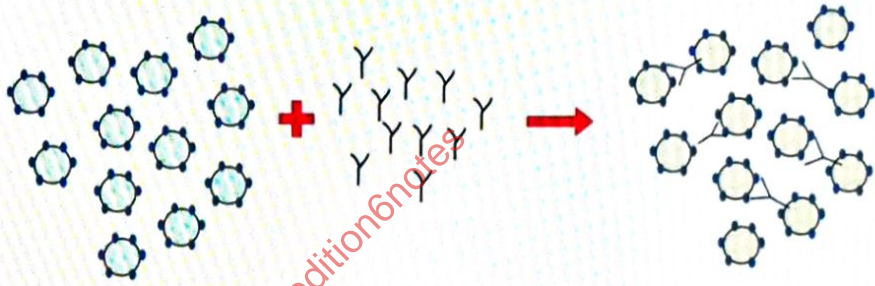
RBCs become clumped.

Treponema pallidum particle agglutination test :

Gelatin particles are coated with treponema pallidum antigens mixed with patient's serum.

Agglutination of gelatin particles.

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Indirect ELISA/CLIA :

ELISA wells with T. pallidum antigens.

Serum of the patient is added.

The antibodies bind.

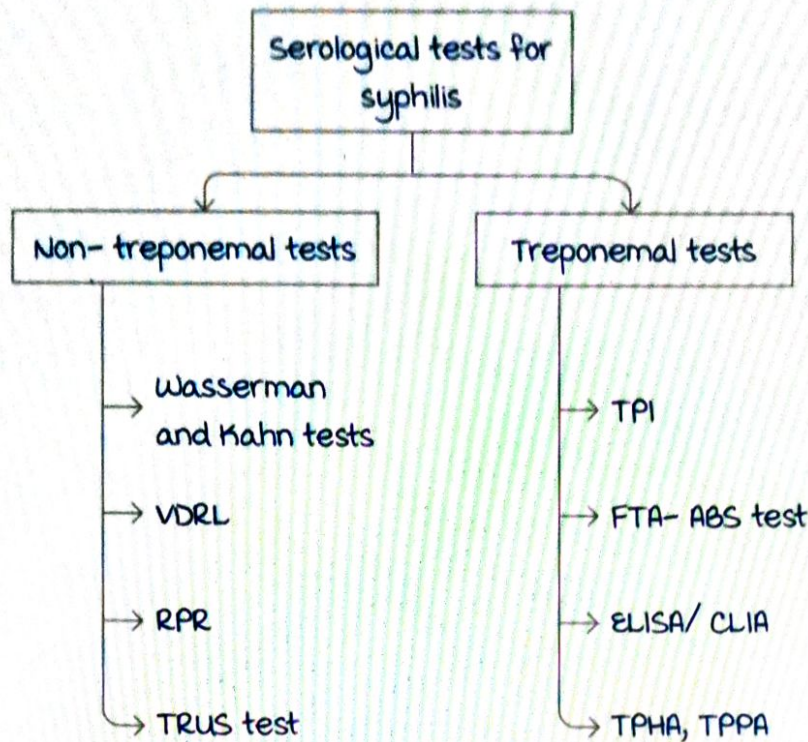
Well is washed and anti-human antibodies, conjugated with enzymes.

The substrate is added, and the enzyme breaks it down.

Look for colour change/ emission of light.



Active space



Serology

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00:55:32

Serology becomes positive 10-14 days after the appearance of chancre.

1st to become positive : FTA-ABS.

Diagnosis of syphilis : 2 step approach.

Screening test : Non-treponema test → VDRL, RPR, TRUS test.

Confirmatory test done on positive/ reactive screening test, with treponema tests → TPHA/ TPPA (most commonly used tests).

Follow up after treatment : VDRL/ RPR (whichever test was used for screening is used for follow up) → 4 fold fall in antibody titers is looked for after 6 months (1:64 to 1:16).

- Treponemal test not used → remain positive years after treatment.

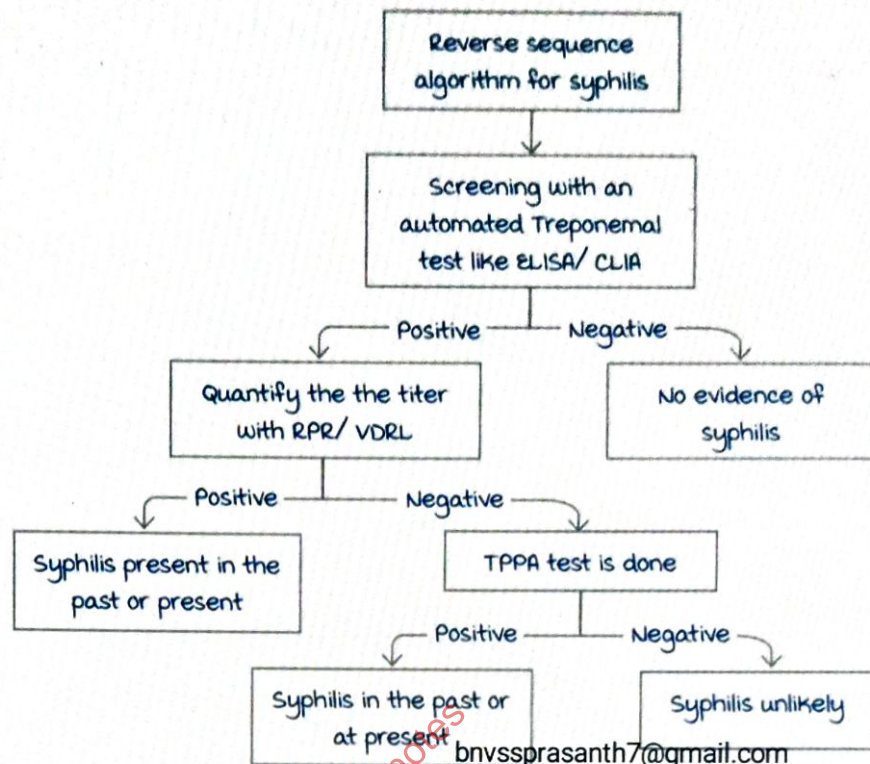
For the diagnosis of syphilis :

most sensitive test : FTA-ABS test.

most specific test : TPHA > TPPA.

Active space

Reverse sequence algorithm syphilis :
Done in high throughput laboratories.



Neurosyphilis :

Sample used is CSF.

Treponemes are usually absent in the CSF specimen.

most specific test : VDRL.

most sensitive : FTA- ABS test. Can give false positive test.

Treatment :

Early syphilis :

Benzathine Penicillin 2.4 million units Im single dose.

Allergic to Penicillin → Doxycycline or Tetracycline can be used.

- Doxycycline 100mg BD orally for 14 days.
- Tetracycline hydrochloride 500mg QID orally for 14days.

Late latent or syphilis of unknown duration or non- neurological tertiary syphilis :

Benzathine Penicillin 2.4 million units Im at weekly intervals x 3 (total of 7.2 million units).

In case of allergy to penicillin : Doxycycline or tetracycline.

- **Doxycycline** 100 mg BD orally for **28 days**.
- **Tetracycline** hydrochloride 500mg QID orally for **28 days**.

Neurosyphilis, ocular or otic syphilis :

Aqueous crystalline penicillin G 18- 24 million units per day for 10- 14days.

Procaine penicillin G 2.4 million units IM + Probenecid QID orally for 10- 14days.

Allergic to penicillin → **Desensitized** and **treated** with penicillin.

In pregnancy → **Desensitize** the patient and treat with stage appropriate dose.

Non- venereal treponematosi s (NVT)

01:06:12

Also called as **endemic treponematosi s**.

Caused by treponemes morphologically and antigenically similar to *T. pallidum* subsp. *pallidum*.

Seen in **rural areas** of **developing nations**.

Transmission : **Skin to skin contact** with **exudates from lesions/ fomites/ flies**.

Like syphilis, can cause **severe late manifestations** years after initial infection.

Unlike syphilis → **No CNS involvement** or transplacental transmission.

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Yaws :

Also called **Framboesia**.

IP : **~3 weeks**.

Patient comes with a lesion called **mother yaw/ Framboesioma**.

- **Ulcerating skin lesion** associated with regional lymphadenopathy.

Resolves **spontaneously** if untreated.

Few weeks- months later → secondary lesions similar to mother yaw, which relapses and remitted for several months- years.

10% develop gumma of skin and bones → gondou, saber shins, gangosa.

Gondou : Severe hypertrophy of maxillary bones.

Gangosa : Severe destruction of nasal cartilages.

No CNS or CVS manifestations.

most common NVT.

Eradicated from India in 2006.

Treatment :

Single dose of Azithromycin 30mg/kg.

Alternative : Benzathine penicillin 6 lakh units IM (≤ 10 years of age) or 1.2 million units (>10 years of age).

Endemic syphilis/ Bejel :

Reported from Africa, Southeast Asia, Middle east.

IP : ~3 weeks.

Patient presents with a primary lesion that often goes unnoticed → oral mucus patch that resolves spontaneously.

Few weeks later presents with rash and mucous patches.

Gummas of skin and bone after months- years → bone deformity and gangosa.

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Treatment :

Single dose of Azithromycin 30mg/kg.

Alternative : Benzathine penicillin 6 lakh units IM (≤ 10 years of age) or 1.2 million units (> 10 years of age).

Pinta :

IP : ~3 weeks.

Presents with papule/ plaque with regional lymphadenopathy.

Does not resolve spontaneously.

Remains and increases in size to become hyperkeratotic

pigmented lesions.

In the next 15- 30 years → disseminated pintids.

- Hyperpigmented dyschromic lesions that contain treponemes.
- Achromic lesions which do not contain treponemes.

No gumma, CVS or CNS manifestations.

Treatment :

Single dose of Azithromycin 30 mg/kg.

Alternative : with Benzathine penicillin 6 lakh units IM (≤ 10 years of age) or 1.2 million units (>10 years of age).

@marrowedition6notes

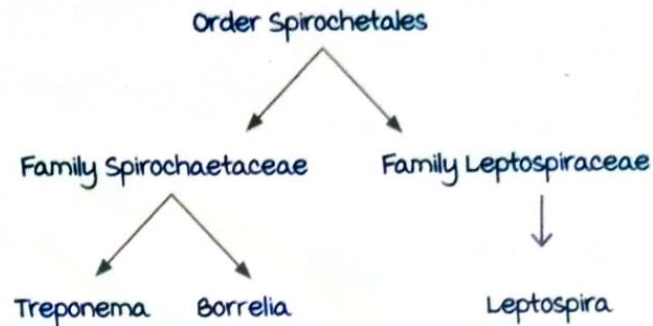
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Active space

LEPTOSPIRA AND BORRELIA

Introduction

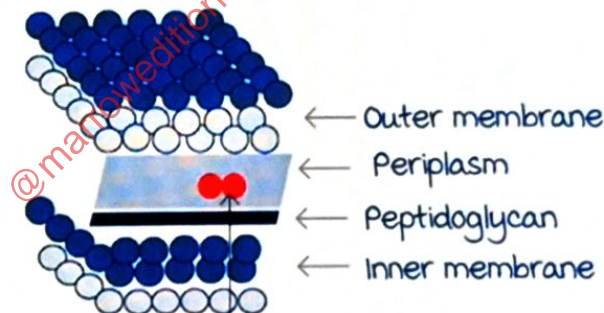
00:00:36



Endoflagellae :

All spirochetes are motile by endoflagellae present in the periplasmic space.

Endoflagellae are also called polar endoflagellae/axial filaments.



Endoflagellum rotates while attached to one end of the protoplasmic cylinder.

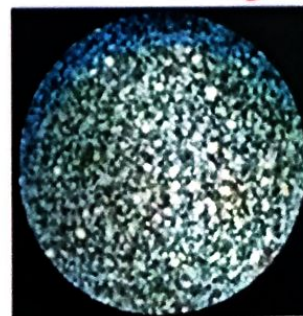
Leptospira

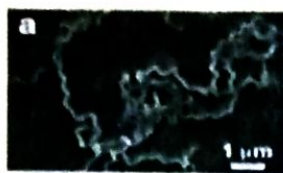
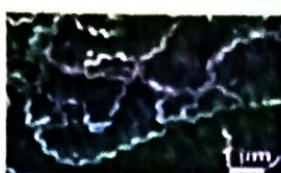
00:01:54

10 pathogenic species → most common → *L. interrogans*.

Further divided into :
23 sero groups (based on antigens)

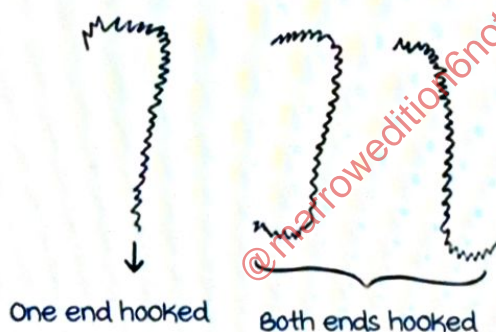
> 250 serotypes/serovars.



Sputter coat
(SEM)Ionic Liquid
(SEM)Negative Stain
(TEM)

morphology :

- Gram negative spirals.
- 6 - 20 μ long.
- 0.1 μ broad \rightarrow too slender to be seen by light microscope (resolution : 0.2 μ). Hence, they can be visualised by dark field microscope.
- Endoflagellate.
- Closely wound spiral with 1 or both ends hooked.
- 1 end hooked : umbrella handle/ shepherd crook appearance.



Cultivation :

Leptospira and Borrelia are fastidious.

Serum media :

EMJH
Korthoff's
Fletcher's
Stewart's

These media are either liquid/ semisolid.

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Leptospira is microaerophilic : Requires less amount of O_2

Hence grows just beneath the surface.

Dinger's ring/ disc



Active space

Growth is very slow \rightarrow takes several weeks.

Epidemiology

00:06:47

very common zoonotic infection.

most important reservoir → **Rodents**.

Other reservoirs : wild & domestic animals (dogs, horse, cows).

Leptospira are asymptomatic colonisers of **collecting tubules** of the reservoirs.



Periodically excreted out through urine.

mode of infection :

Abraded skin/ mucous membrane exposed to urine contaminated water/soil.

Occupational risk :

Rice field farmers, sewer workers, mine workers, veterinarians, abattoir workers.

Course of infection :

40% : Asymptomatic.

60% : Symptomatic.

90 - 95% : Anicteric febrile illness.

5 - 10% : **Severe systemic vasculitis (Weil's disease/**

ictero hemorrhagic fever).

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Leptospirosis and Weil's disease

00:11:27

Incubation period : 5 - 14 days (average : 10 days).

Biphasic disease : Stage of bacteraemia and immune phase.

Stage of bacteraemia :

Characterised by non specific symptoms like high grade fever, nausea, myalgia, headache, chills, rigors, conjunctival suffusion (redness).

usually lasts for around 7 days.

Immunological stage (in 2nd week) :

Acute renal failure : Raised BUN, oliguria, haematuria.

Jaundice (due to necrosis of hepatocytes) : Raised bilirubin,

raised liver enzymes.

Haemorrhage : Conjunctival, pulmonary.

70 - 80 % : **Aseptic meningitis**.

Cardiac arrhythmia.

mortality : 5 - 40%

Diagnosis :

Sample :

- Blood : Positive only in 1st week.
- CSF : Positive for first 10 days.
- urine : Positive from 2nd week onwards for several days.

Dark field microscopy (DGM).

Culture takes several weeks.

NAATs : PCR.

Serology - antibodies : most commonly used.

- Positive from second week onwards.
- **Gold standard** : **Microscopic agglutination test (MAT)**.
- DGM is used to observe agglutination of leptospires in the presence of specific antibody in serum.
- Done only in reference laboratory because of the difficulty in storing the leptospires.
- Other tests for antibodies detection :
Igm ELISA/ latex agglutination test/ ICT. Once these tests are positive, confirmation by MAT is necessary.

Leptospirosis associated with 3R's :

Rats.

Rice fields.

Rainfall.

Also known as **hepatorenal syndrome/7 day fever**.

Treatment :

Anicteric febrile illness :

- Oral → Doxycycline x 7 days (or)
- Ampicillin x 7 days (or)
- Amoxicillin x 7 days.

weil's disease : IV Penicillin/ Ampicillin/ Ceftriaxone/
Doxycycline for 7 days.

Prophylaxis for occupational risk : Doxycycline 200 mg
weekly.

Borrelia

00:21:26

morphology :

- Gram negative spirals.
- 0.3 - 0.5 μ broad : Light microscopy is possible.
5ca2793ec88d500486113130 8 - 30 μ long.
- motile.
- Cultivation on complex media : Barbour Stoenner
Kelly's medium (BSK/ modified Kelly's medium).
Slow growth.
- Diseases : Vincent's angina (fusospirochetosis).
Relapsing fever.
Lyme's disease.

Lyme's disease

00:23:30

most common vector borne disease in USA and Europe.

Etiology : *Borrelia burgdorferi sensulato* : Group of bacteria
which are :

- | | |
|-------------------------------|----------|
| • <i>B. burgdorferi</i> → USA | } Europe |
| • <i>B. garinii.</i> | |
| • <i>B. afzelii.</i> Asia | |

vector : Ixodid ricinus complex (hard tick).

Tick must be attached to human skin for atleast 24 - 48
hours for transmission of pathogen.

Reservoir : Rodents, deer → Play
an important role in survival of ixodid
ticks.



Incubation period : 3 - 32 days.

Primary stage : Starts when the hard tick bites.

10% of infected : Asymptomatic.

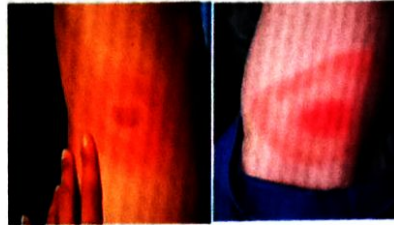
Rest 90% develop disease.

Typical lesion : **Erythema chronicum migrans (ECM)**.

Starts as small red macule at the site of bite → Gradually expands with central clearing.

Also known as **Bull's eye rash**.

Resolves spontaneously in couple of weeks.



Secondary stage : Stage of dissemination/new great imitator.

Develops days to weeks later after ECM.

Characterised by :

- 2° **annular lesions** (ECM like lesions at multiple sites).
- migratory arthralgia & myalgia.
- Fever with malaise.
- Generalised lymphadenopathy.

15% people develop **frank neurological abnormalities** like :

- Aseptic meningitis : Lymphocytosis, raised protein, normal glucose in CSF.
- Encephalitis.
- Cranial neuritis (B/L VII nerve palsy).
- Cerebellar ataxia.

5% develop **frank myocarditis**.

Characterised by **fluctuating AV Blocks**.

Brief and resolves in 2 weeks.

Others : Uveitis, iritis.

Rare, seen in Europeans : **Borrelial lymphocytoma** (lesion seen on ear or breast).

Tertiary stage : Stage of persistence.

Develops months to years after ECM.

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Primary stage : Starts when the hard tick bites.

10% of infected : Asymptomatic.

Rest 90% develop disease.

Typical lesion : **erythema chronicum migrans (ECM)**.

Starts as small red macule at the site of bite → Gradually expands with central clearing.

Also known as **Bull's eye rash**.

Resolves spontaneously in couple of weeks.



Secondary stage : Stage of dissemination/new great imitator.

Develops days to weeks later after ECM.

Characterised by :

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Tertiary stage : Stage of persistence.

Develops months to years after ECM.

Site of persistence	manifestations
Joints	Chronic symmetric polyarthritis (remitting and relapsing). MC affected: knee joints. Synovial fluid examination: PMNs are raised in synovial fluid.
CNS	Cranial nerve palsies. Cerebellar ataxia. Polyradiculopathy.
Skin (Europeans)	Acrodermatitis chronicum atrophicans : Purplish red atrophic lesions in dorsum of hands and feet.

Diagnosis and treatment

00:36:07

Diagnosis :

skin biopsy, blood, CSF, synovial fluid.

methods :

Giemsa/gram staining : To demonstrate gram negative spirals.

Culture in BSK medium.

PCR.

Serology : most commonly used method : Two step approach.

Screening by ELISA.

↓ Positive

Confirmation by Western blot.

Treatment :

No CNS/CVS involvement : Drug of choice is Doxycycline.

ECM : 2 weeks.

Joint/acrodermatitis chronicum atrophicans : 4 - 8 weeks.

CVS/CNS involved : DOC → IV Penicillin/Ceftriaxone/

Cefotaxime for 2 - 4 weeks.

Other tick transmitted infections :

Protozoal :

Mnemonic : **BELQRST**.

Babesiosis.

Ehrlichiosis.

Lyme's disease.

Q fever → only in **animals**. Q fever in humans transmitted by inhalation, unpasteurised milk.

Relapsing fever → only disease transmitted by **soft tick**.

Spotted fever rickettsiosis.

Tularaemia.

Viruses :

Flaviviruses :

- Kyasanur forest disease (KFD).
 - Russian spring summer encephalitis (RSSE).
 - Powassan virus.
- } Tick borne encephalitis

Bunyaviruses :

- Crimean congo haemorrhagic fever.
 - Ganjam virus.
 - Bhanjavirus.
- } Seen in India

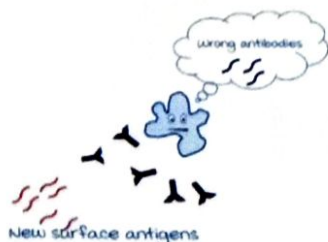
Reovirus : Colorado tick fever virus.

Relapsing fever

00:42:36

Reason for relapses :

Antigenic variations in variable surface antigens, which prevents antibodies from neutralising them.



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Active space

Before
feedingAfter
feeding

Body louse

	Louse borne relapsing fever	Tick borne relapsing fever
Vector	<i>Pediculus humanus corporis.</i>	<i>Ornithodoros</i> species.
Species	<i>Borrelia recurrentis.</i>	Caused by 15 species of <i>Borrelia</i> , <i>B. hermsii.</i> <i>B. turicata.</i> <i>B. parkeri.</i> <i>B. duttoni.</i>
mode of infection	Crushing of louse against skin. ↓ Feces inoculation.	Tick bite.
Reservoir	Strict human reservoir.	Rodents, squirrels
Severity of symptoms	more severe. 5ca2793ec88d500486113130	Less severe.
No. of relapses	1 - 2	5 - 8
mortality	4 - 40%	5 - 8%

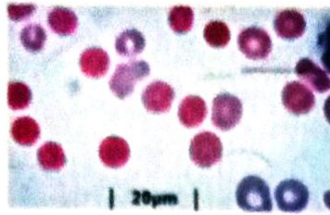
Clinical features :

Incubation period : 2 - 18 days.

Remitting relapsing fever (symptomatic for 3 - 5 days, asymptomatic for the next 7 - 9 days and then symptomatic again).

Headache, myalgia, arthralgia, chills ± lymphadenopathy.

Severe cases : **Bell's palsy**
Lymphocytic meningitis
 Hepatosplenomegaly
 Pulmonary symptoms.



Borrelia floating
around in wet
mount

Diagnosis :

Sample : Blood collected during febrile period.
 CSF.

Wet mount → DFM/ Phase contrast
 microscopy.

Giemsa stain of buffy coat of blood.

Culture : BSK media. **most specific.**

PCR : **most sensitive.**

Serology : Not useful because of antigenic variation.

Relapsing fever is one of the causes of febrile positive non
treponemal tests (biological false positive).

Treatment :

Louse borne relapsing fever :

- **DOC : Tetracycline** PO (or)
 - Doxycycline PO (or)
 - Erythromycin PO (or)
 - Procaine penicillin IM.
- } Once

Tick borne relapsing fever :

- Tetracycline (or)
 - Doxycycline PO (or)
 - Erythromycin PO.
- } 10 days

CNS manifestations :

- **IV Penicillin** or **IV Ceftriaxone** for 10 - 14 days.

Q. A 69 year old man was admitted to the hospital with a 4 year history of fibrosing skin lesions on the back of his right hand and above his elbow on his right arm. The skin lesions started with a bluish discoloration of the skin, followed by gradual epidermal atrophy (figure). The patient's history indicated a risk of tick bite. He lived in an area endemic for tick borne diseases and his hobby was mushroom picking. What is likely cause of the skin lesions ?

- A. *Leptospira interrogans*.
- B. *Bacillus anthracis*.
- C. *Borrelia afzelii*.
- D. *Borrelia recurrentis*.



Ans. Tick borne skin lesion : Likely to be Lyme's disease. *Borrelia afzelli* causes Lyme's disease in India & Europe.

@marroweditionsnotes

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Active space

CHLAMYDIA AND LEGIONELLA

Chlamydia
Legionella
Mycoplasma } Important causes of community acquired pneumonia which are atypical.

Campylobacter : Common cause of food poisoning. 5ca2793ec88d500486113130

Chlamydia

00:01:31

Gram negative coccobacilli.

Non-motile, non-sporing.

Obligate intracellular bacteria.

Filtrable bacteria (0.2- 0.3 μ).

Contain DNA and RNA, hence classified as bacteria.

Energy parasites (They lack enzymes for ATP synthesis).

Basophilic viruses (They form basophilic inclusion bodies).

PLT agents (Psittacosis, LGV, Trachoma).

All species of chlamydia share same LPS antigen but have species specific outer membrane proteins.

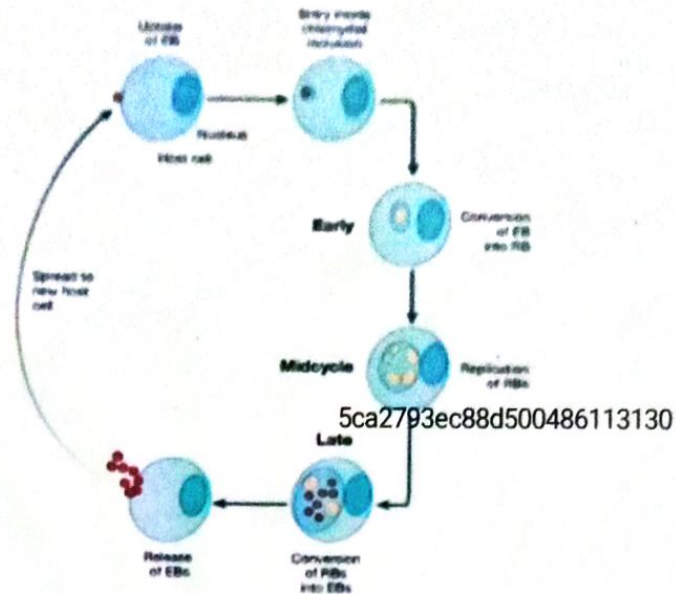
Lacks peptidoglycan.

Life cycle :

Biphasic :

- **Elementary body :**
Extracellular, infectious.
metabolically inactive.
Binds to receptors on cell.
Inhibits fusion with Lysosome.
- **Reticulate body :**
Intra cellular.
metabolically active.
Large : Covers the nucleus like a cloak/ mantle.
Binary fission : Produce 100- 500 elementary bodies.

Active space



Chlamydia : Stains

00:09:23

Gram stain is not used.

Stains used :

- Giemsa.
- Giminez.
- Castaneda.
- machiavello.

C. trachomatis inclusion bodies contain Glycogen (Iodine stain can be used).

Cultivation :

Non-cultivable on cell free media.

Grown in :

1. Laboratory animals.
2. Yolk sac of Hen's egg.
3. Cell lines : Irradiated/antimetabolite added, to make it intracellular.

C. trachomatis + *C. psittaci* : Hela & mc Coy.

C. pneumoniae : Hep-2, Hela, McCoy, Vero, BHK (Baby Hamster Kidney)

Chlamydia trachomatis

00:13:23

15 serotypes :

A, B, Ba, C : Trachoma.

L1, L2, L3 : Lymphogranuloma venereum.

D to K : Genital chlamydiae.

D-K serovars: Treatment

00:24:43

Uncomplicated Genital chlamydiae :

- Treatment : Azithromycin 1g single dose.
- Alternative : Doxycycline
or
erythromycin } x 7 days

Complicated Genital chlamydiae : PID, proctitis, epididymitis.

- Doxycycline/ Erythromycin (1- 2 days).
- Neonatal conjunctivitis : Erythromycin (1- 2 days).
- Adult conjunctivitis : 1g Azithromycin (Single dose).

Lymphogranuloma venereum (L1,L2,L3)

00:26:51

Lymphogranuloma venereum (L1, L2, L3) L1-3-30 D	
Primary	Painless papulo-vesicular genital / anal lesion.
Secondary	Regional LNAP → matting → suppurate sinus tracts. Groove's sign (groove of LN). Fever, malaise. metastasis - meningitis, pericarditis, arthritis.
Tertiary	Elephantiasis of genitalia.
Diagnosis	Miyagawa's granulocorpuscles. Cell line culture is more sensitive. NAATS : most (15-50%) sensitive. High titer of circulating antibodies with titers above 1:64 in CFT and above 1:512 by IF.
Treatment	Doxy BD for 21 days or Erythromycin x 21 days.

D-K serovars: Treatment

00:24:43

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Lymphogranuloma venereum (L1,L2,L3)

00:26:51

Lymphogranuloma venereum (L1, L2, L3) L.P-3-30 D	
Primary	Painless papulo-vesicular genital / anal lesion.
Secondary	Regional LNAP → matting → suppurate sinus tracts. bnvssprasanth7@gmail.com Groove's sign (groove of LN). Fever, malaise. metastasis - meningitis, pericarditis, arthritis.
Tertiary	Elephantiasis of genitals.
Diagnosis	Miyagawa's granulocorpuscles. Cell line culture is more sensitive. NAATS : most (15-50%) sensitive. High titer of circulating antibodies with titers above 1:64 in CFT and above 1:512 by IF.
Treatment	Doxy BD for 21 days or Erythromycin x 21 days.

Active space

Chlamydia psittaci & Chlamydia pneumoniae

00:29:20

Chlamydia psittaci :

Reservoir : **Psittacine** (urine, feces, oral secretions) &
non-psittacine birds.

People at risk : Vets, Pet shop owners, Abbatoir/
slaughter house workers.

MOI : Inhalation of aerosols containing urine, feces, oral
secretions of birds.

Psittacosis/ Ornithosis : Atypical pneumonia → Septicemia
→ meningitis, endocarditis,
myocarditis, pericarditis, hepatitis,
glomerulonephritis.

Diagnosis : **Levinthal Cole Lillie (LCL) bodies.**

Serology (MC used).

DFAT : Antigen detection.

Culture : BSL-III lab.

Treatment : Doxycycline/ Erythromycin (3 weeks).

Chlamydia pneumoniae : Common cause of atypical
pneumonia. Strict human pathogen.

TWAR strain.

1 serotype only.

Transmitted via aerosols.

Causes : Pharyngitis, sinusitis, atypical pneumonia.

Chronic infection : Atherosclerosis.

Adult onset asthma.

Important points :

- Chlamydia : Lacks Peptidoglycan in cell wall, cannot
synthesise ATP.
Obligate intracellular.
Basophilic viruses/ PLT agents.
Share same LPS antigen.

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Active space

Legionella pneumophila

00:38:46

Gram negative pleomorphic (poorly gram stained).

motile (polar flagella).

Strict aerobe.

Cultivation :

- Fastidious.
 - High humidity.
 - Iron & Cysteine.
 - pH : 6.9.
- Buffered charcoal yeast extract.

Reservoir : Aquatic environment.

- Natural :
Rivers, streams, oceans, hot springs.
Intracellular in free living amoeba.
Viable non-cultivable state (sometiomes).
- Artificial :
Cooling towers, decorative fountains,
plumbing systems.

MOI : Inhalation of aerosols.

microaspiration of aerosols.

MC serotypes : 1 (80- 90% infections), 2, 6.

Legionellosis :

1. Pontiac fever : mild influenza like fever.
2. Legionnaire's disease : Atypical pneumonia.

10- 15% : Nosocomial pneumonia.

2- 13% : Community acquired pneumonia.

IP : 2- 10 days.

Presentation : Fever, cough, dyspnea, diarrhea,

mortality : 10- 15%.

Risk factors : male.

Smokers.

Age > 50.

DM.

ESRD.

Solid organ transplant recipients.

Hairy cell leukemia.

Lung carcinoma.

Diagnosis : Leucocytosis.

X-ray chest : Lobar/ segmental/ patchy infiltration

Sputum, tracheal aspirate, bronchial washing, BAL

Test (specimen)	Sensitivity, specificity	Comment
Gram stain (sputum, tracheal aspirate)	Poor sensitivity	
Culture (sputum, tracheal aspirate)	20- 90% 100%	Always to be done.
DFAT (sputum, tracheal aspirate)	20- 50% 99%	
NAATs	70- 95% ~ 99%	Expensive : Available in research labs.
ELISA (urine)	60- 95% > 99% : Only for serogroup 1	mc ordered.
Serology (Paired serum) hnyssprasanth7@gmail.com	@marroweditions60765	Not useful in acute disease

For optimal testing, more than 1 type of test is used. PCR, sputum culture & urine antigen testing are the preferred tests.

Treatment : Levofloxacin/ Azithromycin.

Alternative : Ciproflox/ moxiflox/ Doxycycline.

Very ill patients : Axitromycin + Fluoroquinolones

+/- Rifampicin.

MCQs :

Q. A 70 yr old man presents to the emergency department with a fever of 103.5° F, a dry cough, tachypnoea, and chest pain. History reveals he has been smoking since he was a teen. He mentions that several people at the assisted living community where he resides have had similar symptoms. A chest X-Ray showed diffuse bilateral infiltrates. Over the next 3 days, his cough worsened, his respiratory rate went up to 26/min and oxygen saturation was 90% on room air. He was intubated & transferred to the ICU. Meanwhile, the sputum sample isolated organisms that grew on buffered charcoal yeast extract agar (fig). Which of the following properties is consistent with the above organism ?



- A. Capsulated.
- B. No cell wall.
- C. Requires iron and cysteine for growth.
- D. Serpentine growth in vitro.
- E. Bacitracin sensitive.

Organism : *Legionella pneumophila*.

MYCOPLASMA AND CAMPYLOBACTER

Mycoplasma

00:00:08

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Pleuro Pneumonia Like Organism (PPLO).

Smallest pathogenic bacteria (0.2μ)

Filtrable through the pore size of standard bacterial filters.

Only bacteria which lacks a cell wall.

Pleomorphic.

Resistant to cell wall acting agents : Lysozymes and

Antibiotics acting on cell wall (Penicillin and β -Lactams).

Only bacteria with sterols in cell membrane - **Jumping Joker.**

Lack enzymes for protein synthesis/ Fatty acid synthesis/

Sterol synthesis - **Fastidious.**

Sterols are hence essential

growth factors.

media : PPLO broth (Primary Isolation)



PPLO Agar.

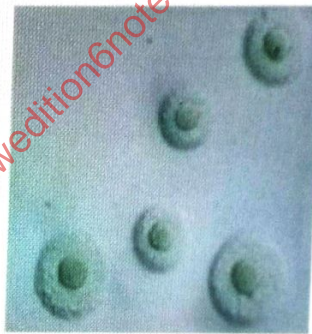
Selective agents used in growth

of mycoplasma : **Penicillin and Thallium Acetate.**

No Turbidity on broth medium.

Fried egg colonies/ mulberry shaped Colonies.

Diene's Stain : used to demonstrate colonies when they are growing on the surface of the media.



Mycoplasma pneumoniae

00:05:52

Eaton's Agent.

Strictly human pathogen.

Affects both Immunocompetent and Immunocompromised.

Transmitted by : **Aerosols.**

SAR : **70 - 80%.**

It causes Pharyngitis, Tracheobronchitis, Otitis media.

10 - 13 % cases : **Primary Atypical Pneumonia.**

Also called **walking Pneumonia**.

MC cause of community acquired atypical Pneumonia.

It can trigger cold agglutinin disease.

X-ray Chest: **Streaks of Interstitial Infiltration**.

Sputum: Bronchial washings.

Stain: Giemsa Stain.

Culture: 1 - 2 weeks.

NAAT: most sensitive method which is preferred.

Serology, Complications and Treatment of Mycoplasma

00:10:43

Serology:

1. Heterophile agglutination Test:

- Cold Agglutination Test: $\geq 1:32$.

Igm antibodies + Human blood group 'O' RBCs

Agglutination occurs at 4°C and reversible at higher temperatures.

- Streptococcus M test: $\geq 1:20$.

Antibodies against group F streptococcus.

2. Specific mycoplasma Antibodies: Forms later and isn't useful.

Complications:

Dermatological: Rashes, SJS.

Neurological: meningitis, Encephalitis, Transverse myelitis.

Others: AIHA, Thrombocytopenia, Pancytopenia, Hepatitis,

Pericarditis, Glomerulonephritis, Pancreatitis.

Treatment:

macrolides (Azithromycin)

Alternative Doxycycline, Levofloxacin or moxifloxacin.

Campylobacter

00:18:35

Gram (-) Curved rod/ Spiral/ Gull wing forms.

Amphitrichous/ Lophotrichous Flagella.

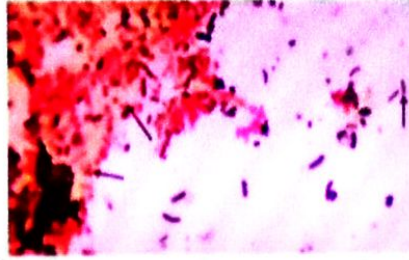
Darting motility.

Microaerophilic - 5% O_2 .

C. Jejuni - MC strain. Optimum temperature: 42°C .

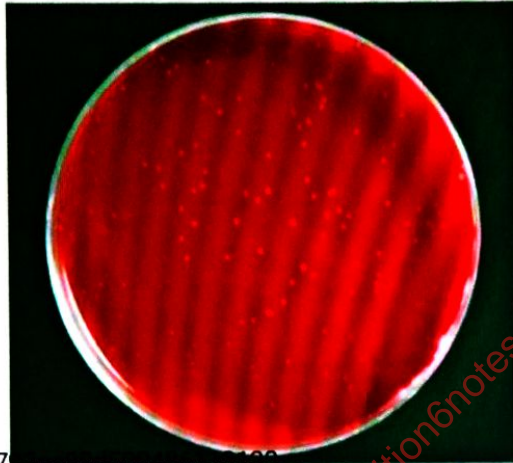
Biochemical Reactions for Campylobacter:

- Catalase (+).
- Oxidase (+).
- Urease (-).
- No utilization of sugars.



medium used :

1. Skirrows (Vancomycin + Polymyxin + TMP).
2. Butzler.
3. CVA. (Cefoperazone Vancomycin Amphotericin B).
4. Campy BAP.



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Reservoirs and complications of Campylobacter

00:22:56

Reservoir : GI flora of cows, Sheep, Dogs, Cats and birds.

mode of Infection :

- Poorly cooked Poultry.
- Raw milk.
- Handling of animals.

Incubation Period : 2 - 7 days.

Symptoms : **Invasive Diarrhea** → Fever, Abdominal cramps
(Pseudo-appendicitis), Bloody diarrhea.

Complications :

Local → Cholecystitis, pancreatitis.

Systemic (with Campylobacter fetus) → meningitis and
Endocarditis.

Active space

Diagnosis and treatment of campylobacter

00:26:06

Diagnosis : Stool microscopy for Pus cells +RBC's.

Culture : Gold Standard.

Treatment :

Severe Fever > 1 week : **Azithromycin (DOC)**.

Fluids and electrolytes.

Treatment of Invasive disease : **Gentamycin**.

Guillain Barre Syndrome (GBS) :

- molecular mimicry.
- Epitopes on Campylobacter can act as auto-antibodies.



Bind myelin Sheath of PNS
and causes destruction.

- **20 - 40%** : Preceding sub-clinical/Clinical Campylobacter infection.

MCQs :

Q. A 21 yrs old student complained of malaise, low-grade fever, and a harsh cough. CXR revealed a diffuse interstitial pneumonia in the left lobe of the lung. The WBC count was normal. The student had been ill for a week. The following laboratory data were available within 2 days.

Cold agglutinins - 1:64; Complement fixing antibodies negative; viral culture - pending, but negative to date; bacterial culture of sputum on blood agar and MacConkey's agar - normal oral flora.

In order to confirm the diagnosis, which procedure could be ordered to achieve a specific and sensitive diagnosis?

- bnvssprasanth7@gmail.com
- A DNA probe to the 16S ribosomal RNA of an organism lacking a cell wall.
 - Another viral culture in 1 week.
 - A repeat CF test in 5 days.
 - A repeat cold agglutinin test.

Q. 5 yr old boy presents with diarrhoea, malaise, fever, and abdominal pain. The stools were positive for occult blood and fecal leukocytes. microscopy of the stool also reveals the

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presence of curved bacilli that moved about on a slide in a darting motion. The etiologic agent, a gram negative, curved rod, grew on specialized medium in microaerophilic conditions. Which one of the following organisms is most likely responsible for the disease?

- A. *Vibrio cholerae*.
- B. *Campylobacter*.
- C. *Shigella*.
- D. ETEC.

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HACEK, ACINETOBACTER AND FRANCISELLA

HACEK

00:01:19

- Hemophilus species (MC → *H. para influenzae*)
- Aggregatibacter actinomycetemcomitans, A aphrophilus.
- Cardiobacterium hominis
- Eikenella corrodens
- Kingella kingae

morphologically : Gram -ve, **Pleomorphic bacilli**.
Non-motile, Non-sporing.

Eikenella corrodens :

Twitching motility → Produces pitting on agar.
Capnophilic (Grows in the presence of oxygen)
Fastidious → No growth on MacConkey agar.
Grow in Blood agar → Colonies 3 - 30 days.

Normal oral flora

Occurs In periodontitis, dental manipulation without antibiotic cover, brushing



Bacteremia : Nasal valve / Prosthetic valve endocarditis
(0.8-6% SABE)

Causes **Needle licker's osteomyelitis**. (Except Kingella)
Human bite infections.

HACEK endocarditis → Blood culture can be negative sometimes.

Causes **Culture negative endocarditis** → Patient has to be kept admitted for a longer time to assess & avoid the same.
Excellent Prognosis.

DOC → **Ceftriaxone**.

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Kingella → **Aggressive course**.

Affects young people.

Emboli are common.

MC → *A. actinomycetemcomitans*.

Least common cause → *Eikenella corrodens*.

Acinetobacter

00:10:24

morphology :

Gram negative cocco-bacillus (sometimes in pairs)

Non-motile, non-sporing.

Obligate Aerobe.

Biochemical tests : Catalase positive, oxidase negative.

Non-fermenters/ do not utilise sugars.

Culture : Simple media. MacConkey - Non lactose fermenting.

most virulent species : A. baumannii.

Soil and water saprophyte.

Present in hospital environments - sinks, bed rails, mops,
supply carts.

Causes Opportunistic nosocomial infections.

Infections caused :

- Ventilator associated pneumonia
- Blood stream infections
- meningitis following neurosurgery
- Burn & Surgical site infections
- Post catheterisation UTI.

Multi-drug resistant bacteria

Chromosomal & plasmid mediated β lactamases secreted.

Amp C β lactamase - Resistance to penicillin and 1,2,3,4
Cephalosporins.

Fluoroquinolone resistance due to altered drug targets.

Aminoglycoside resistance - aminoglycoside modifying
enzymes.

For Pan-drug resistant : Use Polymyxin or Colistin.

They are categorised as **ESKAPE** group.

E. faecium

S. aureus

K. pneumoniae

Acinetobacter

P. aeruginosa

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Enterobacterium.

Carbapenem resistant Acinetobacter baumannii (CRAB) are
critical priority list.

Francisella tularensis

00:19:29

Category A bioterrorism agent.
Biosafety level III pathogen.

morphological : Gram -ve, pleomorphic bacillus

Safety pin appearance on bipolar staining.

Non-motile, non-sporing, Capsulated.

Strict Aerobes.

Nutritionally fastidious. (No growth on macconkey)

Requires Cysteine to grow → Francis cysteine dextrose blood agar.

Also grows on Buffer charcoal yeast extract agar.

4 varieties :

- F. tularensis subspecies tularensis (most virulent)
- F. tularensis subspecies holartica (ASIA)
- F. tularensis subspecies novicida
- F. tularensis subspecies mediastica

Reservoir : Small mammals like mice, rats, rabbits, hares, musk rats.

mode of Infection :

Bite of arthropods like ticks, deer fly, mosquito.

Abraded skin/ mucosal membrane exposure to animal tissues/ water contaminated animal carcasses, excreta.

Bites of reservoir hosts.

1. Ulcero-glandular tularemia (mc)
Painful ulcer + tender regional lymphadenopathy.
2. Glandular tuleremia.
3. Occulo-glandular tularemia
4. Inhalation of aerosols (Pneumonic type)
5. Ingestion of contaminated food/ water (pharyngeal tularemia)
6. Causes exudative pharyngitis + cervical lymphadenopathy.
7. Typhoidal type : Follows any exposure.



Lethal dose : 10 - 50 bacteria.

Diagnosis of tularaemia

00:28:39

Lymph node aspirate.

Ulcer swab/biopsy

Conjunctival swab/scrapings.

Blood & bone marrow (typhoidal type)

Antigen detection by immunofluorescence.

Culture.

PCR (most sensitive).

Serology (most common) - agglutination tests.

> 1:160 → Significant.

Drug of choice: Streptomycin/ Gentamicin.

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Active space

PASTEURELLA, SPIRILLUM AND STREPTOBACILLUS

Pasteurella multocida

00:00:08

Gram negative pleomorphic bacillus (**Safety pin appearance**)
due to bipolar staining

Non motile, non sporing.

Fastidious organism (Grows only on blood agar).

It is a normal respiratory / GI tract flora in wild and domestic birds and animals. It causes **avian cholera**, haemorrhagic septicaemia in birds & animals. It is the MC cause of infection following a dog or cat bite.

Within 24 hours : Severe inflammation at site of bite + swelling and tenderness + purulent discharge.

It may progress to abscess, tenosynovitis, osteomyelitis & septic arthritis.

In immunodeficiency/ liver disease/ malignancy :

Widespread infection → Pneumonia, meningitis or endocarditis.

Drug of choice : **β lactams**.

Spirillum minus

00:04:36

Gram negative spiral shaped organism.

It has **amphitrichous flagella** (1-7 polar flagellae).

Length : 3-5 microns.

It is non-cultivable on cell free media.

Culture : Intraperitoneal guinea pig or mice inoculation.

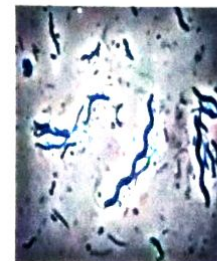
It forms the normal flora in mice and rats.

Rat bite fever/ Sodoku :

Bite of infected rodent → wound heals → 1 - 4 weeks later :

Local ulcer + regional lymphadenopathy + fever & chills + blotchy (violaceous/ reddish) rash all over the body → Resolves in few day.

It can remit & relapse for 1 - 2 months if left untreated.



Complication : Endocarditis.

DOC : Penicillin G or Ceftriaxone for 10 - 14 days.

Streptobacillus moniliformis

00:09:08

Gram negative pleomorphic bacillus.

Non motile, non sporing, non-capsulated

Fastidious (Grown on blood agar).

When grown on broth medium : Flocculent puff balls of growth.



It is the normal flora in rats, mice, guinea pigs, squirrels.

Rat bite fever :

Infection acquired following infected bite/ scratch

After an incubation period of 10 days: Systemic symptoms (Fever, headache, vomiting, arthralgia).

2 - 3 days later: macular/ pustular rash on palms, soles and extremities + migratory asymmetric oligo/ polyarthritis.

The symptoms may remit & relapse for a few months.



Haverhill fever/ erythema arthriticum epidemicum : Occurs following ingestion of food or water contaminated with rodent faeces :

Rat bite fever symptoms + severe vomiting + pharyngitis.

DOC : Penicillin G or Ceftriaxone for 10 - 14 days.

STERILIZATION AND DISINFECTION : PART 1

Physical methods

00:00:30

Sterilization	Disinfection	Anti sepsis
Elimination of all forms of life (microorganisms) including spores.	Elimination of the pathogenic microorganisms from inanimate objects. (Spores may survive). Disinfectants.	Disinfection of live tissues. Anti septics.

Order of susceptibility :

most to least difficult to eliminate,

Prions → Bacterial spores → Cysts of protozoa → Non enveloped viruses → mycobacteria → Gram positive bacteria → Gram negative bacteria → Enveloped viruses (**most easy to eliminate**).

Non enveloped viruses lacking lipid envelope are **more resistant** to sterilization & disinfection as compared to enveloped viruses as **lipid envelope** gets **easily dissolved** in alcohol & other solvents. **Gram positive bacteria** are **more resistant** than gram negative bacteria. Outer membrane of gram negatives is made of phospholipids.

methods of sterilization & disinfection :

Physical methods	Chemical methods
Heat. Radiation. Filtration.	Alcohols. Aldehydes. Phenols. Halogens. Surface acting agents. Hydrogen peroxide. Ethylene oxide. Peracetic acid.

Physical methods :

Heat : 2 forms. Dry & moist heat.

Dry heat	moist heat
Red heat. Incineration. Hot air oven.	Pasteurization. Serum Bath. Vaccine Bath. Inspissation. Tyndallization. Autoclave.

Dry heat

00:06:16

Heating up air and sterilizing.

mode of action :

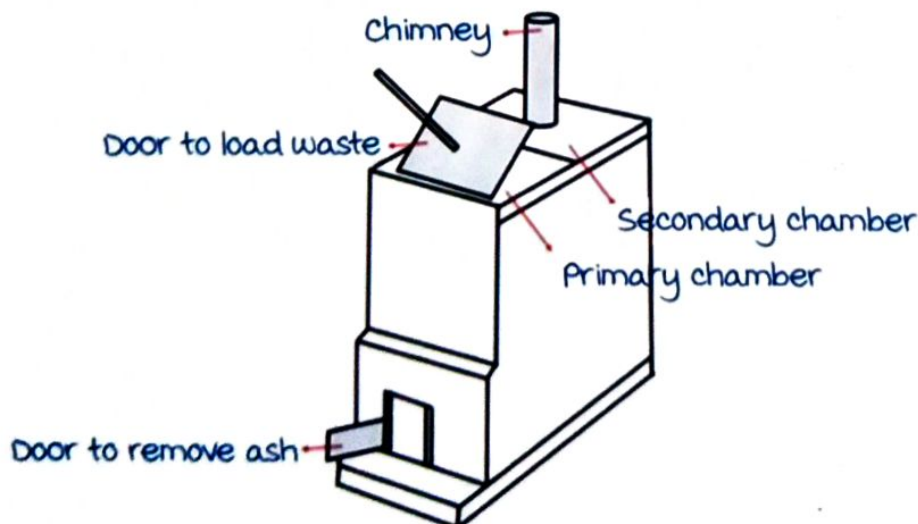
- Denaturation of proteins of microorganisms.
- Oxidative damage.
- Raises the levels of electrolytes to toxic levels.

Red Heat : Sterilization of

- Inoculating loops
- Straight wires.
- Tips of forceps.
- Searing spatulas.



Incineration :



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Sterilization & reduction in volume of infectious hospital waste (by 80 - 85%) before it is sent for final disposal.

Includes :

- All animal carcasses.
- Human pathological material.
- Soiled beddings & dressings.
- Expired cytotoxic drugs.

Incinerator : 2 chambers.

Primary chamber : 600 - 700 °C.

Secondary chamber : 1000 - 1100 °C.

Hot air oven

00:11:34

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Introduced by Louis Pasteur, father of modern microbiology.

Temperature : 160 °C for atleast 1 hour.

Duration can be increased depending on the load upto 2 hours.

Alternate temperatures : 150° C for 2 hours/ 170° C for 40 minutes.

Uses :

- Sterilization of glass ware : test tubes, pipettes etc.,
- All kinds of metallic instruments : sharp & non sharps.
- Oils, jellies, powders, waxes since steam cannot penetrate through these substances.
- Cotton swabs.



Controls of hot air oven/ efficacy testing :

Physical method	Chemical method	Biological method
Incorporation of a thermocouple inside. Senses temperature & alters it accordingly.	Involves using a tube/ special equipment : Browne tube No.3 with chemical agent that changes color when desired temperature is reached for desired amount of time.	Spores of Bacillus atrophius or Clostridium tetani . Special strips that contain these spores are exposed to cycles and tested if the spores had been killed.

Moist heat

00:16:12

Effective at lower temperatures.

mode of action : Denaturation & coagulation of proteins.

Pasteurization : Disinfection of milk, juices, jams, sauces, cream, mayonnaise.

methods :

Holder method	Flash method	ultra high temperature method
moist heat at 63 °C for 30 minutes .	72 °C for 15 - 20 seconds .	149 °C for 0.5 seconds .
<i>Coxiella burnetti</i> can survive holder method.	↓ Rapid cooling to < 13 °C .	Used in western countries.

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efficacy of pasteurization :

Phosphatase Test	Coliform Test
<p>Principle : Raw milk contains phosphatase that is denatured by pasteurization.</p> <p>mc method.</p> <p>Pasteurized milk + substrate for phosphatase.</p> <p>If properly pasteurized, there will be no color change.</p> <p>↓</p> <p>Takes only 2 hours.</p>	<p>Principle : Raw milk contains lots of coliforms → normal commensals that are present in animals & human GIT. E.g., E.coli, Klebsiella, Enterobacter.</p> <p>Better method.</p> <p>Test for live coliforms. mc Conkey broth + pasteurized milk.</p> <p>↓ overnight incubation.</p> <p>Absence of acid/ gas production & no change in mc Conkey's agar: Adequate.</p>

Serum Bath :

Inactivation of bacteria in serum specimens. **56 °C for 1 hour.**



Serum bath/ vaccine bath.

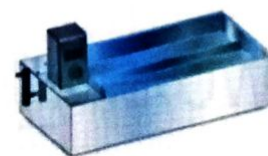
Vaccine Bath :

Inactivation of bacteria from vaccine specimens. **60 °C for 1 hour.**

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Inspissation :

Sterilization of certain culture media that contain egg or serum. **80 - 85 °C for 30 minutes for 3 consecutive days.**



Inspissator

E.g., LJ medium, Loeffler's serum slope:

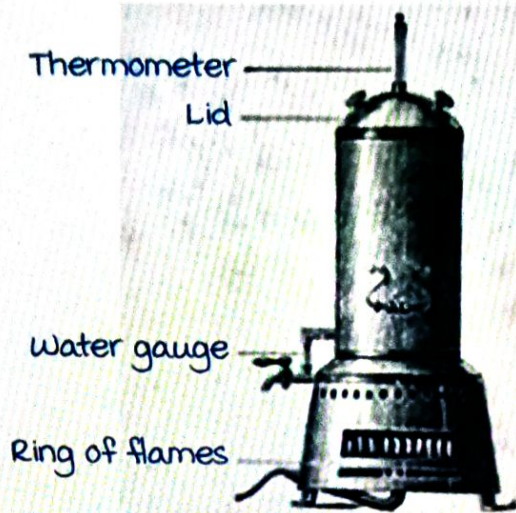
Active space

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Tyndallization : Koch & Arnold steam sterilizer.

moist heat at 100 °C for 20 minutes for 3 consecutive days.

uses : Sterilization of urea, sugar solutions & certain culture media like TCBS/ XLD/ DCA media & Selenite F broth.



Koch's steam steriliser

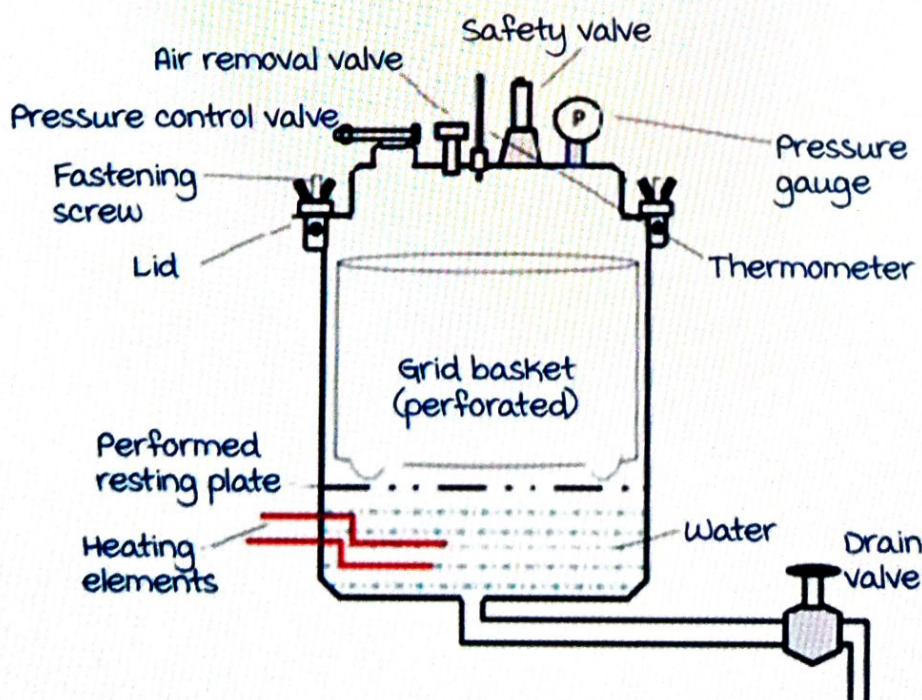
Autoclave

00:26:43

moist heat under pressure.

121 °C for 15 - 20 min at 15 pounds per square inch (psi) or

134 °C for 3 min at 30 psi.



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382 Hospital
Infection
Control

36

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Uses : Sterilization of

- Microbiological culture media before & after use.
- Dressings, hospital linen.
- Non sharp metallic instruments.
- Plastic & rubber objects.
- Aqueous solutions.
- Glasswares.

Autoclave	Image
Vertical autoclave.	
Small autoclave used in labs.	
Horizontal autoclave.	
Horizontal high capacity autoclave	

Active space

efficacy of autoclave :

Physical method	Chemical method	Biological method
Thermocouple	Browne's tube no.1 or Bowie Dick tapes.	Spores of <i>Geobacillus</i> <i>stearothermophilus</i> .

Radiation

00:33:02

mechanism of action : DNA damage :

Non ionizing	Ionizing
UV rays.	Gamma rays/ Cold sterilization :
	Generated by nuclear disintegration of radio isotopes Cobalt (Co) or Caesium (Cs).
Low penetrating power.	Very high penetrating power.
Bactericidal. Cannot kill spores.	Sporicidal.
Surface disinfection of hospital corridors, labs & biosafety hoods.	Commercial sterilization :
Special lamps in corridors that are switched on after the corridor is evacuated.	Disposable gloves, syringes, petri plates, IV sets, foleys catheter, implants, prosthesis, contraceptive devices, sutures, gauze, cotton swabs.
	Efficacy tester : Spores of <i>Bacillus pumilus</i> .


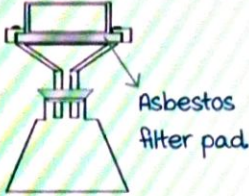


Filtration :

For heat sensitive liquids like serum solutions, sugar solutions, antibiotic solutions.

Pore size of standard bacterial filters : 0.2 - 0.45 μ .

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Types of filters :

Type	Shape	made of	Notes
Earthenware filters.	Candles.	Porcelain or diatomaceous earth.	E.g., Berkefeld filters & Mandler's filters. 
Asbestos filters.	Discs.	magnesium trisilicate.	E.g., Seitz filters. Rarely used due to carcinogenic potential. 
Sintered glass filters.	Discs.	Fusion of powdered glass.	multiple use. Expensive. 
membrane filters (MC).	Discs.	Cellulose esters/ polycarbonate	market name : millipore. Single use discs. very cheap. 

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efficacy for standard bacterial filters : Ability to filter out *Serratia marcescens* / *Brevendimonas diminuta*.

Summary

00:41:31

Physical methods		
Heat	Radiation	Filtration
Dry heat. moist heat.	Ionizing. Non ionizing.	Earthenware filters Asbestos filters. Sintered glass filters. membrane filters.

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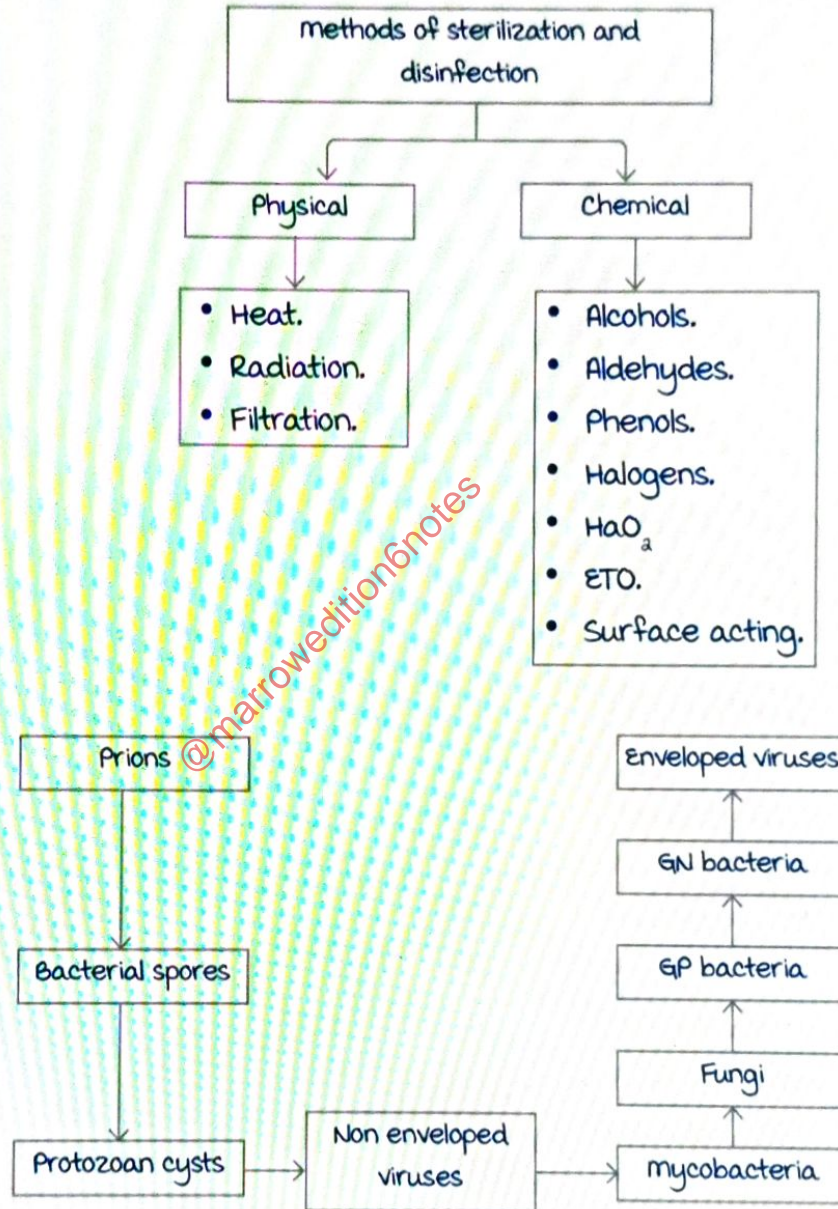
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STERILIZATION AND DISINFECTION : PART 2

Chemical methods

00:00:14



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most resistant to kill → Prions.

most easy to kill → Enveloped viruses.

All the chemical agents are broadly classified into 3 types :

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	Bacteria	Fungi	myco bacterium	Viruses	Spores	Groups
High level	+	+	+	+	+, dont Kill large no. of spores.	ETO, plasma (vapourized H ₂ O ₂ , Peracetic acid
Inter mediate level	+	+	+	+, enveloped viruses, variable action against non enveloped viruses	-	Phenols, alcohols and halogens. Phenols and aldehydes are active in the presence of organic matter.
Low level	+	+	+, variable action	variable action	-	Surface acting agents

Ethylene oxide (ETO)

00:05:39

High level disinfection.

Low temperature sterilization → Heat sensitive and moisture sensitive objects and instruments.

MOA → Alkylating agent.

Excellent penetration power → Lumen, scopes - rigid and flexible.

Sweet smelling ethyryl gas above 10° C.

Available in the form of preformed cylinders, i.e., Pure (100% of ETO gas).

Also available in (91% of ETO + 9% of CO₂/HCFC).

Use :

Sterilization of all types of scopes, blankets, beddings, all complex apparatuses (heart lung machine, respirators, dental equipments, all rubber and plastic objects).

Disposable gloves, syringes, etc.

Efficacy → *Bacillus atropheus*.

Temperature → 37-63° C.

One cycle time → Ranges from 18-24 hours.

Long cycle time is due to special steps.

Active space

Steps :

1. Pre conditioning and humidification (few hours).
2. Introduction of gas.
3. Exposure to gas.
4. Removal of gas
5. Air washes (sterile) : To remove toxic ETO residues.

Disadvantages :

- Carcinogenic → Exposure monitoring required
- Irritant to eyes and mucous membranes.
- Inflammable.
- Leaves toxic residues.

ETO vapour monitor



Hydrogen peroxide

00:11:08

High level disinfection.

AKA Plasma sterilization.

Vapourized form.

Low temperature sterilization → Heat sensitive objects.

MOA → Oxidation (toxic oxygen radicals are generated).

59% vapourized H_2O_2 gas.

STERRAD :

1. Deep vacuum is created.
2. Aerosolized H_2O_2 is introduced.
3. Converted into gas.
4. Radiofrequency waves are applied.
5. Generation of toxic oxygen radicals.
6. Vacuum and exhaust.

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Cycle time → 75 minutes (very short).

Advantages → No toxic residues.

Disadvantage :

- All the objects placed should be dry.
- Not compatible with most packaging material (paper, cotton and linen all have cellulose that absorbs H_2O_2 and thereby prevents contact).
- Special material for wrapping.

Active space

Temperature → 37-44° C.

efficacy → Spores of bacillus stearothermophilus or spores of bacillus atrophaeus.

used for all metallic instruments.

Aldehydes

00:15:17

High level of disinfection.

MOA → Alkylating agents.

Also cause DNA damage to micro organism.

Formaldehyde/formalin → Available as 37% liquid.

Basically used for fumigation.

used for sterilization of wards and OT.

Gaseous formalin is generated by heating it, and then the formed formalin is neutralized by ammonia vapour.

Fumigation is now gradually replaced by fogging.

Fogging is nothing but vapourized H_2O_2 .

Other uses :

Preservation of anatomical specimens.

Duckering (process of killing of bacillus anthracis spores from animal wool or animal hide).

Glutaldehyde → Cidex (conc. of 2%).

High level of disinfection.

used in sterilization and disinfection of heat sensitive apparatuses/instruments such as scopes, dialyzers, anaesthetic equipments, ET tubes.

In case of sterilization → 6-8 hours required.

In case of disinfection → 25 minutes is suffice.

Ortho-phthaldehyde :

OPA-Cidex → 0.55%.

Absolutely similar to Cidex.

Advantages over Cidex :

- Active at wide pH range (unlike cidex needs to be alkalinized).
- Less irritant.
- Has better mycobactericidal activity.
- Better odour.

Cidex and OPA cidex → maintain their ability to kill for atleast 2 weeks.

Halogens

00:21:02

Intermediate level of disinfection.

MOA → Oxidizing agents.

2 commonly used → Chlorine compounds and iodine compounds.

Chlorine compounds :

a. Chlorine tablets → used for water disinfection (water supplies, swimming pools).

b. Sodium hypochlorite (household bleach) →

Available in hospital at a concentration of 5-6%.

Used in disinfection of infectious hospital spills.

If spill is < 10 mL → Dilute sodium hypochlorite 1 : 100 in water.

If spill is > 10 mL → 1 : 10 dilution in water.

Iodine compounds

Uses :

Skin antiseptics.

Tincture of iodine and betadine (povidone iodine).

Iodine has been absorbed on to neutral carrier.

Neutral carrier molecule : Polyvinyl pyrrolidone.

This is done for residual action.

Phenols

00:24:28

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Intermediate level of disinfection.

MOA → Precipitation of proteins and cell membrane damage.

3 commonly used phenols :

- Phenol → 5%.
- Cresol → 1-4%.
- Lysol → 2-5%.

These are used for surface disinfection of **worktops, benches and hospital floors.**

All these are **corrosive to skin**, never used as skin antiseptics.
Can be used as skin antiseptics if modified.

modified phenols → **Chlorinated biphenolics.**

This includes :

- Chlorhexidine.
 - Hexachlorophene.
 - Chlorxylenol.
 - Triclosan.
- } Skin antiseptics and hand
disinfectants

Alcohols

00:27:03

Intermediate level disinfection.

MOA → Coagulation of proteins and dehydration of cells.

Concentration : **60-80%** and **ideal is 70%**.

3 alcohols in use :

Ethyl alcohol/spirit → Skin antiseptics.

Isopropyl alcohol → Similar to spirit, used in hand disinfection and thermometer disinfection.

Methyl alcohol → Killing fungal spores in biosafety hoods.

Surface acting agents :

Low level disinfection.

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Altering surface tensions leading to disruption of cell membranes.

Eg :

Cationic → Cetyltrimonium bromide and benzalkonium chloride.

Can be used for antiseptics.

Anionic → Soaps.

Testing disinfectants

00:31:20

New chemical agent is used or introduced for the purpose of disinfection or sterilization, efficacy against phenol is tested. i.e., phenol coefficient.

This is used to **find out efficacy of any disinfectant.**

- Reidel walker test.
- Chick martin test.

Efficacy of the test disinfectant in killing *S.aureus/S.typhi* is compared with efficacy of phenol in killing *S.aureus/S.typhi*.

Dilution of disinfectant and phenol are prepared :

Add standard inoculum of *S.aureus/S.typhi* in each of dilution. At regular intervals, each dilution is subcultured at 2.5, 5, 7.5 and 10 minutes.

Incubate subculture plates at 37° C for 48-72 hours.

2 days later :

Dilution of disinfectant which disinfects the suspension in a given time

Dilution of phenol which disinfects the suspension in same time

If 1 : 100 dilution of the test disinfectant killing all *S.aureus* in 5 minutes.

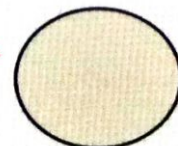
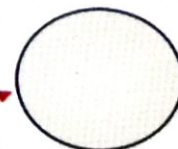
Check dilution of phenol, Killing same *S. aureus* inoculum in 5 minutes.

In use test of maurer → Checks the contamination of disinfectants being used.

1 mL of test + 9 mL of diluent (inactivator).

↓
10 drops of 0.02 mL into
2 plates

↓
Incubate 1 plate at 37° C for 3 days and other plate at 28° C for 7 days.



↓
5 or more colonies on either plates indicate contamination.

Spaulding classification

00:36:34

Earle H. Spaulding devised a rational approach to disinfection and sterilization of patient-care items and equipment. Medical devices are assigned to one of three categories:

- Critical.
- Semi-critical.
- Non-critical

Critical : Come in contact of sterile tissues/vasculature.

Surgical instruments, prosthesis, implants, cardiac catheters, IV cannulas.

Sterilized either by heat/ γ radiation or HLD.

Semi critical : Come in contact with mucous membranes.

Respiratory therapy equipments, all GI scopes, esophageal manometry probes, bronchoscopes and cystoscopes.

Sterilized or HLD, except for thermometers.

Thermometers \rightarrow IMLD.

Non critical : Just in contact with intact skins.

Eg : Stethoscopes, BP cuff, bed pans, hospital furnitures.

LLD is adequate.

Inactivation of prions :

Critical devices and semicritical devices contaminated with high-risk tissue (i.e., brain, spinal cord, or eye tissue) from high-risk patients (e.g., known or suspected infection with prion disease) require special prion reprocessing

method 1 : Soak with 1 normal sodium hydroxide.

Heat in autoclave at 121° C for 30 minutes.

After that rinse it.

Followed by routine sterilization.

method 2 : Immerse with 1 normal sodium hydroxide/25% of sodium hypochlorite for 1 hour.

Rinse it.

Autoclave it for 1 hour.

Followed by routine sterilization.

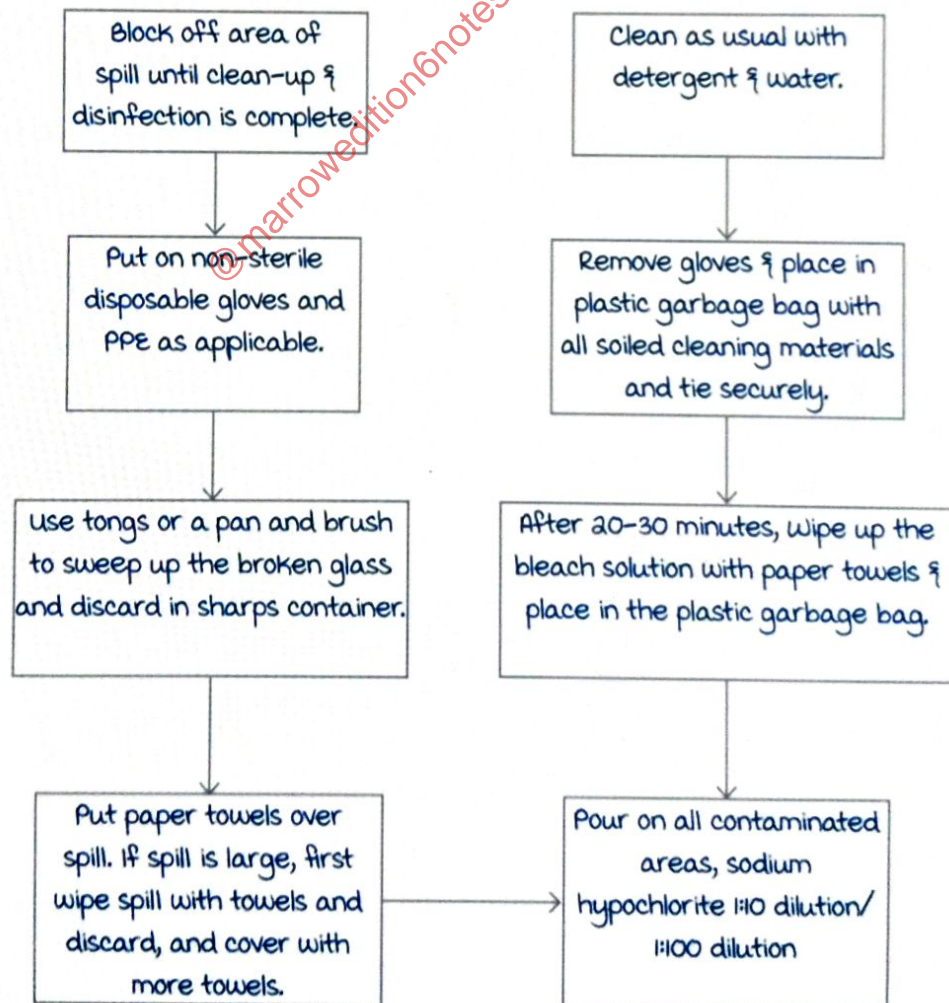
Active space

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Petroleum jelly, dusting powder, paraffin	Hot air oven
metallic instruments (sharp)	Hot air oven
metallic instruments (non-sharp)	Hot air oven/ autoclave
Glassware	Hot air oven/ autoclave
microbiological culture media	Autoclave
Rubber ware & plastic ware	Autoclave
All sutures (except catgut)	Autoclave, Gamma rays
Disposable syringes, IV cannula, Foley's catheter	Gamma rays & ETO
Bone grafts, prosthesis	Gamma rays
Heart lung machines, respirators	ETO

Infectious spill management

00:45:18



Wash hands thoroughly with soap and water.

Active space

STANDARD AND TRANSMISSION BASED PRECAUTIONS

In 1985, Centres of Disease Control (CDC) made universal precautions in view of the HIV epidemic.

In 1987, they introduced body substance isolation precaution.

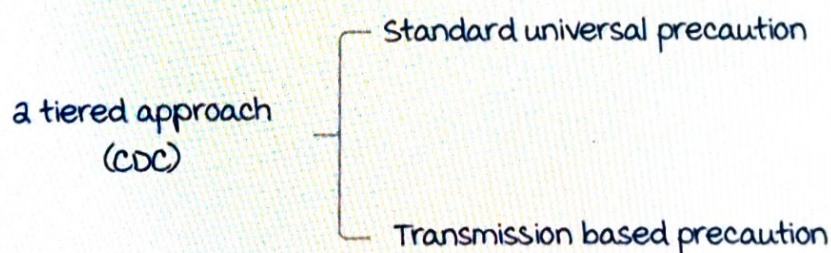
In 1997 both universal precaution and body substance isolation together formed Standard and transmission based precautions.

Healthcare associated infections/HCAI :

Definitions :

- Infection acquired by a patient in a hospital or any other healthcare facility in whom the infection was not present or incubating at the time of admission.
- Includes infections acquired in the hospital but manifest after discharge.
- Occupational infections among staff of the facility.

Infection prevention & control in the hospital 00:03:40



Standard precautions :

Patients, their blood, body fluid, secretion, excretion, mucous membrane and non-intact skin are infectious.

Infection prevention and control practices are meant to decrease the risk of transmission of blood borne and other pathogens from recognised and unrecognised sources that must be used for all patients, at all times, in all situations, whatever be the infectious state of patient.

methods to prevent hospital acquired infections :

Hand hygiene : Largest pillar.

It prevent infection from patient to health worker and vice versa

WHO recommends 5 moments of hand washing.

1. Before touching the patient.
2. After touching the patient.
3. Before any procedure.
4. After any body fluid exposure.
5. After touching patient surrounding.

modalities of handwashing :

Alcohol based handrub.

1. Easily available.
2. No requirement of water and sink.
3. more efficient in destroying microorganisms.
4. Less drying of skin.

Hand wash with soap.

1. Hand wash with soap is mandatory when hand is visibly soiled.
2. After use of toilet.
3. Before touching food.
4. Handling patient with diarrhoea or incontinence.

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Steps of handwashing with soap

00:11:41

Ideal hand wash takes about 40 to 60 seconds.

1. Wet hands with water .
2. Take adequate soap to cover all surfaces.
3. Rub hands palm to palm.
4. Left dorsum with right palm with finger interlaced and vice versa.
5. Palm to palm with fingers interlaced
6. Back of fingers to opposing palm with fingers interlocked and vice versa
7. Rotational rubbing of left thumb with right palm and vice versa.
8. Rotational rubbing of centre of left palm with right fingers and vice versa.
9. Rinse with water.
10. Wipe hands with single use towel , and close tap with same towel, and then discard the towel.

Steps of handwash

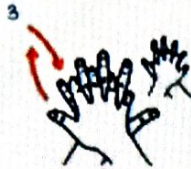
Duration of the entire procedure : 20-30 seconds



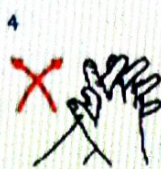
Apply a palmful of the product in a cupped hand, covering all surfaces.



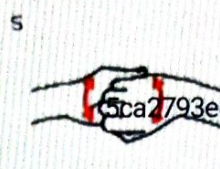
Rub hands palm to palm.



Right palm over left dorsum with interlaced fingers and vice versa.



Palm to palm with fingers interlaced.



Backs of fingers to opposing palms with fingers interlocked.



Rotational rubbing of left thumb clasped in right palm and vice versa.



Rotational rubbing, back and forth with clasped fingers of right hand in left palm and vice versa.



Once dry, your hands are safe.

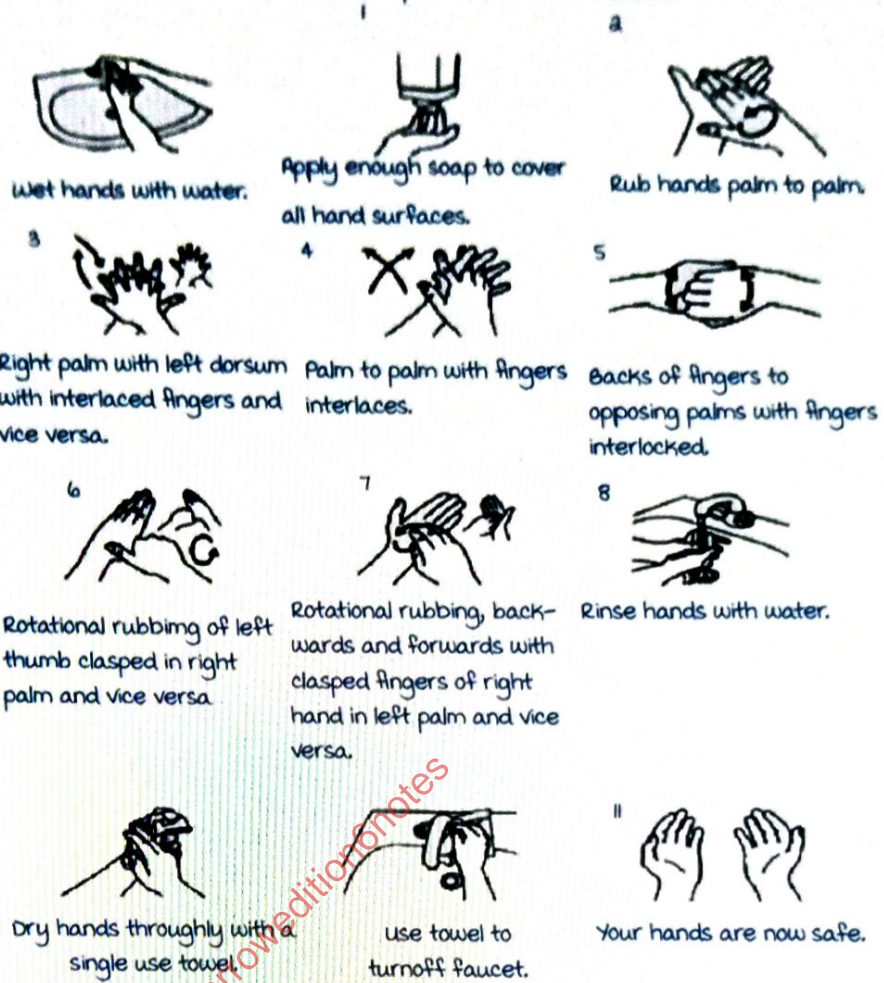
Steps of hand rub with alcohol

00:13:07

1. Duration is about 20 to 30 seconds.
2. Take palm full of alcohol to our cupped hands.
3. Cover all surfaces of hand with solution.
4. Rub hands palm to palm.
5. Left dorsum with right palm with finger interlaced and vice versa.
6. Palm to palm with fingers interlaced and vice versa.
7. Back of fingers to opposing palm with fingers interlocked and vice versa.
8. Rotational rubbing of left thumb clasped with right palm and vice versa.
9. Rotational rubbing of centre of left palm with right fingers and vice versa.
10. Finally hands are dry.

Wash hands when visibly soiled otherwise, use handrub.

Duration of the entire procedure: 40-60 seconds



PPE

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00:14:29

Barrier used by healthcare workers either alone or in combination to **protect** mucosal membranes, airways, skin and clothing from contact with infectious agents.

PPE can be gloves, gowns, apron, goggles, face shield and mask.

Instructions to use PPE.

Assess the risk of exposure to body substances of contaminated surfaces **before** any healthcare activity and appropriate PPE.

Should be used as a routine.

PPE should not be shared.

No contact with PPE with contaminated surface.

Proper disposal after use.

Active space

PPEs always should be changed in between patients or change of duties.

It is mandatory to follow hand hygiene after removing PPE.

Commonly used PPEs :

5ca2793ec88d500486113130 gloves	Facial protection	Gown/Aprons
<p>worn whenever healthworker anticipates contact with blood, body fluids, excretion, secretion, non-intact skin.</p> <p>Single use only.</p> <p>Clean and nonsterile Sterile for invasive procedure. Hand hygiene after removing gloves.</p>	<p>Eg : mask, goggles, face shield.</p> <p>when you anticipate during a procedure on patient that there is a likelihood of generation of droplets, splashes, spray of blood, body fluid excretion, secretion.</p>	<p>Protect clothing and skin</p> <p>used when you anticipate splash or spray of body fluids</p>

Respiratory hygiene or cough etiquette :

Patients, HCWs and visitors must cover nose and mouth while coughing, sneezing or blowing their nose with tissues.

These tissues should be properly disposed in dustbin.

Patients who are constantly coughing must wear a surgical mask.

In common waiting areas, coughing patients should have a spatial separation of more than 3 feet from others.

Safe injection practices

00:22:58

Safest possible manner of all parenteral injection.

1. Sterile disposable needle syringe for every injection.
2. Single dose vials preferred over multidose vials.
3. Rubber septum on a medication vial to be cleaned by alcohol before piercing.
4. All injections to be give using aseptic technique.

Appropriate handling and disposal of sharps :

Precautions to be used with sharps.

1. Avoid unnecessary uses of sharps.
2. Never pass sharps directly from one to another. (hand to hand).
3. Never recap needles (no reuse).
4. Never leave needles or any kind of sharps on trolley or bedside tables.

5. Disposal of sharps in a puncture proof container by a person handling the sharp.

Decontamination of patient linen :

must be changed daily.

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In case of any soiling by blood or body fluids, it needs to be changed immediately.

Changing of linen to be done with minimal agitation.

Patient with HIV, Hepatitis B, Hepatitis C or any MRSA infection

→ All linen should be decontaminated by autoclaving or 7% lyzol or 1% sodium hypochlorite → Sent to laundry.

Environmental cleaning and disinfection

00:30:05

Regular cleaning of environmental surfaces like bed side rail tables, floors, handle toilet seat.

Hospital waste management :

Proper segregation of waste → Disposal.

Transmission based precaution :

Added when standard precaution is insufficient.

They are :

1. Contact precautions.
2. Droplet precautions.
3. Airborne precautions.

Contact precaution :

Used for infection or with direct contact with the patient or indirect contact with the patient surroundings.

Guidelines :



Sign must be put outside the patient room

Active space

Isolation	mandatory PPE	Patient care equipment	Transport
1. Separate room. 2. If not possible keep them away from immunocompromised patient.	1. Gloves, facial protection, and gown if splash or spilling of body fluid expected. 2. When HCWs go to the patient's side, they must wear PPE.	1. Disposable if affordable. 2. Dedicated to the same patient. 3. Disinfect the patient care equipment before and after use of a patient.	1. Cover the area of infection. 2. Change PPE before transport of the patient.

Contact precautions are used in :

1. Multidrug resistant infections : MRSA, VRE, VRSA.
2. Skin and soft tissue infections : Impetigo, herpes zoster, chickenpox, draining abscess, leprosy or SSSS.
3. Diarrheal diseases : Rotavirus, Norovirus, C. difficile.
4. GI infections : HAV, HEV, polio and patients who are incontinent.
5. Conjunctivitis.
6. Congenital rubella syndrome.

Droplet precautions

00:40:20

When transmitted by secretions **more than 5 microns** in size generated by cough, sneeze, talk or singing.

Guidelines :

Isolation	mandatory PPE	Transport
1. Isolation in bedroom is the best 2. If not possible cohort with similar patients, with separation of $\geq 3m$	1. Mask 2. mask discarded on exit and followed by hand hygiene. 3. Facial protection / gown if splashes or spray are anticipated.	1. Transportation best avoided or limited. 2. If mandatory, ask patient to wear mask, to follow cough etiquette.

Droplet precaution is used in : mnemonic : **SPIDERMAN**.

Scarlet fever, **S**treptococcus pharyngitis.

Pertussis, **P**neumonic plague.

Influenza

Diphtheria

Epiglottitis, **E**bola

Rubella, **R**SV.

Mumps, **M**ycoplasma pneumoniae, **M**RSA, **V**RE

→ Add contact precautions

Adenovirus pharyngitis.

Neisseria meningitides.

Airborne precautions :

Transmitted in a droplet nuclei ≤ 5 micron, they remain suspended in air for many hours.

Isolation	mandatory PPE	Transport
1. Negative pressure room (airborne infection isolation room). 2. Door absolutely closed, with special outlet. 3. 6-12 air exchange/hr	1. FIT tested N95 respirator or powdered air purifying respirator, discarded on exit of room followed by hand hygiene. 4. Gown/facial protection used if splashes anticipated.	1. Transport limited if mandatory, mask on patient. 2. Change your own N95. 3. Ask patient to follow cough etiquette. 4. Limit movement.

Used in measles, TB, disseminated zoster, Avian flu, SARS, Small pox.

Also Follow contact precaution : Disseminated zoster, Avian flu, SARS, Small Pox.

Q : Select all of the conditions that warrant airborne precautions :

- A. Norovirus.
- B. Hepatitis A.
- C. Varicella.
- D. measles.
- E. Disseminated herpes zoster.
- F. TB.
- G. Whooping cough.
- H. RSV.

Answer : C, D, E and F.

Q : A patient is diagnosed with Hep A. The patient is incontinent. What type of precautions will you initiate?

- A. Standard.
 - B. Contact.
 - C. Droplet.
 - D. Airborne.
1. A only.
 2. A and B.
 3. A and C.
 4. A and D.

Answer : 2.