LECTURE V

The causative agents of dental bacterial infections

Staphylococcus, *Streptococcus*, *Neisseria*, *Proteus*, *Klebsiella*, *Clostridium*, *Bacteroides*, *Fusobacterium*, *Corynebacterium*, *Mycobacterium*, *Actinomyces*, *Treponema*, *Leptospira*, *Mycoplasma genus*

- *The cocci as a causative agents*
- *of pus inflammation proses*

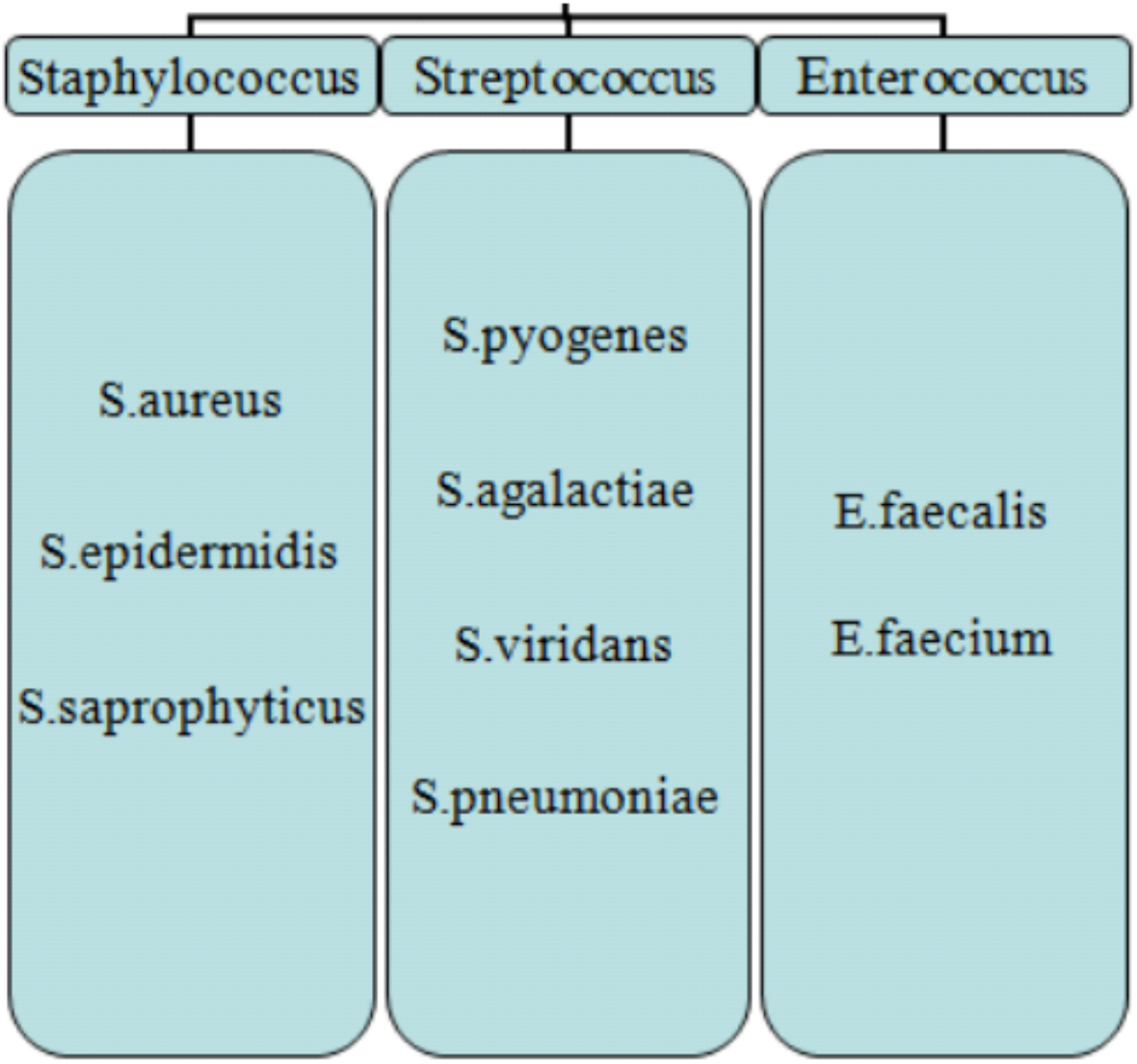
- *Aerobic gram-positive cocci:*

- *- family Micrococcaceae (genes Staphylococcus,*
- *Micrococcus, Stomatococcus)*

- *- Streptococcaceae family (genes Streptococcus,*
- *Enterococcus, Aerococcus, Leuconostoc,*
- *Pediococcus, Lactococcus)*

- *Aerobic gram-negative cocci:*
- *- cocci of the family Neisseriaceae (genus*
- *Neisseria)*

Gram positive cocci



Staphylococcus

- Most important genus in Family *Micrococcaceae*
- *Other genera*
  - *Stomatococcus*
  - *Micrococcus*

Staphylococci

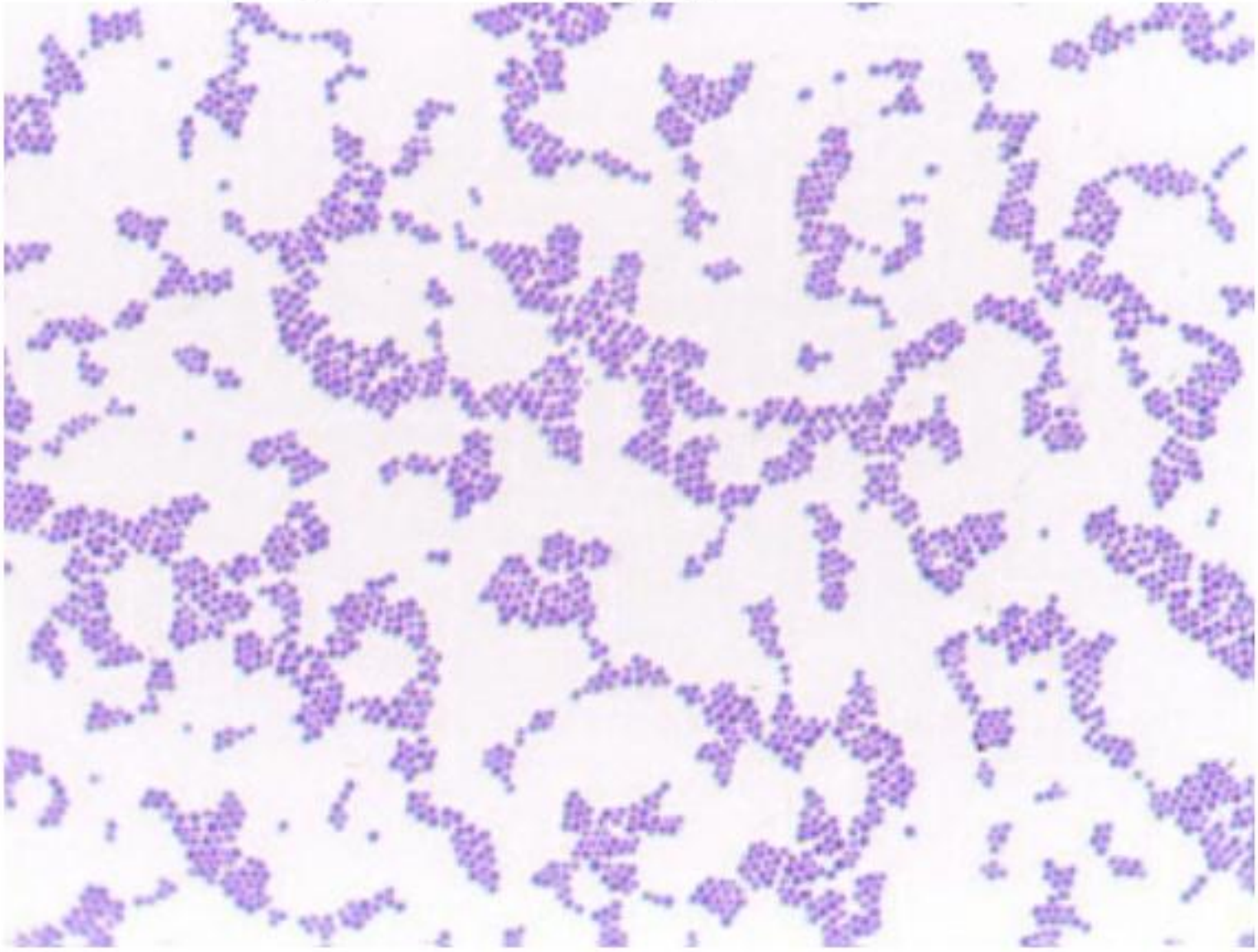
- Family - *Micrococcaceae*
- Genus - *Staphylococcus*
- Species - 27 species are known, 14 species are found in the human body, 3 of them are pathogenic: *S.aureus, S.epidermidis, S.saprophyticus*

- The greatest clinical value is *S.aureus*.

Habitat

- ***S. aureus***
  - anterior nares 50-75% healthy people
  - skin & mucous membranes
  - hospital environment
- ***S. epidermidis* & others**
  - resident skin flora, gut, upper respiratory tract
- ***S. saprophyticus***
  - Urinary tract in young women

Staphylococci, by gram stained

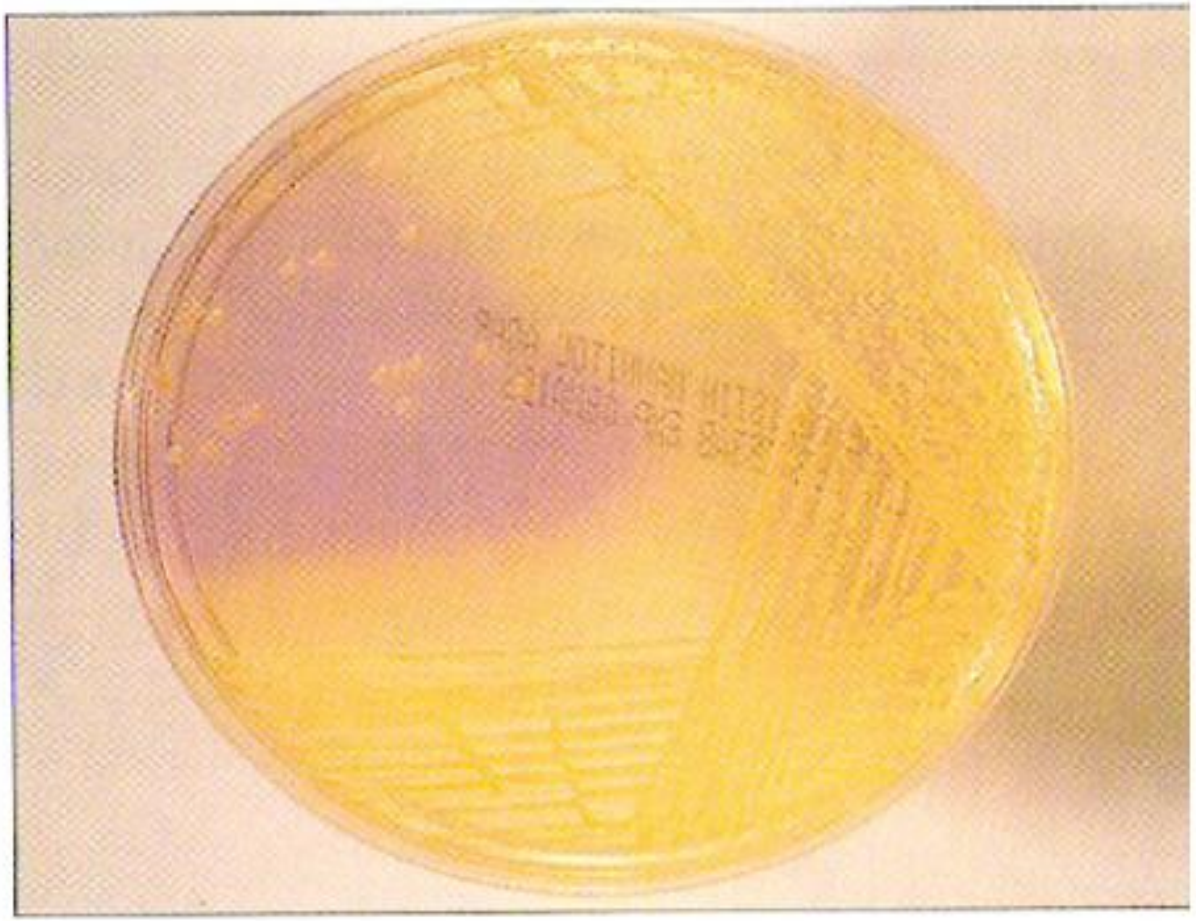


Staphylococcus aureus

culture on blood agar

Staphylococcus aureus

lecithinase test on yolk-salt agar



Differential signs of *staphylococci*

Oxidase	Negative							
Novobiocin	Susceptible							
Coagulase	Negative	Positive[1] – variable[2] – negative[3]		Negative				
Species group	Hyicus-Intermedius			Epidermidis-Aureus				
Cluster group	Muscae	Hyicus	Intermedius	Aureus	Epidermidis	Warneri	Haemolyticus	Lugdunensis
Species	*S. muscae* *S. microti* *S. rostri*	*S. hyicus*[2] *S. agnetis*[2] *S. chromogenes*[3] *S. felis*[3]	*S. intermedius*[1] *S. delphini*[1] *S. lutrae*[1] *S. pseudintermedius*[1] *S. schleiferi ssp. schleiferi*[3] *ssp. coagulans*[1]	*S. aureus ssp. aureus*[1] *ssp. anaerobius*[1] *S. simiae*[1]	*S. epidermidis* *S. capitis ssp. capitis* *ssp. urealyticus* *S. caprae* *S. saccharolyticus*	*S. warneri* *S. pasteuri*	*S. haemolyticus* *S. devriesei* *S. hominis ssp. hominis* *ssp. novobiosepticus* *S. jettensis* *S. petrasii ssp. croceilyticus* *ssp. petrasii*	*S. lugdunensis*

Oxidase	Negative						Positive
Novobiocin	Susceptible		Resistant				
Coagulase	Negative						
Species group	Auricularis	Simulans	Saprophyticus				Sciuri
Cluster group	Auricularis	Simulans-Carnosus	Pettenkoferi-Massiliensis	Saprophyticus	Cohnii-Nepalensis	Arlettae-Kloosii	Sciuri
Species	*S. auricularis*	*S. simulans* *S. carnosus ssp. carnosus* *ssp. utilis* *S. condimenti* *S. piscifermentans*	*S. pettenkoferi* *S. massiliensis*	*S. saprophyticus ssp. saprophyticus* *ssp. bovis* *S. equorum ssp. equorum* *ssp. linens* *S. gallinarum* *S. succinus ssp. succinus* *ssp. casei* *S. xylosus*	*S. cohnii ssp. cohnii* *ssp. urealyticus* *S. nepalensis*	*S. arlettae* *S. kloosii*	*S. sciuri ssp. sciuri* *ssp. carnaticus* *ssp. rodentium* *S. fleurettii* *S. lentus* *S. stepanovicii* *S. vitulinus*

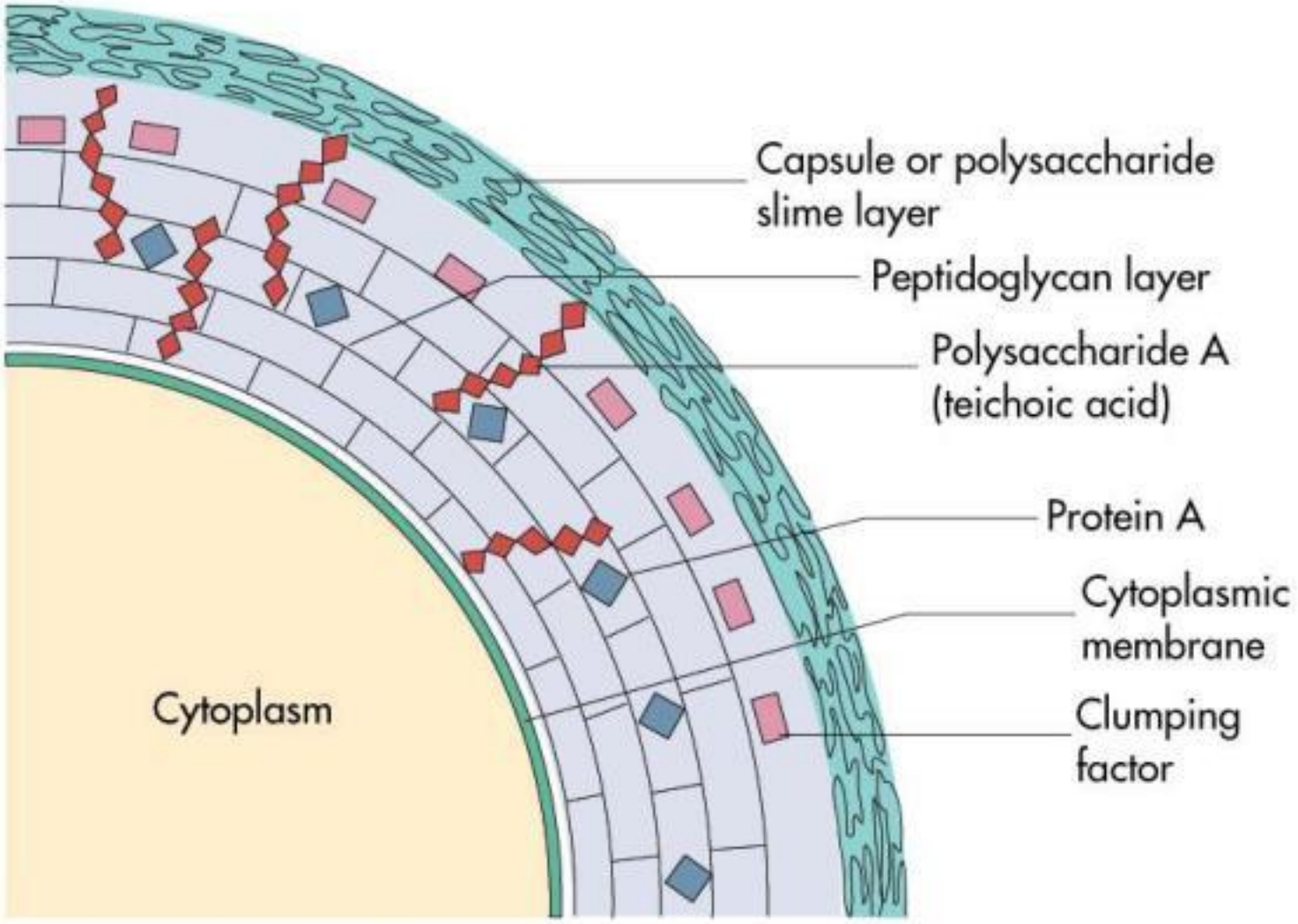
Antigenic structure

- About 30 antigens
- Common for all types of protein antigen
- Protein A is species-specific for *S.aureus*.
- Polysaccharide antigens for serological specificity
- Polysaccharide A - ribitteichoic acid, found *in S.aureus*
- Polysaccharide B - glycerine-teichoic acid, found in *S.epidermidis*.

Antigenic Structures & Virulence Factors of *S. aureus*

- Cell wall peptidoglycan
  - elicits production of IL-1 and opsonic antibody
  - PMN chemotaxis “pyogenic”
  - induces sepsis
  - activates complement
  - teichoic acid binds fibronectin on host cells
- Protein A - binds Fc of IgG
- Capsule (some strains) antiphagocytic

Cell Wall of S. aureus



S. aureus Soluble Virulence Factors

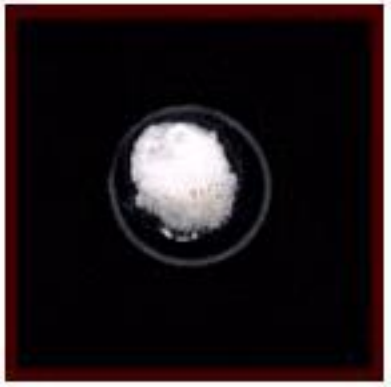
- Catalase - reduce phagocyte killing - remove H_2O_2
- Coagulase - clots plasma (free & bound)
- Hyaluronidase - destroys connective tissue
- Beta lactamase - destroys beta lactam drugs
- Altered Penicillin binding proteins (PBP2')
- Fibrinolysin
- Lipases
- Nucleases

Catalase Test for Distinguishing Staphylococci from Streptococci

$$H_2O_2 \xrightarrow{catalase} O_2 + H_2O$$

Bubbles

Strep. Negative



Staph. positive

Tube Coagulase Test

- Free coagulase secreted by *S. aureus* but not CNS
- Clots rabbit plasma

S. aureus Soluble Virulence Factors

- Cytotoxins & leukocidins
  - lyse white blood cells (Panton-Valentine)
  - release lysosomal enzymes → damage tissue
- Exfoliatin
  - interrupts intercellular skin junctions
  - "Scalded Skin Syndrome"
- Toxic Shock Toxin
  - stimulates T cells → cytokines,
  - endothelial damage → rash
  - "Toxic Shock Syndrome"
- Enterotoxins
  - stimulate vomiting by interaction with GI neural receptors (food poisoning)

S. aureus Diseases

Skin and soft tissue infections
- Furuncles
- Carbuncles
- Wound infections
- Cellulitis
- Impetigo
- Bacteremia

Endocarditis

CNS Infections
- Brain abscess
- Meningitis - rare
- Epidural abscess

Impetigo

S. aureus Diseases

Pulmonary Infections
- embolic
- aspiration

Musculoskeletal
- osteomyelitis
- septic arthritis

Genitourinary Tract
- renal carbuncle
- lower UTI

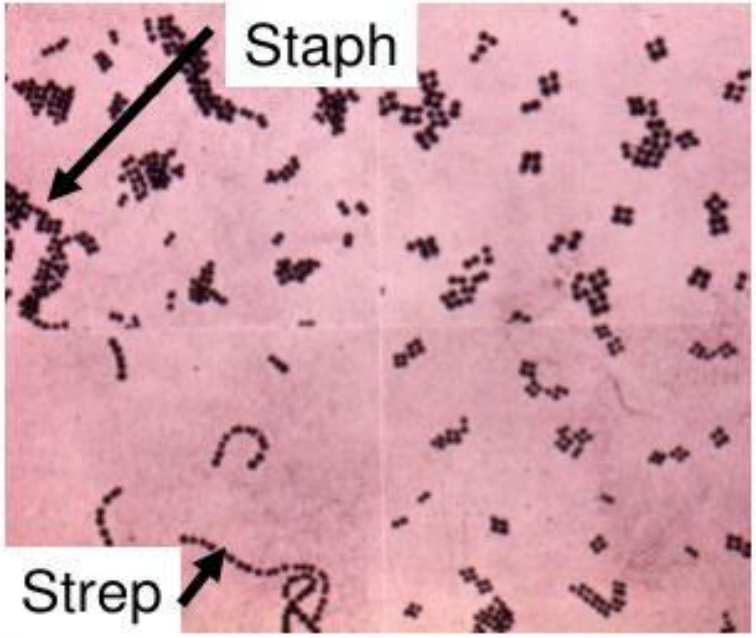
Toxin mediated diseases
- scalded skin syndrome
- food poisoning
- toxic shock disease

Toxic shock

Scalded skin

Streptococcaceae

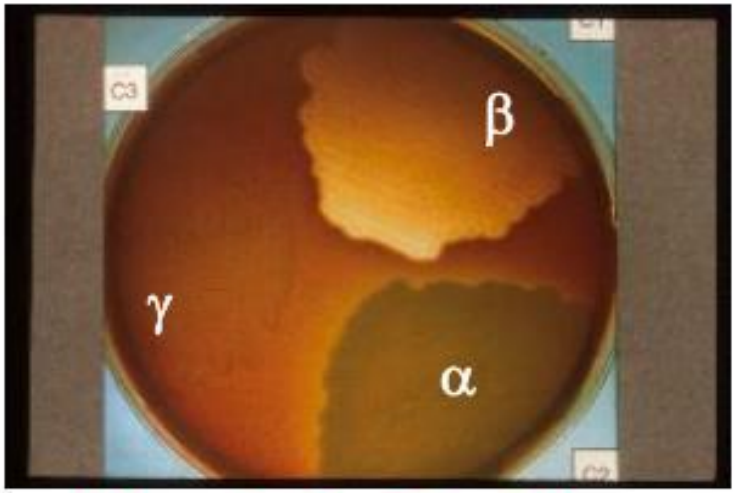
- *Streptococcus*
- *Enterococcus*



Gram stain of staph (clusters) and strep (chains)

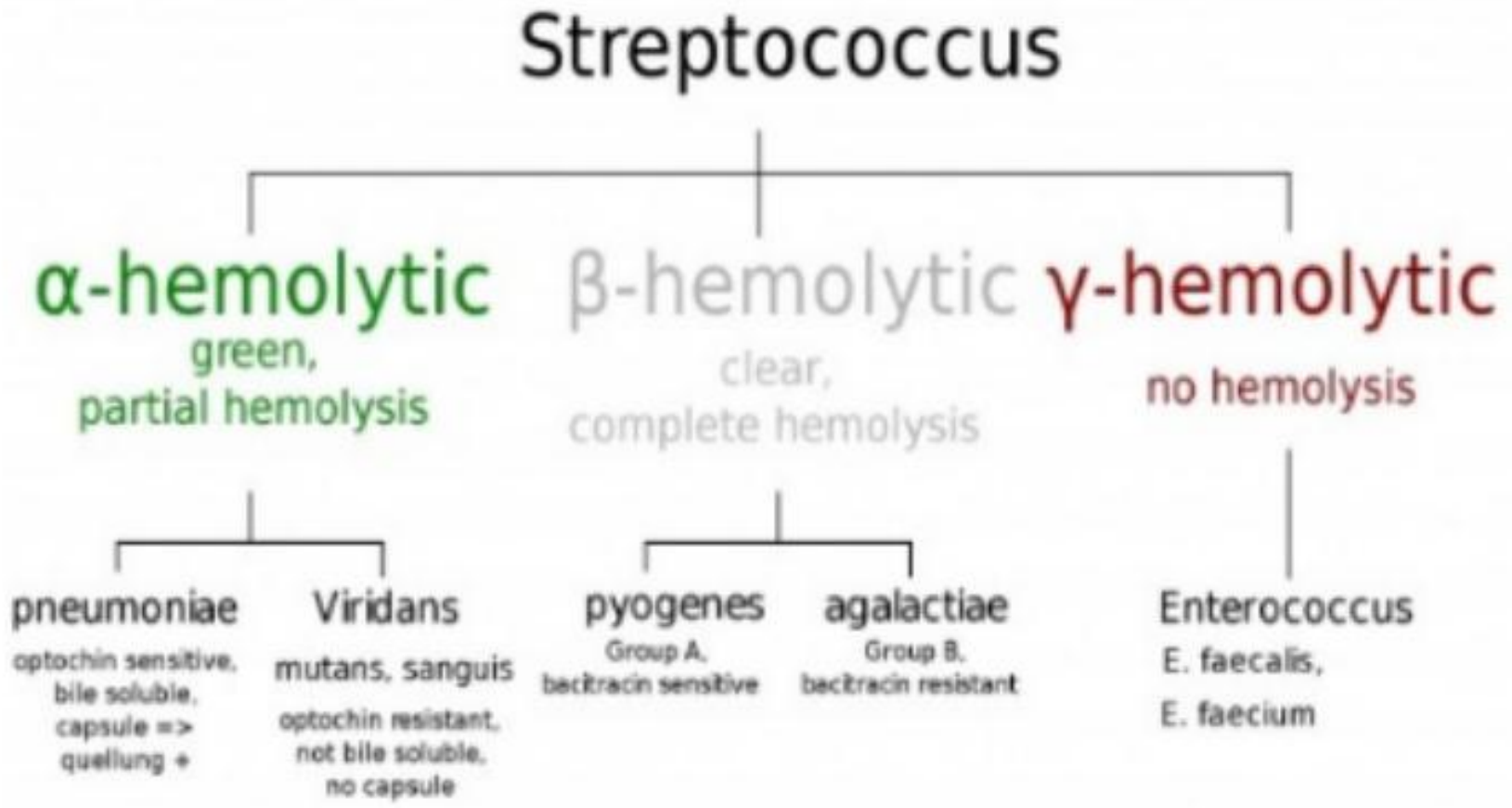
Streptococcus Classification

- Hemolysis
  - beta
  - alpha
  - gamma
- Lancefield Groups
  - *(*A-T- β hemolytic*)*
  - group-specific cell wall polysaccharide
- Species
  - phenotypic biochemical reactions

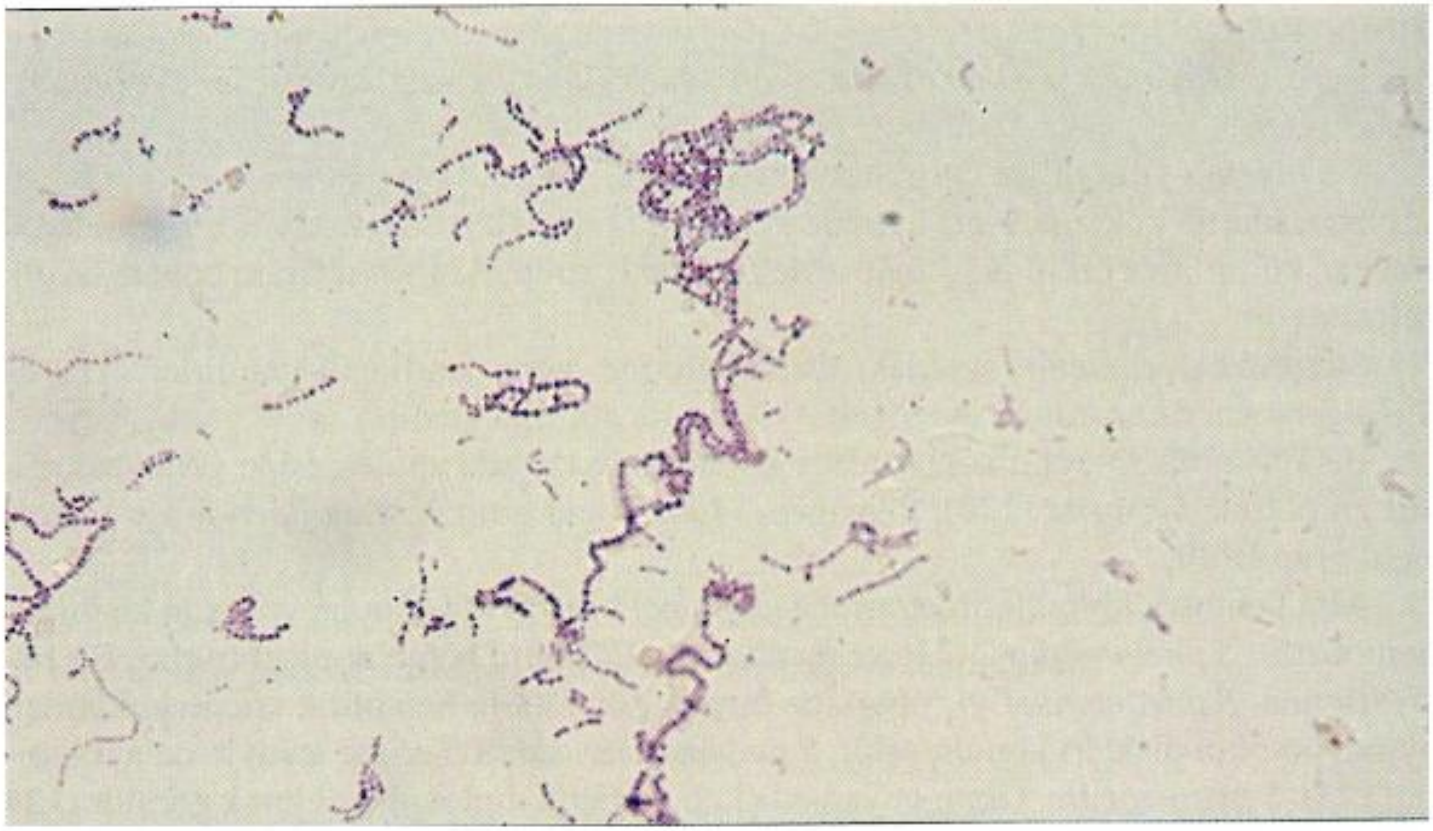


Hemolytic Reactions

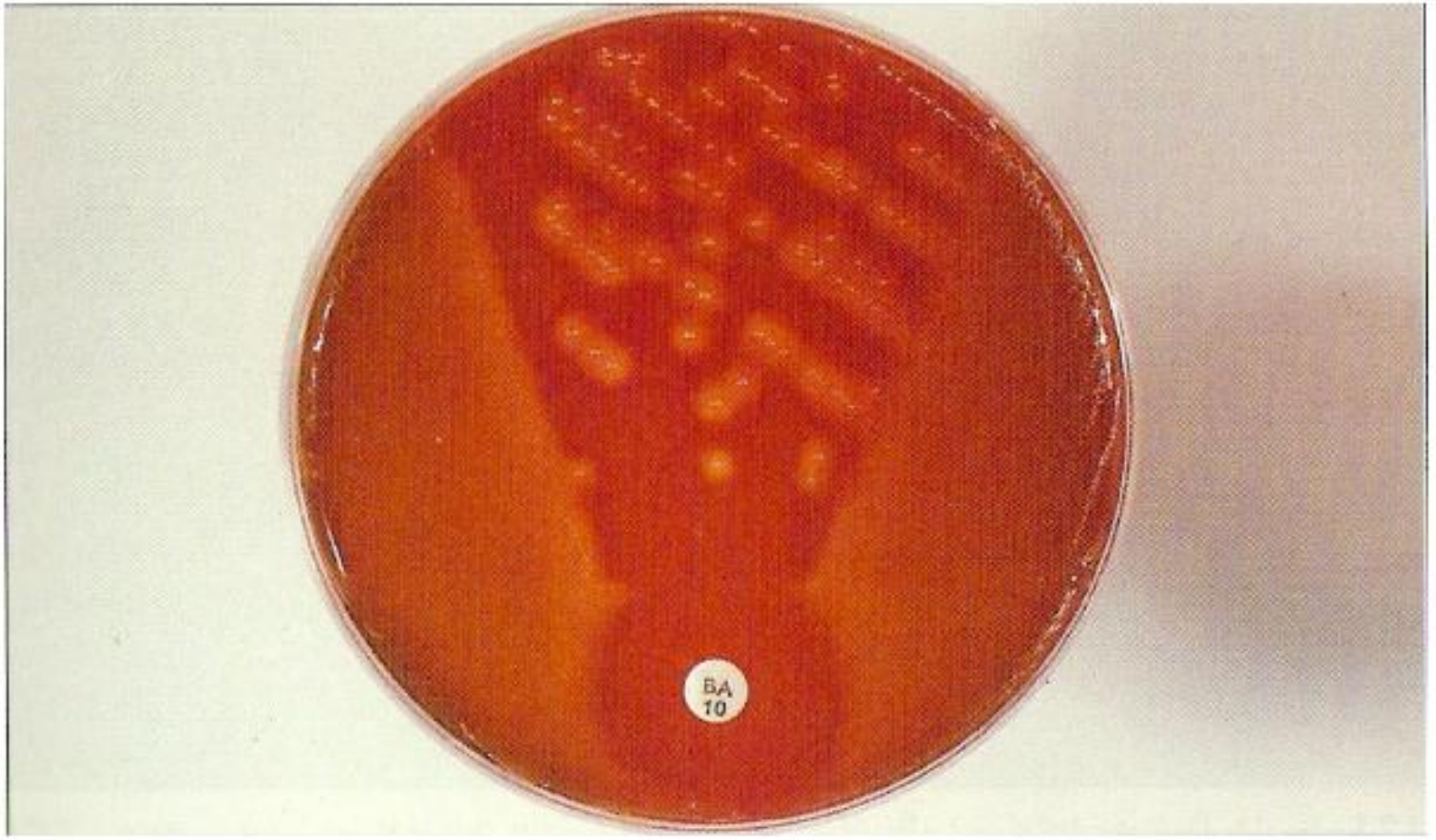
Classification of streptococci



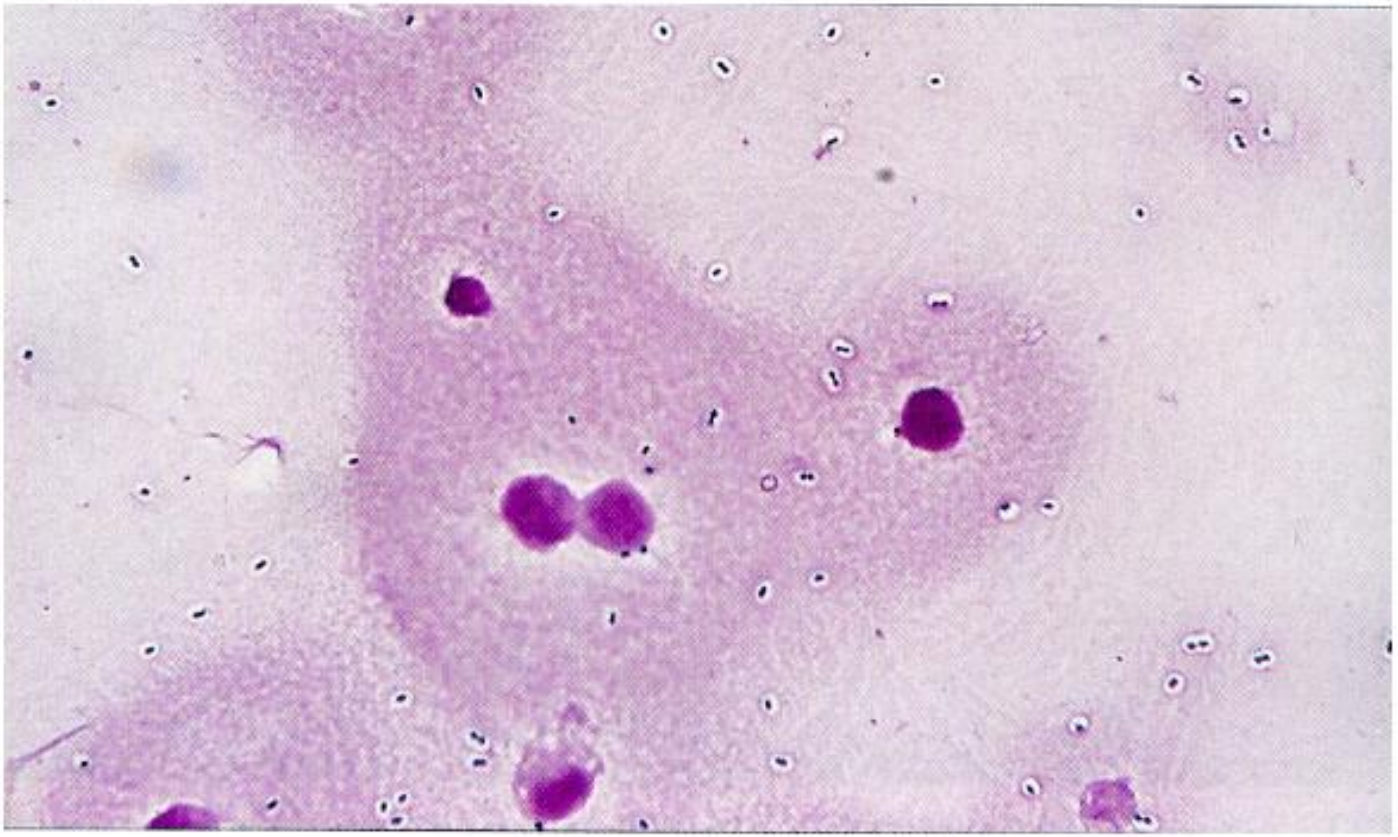
Streptococcus pyogenes



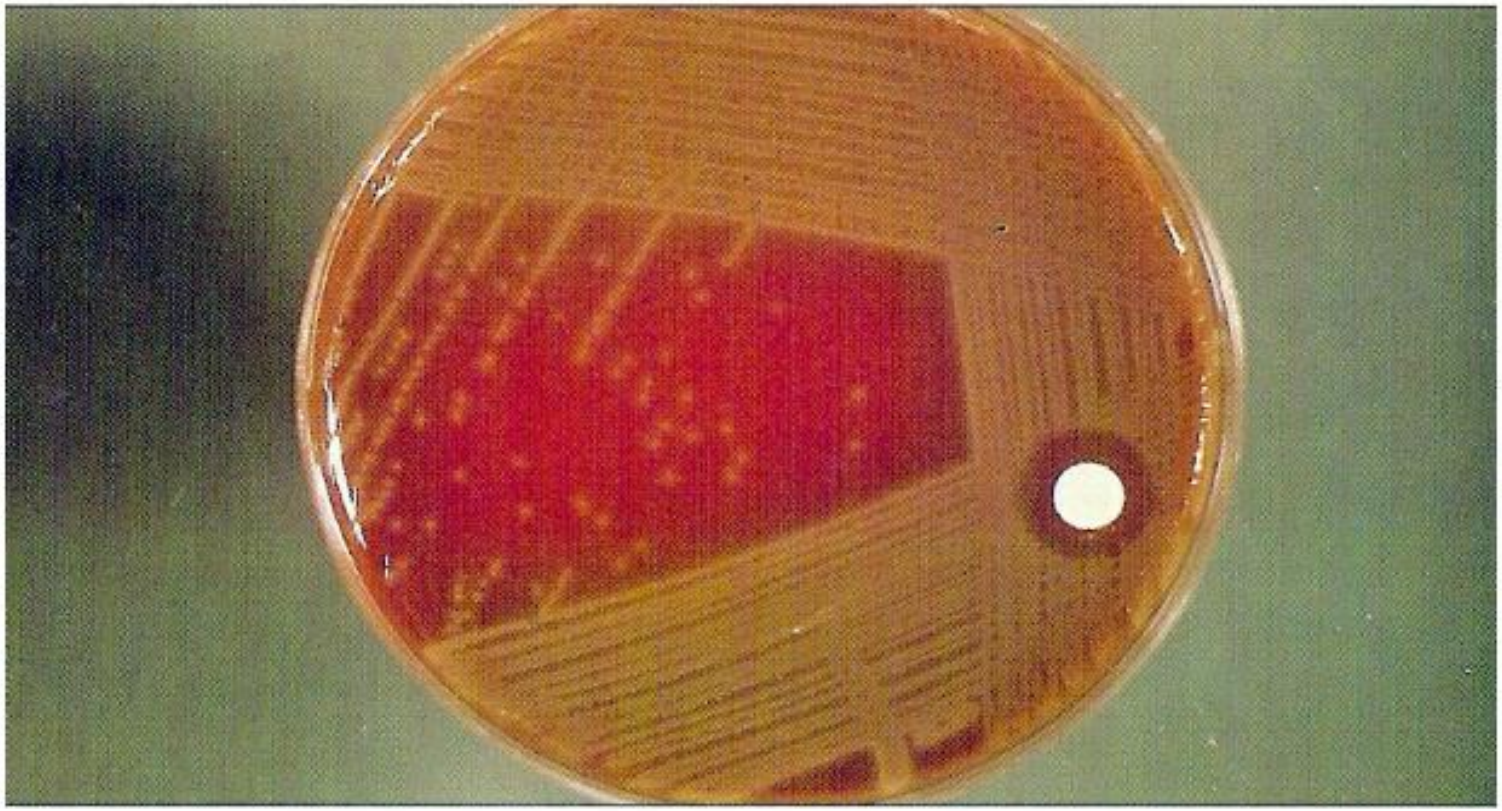
Streptococcus pyogenes on blood agar



Streptococcus pneumoniae



Streptococcus pneumoniae on blood agar

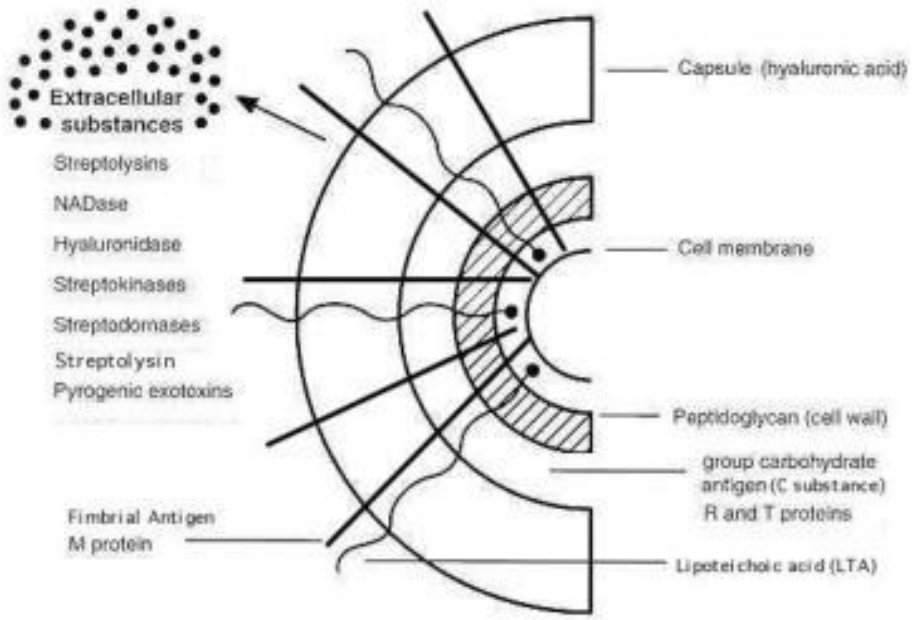


Streptococcus Habitat

- Skin, mucous membranes, respiratory tract and GI/GU tracts, depending on species
- 20% of children may carry GAS in their pharynx during winter months.
- *S. pneumoniae* is commonly isolated from the respiratory tract of asymptomatic carriers.
- Enterococci in gut flora are are important pathogens in hospitals where they are selected by high antibiotic usage.
- Organisms spread by droplets, direct contact and fomites.

Antigenic Structure & Virulence Factors of *S. pyogenes*

- Hyaluronic acid capsule - antiphagocytic
- Hyaluronidase - tissue penetration
- Group specific cell wall antigen distinguishes from B,C,D,F,G, etc.
- Beta hemolytic



Antigenic Structure & Virulence Factors of *S. pyogenes*

- **M Protein**
  - Virulence factor present on pilus with teichoic acid
  - Organisms lacking it are readily opsonized and phagocytized
  - Binds fibrinogen, fibrin & degradation products forming dense coating on the organism's surface, blocking complement
  - Antibody against M protein is an important protective mechanism, but repeated infections with strains possessing one of over 80 different serotypes can occur
  - Autoantibody target-Acute Rheumatic Fever

Antigenic Structure & Virulence Factors of *S. pyogenes*

- Protein F - facilitates attachment by binding fibronectin
- Protein G - binds Fc portion of antibody
- Diphosphopyridine nucleotidase (DPNase) – enzyme kills WBCs
- C5a peptidase

Erysipelas

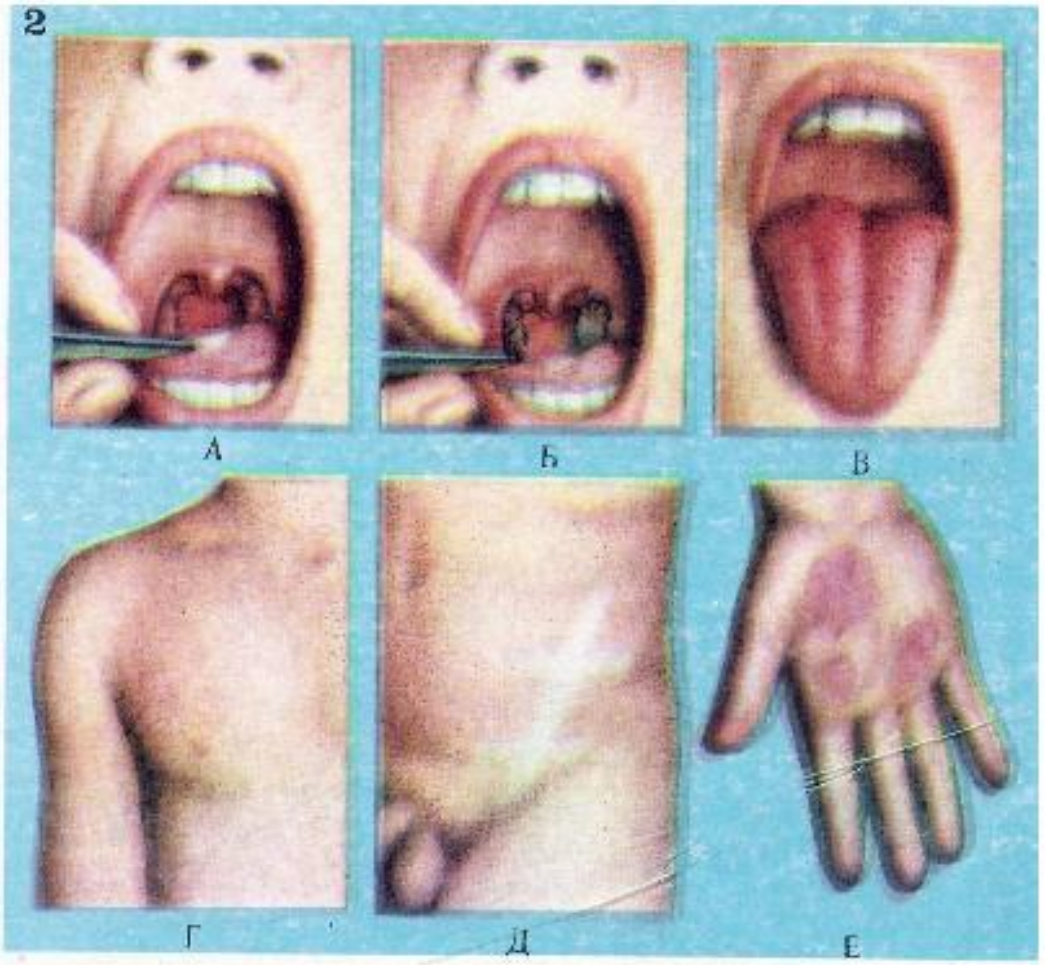
Antigenic Structure & Virulence Factors of *S. pyogenes*

- Erythrogenic Toxin "Scarlet Fever"
- Streptokinases
  - transform plasminogen to plasmin
  - digest fibrin
- DNAase
  - depolymerizes DNA
    antibody used to follow pyoderma
- Hemolysins "Streptolysins"
  - Important immunogens
  - Antibody against streptolysin O used to follow course of pyoderma
  - Streptolysin S - β hemolysis

Strep. cellulitis

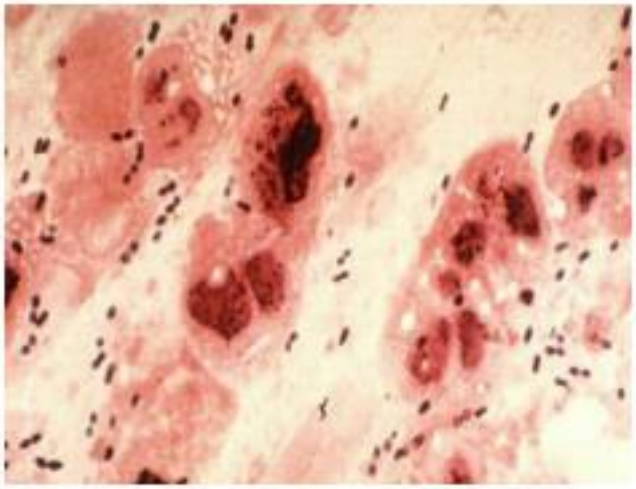
Erysipeloid

Scarlet fever

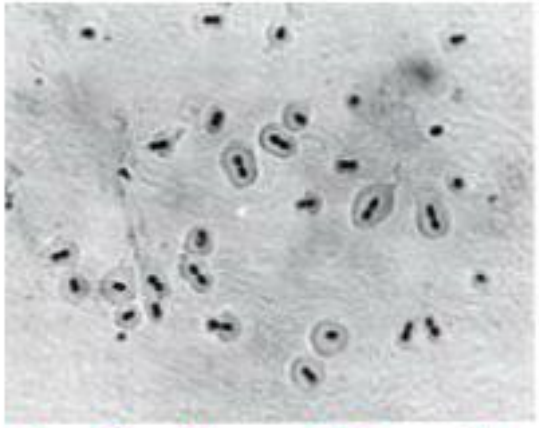


S. pneumoniae Virulence Factors

- Antiphagocytic capsule – immunogen
- PspA: inhibits opsonization
- Autolysin – release cell components
- Pneumolysin
  - Cytotoxic – inhibit cilia, wbcs
  - lyses RBCs
  - activates classic complement path.
  - stimulates cytokines → tissue damage & purulent inflammation
- Hydrogen peroxide - tissue damage
- Surface protein adhesins
- Neuraminidase
- IgA protease
- Peptidoglycan
  - activate alternate complement
  - cytokine release
- Transformation– antibiotic resistance
- Intracellular invasion

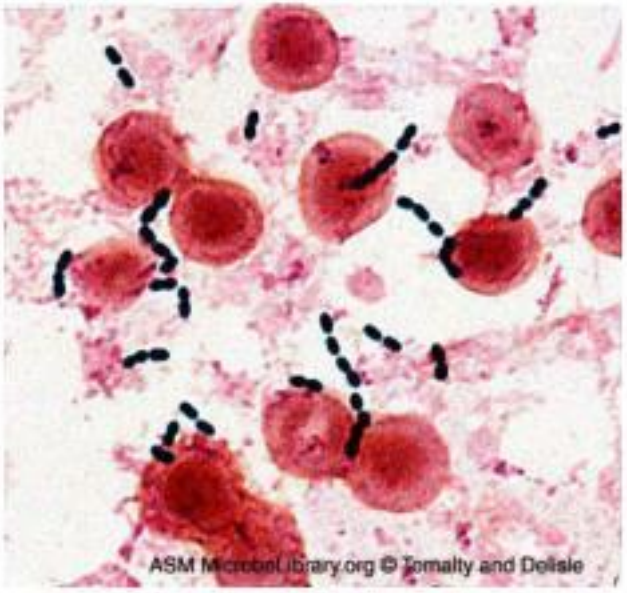


sputum - pneumonia



Capsule Quellung Reaction

Enterococcus

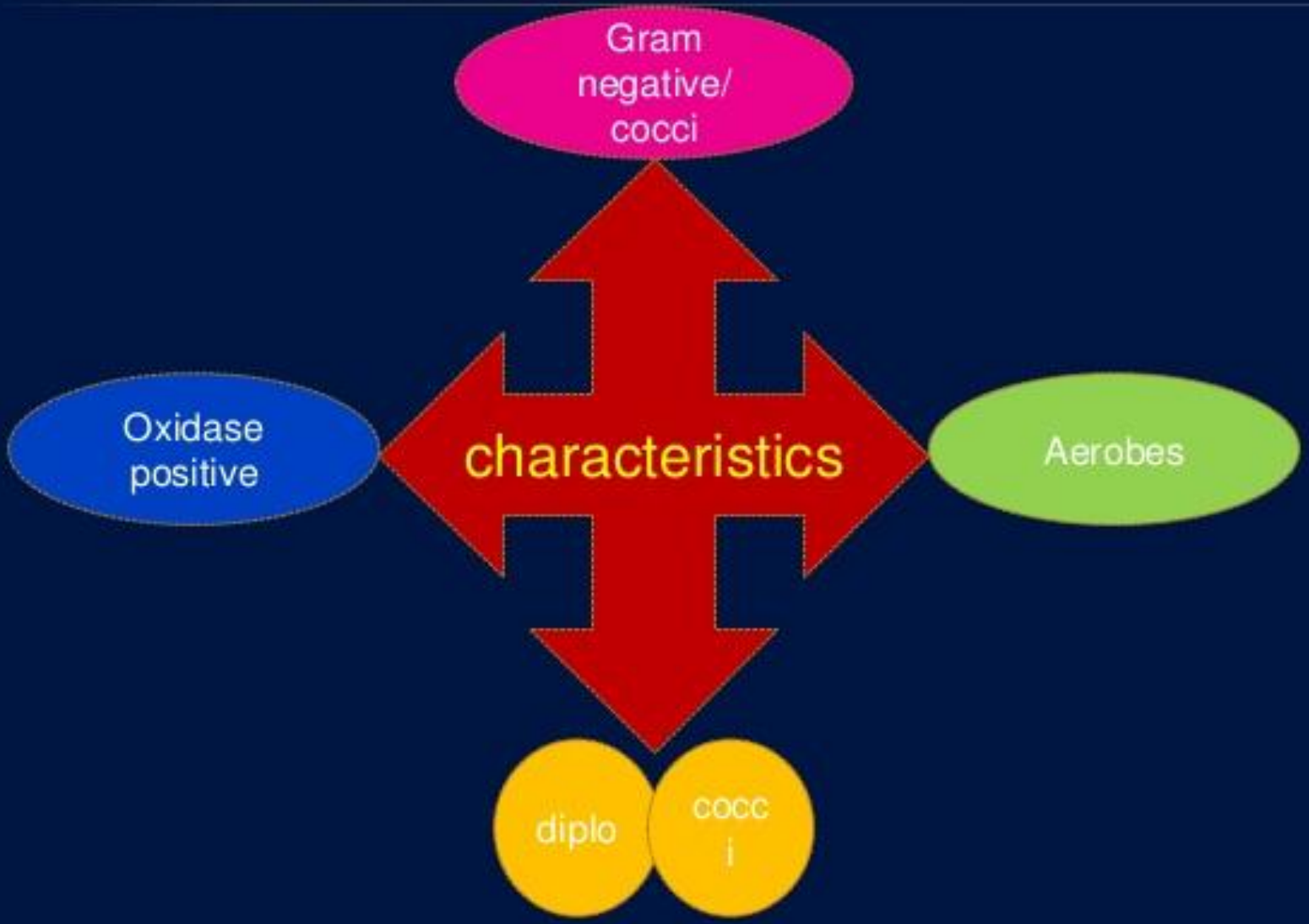


- At least 12 species
- Usually non-hemolytic
- *E. faecalis* most common
- Distinguish from streptococci by:
  - esculin hydrolysis
  - growth in 6.5% NaCl
  - PYR hydrolysis (Group A β strep. are +)
- Enteric flora
- Opportunist - nosocomial pathogen
- Intrinsic antimicrobial resistance
- *E. faecium* - vancomycin-resistance
- Abscesses, urinary tract, endocarditis, abdominal/pelvic, bacteremia, wound infections

Aerobic Gram-negative cocci

- Family - *Neisseriaceae*
- Genus - ***Neisseria***
- Species – ***Neisseria meningitidis, Neisseria gonorrooeae,*** *N.flava, N.subflava, N.perflava, N.sicca, N.mucosa* etc.

CHARACTERISTICS:



CONTAINS TWO IMPORTANT PATHOGENS

- *Nesseria meningitidis*
- *Nesseria gonorrhoeae*

The intracellular presence of these bacteria inside polymorphs in patient sample is a characteristics finding

IMPORTANT DIFFERENCE BETWEEN
N.gonorrhoeae & N. meningitidis

N. gonrrhoeae

N. meningitidis

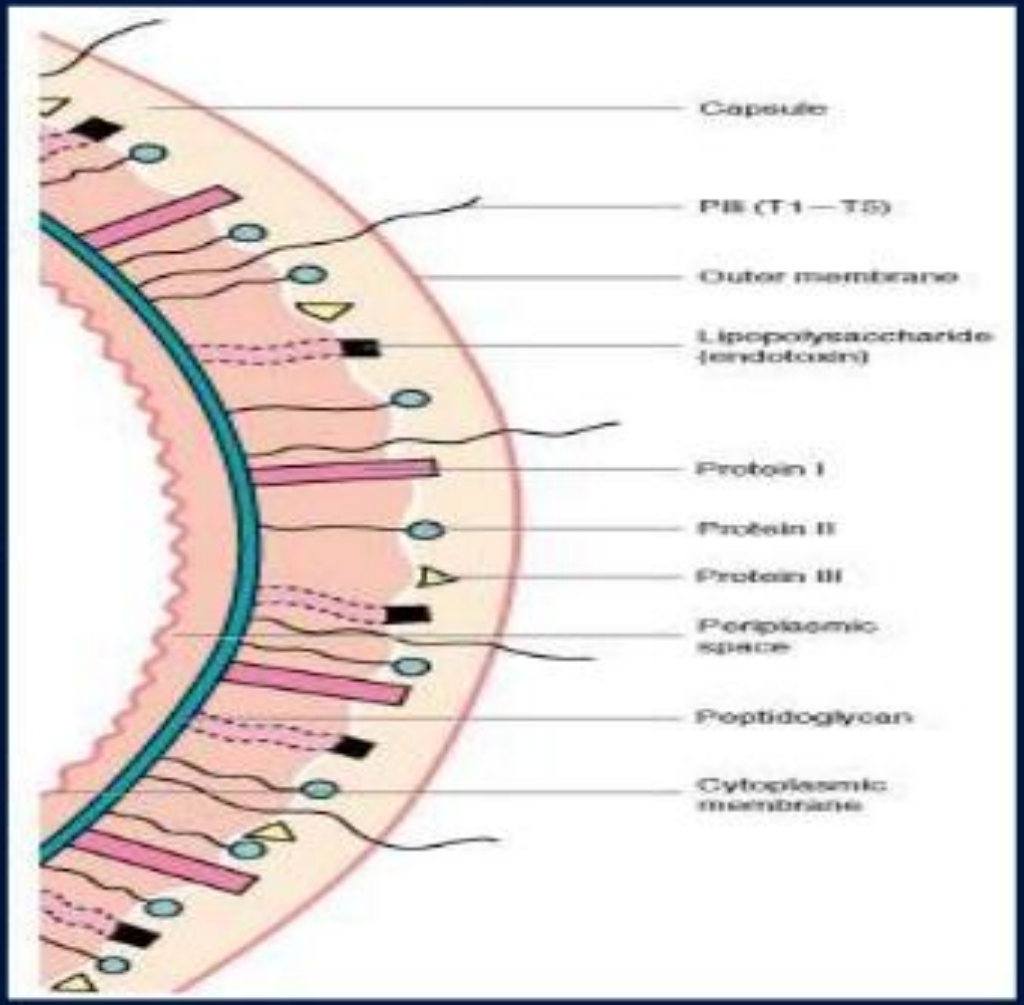
Note:both can be differentiated by biochemical tests using serum sugar

Neisseria meningitidis growing on chocolate agar

Neisseria meningitidis growing on sheep blood agar

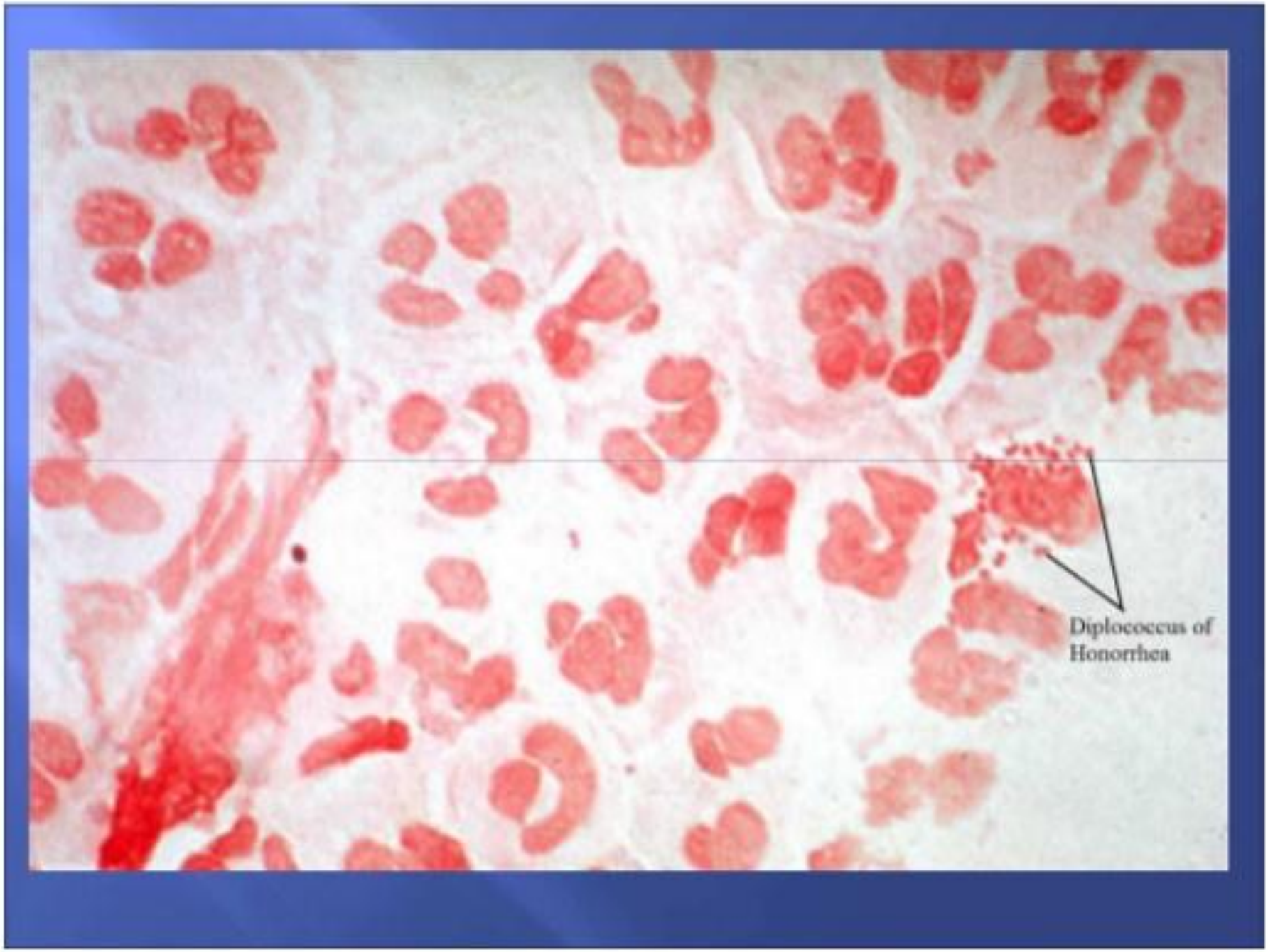
VIRULENCE FACTORS

- **Fimbrae** (common pili)-
- **Lipooligosaccharide**:
- **Capsule**
- **Cell membrane proteins**
- **IgA protease-**



Hemorrhage in the adrenal glands in Waterhouse-Fridericksen syndrome

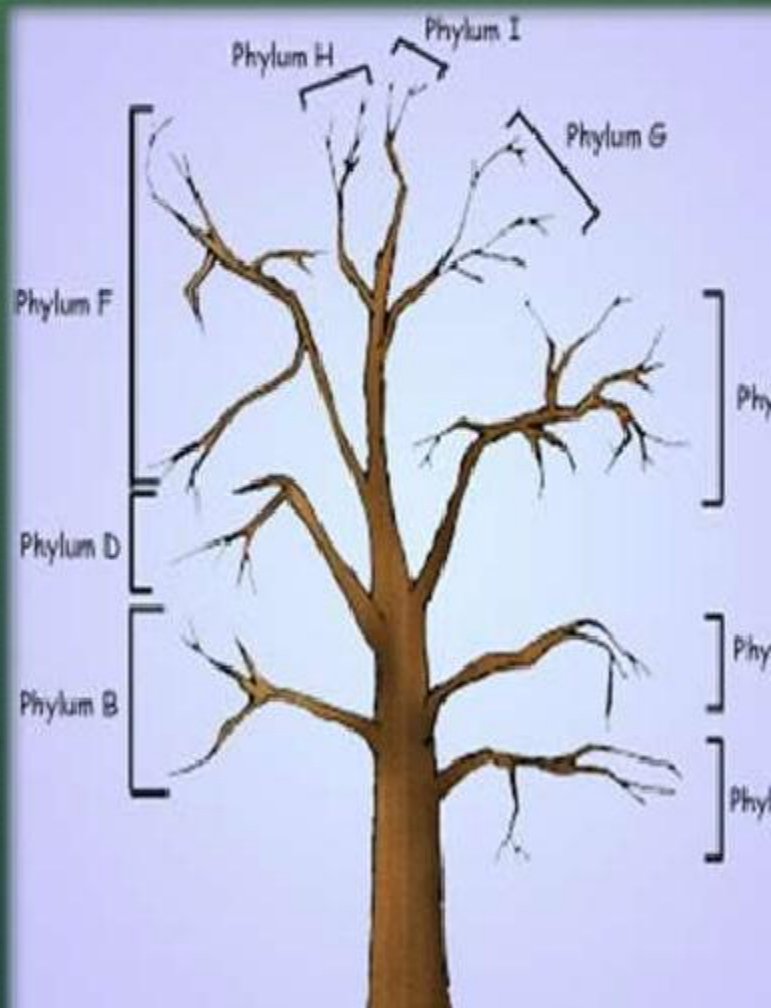
Meningococcal disease is favoured by defieciency of the terminal complement components (C_5-C_9)


Diplococcus of
Honorrhea

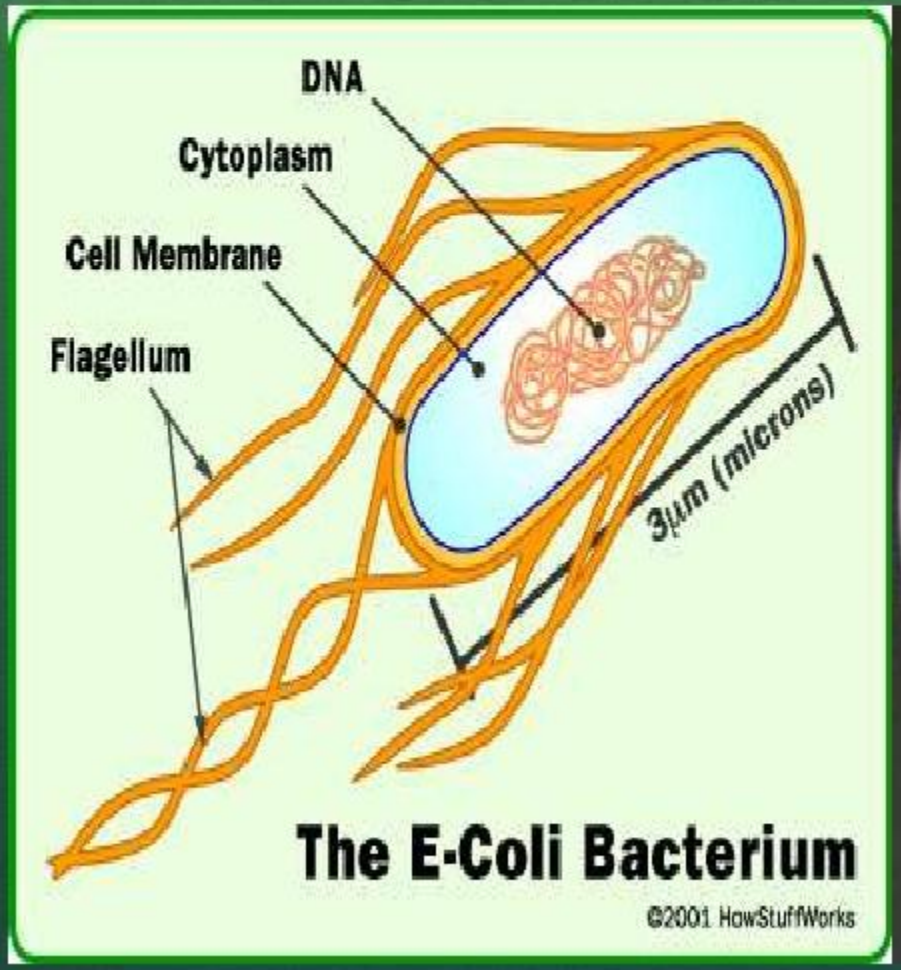
Meningococcemia showing striking involvement of the extremities with relative sparing of the skin of the child's body surface.

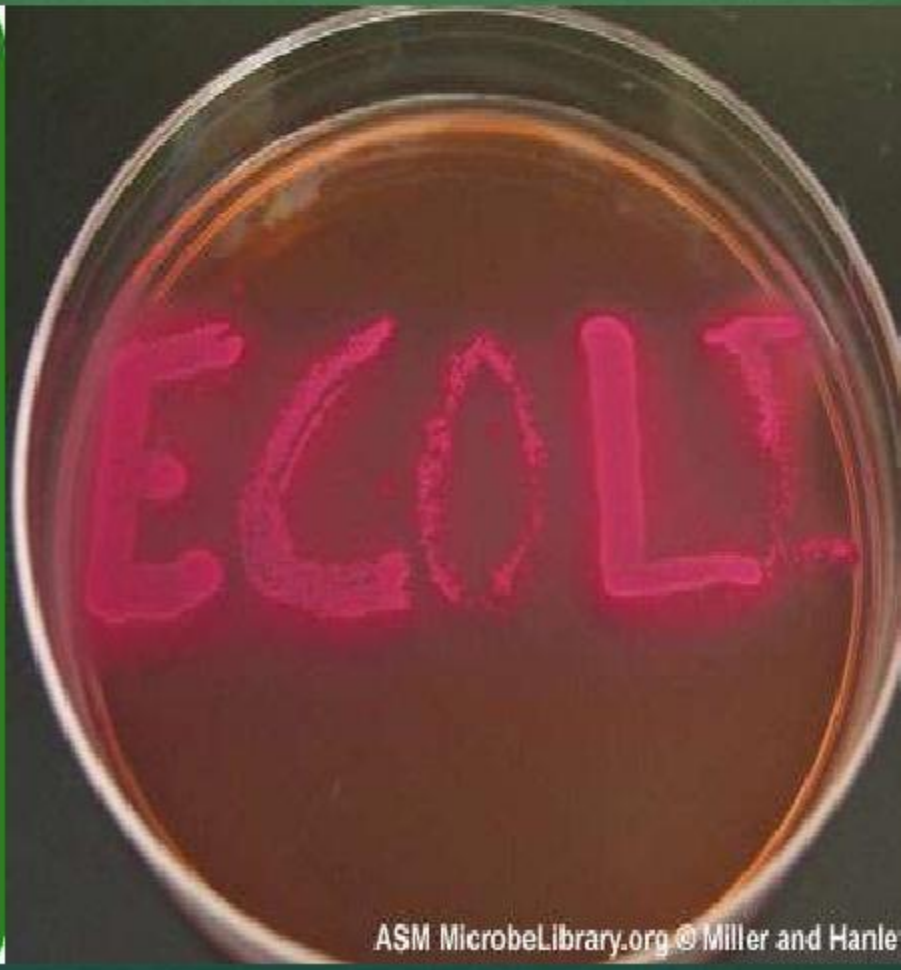
Classification

- Domain: Bacteria
- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class: Gamma Proteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: Escherichia
- Species: Escherichia coli (E. coli)



E.coli


DNA
Cytoplasm
Cell Membrane
Flagellum
3µm (microns)
The E-Coli Bacterium
©2001 HowStuffWorks


E.COLI
ASM MicrobeLibrary.org © Miller and Hanle

Identification of *Enterobacteriaceae* Biochemical reactions

- Oxidase test
  - **All members of *Enterobacteriaceae* are oxidase negative**
  - ***Pseudomonas* is oxidase positive**
- O/F test
  - **All members of *Enterobacteriaceae* are O+/F+**
  - ***Pseudomonas* is O+/F-**
- Nitrate reductase
  - All members of Enterobacteriaceae are nitrate reductase positive
  - *Pseudomonas* is nitrate reductase negative

E.coli
Biochemical Characters,

Glucose,Lactose,Mannitol,Maltose

fermented. with A/G

I,M,Vi,C tests.

Indole +

Methyl Red +

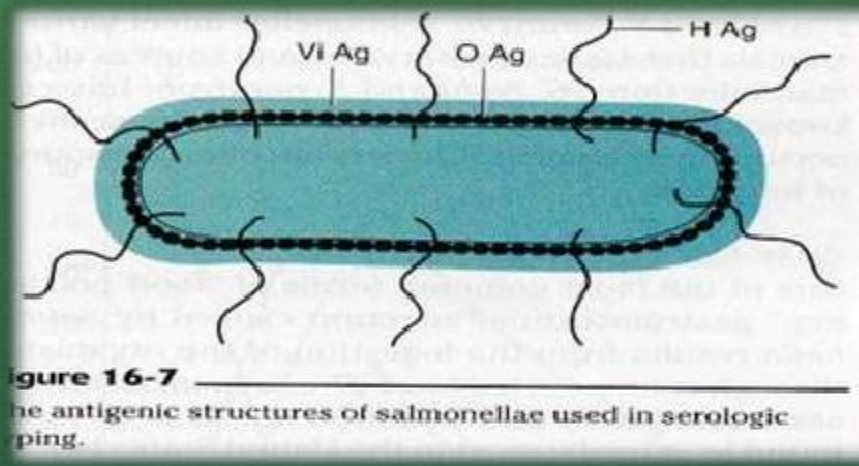
Voges Proskauer – ve I,M,Vi,C tests.

Citrate –ve

Urease not produced.

E.coli
Antigenic Structure

- Somatic 0 170
- Capsular K 100
- Flagella H 75
- Virulence factors



igure 16-7
he antigenic structures of salmonellae used in serologic
ping.

Surface Antigens Toxins

O Endotoxic activity

K protects against the phagocytosis

Fimbriae promote virulence (important in UTI)

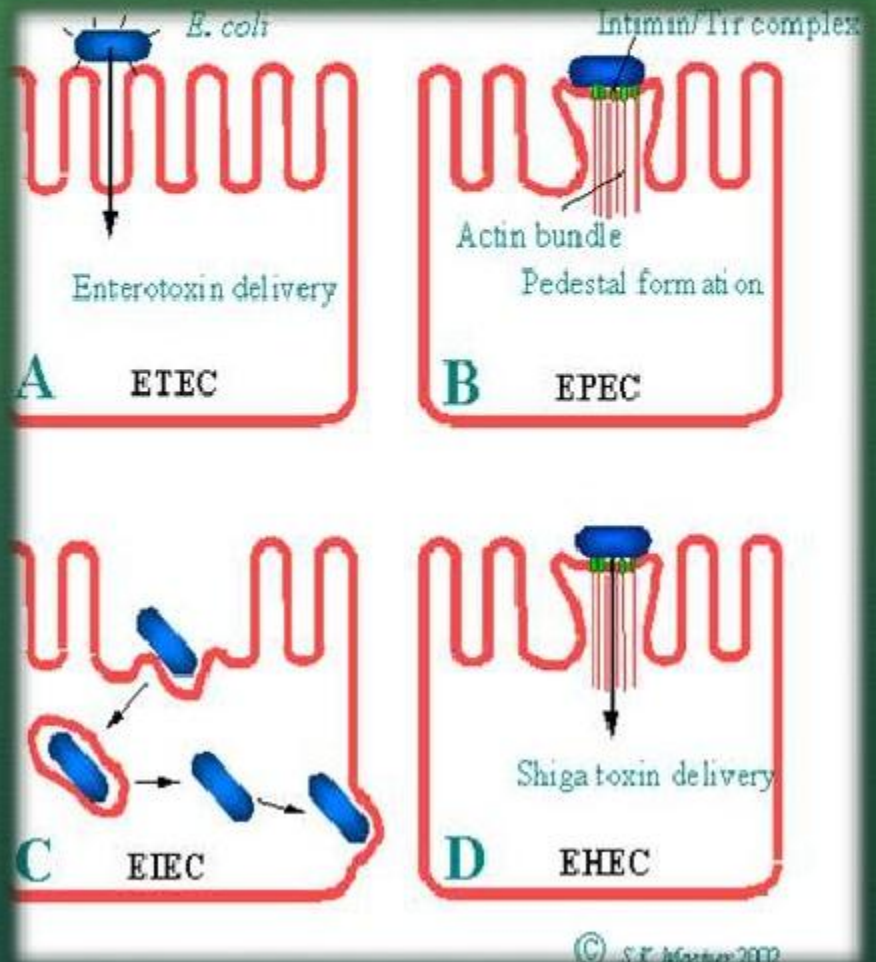
Toxins and E.coli

- E.coli produce Exotoxins
- Hemolysins, Enterotoxins causes Diarrheas,
- Important toxins produces.
- Heat labile HL Heat stable HS

Vero toxins VT Like Shigella toxins

Mechanism of action of Toxins

- Increased cAMP alters the activity of sodium and chloride transporters producing an ion imbalance that results in fluid transport into the bowel



Classification of E.coli

1. Enteropathogenic EPEC
2. Enterotoxigenic ETEC
3. Enteroinvasive EIEC
4. Enterohemorrhagic EHEC
5. Enteroaggresive EAEC

Enteropathogenic E.coli

- Causes diarrheal disease in children,
- EPEC O26/O11
- Produce Verocytotoxin
- Infantile enteritis, Involves upper part of Intestine
- Brush border of the intestine is lost
- Intimacin – EPEC adhesion factor.
- Frequent in summer months
- Poor hygiene predisposes.

Enterotoxigenic E.coli

- Causes travelers diarrhea
- Water contaminated with Human and Animal feces predisposes.
- Laboratory Diagnosis

Demonstration of Enterotoxin LT and ST

Tissue culture tests,

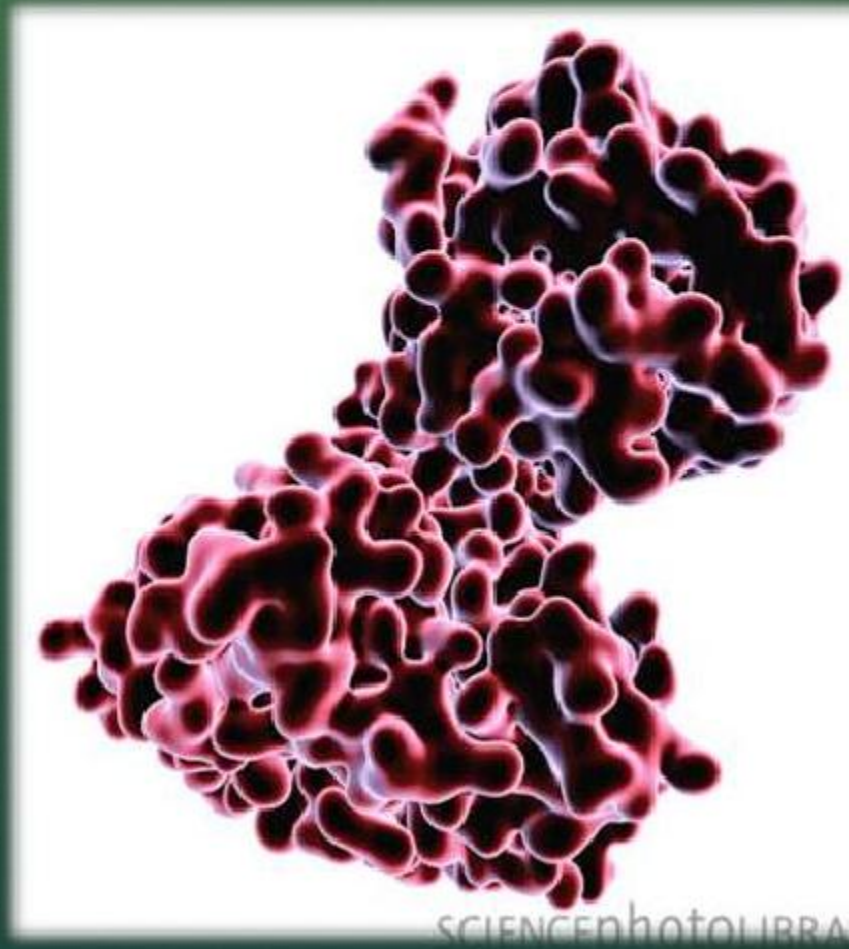
ELISA

Passive agglutination tests.

Animal experiments in Rabbit ileal loop test.

Entero invasive E.coli

- Some are non motile strains,
- Atypical resembles like Shigella.
- Clinically mild diarrhea
- Sereny test positive animal Rabbit.
- ELISA



EHEC (contd)

- Culture
- DNA detection methods.
- Cytotoxic effects on Vero cells.
- Detection with monovalent sera O157/H7

Enterohemorrhagic bacteria Escherichia coli (EHEC)

Most Escherichia coli (E.coli) strains are harmless.

But some, like enterohemorrhagic **E. coli (EHEC)**, are a hazard to human health and life.

Incubation period:

three to eight days

E. coli (EHEC), once in th human stomach, begins p ing toxins that cause serio illnesses

Symptoms caused by E. coli (EHEC)

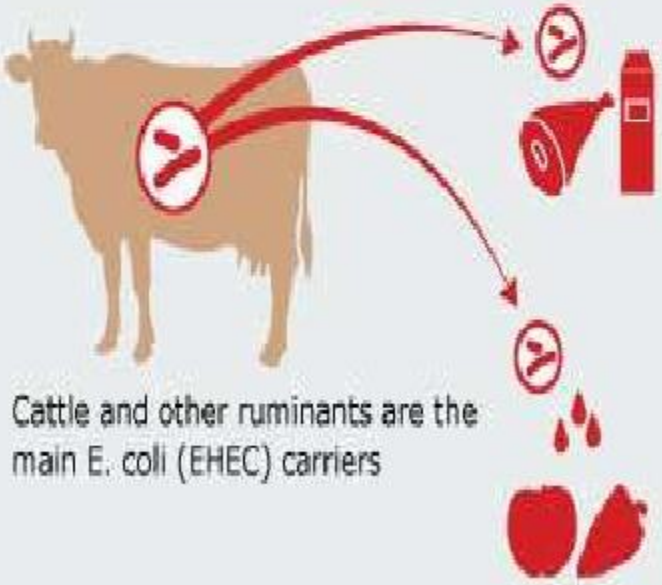
- Stomach muscle spasm
- Diarrhea (sometimes bl diarrhea)
- Fever
- Vomiting

Complications:

hemolytic uremic syndrom

Death rate: 3-5 %

INFECTION SOURCES



Cattle and other ruminants are the main E. coli (EHEC) carriers

Uncooked meat and raw milk

The bacteria die when food is exposed to heat (70°C and higher)

Fruit and vegetables (droppings of sick animals find their way into water bodies that in turn feed the soil)

Enteroaggresive E.coli EAEC

- Can cause Diarrhea Detect by Culture methods
- Brick-like aggregates on cell surfaces
- Mucus biofilm inhibits fluid absorption
- Diarrhea
- Detection of Enterotoxin

Culturing for E.coli

- Mid stream sample/semi quantitative culturing (Kass et al) >_ 1.00,000/ml of urine. (significant Bacteriuria)
- Urine should not be kept in wards for > 2 hours and to be preserved at 4 c
- Culture by standard loop method.
- Fixed volume cultured on MacConkey agar Lactose fermenters I M Vi C
- Antibiotic sensitivity tested

Taxonomy :

| Domain = Bacteria
| Phylum = Proteobacteria
| Class = Gammaproteobacteria
| Order = Enterobacteriales
| Family = Enterobacteriaceae
| Genus = *Klebsiella*
| Species =k.pneumonia , k.ozaenae
, k.rhinoscleromatis.

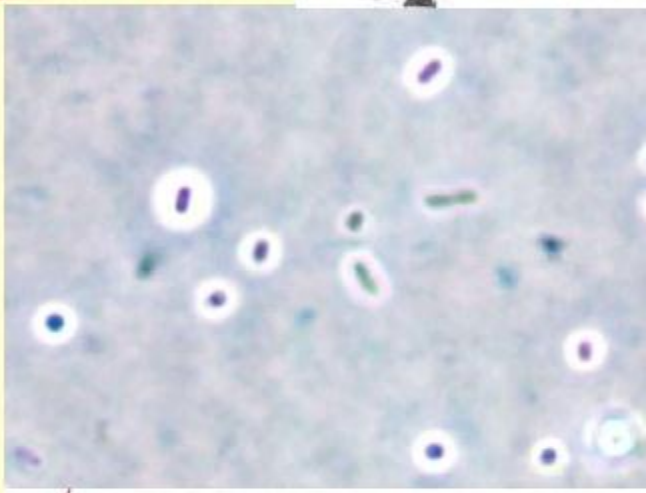
Klebsiella pneumoniae

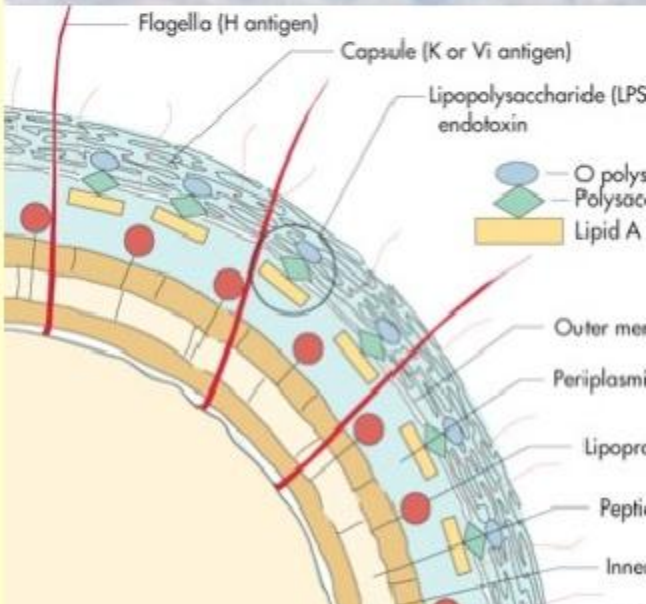
Characteristics:

1. gram-negative
2. Non motile
3. Lactose fermenting
4. Oxidase negative
5. Rod shaped organism
6. Facultative anaerobe
7. Surrounded by thick capsule
8. Act as oppurtunistic human pathogen

Antigenic structure

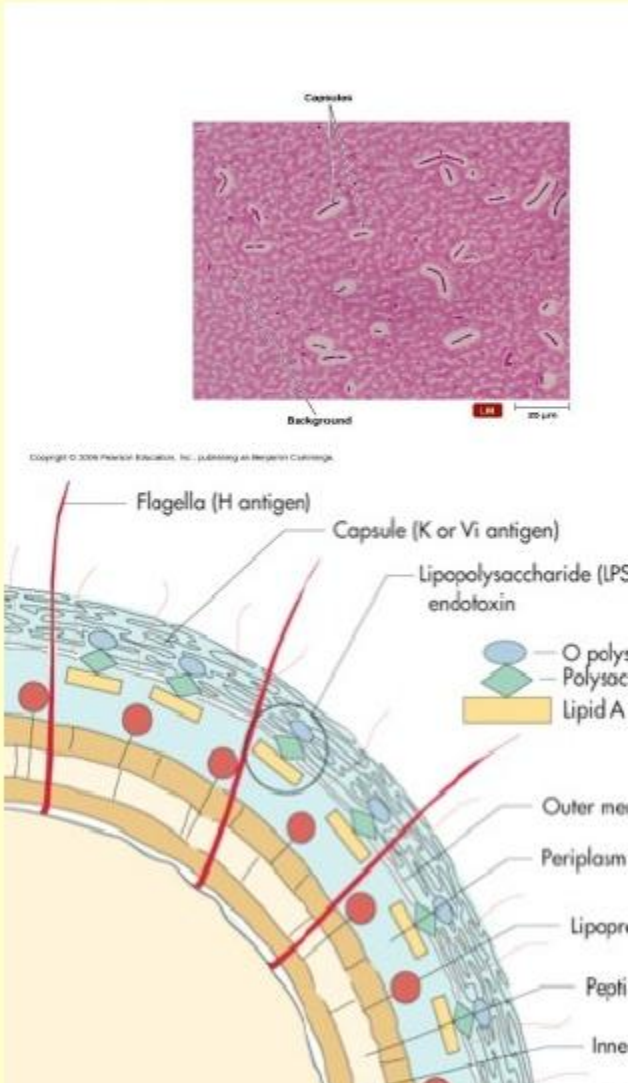
- 80 Capsular (K) antigens
  - Gram stain
  - Capsular ‘swelling’ reactiion
  - CCIE
  - ELISA
- 5 Somatic (O) antigens



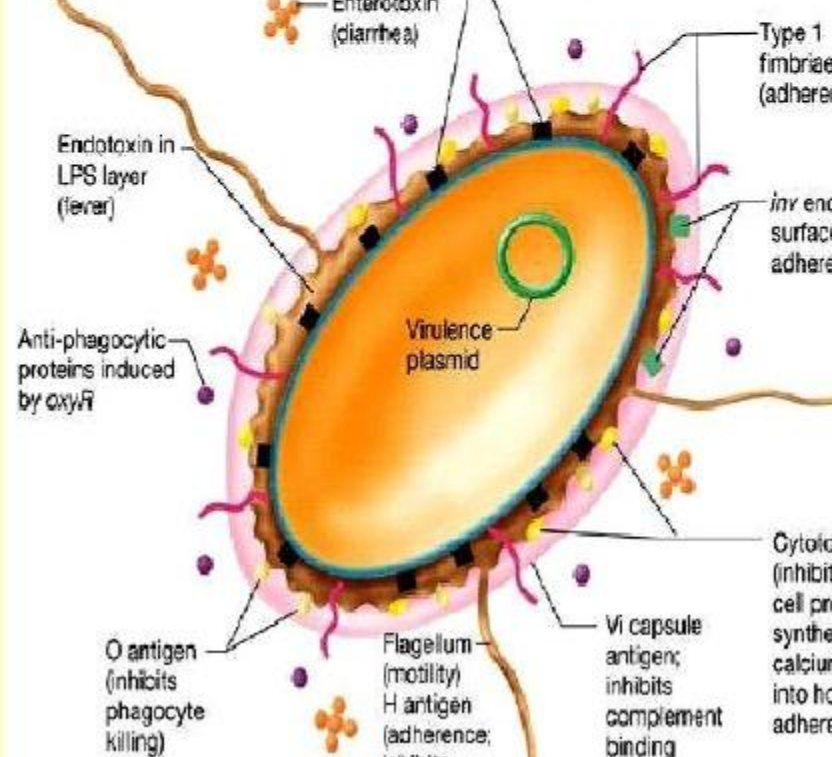


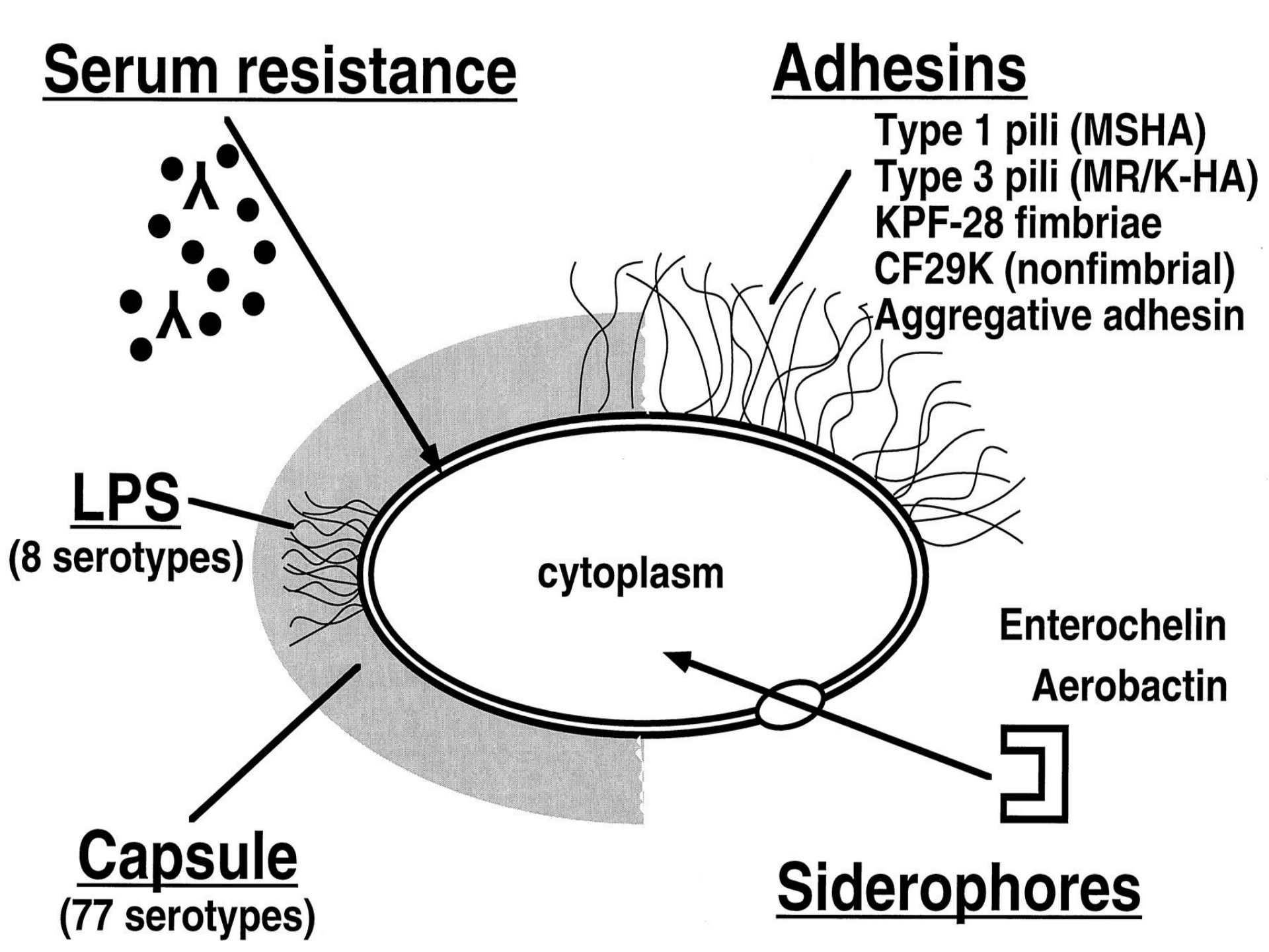
Virulence factors and Pathogenesis

- Capsule
  - Anti phagocytic
  - Prevents from complement mediated bacteriolysis
- LPS
  - Prevent from complement mediated bacteriolysis
- Adhesins (Fimbrial and non-fimbrial)
  - Type-I and Type-III
  - Adhesion to host tissues



- Toxins
  - Heat labile and heat stable toxins
  - Role not well defined
- Enzymes
  - β-lactamase and ESBL




Serum resistance
Adhesins
Type 1 pili (MSHA)
Type 3 pili (MR/K-HA)
KPF-28 fimbriae
CF29K (nonfimbrial)
Aggregative adhesin
LPS
(8 serotypes)
cytoplasm
Enterochelin
Aerobactin
Capsule
(77 serotypes)
Siderophores

Where it is found?

1)Found in the normal flora of the nose, mouth, skin, GI tract and intestines.
2)It is also found in soil and water.

Generally, Klebsiella infections are seen mostly in people with a weakened immune system.

Diseases Caused by Klebsiella :

1) urinary tract infections
2)pneumonia
3) Specticaemia
4) nosocomial infections
5) soft tissue infections.

Some Klebsiella bacteria have become highly resistant to antibiotics.

Klebsiella pneumoniae produce an enzyme known as a carbapenemase (referred to as KPC-producing organisms).

APPEARANCE OF KLEBSIELLA ON CULTURE MEDIA

On blood agar

-slimy appearance of the colonies

On MacConkey agar

- red/pink colonies

Klebsiella pneumoniae and *Salmonella enterica* on MacConkey agar:
lactose + and -

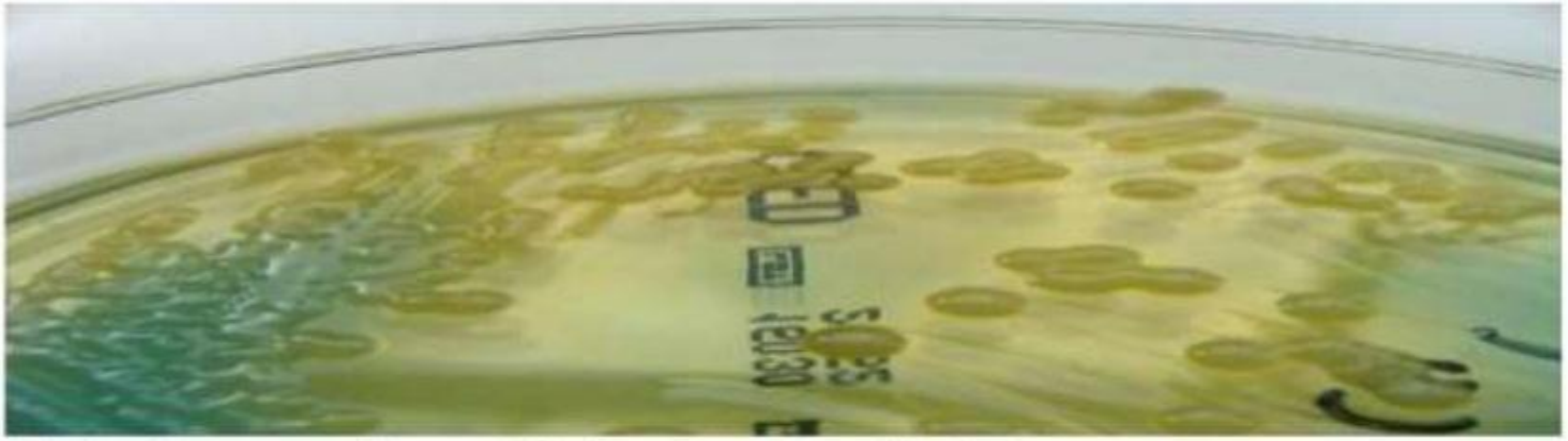
Mucous, lactose positive colonies of *Klebsiella pneumoniae* on MacConkey agar. Cultivation 37°C, 24 hours.

On EMB

- *Klebsiella* species produces large, mucoid, pink to purple colonies with no metallic green sheen on EMB agar.

On CLED AGAR

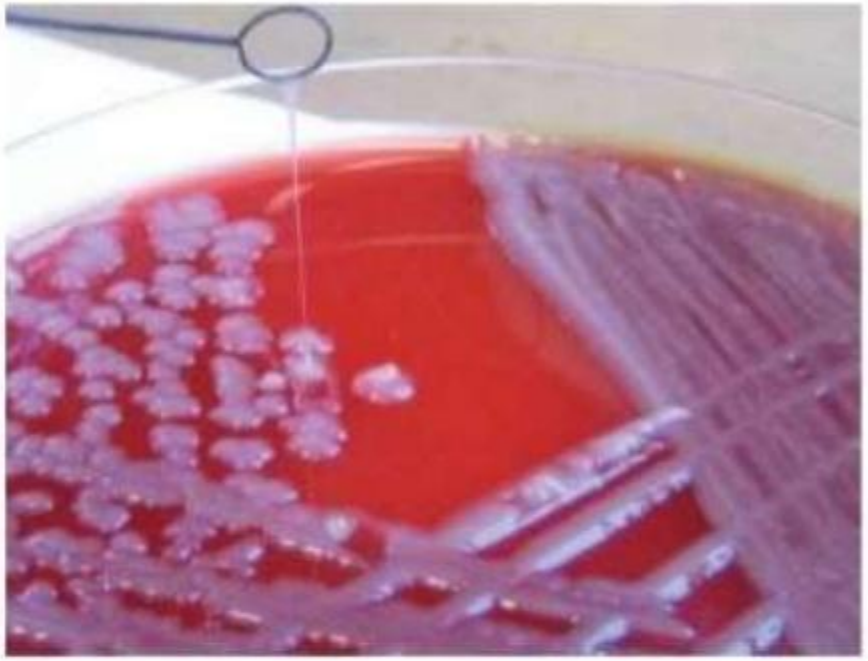
- This medium supports the growth of urinary pathogens and provides distinct colony morphology.



Klebsiella pneumoniae on CLED Agar. Large, mucoid colonies.
Bromothymol blue indicator in the agar changes to yellow due to acidification of the medium due to lactose fermentation by bacterial growth.
Lactose fermenters appear yellow. Non Lactose fermenters remain a translucent blue.

String test

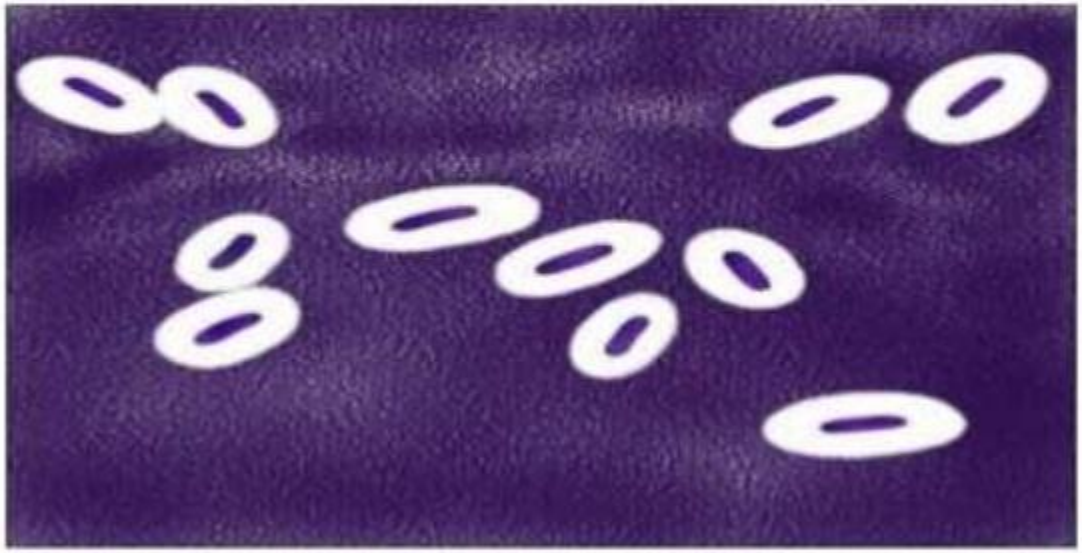
- A colony that stretches more than 5 mm using a standard inoculation loop tests positive for hypermucoviscosity.



Mucoid colony of *Klebsiella pneumoniae* . When colonies were touched with a loop and the loop lifted vertically from the surface of the agar plate, mucoid isolates adhered to the loop as it was lifted from the plate .

India ink capsule stain

-The background will be dark.
-The bacterial cells will be stained purple.
-The capsule (if present) will appear clear against the dark background.



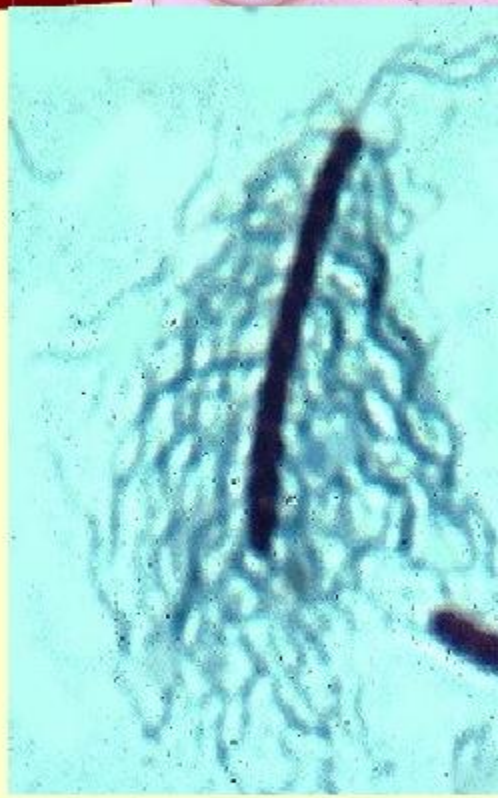
India Ink Capsule Stain of *Klebsiella pneumoniae* showing white capsules (Glycocalyx) surrounding purple cells

IMViC Reactions

	I	M	Vi	C
Escherichia coli	+	+	–	–
Proteus vulgaris	+	+	–	–
Klebsiella pneumoniae	–	–	+	+
Klebsiella oxytoca	+	–	+	+
Enterobacter spp.	–	–	+	+
Serratia marcescens	–	–	+	+
Citrobacter freundii	–	+	–	+
Citrobacter koseri	+	+	–	+

Species of Proteus

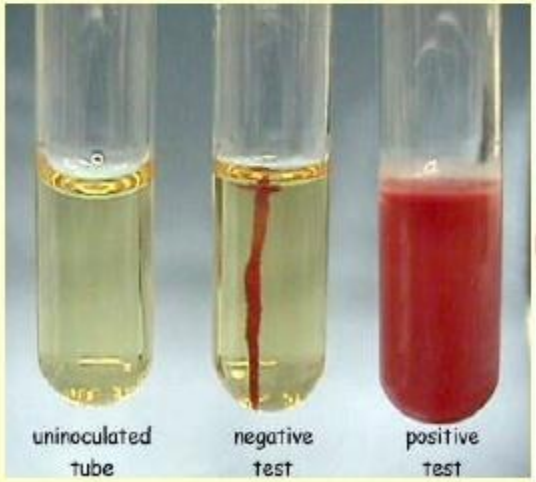
- ***Proteus mirabilis***
- ***Proteus vulgaris***
- *Proteus myxofaciens*
- *Proteus penneri*



Virulence Factors

- Urease activity
- Protease
- Fimbriae
- Haemolysins
- Motility
- Swarming

Motility test



Pathogenicity

- *P. mirabilis* -70-90 %
- UTI – Commonest site
  - Young / elderly patients
  - High concentration of Urea in urine
- Superficial septic lesions
- Meningitis
- Osteomyelitis
- Septicemia
- Otitis media

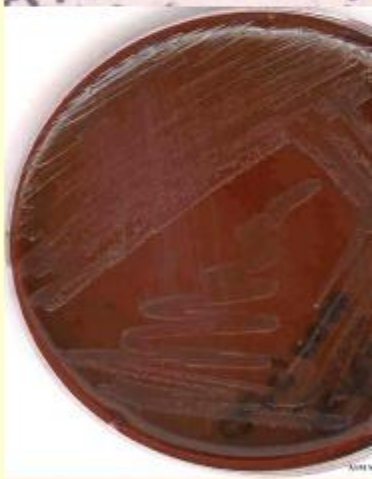
Lab Isolation and Identification

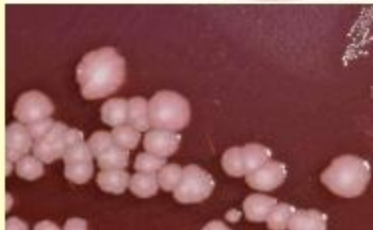
Morphology

- GNR, 1 – 3 um
- Motile-peritrichate flagella

Cultural Characteristics

- Grow well on ordinary media
- Swarming
  - Continuous
  - Discontinuous
- Faint ammonia / fishy odor





Swarming

- Characteristic but not unique
  - *Serratia marcescens*
  - *Vibrio parahaemolyticus*
  - Bacillus
- Continuous swarming
- Discontinuous swarming
- Ascending infection

Anti-swarming Agents

- Increasing Agar concentration 3-4 %
- Incorporation into media of a polyvalent-H anti-sera
- Incorporating growth inhibitors
  - Sulphonamides
  - Chloral Hydrate
  - p-Nitrophenyl Glycerol
  - Neomycin
  - Barbiturates
- Incorporation of
  - Detergents
  - Bile Salts-MacConkey Agar
- Electrolyte Deficiency- CLED

Genus Clostridium

- In Anaerobic spore bearing Gram positive bacilli Spores are wider than the body giving spindle shape
- The name derived from word **Kolster meaning spindle**

Clostridium

***C. perfringens*: gas gangrene; food poisoning**

***C. tetani*: tetanus**

***C. botulinum*: botulism**

***C. difficile*: pseudomembranous colitis**

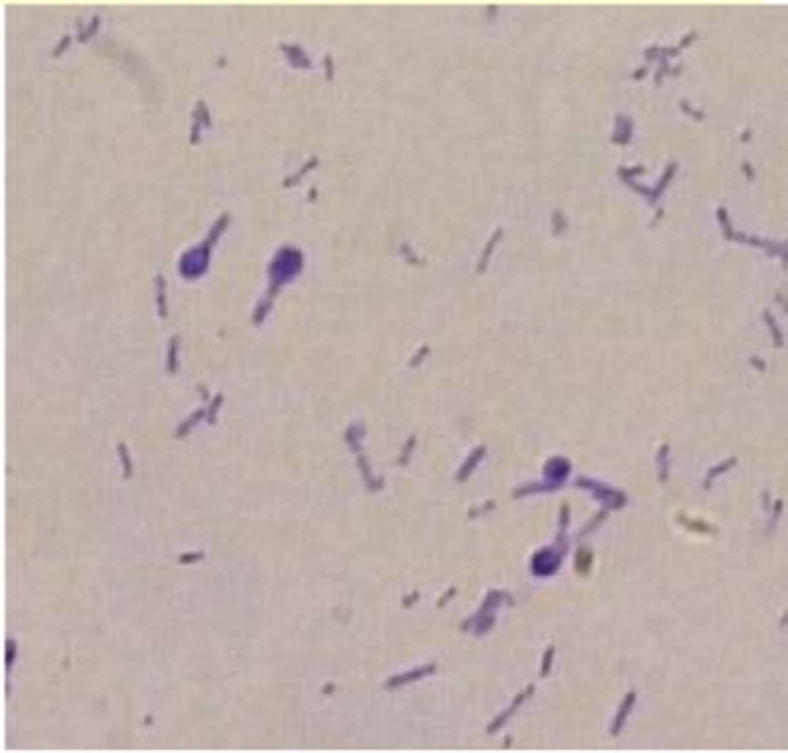
Physiology and Structure

Anaerobic.

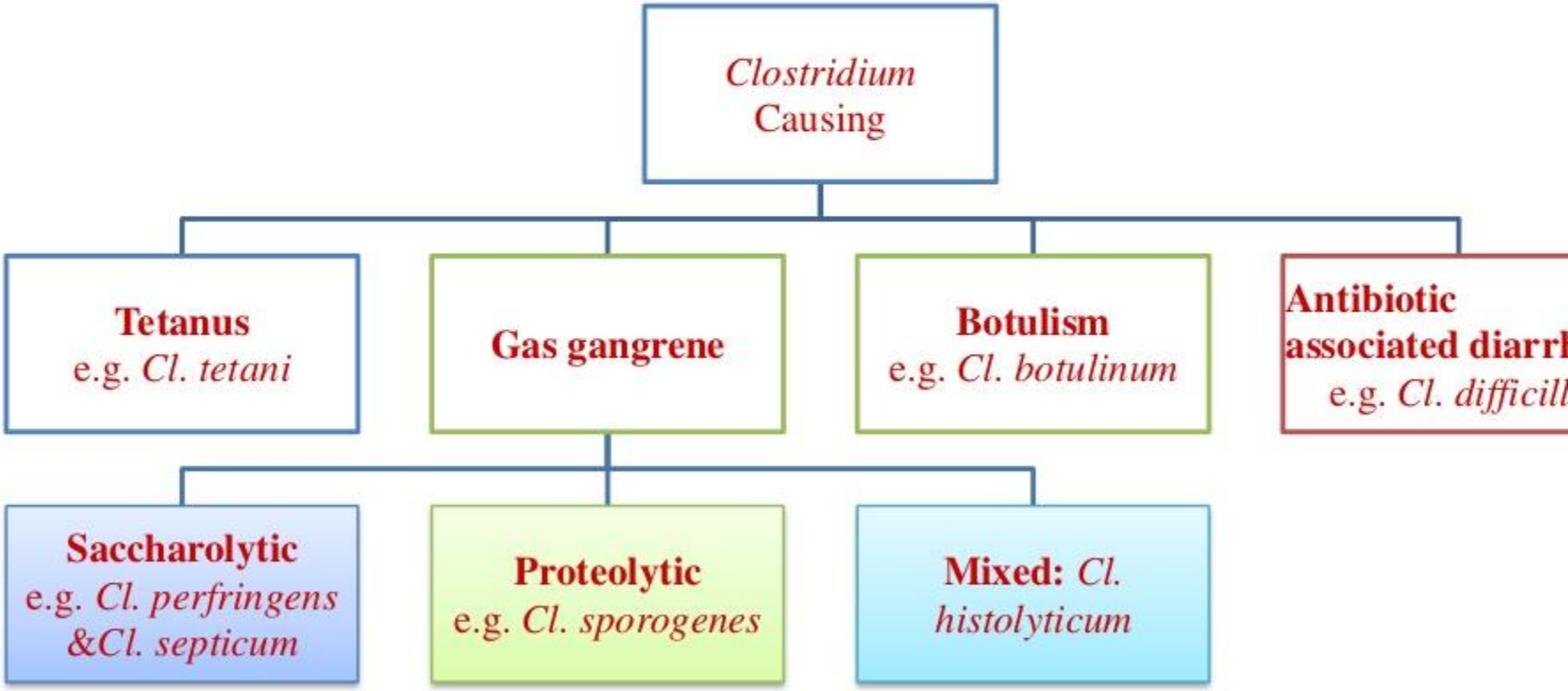
Large gram-positive rods.

The spores are usually wider than the rods, and are located terminally or sub terminally.

Most clostridia are motile by peritrichous flagella.

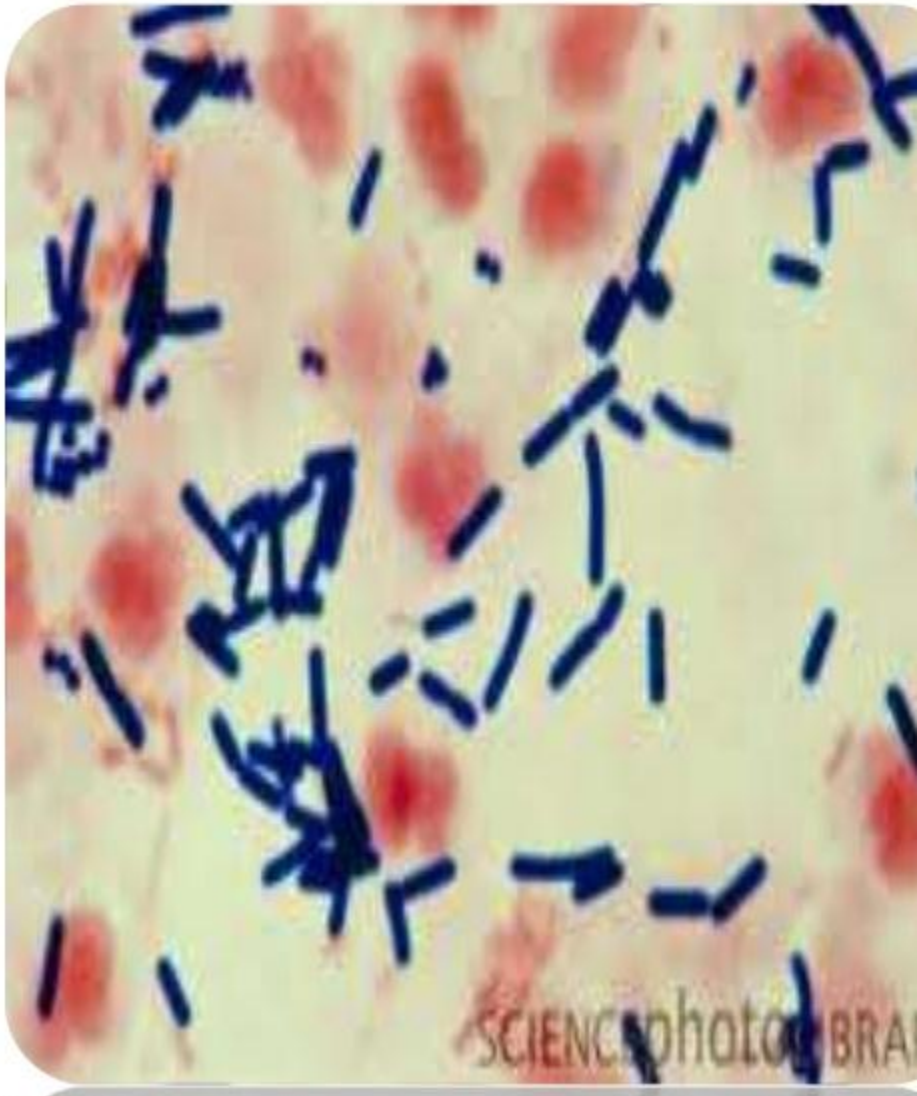


Clostridia of medical importanc



How they appear in Gram staining

- They are Gram positive, but may appear to be Gram negative. All produce spores, which enable the organisms to survive in adverse conditions, for example in soil and dust and on skin.



Clostridium perfringens

- Large Gram-positive bacilli with stubby ends
- **Capsulated**
- **Non motile** Anaerobic
- Grown quickly on selective media
- **Can be identified by** Nagler reaction

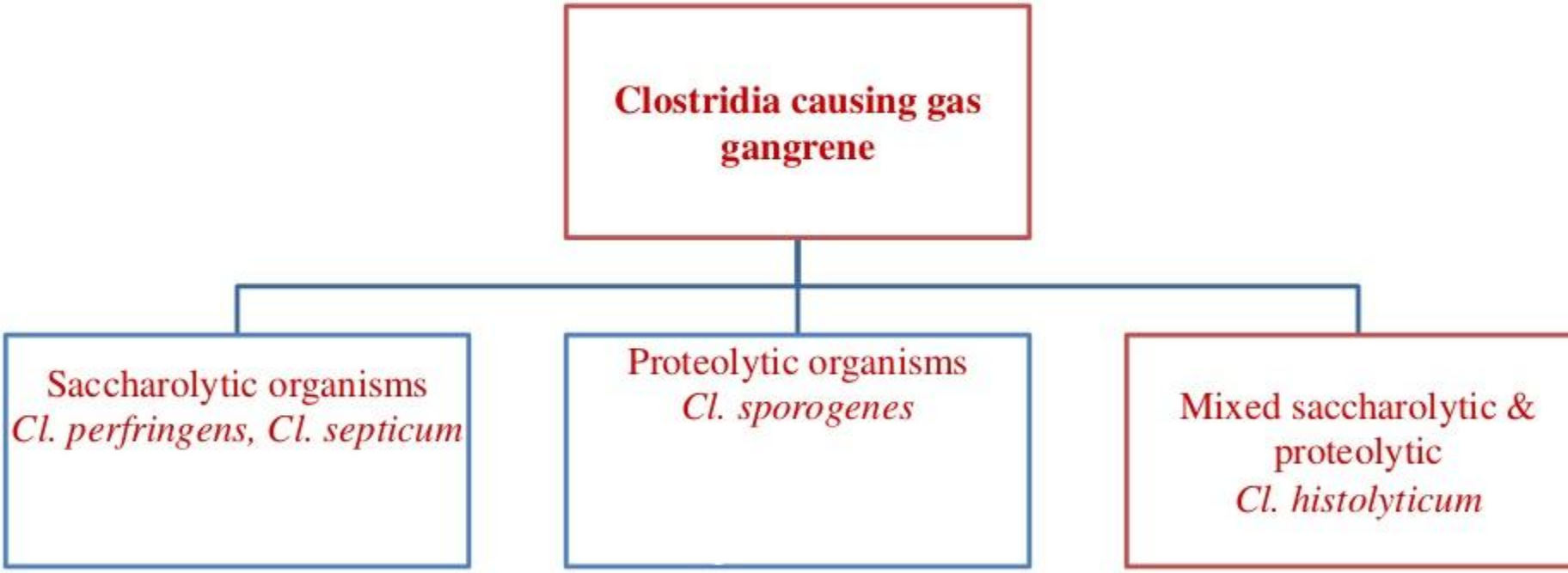
Some Clostridia Produce Gas gangrene

- The organisms associated **with gas gangrene** attack soft tissues by producing toxins and aggressins, and are referred to as histotoxic. C. difficile and some strains of **C. perfringens** produce enterotoxins.

C. perfringens

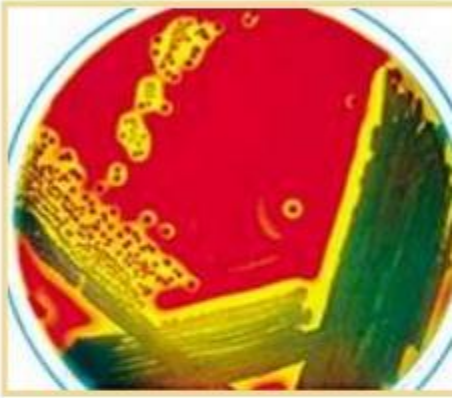
- C. perfringens is a relatively large Gram-positive bacillus (about 4-6 × 1 μm) with blunt ends. It is capsulate and non-motile. It grows quickly on laboratory media, particularly at high temperatures (approximately 42°C), when the doubling time can be as short as 8 min. I

Clostridium Causing Gas Gangren



The Agent

- **Clostridium perfringens**
  - Gram-positive bacteria
    - Anaerobic rod
    - 3-8 x 0.4 – 1.2 mu
  - Found in soil, decaying matter and intestinal tract of mammals
- 5 types (A-E)
  - Types B and D produce the epsilon toxin



Micro & Macroscopic C. perfringen

NOTE: Large rectangular gram-positive bacilli

NOTE: Double zone of hemoly

Inner beta-hemolysis = θ to

Resistance

- Vegetative bacteria is killed like other bacteria
- Cl.perfringens destroyed by boiling
- Cl, botulinum not killed even at 105 c 0 for less than 100 minutes
- All spores are killed at 121 oc in 20 minutes
- Halogens , Glutaraldehyde are effective on spores
- Metronidazole and Pencillin and Chloramphenicol are effective

How Clostridia are Cultivated

- Clostridia grow well on ordinary medium under anaerobic medium

Media used for Cultivation

- Liquid medium for cultivation cooked meat broth
- Thiglyclolate broth
- CMB contain unsaturated fatty acids which take up oxygen
- Proteolytic medium turns the medium black and Saccharolytic medium turn the meat pink

Virulence Factors

- Virulence factors
  - –toxins –
    - alpha toxin – causes RBC rupture, edema and tissue destruction
  - –collagenase
  - –Hyaluronidase
  - –DNase

Toxins

- **The toxins of *Cl. perfringens***
  - **α toxin** (phospholipase C, lecithinase) is the most important toxin
    - Lyses of RBCs, platelets, leucocytes and endothelial cells
    - Increased vascular permeability with massive hemolysis and bleeding tissue destruction
    - Hepatic toxicity and myocardial dysfunction
  - **β-toxin** is responsible for necrotic lesions in necrotizing enterocolitis
  - **Enterotoxin** is heat labile toxin produced in colon → food poisoning

C. perfringens

Clinical Diseases

Gas gangrene

Spores germinate vegetative cells multiply, ferment carbohydrates and produce gas in the tissue. This results in distension of tissue and interference with blood supply the bacteria produce necrotizing toxin and Hyaluronidase, which favor the spread of infection tissue necrosis extends, resulting in increased bacterial growth, hemolytic anemia, then severe toxemia and death.

Incubation: 1-7 days after infection.

Symptoms: Crepitation in the subcutaneous tissue and muscle, foul smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, renal failure, and death.

Can be also caused by other *Clostridium* species.

Clostridial Cellulitis

C. perfringens

Laboratory Diagnosis

Specimens: pus, necrotic tissue, feces, food, etc.

Smears: large gram-positive rods with or without spores, usually in the absence of leukocytes.

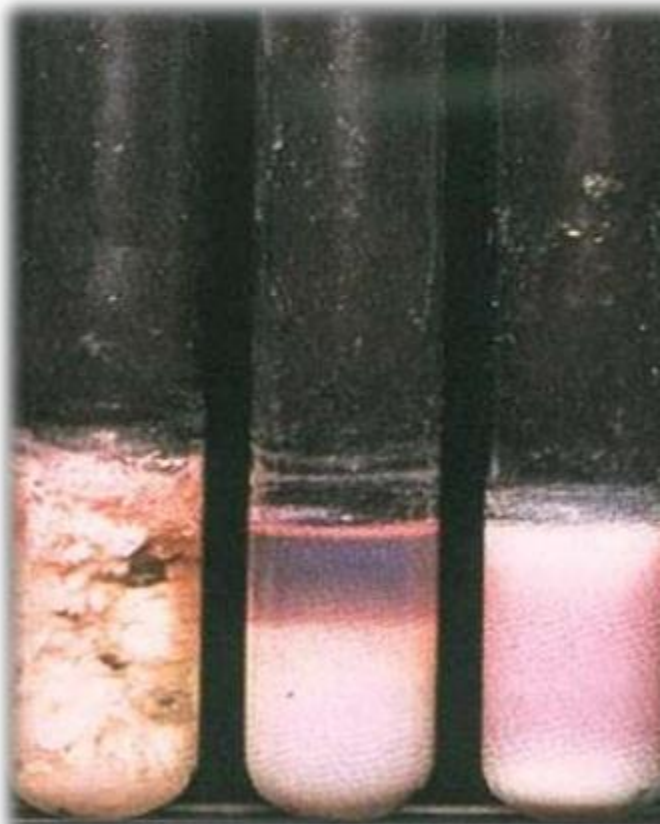
Culture: anaerobic culture on blood plate.

Identification:

"Storming fermentation"-- clot torn by gas in 24 hrs.

Lecithinase test-- precipitate formed around colonies on egg yolk media.

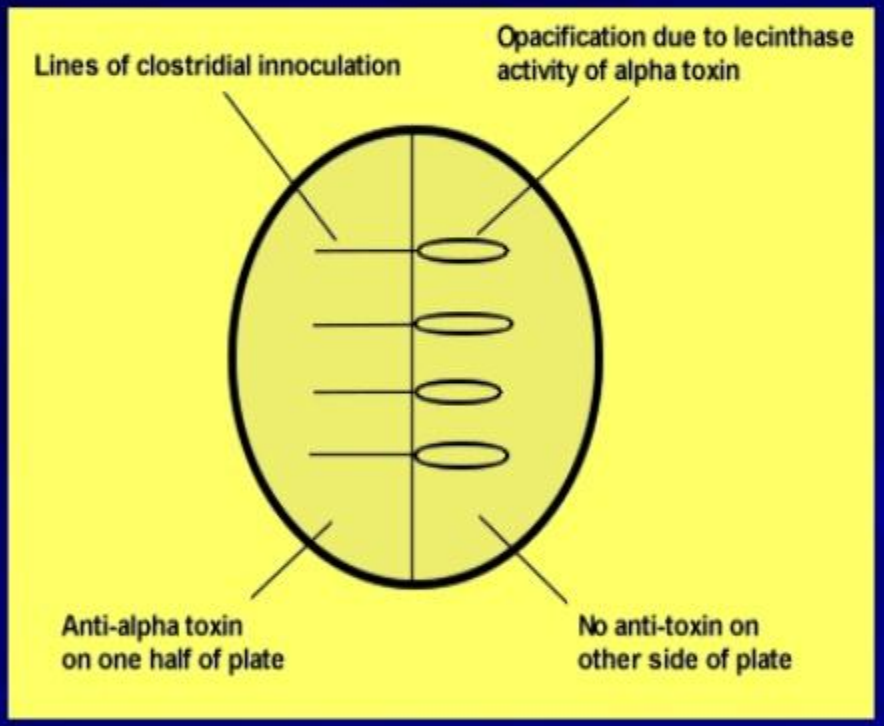
Biochemical tests.



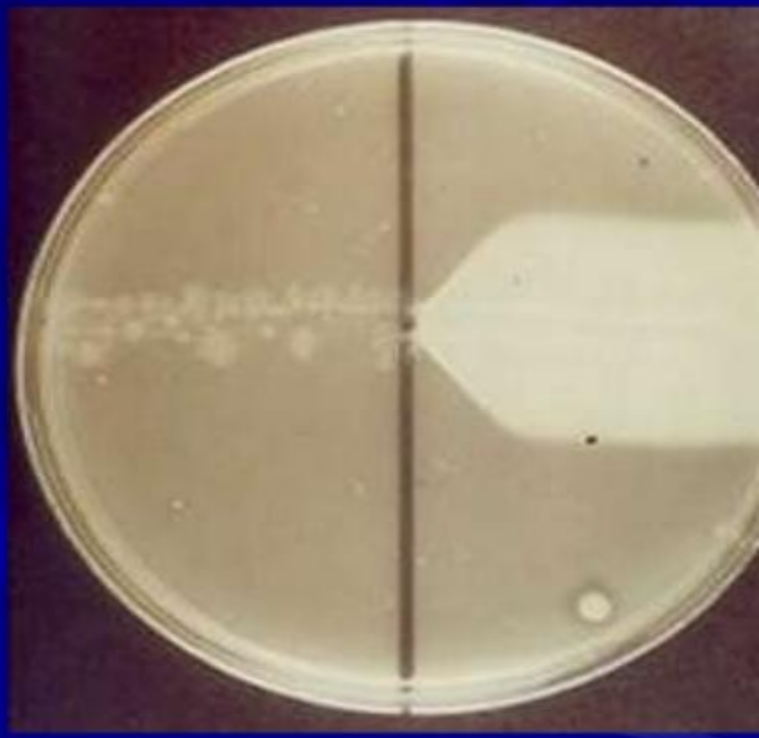
C. perfringens Nagler Reaction

NOTE: Lecithinase (α-toxin; phospholipase) hydrolyzes phospholipids in egg-yolk agar around streak on right. Antibody against α-toxin inhibits activity around left streak.

Nagler Reaction



Procedure of Nagler Reaction



Positive Nagler Reaction

Biochemical Tests

- *Cl. perfringnes* characterized by:
  - ➢ It ferments many carbohydrates with acid & gas
  - ➢ It acidified litmus milk with stormy clot production
  - ➢ Nagler reaction is positive

Clostridium tetani

Anaerobic bacteria of the genus species *Clostridium* it is gram positive, slender bacillus and it has spherical terminal spores giving drum stick appearance

It is non capsulated & motile with peritrichus flagella

It produces a potent biological toxin, tetanospasmin, and is the causative agent of tetanus a disease characterized by painful muscular spasms that can lead to respiratory failure and, in up to 40% of cases, death.

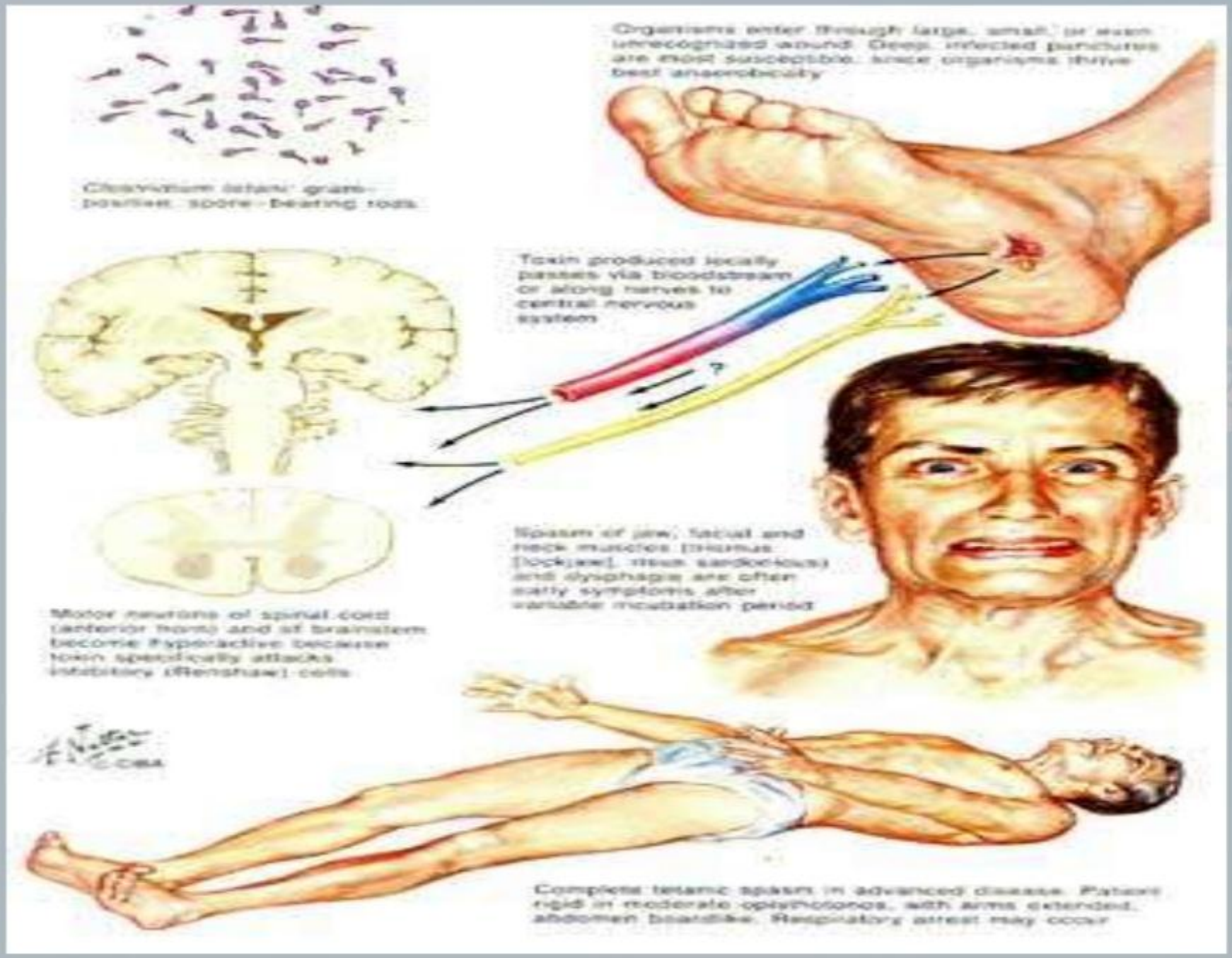
Cl. Tetani

- Soil and GIT
- Terminal spores – drumstick appearance
- Non capsulated, motile except type VI
- Grows on BA,NA,CMB, Thioglycoslate broth
- CMB- Black, BA – Swarming,
- Iridescence – greenish fluorescence on MA
- No sugars fermented

What is Tetanus?

- An infectious disease caused by contamination of wounds from the bacteria ***Clostridium tetani***, or the spores they produce that live in the soil, and animal feces

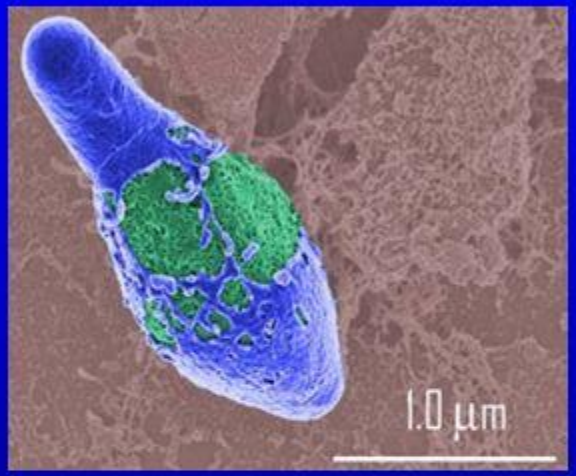
- Infection follows **when spores**
- **become activated and develop**
- into gram-positive bacteria that multiply
- and produce a very powerful toxin (**tetanospasmin**) that affects the muscles.


Clostridium tetani: gram-positive, spore-bearing rods
Organisms enter through large, small, or even unrecognized wound. Deep, infected punctures are most susceptible, since organisms thrive best anaerobically
Toxin produced locally passes via bloodstream or along nerves to central nervous system
Motor neurons of spinal cord (anterior horn) and of brainstem become hyperactive because toxin specifically attacks inhibitory (Renshaw) cells
Spasm of jaw, facial and neck muscles (trismus [lockjaw], risus sardonicus) and dysphagia are often early symptoms after variable incubation period
Complete tetanic spasm in advanced disease. Patient rigid in moderate opisthotonos, with arms extended, abdomen boardlike. Respiratory arrest may occur

Cl. Botulinum

- Botulus = sausages
- Soil, manure, vegetables
- Strict aerobes grow on ordinary media
- Large fimbriate irregular colonies
- GPB with sub-terminal highly resistant spores
- Classification based on toxin produced – 8 types A (most toxic), B, C 1- 3, D, E, F, G

Bacterium of the day: *Clostridium botulinum*



CLOSTRIDIUM BOTULINUM

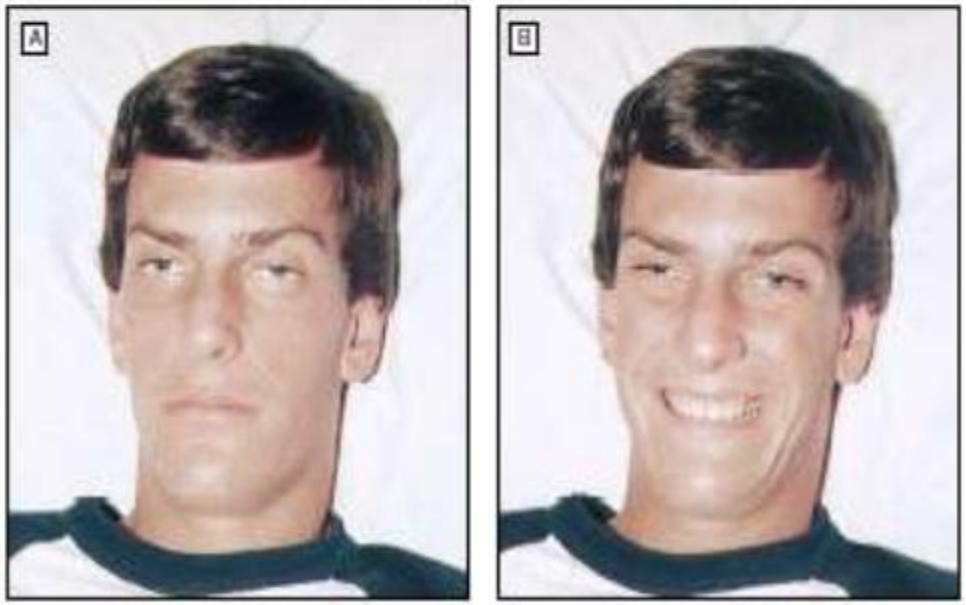
Clostridium botulinum is an anaerobic, Gram-positive, spore-forming rod that produces a potent neurotoxin. The spores are heat-resistant and can survive in foods that are incorrectly or minimally processed.

Foodborne botulism is a severe type of food poisoning caused by the ingestion of foods containing the potent neurotoxin formed during growth of the organism. The toxin is heat labile and can be destroyed if heated at 80°C for 10 minutes or longer .The disease is of considerable concern because of its high mortality rate if not treated immediately and properly. It can happen with inadequately processed, home-canned foods, in meat products, sausages, canned vegetables and seafood productc.

Signs of Food-borne and Wound Botulism

- **Ventilatory (respiratory) problems**
- **Eye muscle paresis/paralysis (extra ocular, eyelid)**
- **Dry mucous membranes in mouth/throat**
- **Dilated, fixed pupils**
- **Ataxia**
- **Hypotension**
- **Nystagmus**
- **Decreased to absent deep tendon reflexes**

Figure 2. Seventeen-Year-Old Patient With Mild Botulism

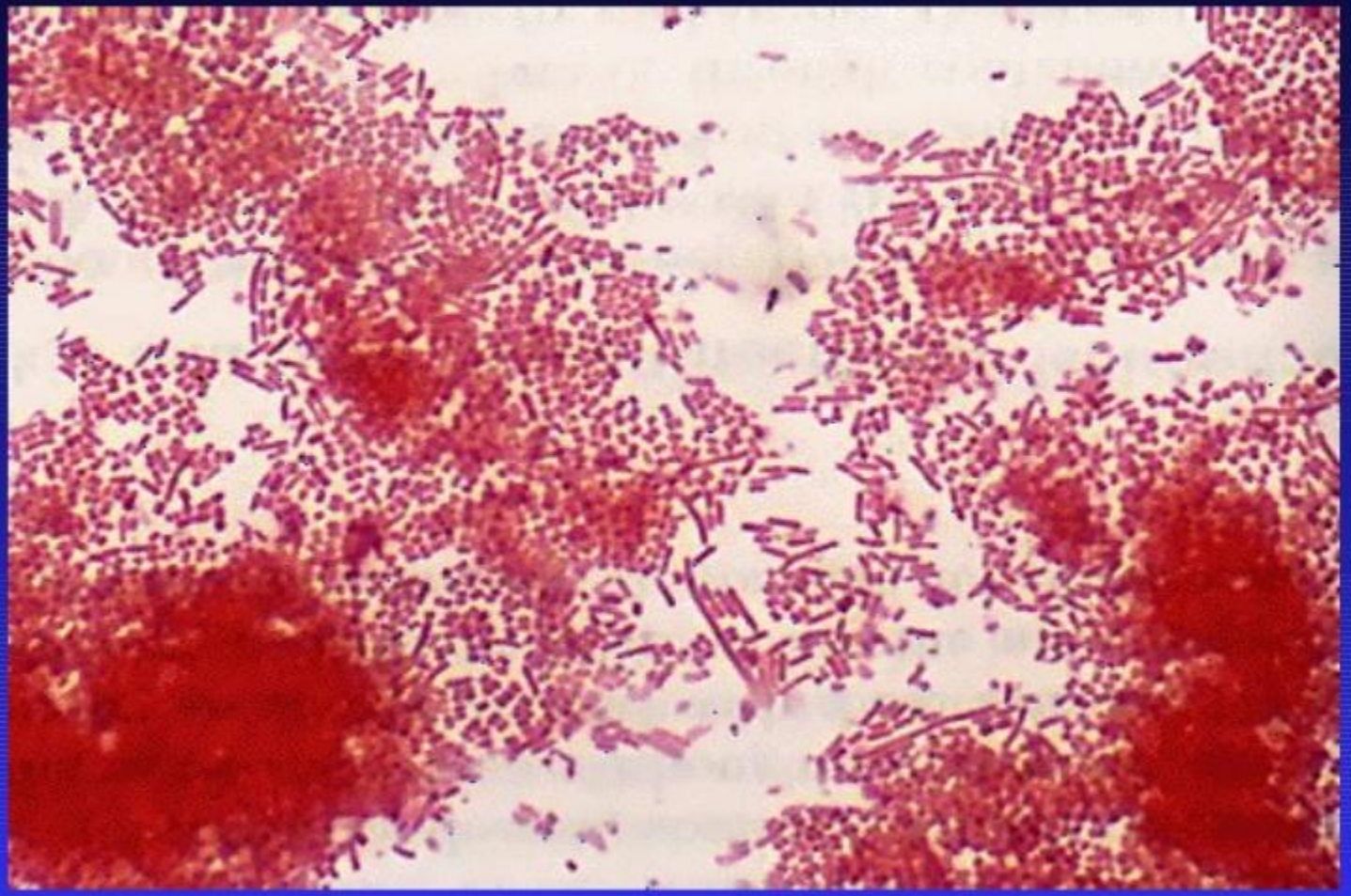


A. Patient at rest. Note bilateral mild ptosis, dilated pupils, disconjugate gaze, and symmetric facial muscles.
B, Patient was requested to perform his maximum smile. Note absent smile creases, ptosis, minimally asymmetric smile.

Bacteroides

- *B. fragilis* is most significant.
- Pale irregularly staining Gram negative bacilli/ coccobacilli with polysaccharide capsule.
  - Pleomorphic
- Normal flora of large intestine & female genital tract. Normal stool contain 11^{10} *B. fragilis organisms per gram.*
- Cause abdominal, lung and brain abscesses and wound infection

Bacteroides



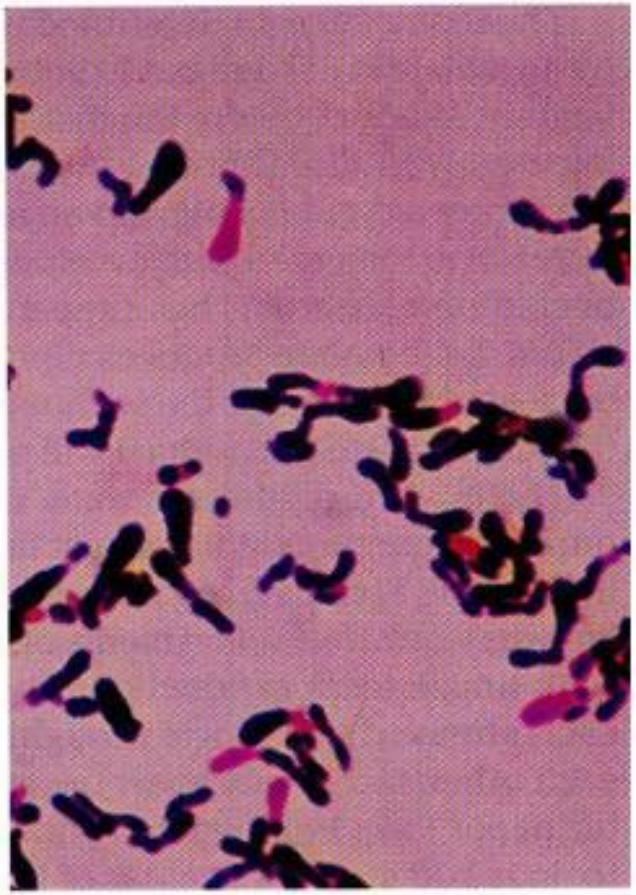
Bacteroides Virulence Factors

- Polysaccharide capsule
- Lipopolysaccharide
- Agglutinin
- Histolytic enzyme
- Oxygen tolerance
- β lactamase

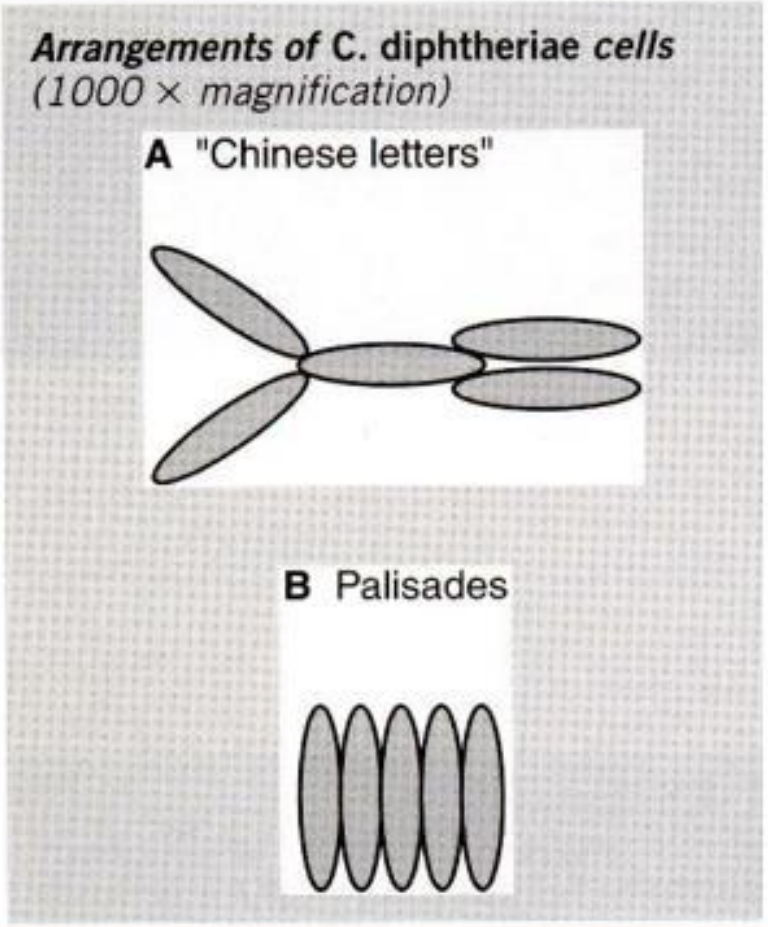
Corynebacterium

- Classification
  - *Corynebacterium diphtheriae* and diphtheroids (look like *C. diphtheriae*) are Gram- positive, club shaped rods.
    - Some are saprophytic
    - Some produce disease in animals.
    - *C. diphtheriae* is the most important pathogen in the group.
- Morphology and cultural characteristics
  - Small G+B; arrangement=palisade or Chinese
  - letters
  - Growth on B.A – raised, translucent, gray colonies

Diphtheroids Gram stain



Arrangement of *C. diphtheria*



Corynebacterium

- Loeffler's agar slant contains serum and egg that enhance the formation of metachromatic granules (polymerized polyphosphoric acid) in *C. diphtheriae*.
  - Also called Babes-Ernst granules.
  - They are visualized by staining with methylene blue.

Biochemical Reactions

- Acid production on

Glucose,Galactose Maltose, Dextrin

Do not produce acid with

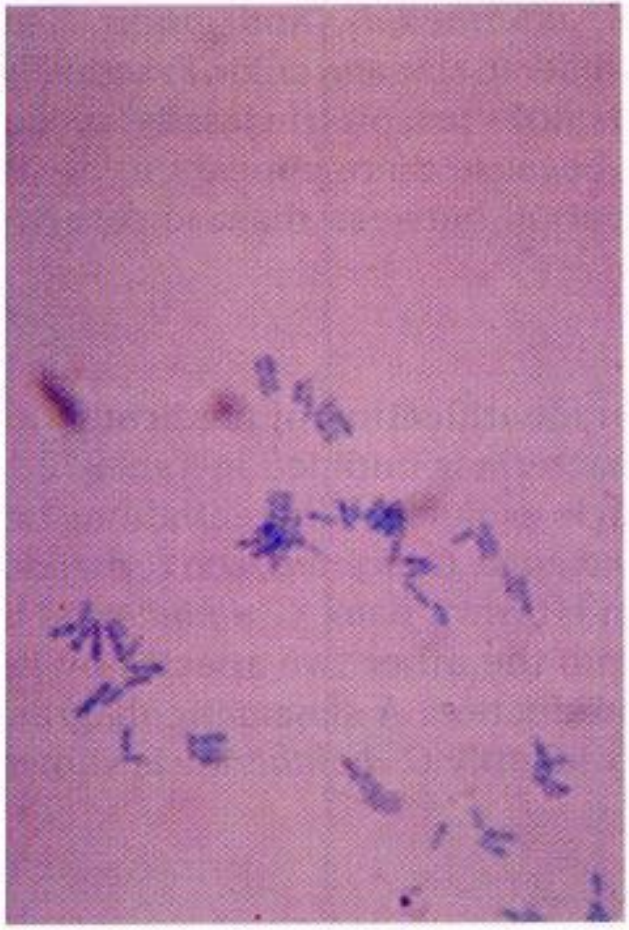
Lactose, Mannitol, sucrose.

All fermentation reactions tested in

Hiss serum sugars

Urease test negative.

Methylene blue stain from Loefflers slant



C. diphtheria toxin

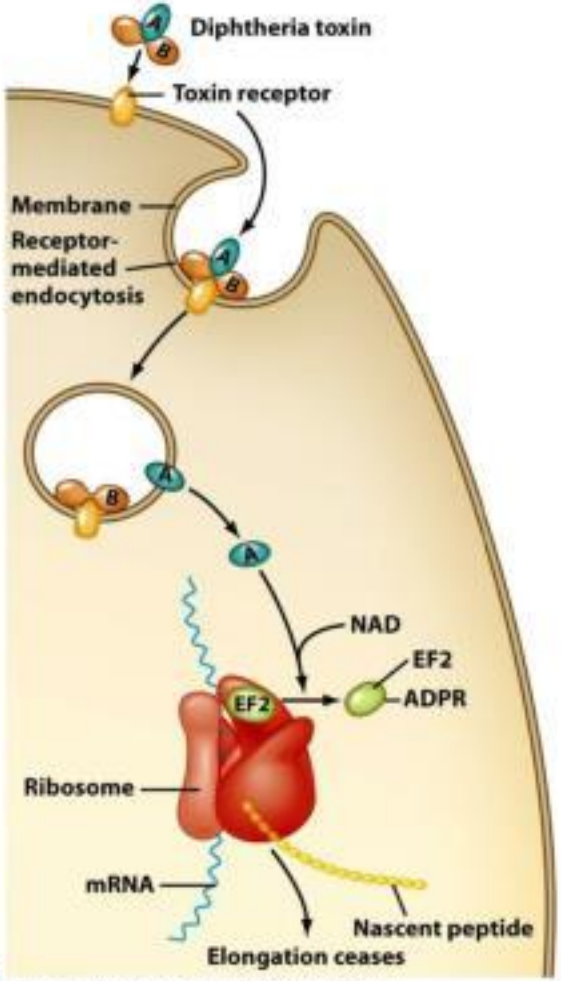
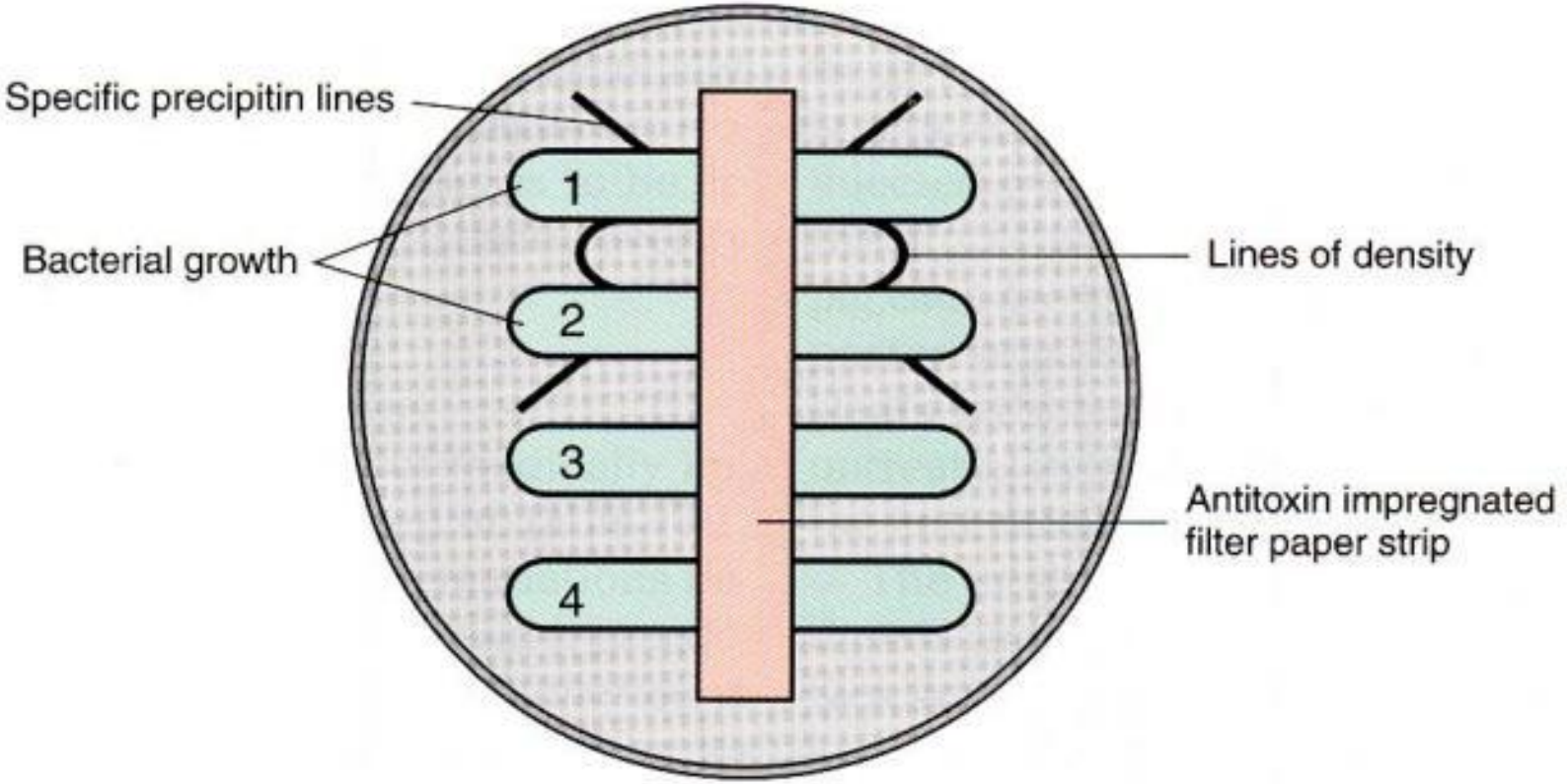


Figure 25.22 Microbiology: An Evolving Science

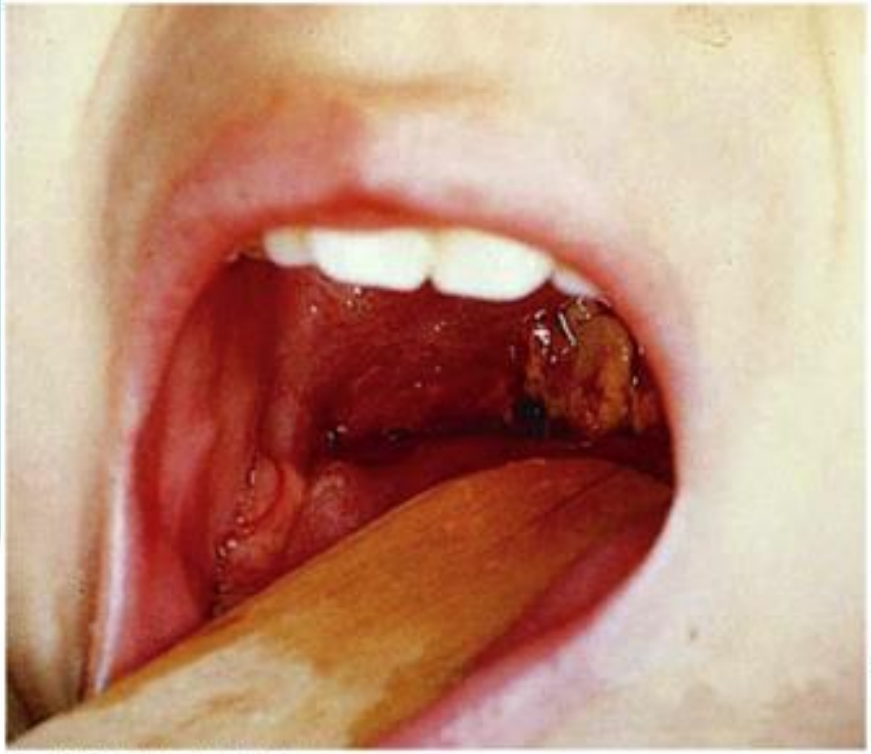
- Toxin enters through receptor mediated endocytosis
- Acidification of endocytic vesicle allows A to dissociate from B
- A enters cycoplasm

Elek plate


Specific precipitin lines
Bacterial growth
1
2
3
4
Lines of density
Antitoxin impregnated
filter paper strip

Diphtheria - pseudomembrane

- This may obstruct the airway and result in suffocation.



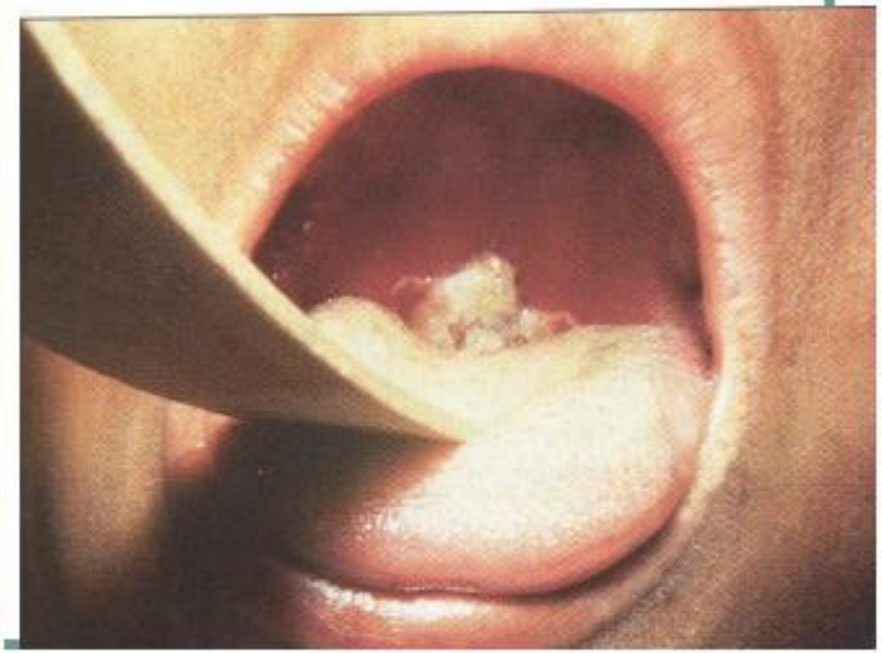


Figure 25.20b Microbiology: An Evolving Science
©Princess Margaret Hospital, Hong Kong

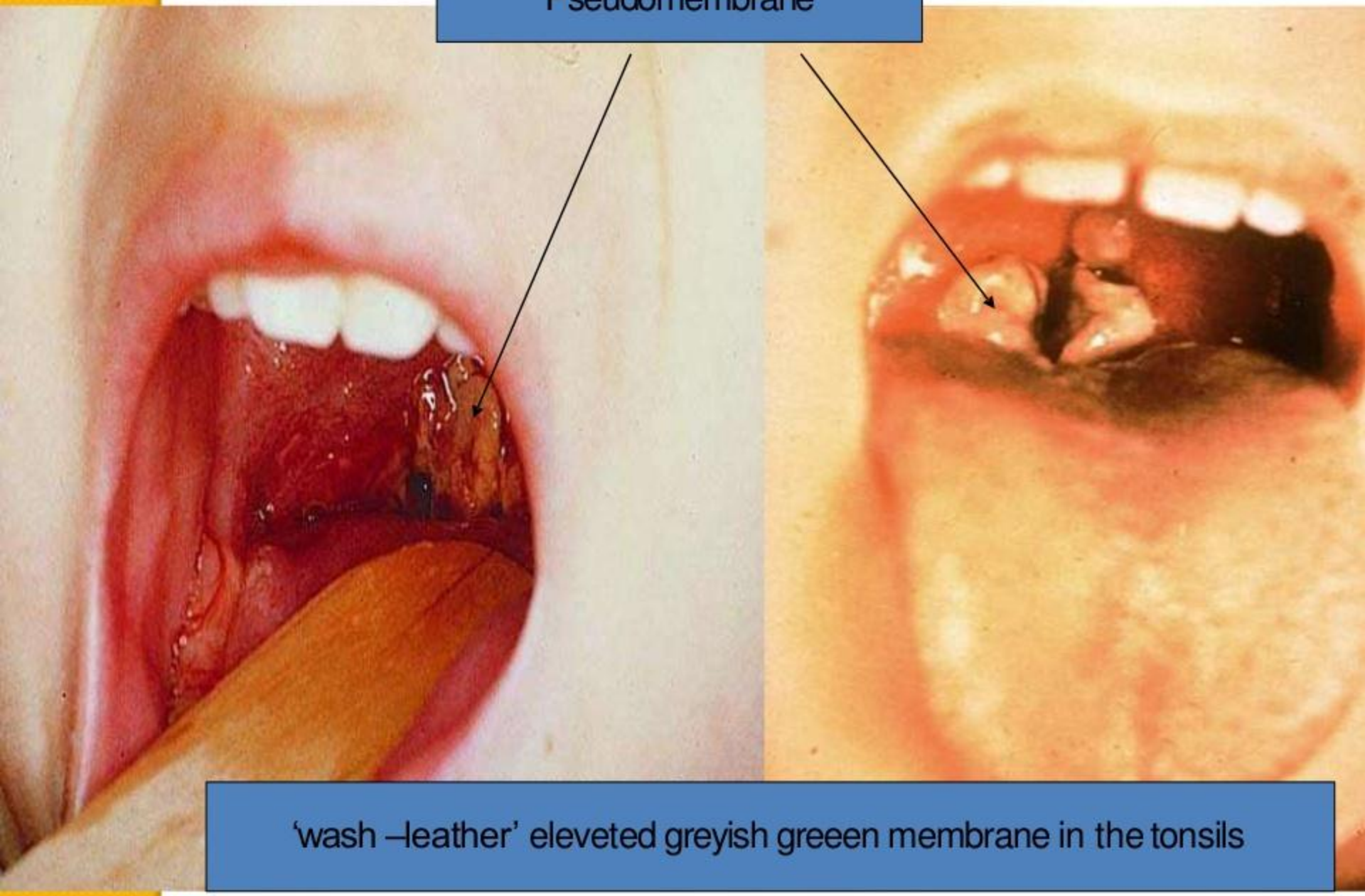
CLINICAL DISEASES

- Incubation period : usually 3-4 days;
- Acute infection : in the form of –
  - **Membranous tonsillitis**
  - **Nasal infection**
  - **Laryngeal infection**
  - **Skin infection -uncommon;**

CLINICAL DISEASES

- Characteristic feature is : 'wash –leather' eleveted greyish greeen membrane in the tonsils with a well defined edge surrounded by a zone of inflammation;

Pseudomembrane

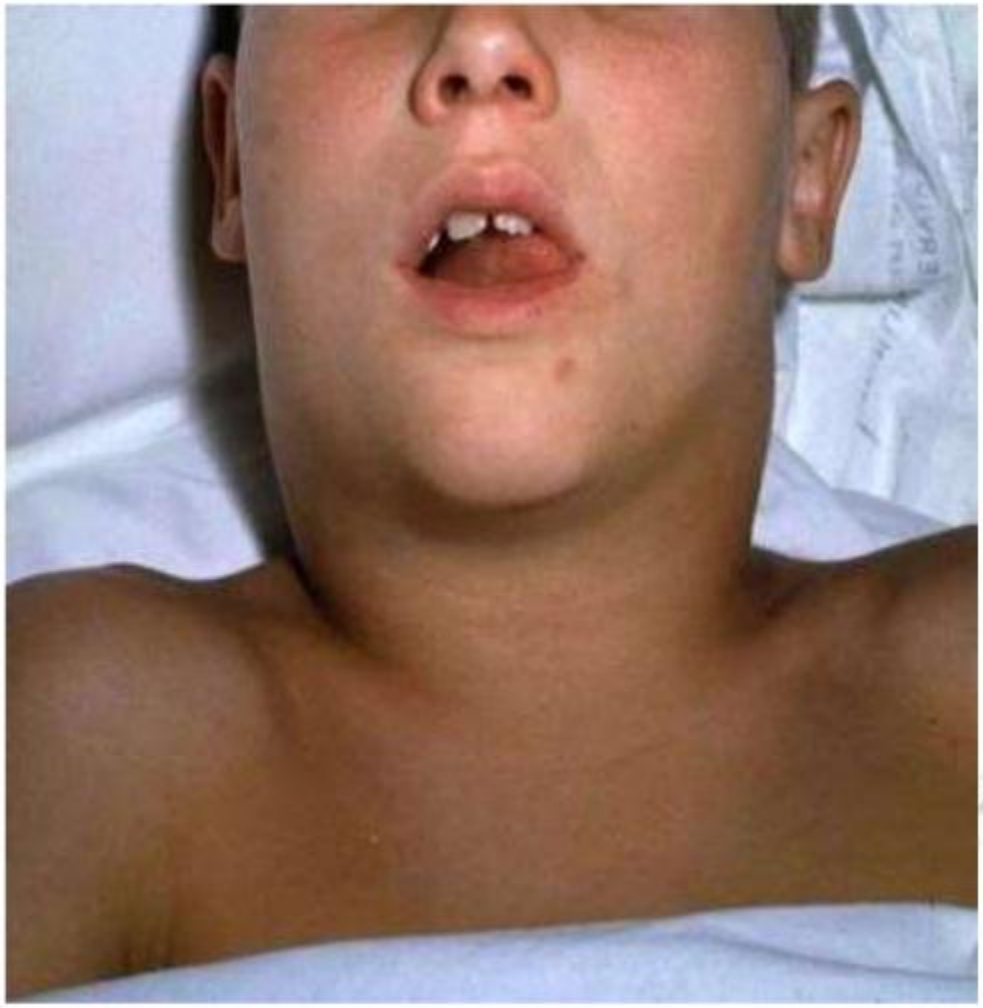


'wash –leather' eleveted greyish greeen membrane in the tonsils

CLASSIFICATION BASED ON CLINICAL SEVERITY

- Malignant or hypertoxic:
  - 'Bull neck' due to marked adenitis in neck;
  - Severe toxemia
  - Circulatory failure
  - Death
  - Paralytic squealae in survivors
- Septic : ulceration, cellulitis and gangrene around pseudomembrane;
- Hemorrhagic: bleeding from the edge of pseudomembrane, epistaxis, purpura etc.

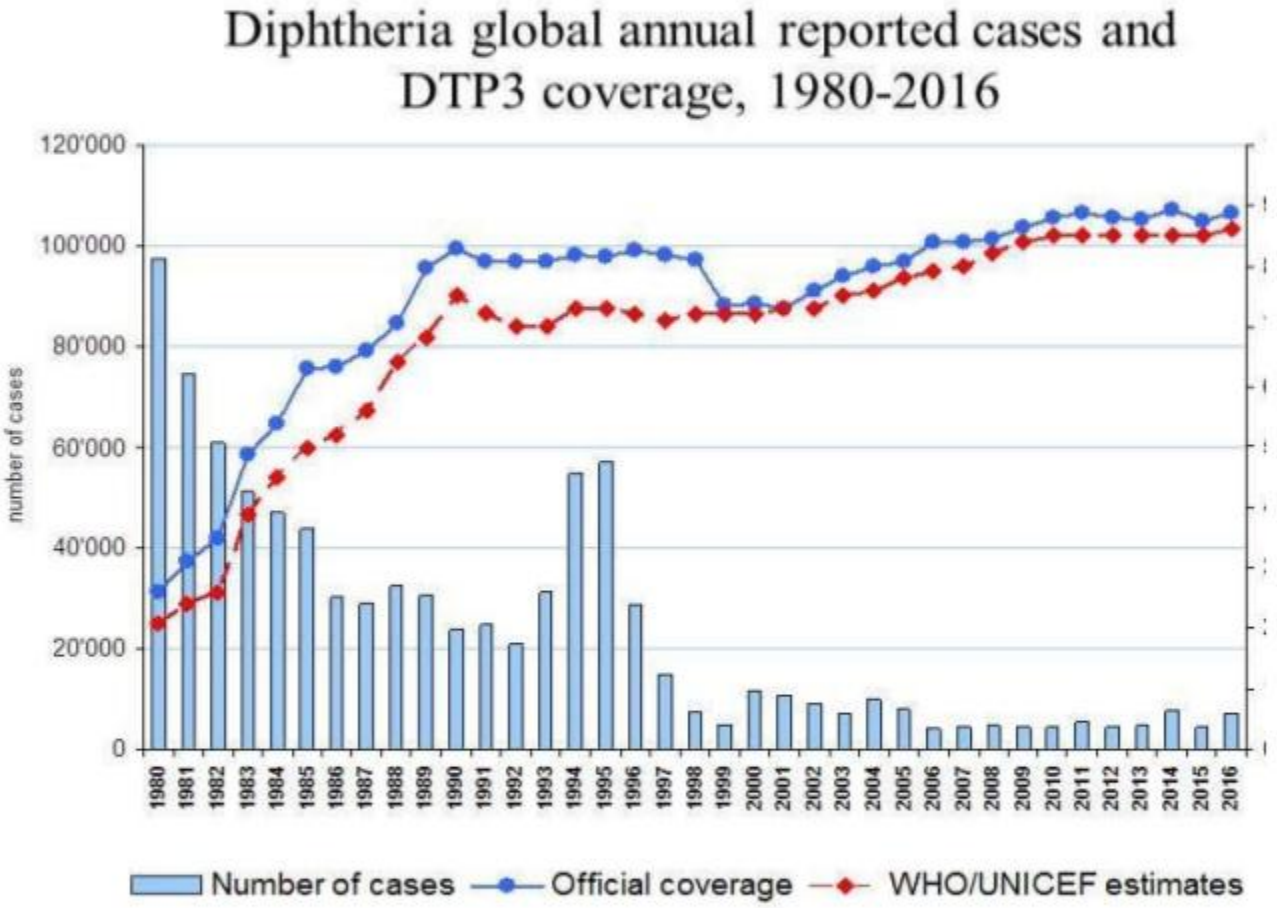
Bull neck : due to cervical adenitis and edema of neck



COMPLICATIONS

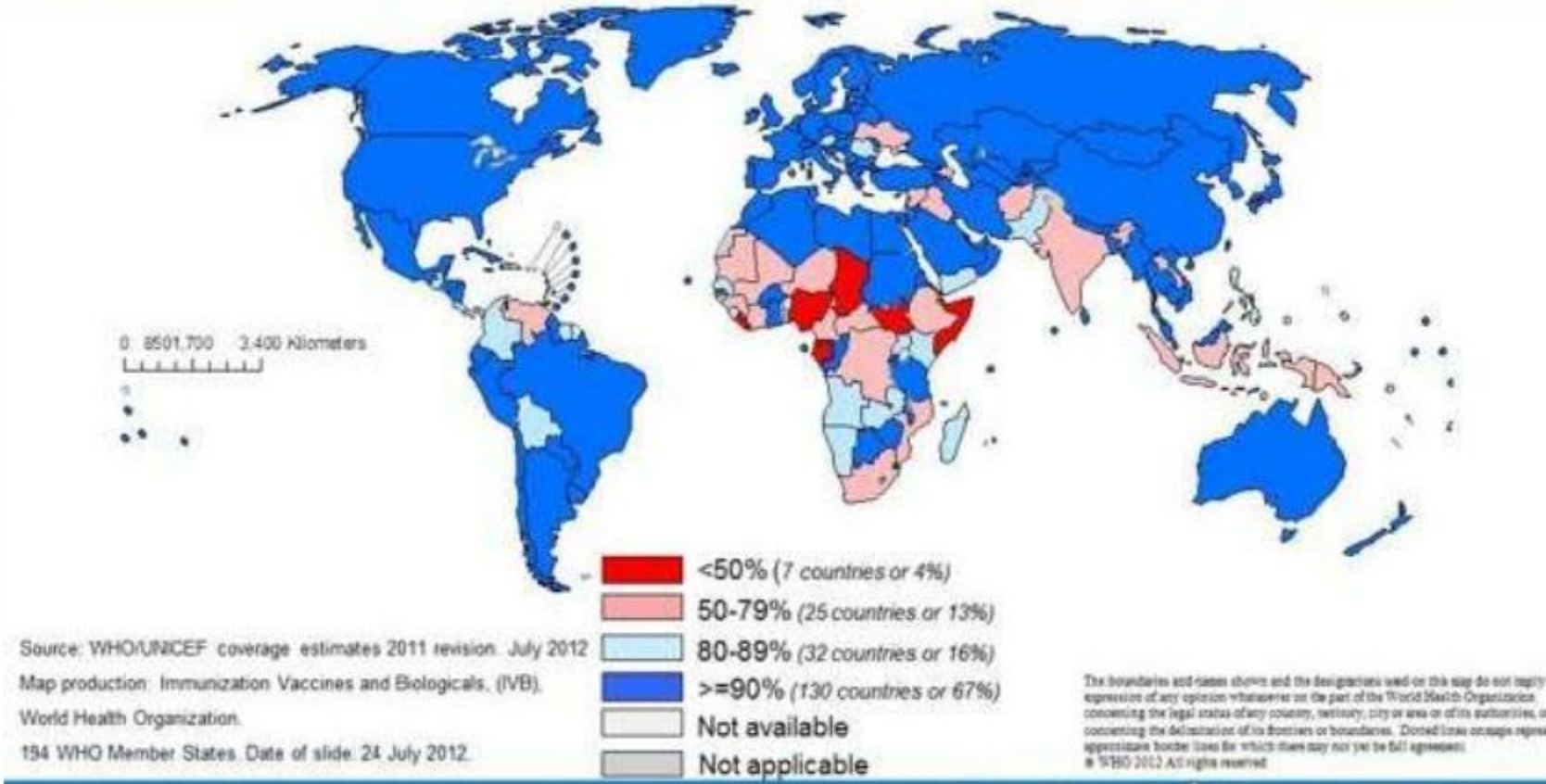
- Asphyxia : due to mechanical obstruction
  - Emergency tracheostomy may be necessary;
- Acute circulatory failure
- Myocarditis
- Postdiphtheritic paralysis-
  - palatine(soft palate) and ciliary (eye muscles) nerves
  - Recovery – spontaneous and complete
- Septic : pneumonia and otitis media
- Relapse : in about 1% of cases

Epidemiology



Source: WHO/IVB database, 2017
194 WHO Member States.
Data as of 19 July 2017

Distribution

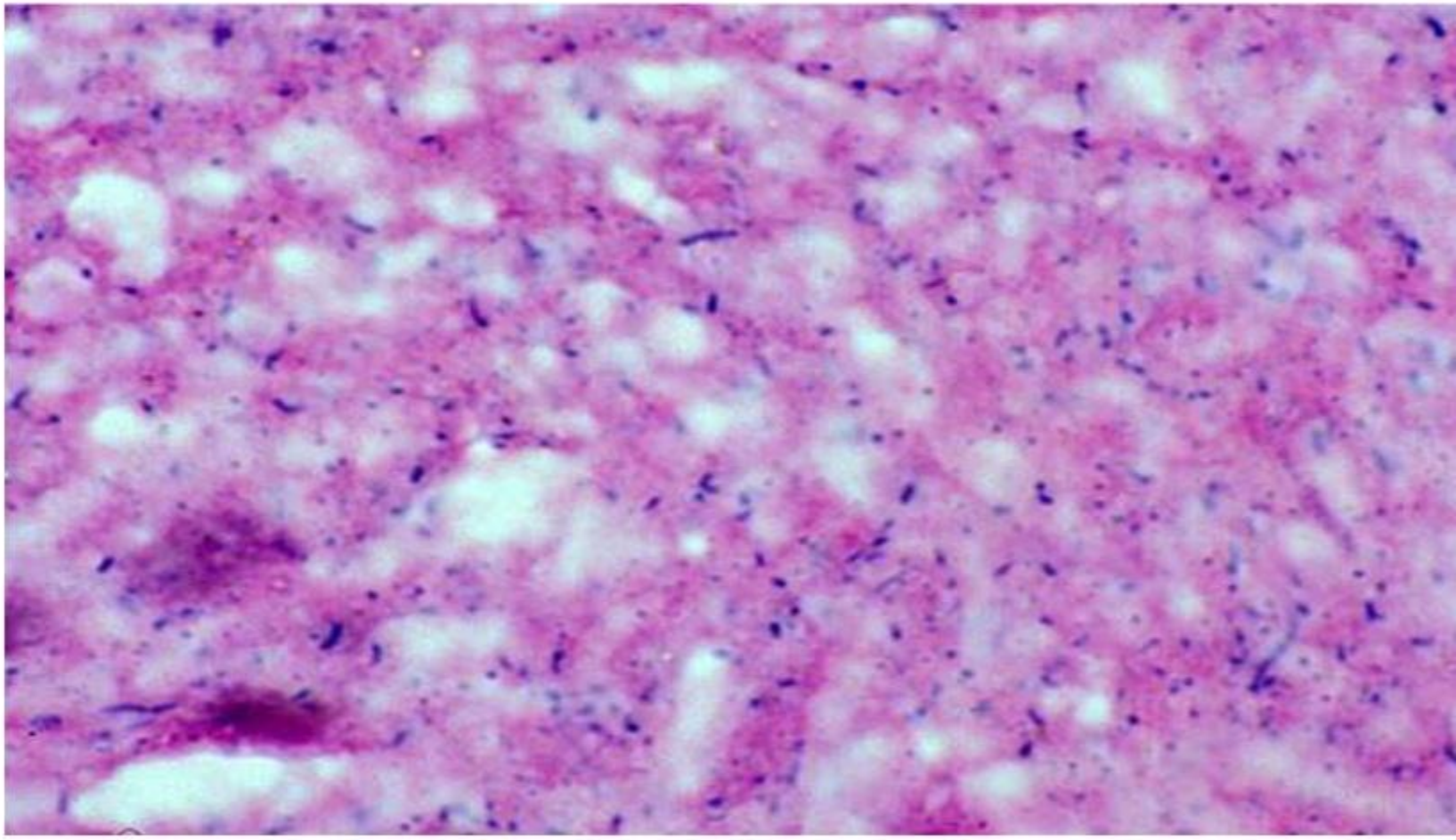


unicef World Health Organization

LABORATORY DIAGNOSIS

- Specimens :
  - Swabs from – nose, throat or other suspected lesions;
- Smear examination: Gram stain
  - shows beaded rods in typical arrangement;
  - Difficult to differentiate from somecommensa corynebacteria normally found in throat;
  - Albert's stain or Neisser's stain is useful for demonstrating the granules;

LABORATORY DIAGNOSIS



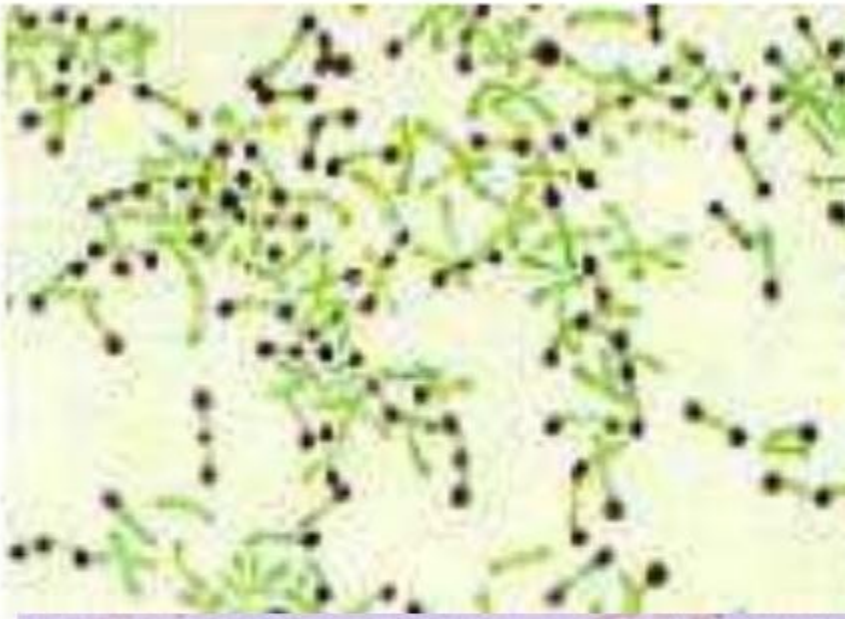
Numbers of large-sized Gram-positive rods are embedded within the pseudomembrane (Gram).

LABORATORY DIAGNOSIS : CULTURE

- If the swabs can not be inoculated promptly, they should be kept moistened with serum;
- Inoculate on :
  - **Loeffler's serum slope**
  - **Tellurite blood agar or Tinsdale medium**
  - **Blood agar (for differentiating Staphylococca Streptococcal pharyngitis that simulate diphtheria);**
- *Tellurite medium is particulary useful for isolating organism from - convalescents, contacts or carrie*

MORPHOLOGY

- Special stains for demonstrating the granules :
  - Albert's stain
  - Neisser's stain
  - Ponder's stain
- The bacilli are arranged in pairs, palisades or small groups; the bacilli lie at various angles to each other, resembling the letters, V or L;
- This is called, "Chinese letter pattern" or "cuneiform pattern";

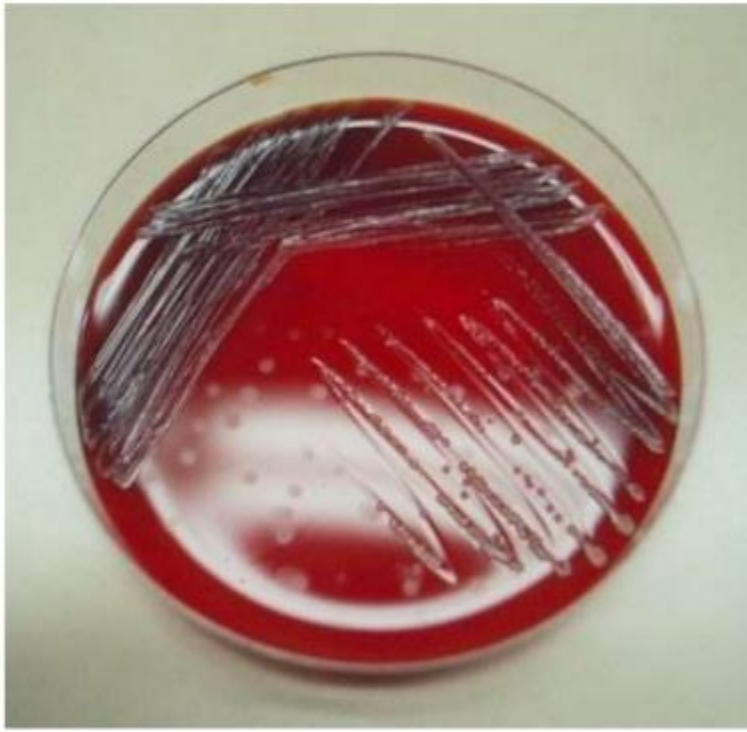


MEDIA FOR CULTIVATION

- Blood agar
- Loeffler's serum slope
- Tellurite blood agar
- Hoyle's tellurite lysed-blood agar
- Tinsdale's medium (cystine added to tellurit
  containing agar)

COLONY CHARACTERISTICS

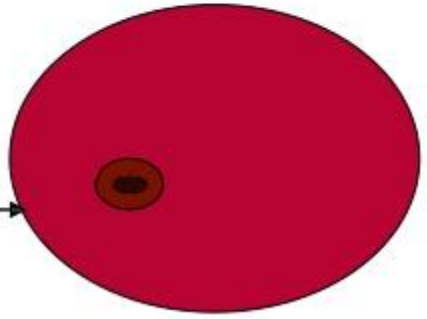
- Blood agar : small, granular and gray with irregular edges; Hemolysis may or may not present;
- Loeffler's serum slope:
  - Very rapid growth;
  - Colonies in 6-8 hrs
  - Initially circular white opaque colonies and acquire yellowish tint on incubation



COLONY CHARACTERISTICS

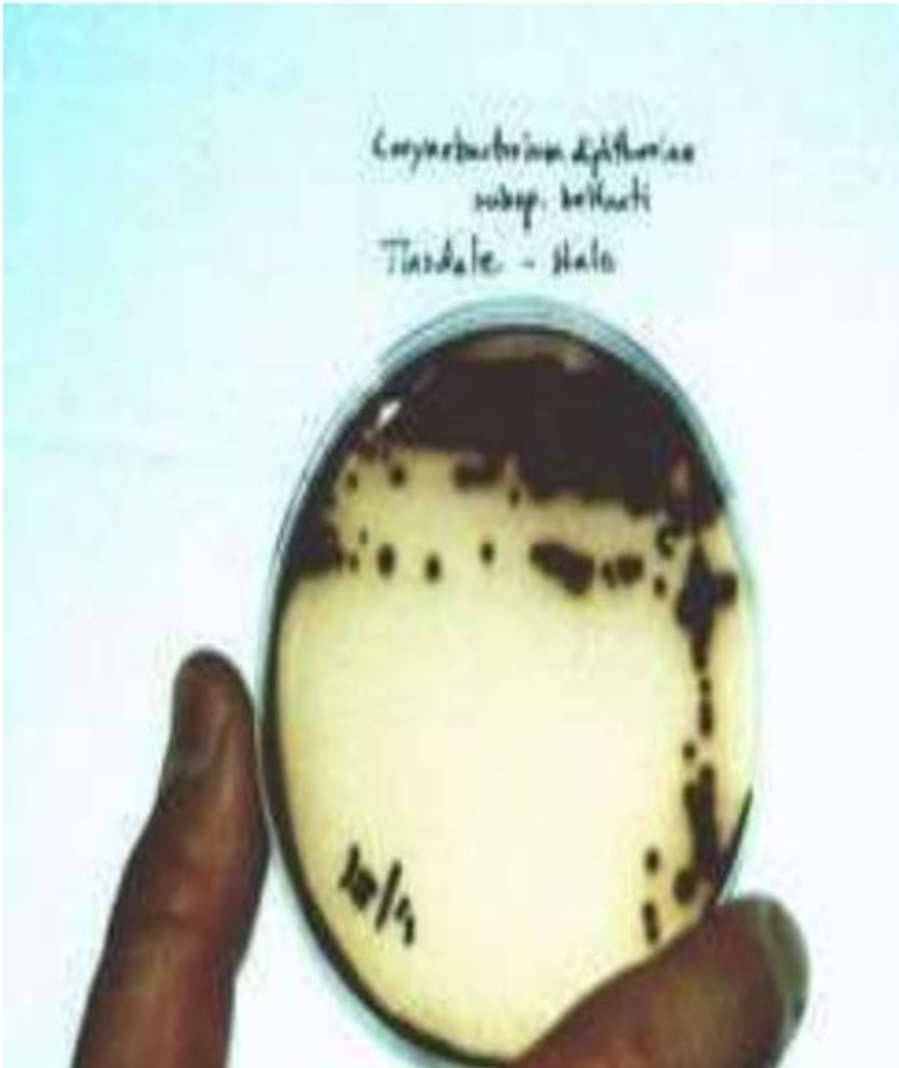
- Tellurite blood agar:
  - Growth slow; colonies seen after 48 hrs;
  - The colonies are brown to black with abrown-black halo because the tellurite is reduced to metallic tellurium;
  - Staphylococcus also produce such colonies

A diagrammatic representation →



COLONY CHARACTERISTICS

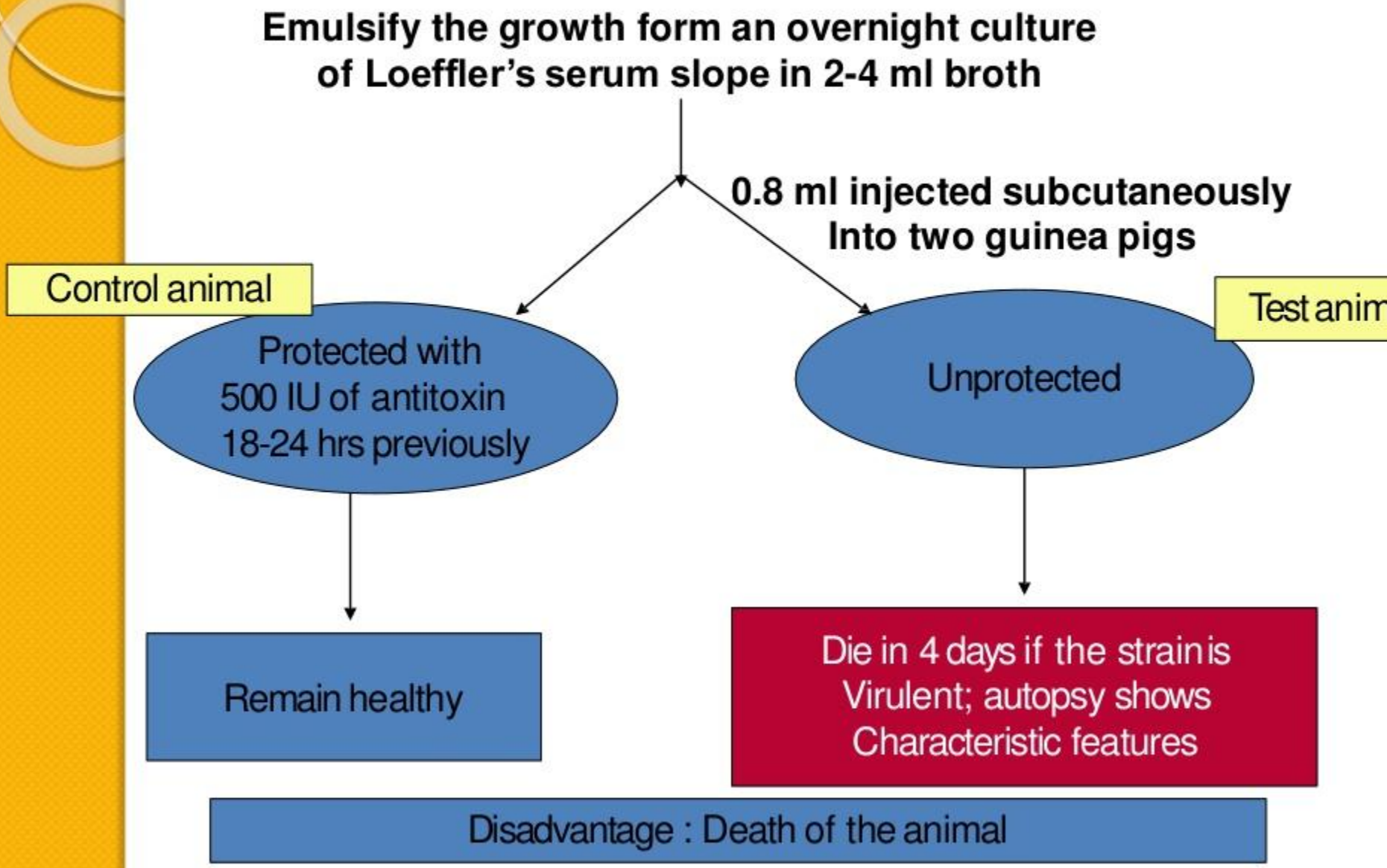
- Tinsdale's medium (also contain cystine in addition to tellurite):
  - **Grey black colonies with dark brown haloes** indicate C.diphtheriae and C.ulcerans (these contain cystinase)



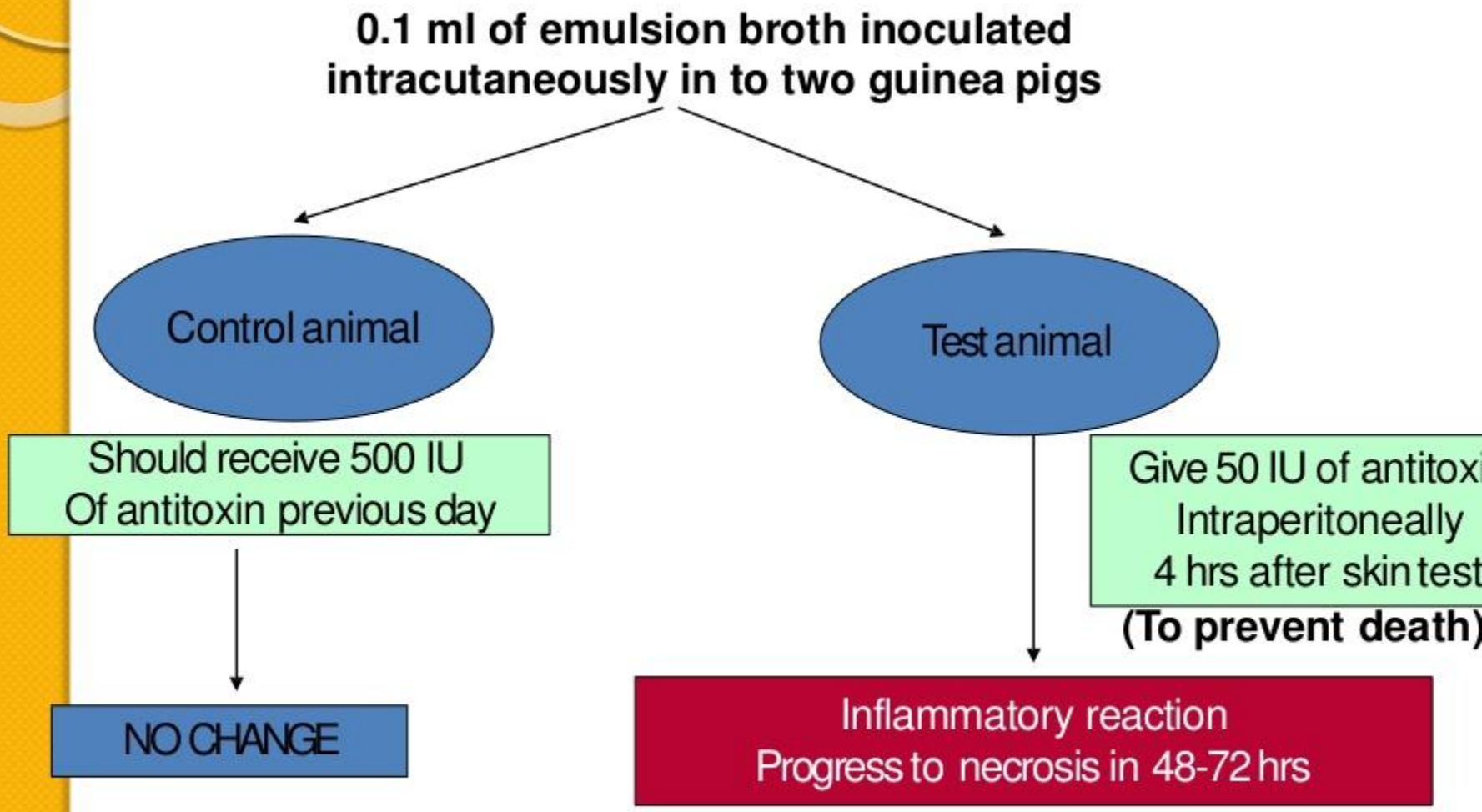
VIRULENCE TESTS

- In vivo methods:
  - Subcutaneous test
  - Intracutaneous test
- In vitro methods:
  - Elek's gel precipitation test
  - Tissue culture test

SUBCUTANEOUS TEST



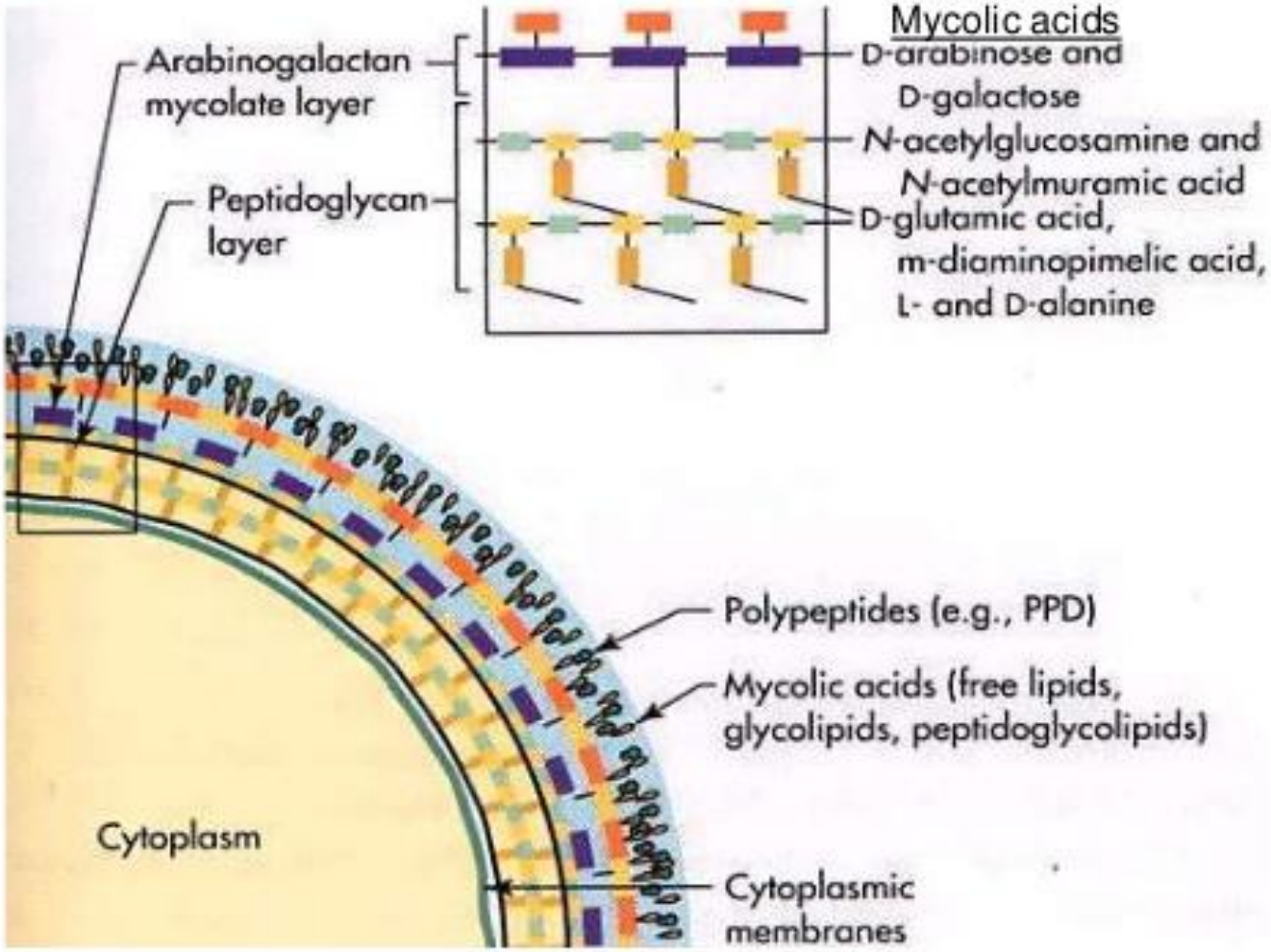
INTRACUTANEOUS TEST



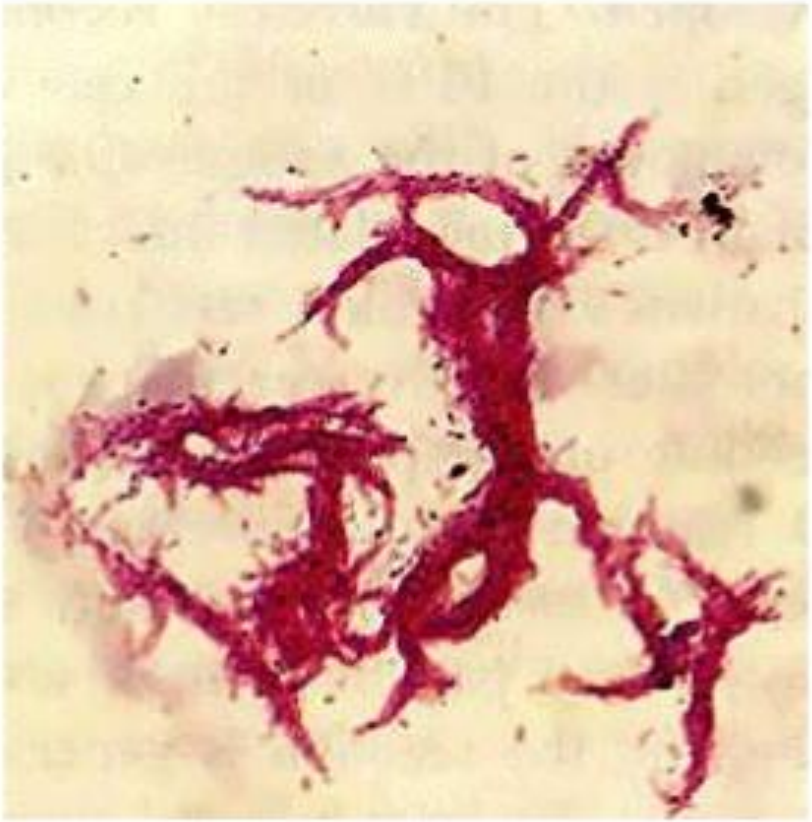
Kingdom:	Bacteria
Phylum:	Actinobacteria
Order:	Actinomycetales
Suborder:	Corynebacterineae
Family:	**Mycobacteriaceae**
Genus:	***Mycobacterium***

- Mycobacterium Tuberculosis
- Mycobacterium Leprae (uncommon)

Lipid Rich Cell Wall



Acid-Fast (Kinyoun) Stain



- Cord growth (Serpentine arrangement) of virulent strains.
- Kinyoun similar to Ziehl-Neelsen Stain

Ziehl-Neelsen Stain Procedure

- 1. Cover with tissue paper or if not then without paper its possible.

- 2. Flood slide with carbolfuchsin, the primary stain, for 3-5 minutes while heating with steam or heating on hot plate.

Continued…

- 3. Remove paper cover, decolorize slide with a mixture of hydrochloric acid and ethanol.

- 4. Counterstain with methylene blue or Malachite green.

Pathogenic Mycobacterium

M. tuberculosis Complex

- M. tuberculosis - Common
- M. leprae - Uncommon
- M. africanum
- M. bovis
- M. ulcerans

} Rare

All are Strictly Pathogenic

MYCOBACTRIUM LEPRAE

Structure and Physiology

- Weakly gram positive
- Strongly acid fast bacilli
- Lipid rich cell wall
- Unable to culture on artificial medium

Virulence

- Capable of intracellular growth
- Disease primarily from host response to infection

Forms of Diseases

- Tuberculoid form of leprosy
- Lepromatous form of leprosy
- Intermediate form of leprosy

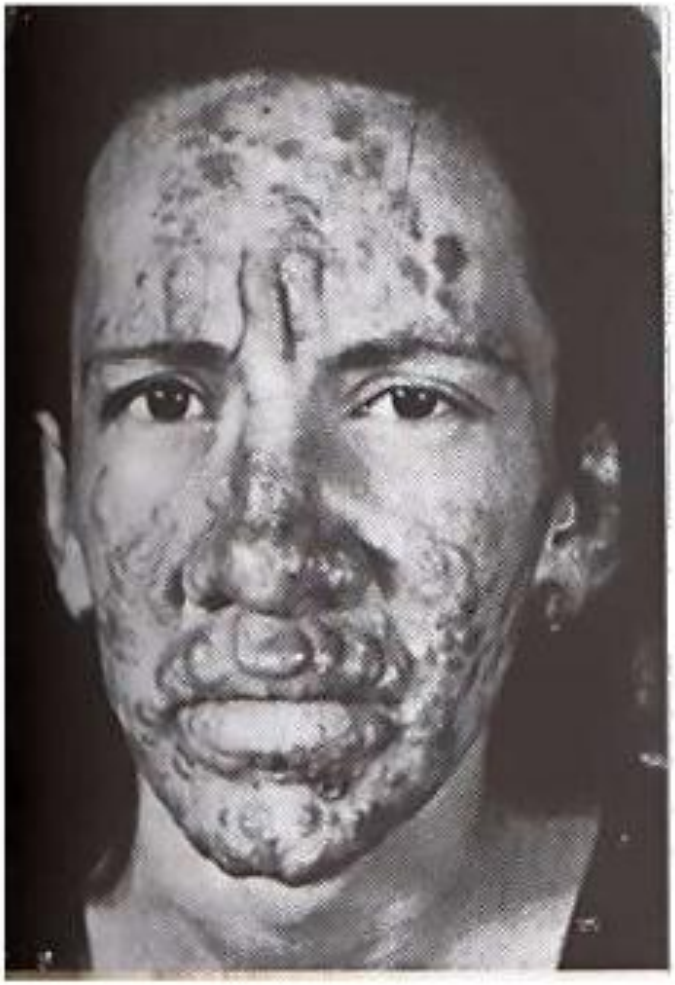
Diagnosis

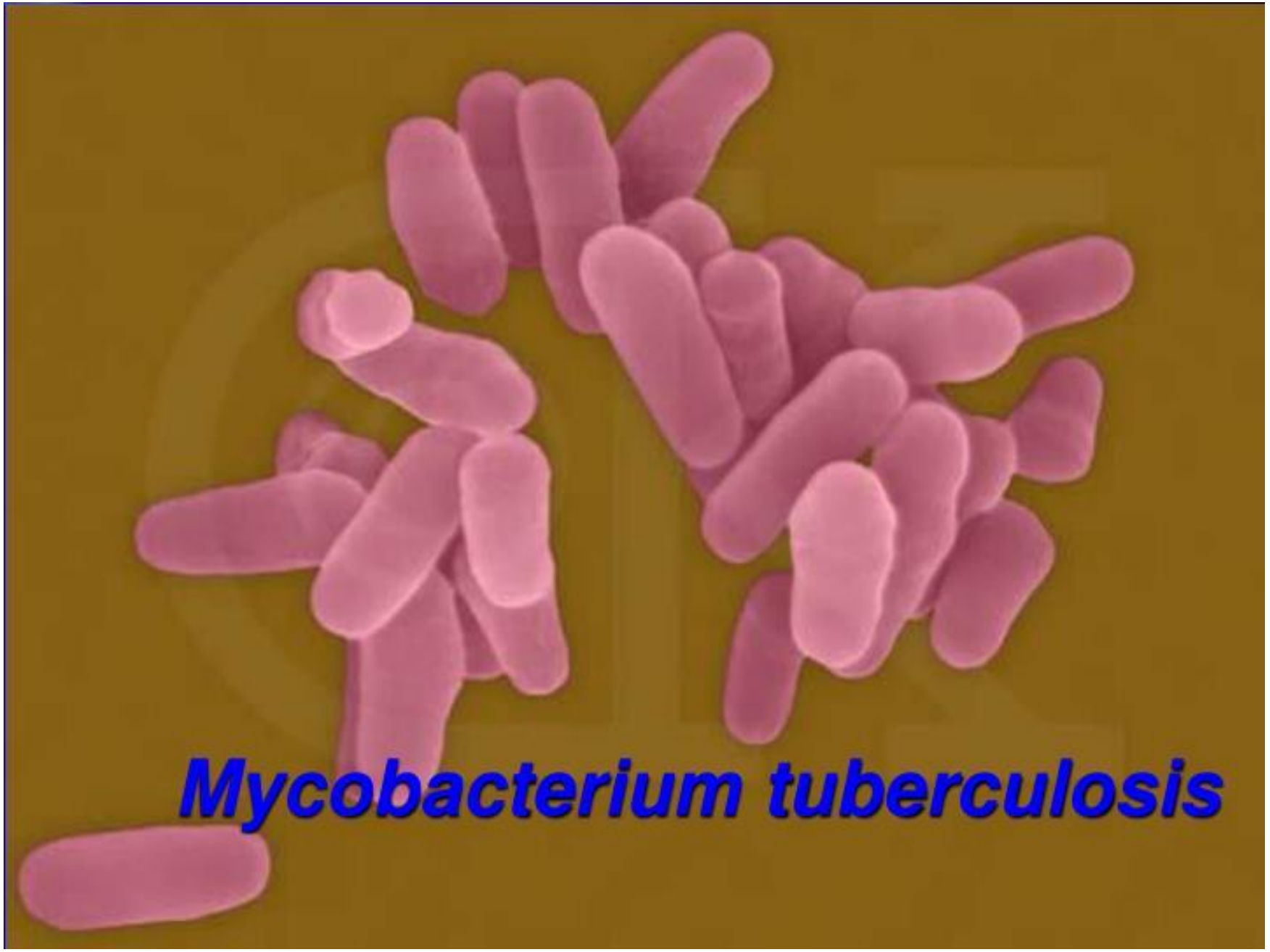
- Microscopy is sensitive for the lepromatous but not for tuberculoid form
- Skin testing is required for tuberculoid leprosy
- Culture cannot be used

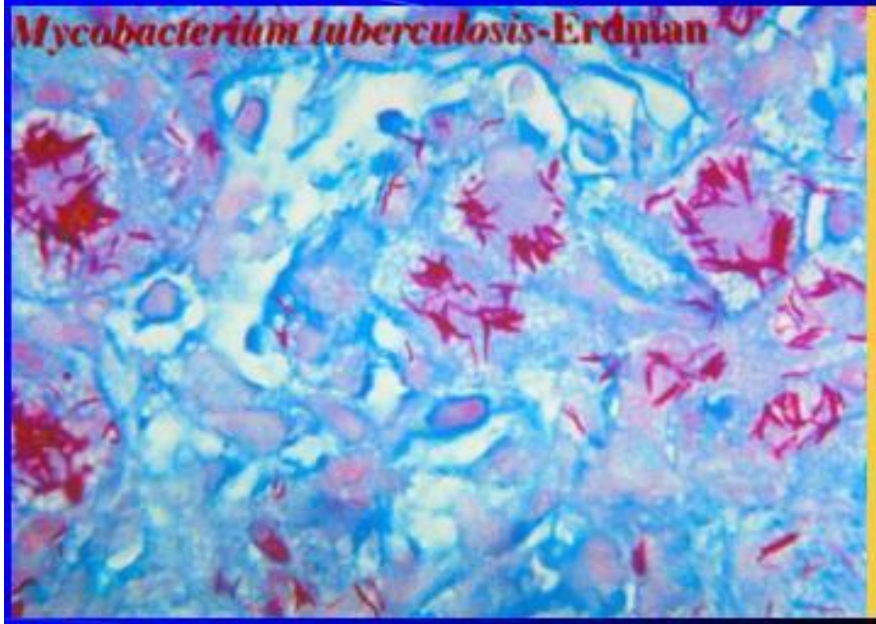
Lepromatous form

Tuberculoid form

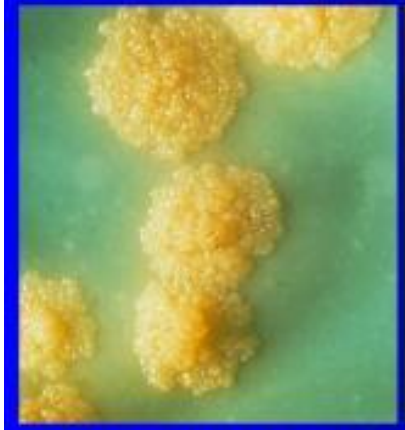
Pre and Post Treatment




Mycobacterium tuberculosis



COLONIAL MORPHOLOGY OF THE TUBERCULOSIS COMPLEX MYCOBACTERIA



EUGONIC GROWTH 14 DAYS
Mycobacterium tuberculosis

DYSGONIC GROWTH 14 DAYS
Mycobacterium bovis

Resistance:

Not sensitive	Sensitive
Dry (highly)	Wet
Chemical disinfectants (more)	Heat(62-63℃,15min)
3%HCL, 6%H_2SO_4, 4%NaOH (15min)	Alcohol (to nonspore-forming bacteria)
Malachite green(1:13000)	UV

Transmission

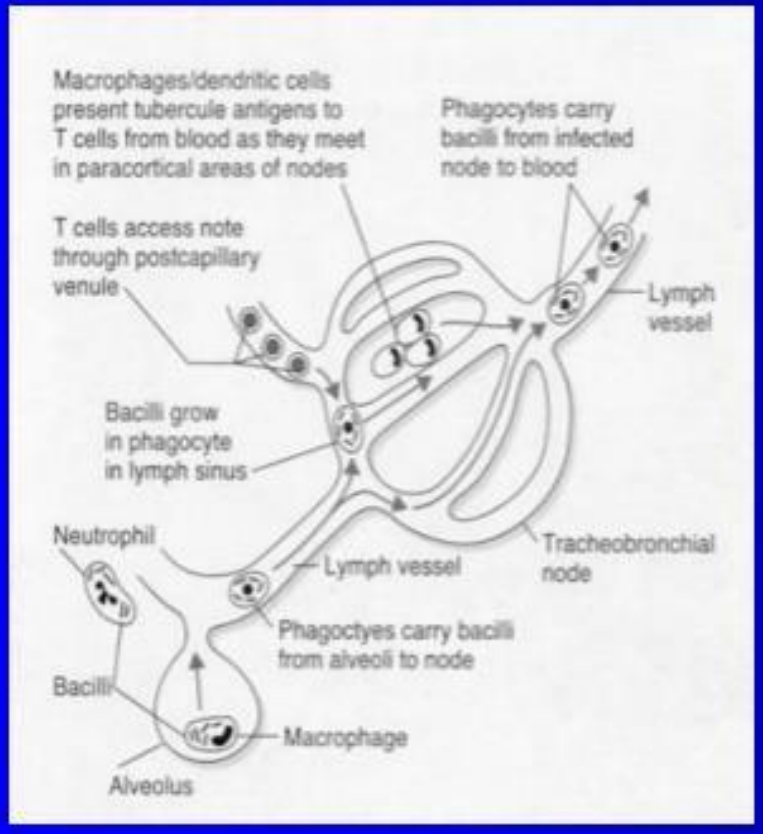
- Through respiratory tract, alimentary tract, injured skin。
- TB in the lungs or throat can be infectious. This means that the bacteria can be spread to other people. TB in other parts of the body, such as the kidney or spine, is usually not infectious.

Pathogenesis

1) lung infection
   - primary infection
   - secondary infection

2) Out lung infection

Primary Tuberculosis



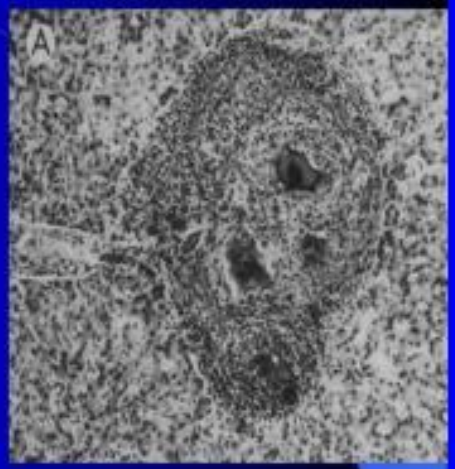
- **The organisms are transmitted among human via aerosol.**
- **TB bacilli lodge in the alveoli or lung alveolar ducts and most of bacilli are phagocytosed by alveolar macrophages.**
- **Macrophages migrate to the hylar lymph node and generate T cell-mediated immune response.**

(can be monitored by tuberculin test)

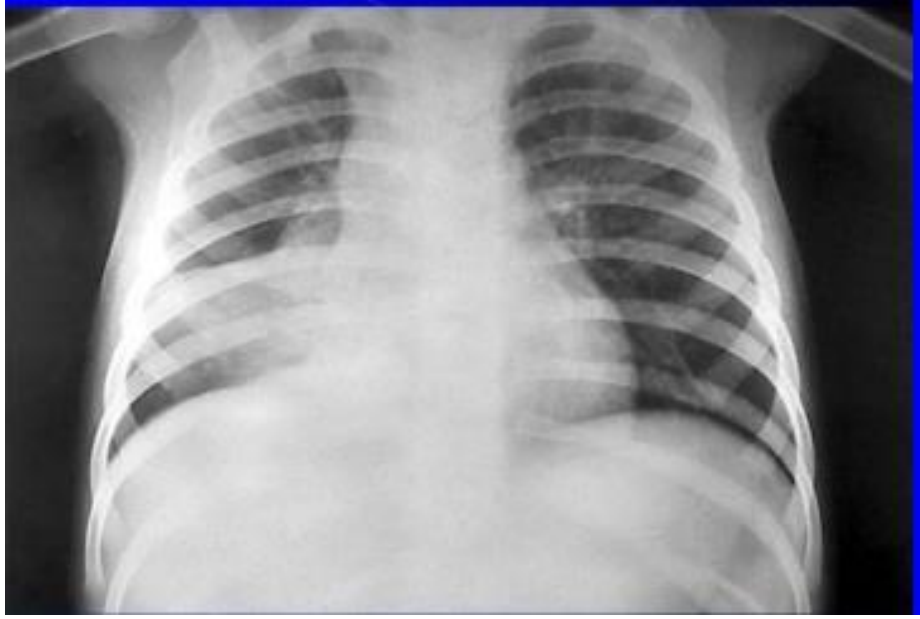
Tuberculin Skin Test

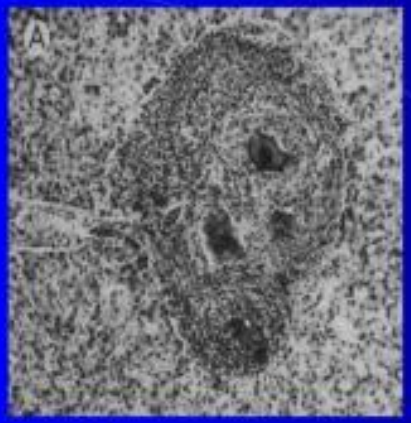
- Tuberculin is a mixture known as purified protein derivatives (PPD) from TB bacilli.
- It is a test for delayed type hypersensitivity. Positive reaction, reddening and thickening (> 5mm) at the site of injection after 2-3 days, indicates cellular immunity to tubercle bacilli.

- Macrophages containing TB bacilli clump together and begin to form tubercles. (granulomatous response)
- With time, the centers of the tubercles become necrotic and form cheesy acellular masses of caseous materials. (caseous lesion)



PULMONARY TUBERCULOSIS





Symptoms:
Activation of macrophages -> cytokine secretion, IL-1: fever,
TNF: lipid metabolism, weight loss, tissue necrosis.
Oxygen radicals: tissue damages
Tissue necrosis -> inflammation -> mucous secretion, destruction of
blood vessels -> frequent cough and bloody sputum

TUBERCULOSIS

Diagnosis

The steps to diagnose TB infection and disease include:

- **A medical evaluation that includes history and risk assessment**
- **The tuberculin skin test**
- **A chest x-ray**
- **A bacteriological examination**

Diagnosis

1. Specimen: sputum, pus, CSF, urine, etc.
2. Microscopic examination: Ziehl-Neelsen stain
3. Concentration: 4%NaOH-3%HCL; 6% H_2SO_4
4. Culture:

   solid culture (2-4 weeks 37℃) ;

   liquid culture (1-2 weeks)
5. Animal inoculation: guinea pig
6. quick Diagnosis: PCR

Mantoux method

When the Mantoux skin test is performed, a needle is injected into the upper skin layer of the patient's arm. The arm is examined 48 to 72 hours after the tuberculin injection in order to evaluate the reaction on the patient's skin. Any swelling that can be felt around the site of the injection, also known as induration, is measured. The diagnosis of TB infection depends on the size of the measured induration and the patient's individual risk factors.

Prevention

- **BCG vaccination for new infants**

  Freeze-drying vaccine

  rRNA vaccine

  eg:south India Chingleput's failure of BCG

- **Find and cure patients**

Table 1. Summary of the protective efficacy of bacille Calmette–Guérin (BCG) against tuberculosis measured in major vaccine trials[a]

Trial/study	BCG vaccine	Protective efficacy (%)	Atypical exposure[b]
Haiti	Montreal	80	?
Canadian Indians	Montreal	80	Low
MRC, UK	Danish and vole[c]	77	(Low)
American Indians	Phipps	75	(Low)
Chicago, USA; high risk	Tice	75	Low
South Africa; miners	Glaxo	37	?
Puerto Rico	Birkhaug	31	(High)
Madanapalle, India	Madras	31	High
Georgia, USA	Tice	0	High
Georgia and Alabama, USA	Tice	14	High
Chingleput, India	Danish and Paris	0	High

[a]Adapted from Ref. 4. Abbreviation: MRC, Medical Research Council.
[b]Population exposure to atypical mycobacteria; parentheses indicate presumed, but not reported, exposure.
[c]*Mycobacterium microti*.

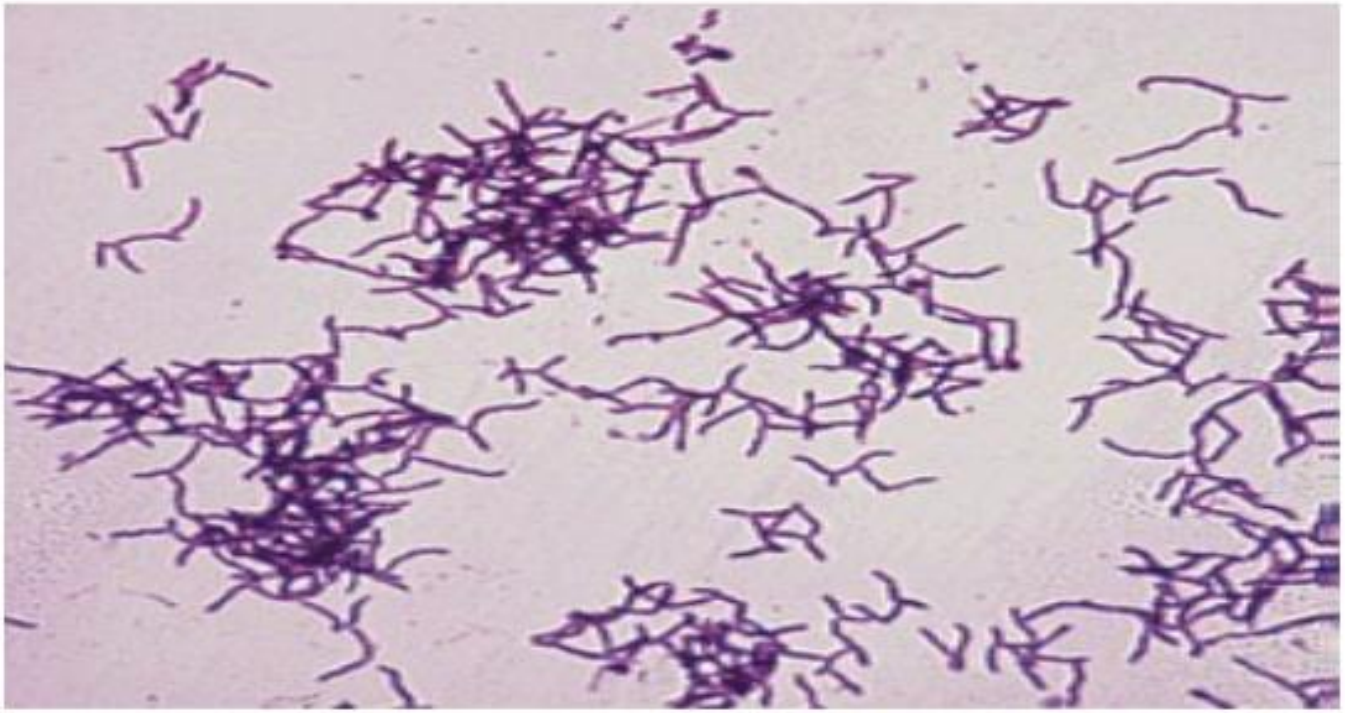
ACTINOMYCES

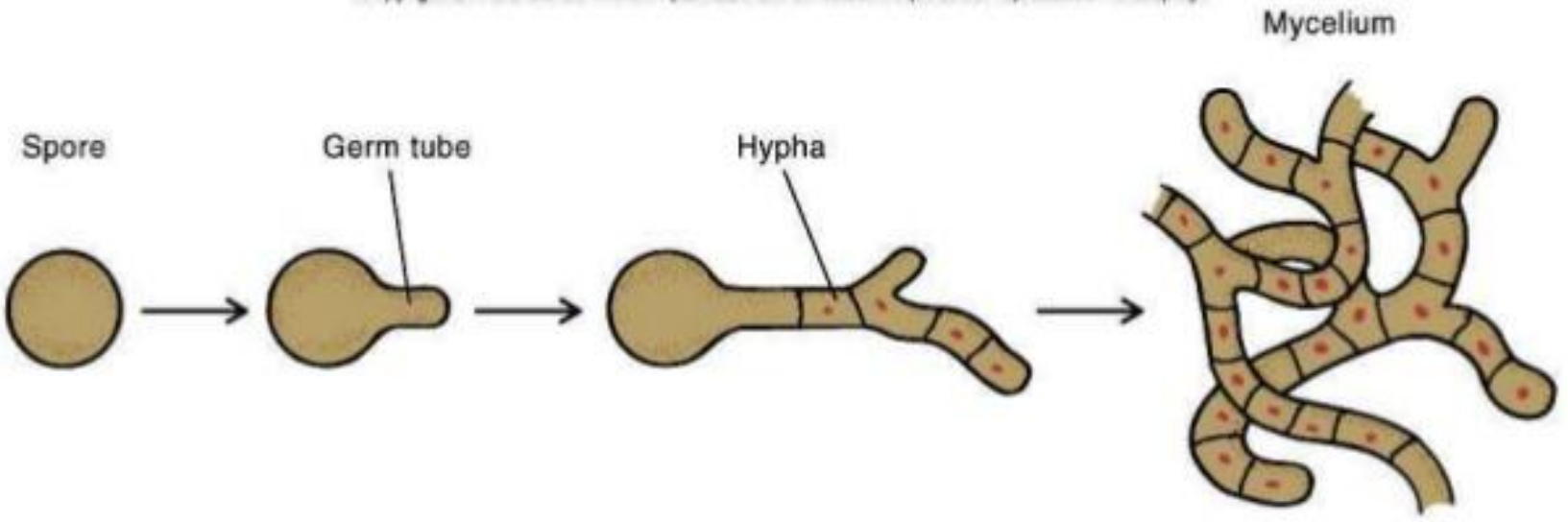
- Anaerobic, filamentous, gram positive bacillus
  - Exhibit true branching
- "Mykes" – Greek for "fungus"
- **Thought by early microbiologist to be fungi because of:**
  - Morphology
  - Disease they cause

Actinomyces

- Strict anaerobe
- Gram positive
- Non-motile
- Non-proteolytic
- Catalase negative

Actinomyces





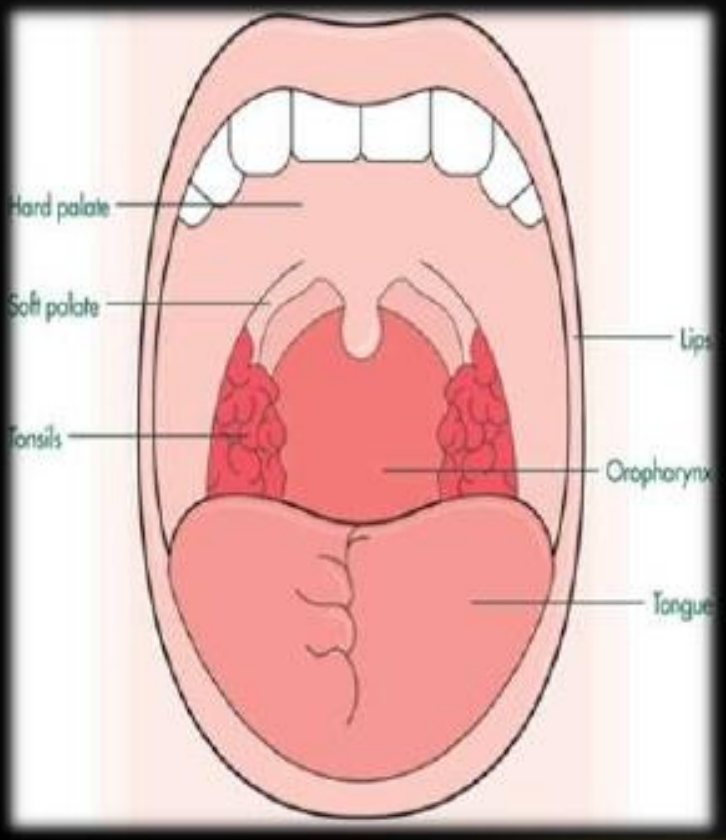
Difference between fungi and Actinomycetes

Actinomycetes are non-motile filamentous gram positive bacteria belonging to the genus of the Actinobacteria class of bacteria.	Fungi are a group of microorganism which includes single cell and complex multicellular organisms such as yeast, mushrooms, moulds, etc.
Actinomycetes are prokaryotic organisms.	Fungi are eukaryotic organisms.
Actinomycetes contain peptidoglycan in their cell walls.	Fungi contain chitin in their cell wall
Actinomycetes filaments are smaller.	Fungi filaments are bigger
GC content in actinomycetes DNA is less than fungi.	Fungi have more GC bases in DNA.

ACTINOMYCOSIS

- *A. israelii* – the commonest
- A .*meyeri*
- A.*naeslundii*
- A.*odontolyticus*
- *A. viscosus*

ACTINOMYCES IS A NORMAL FLORA

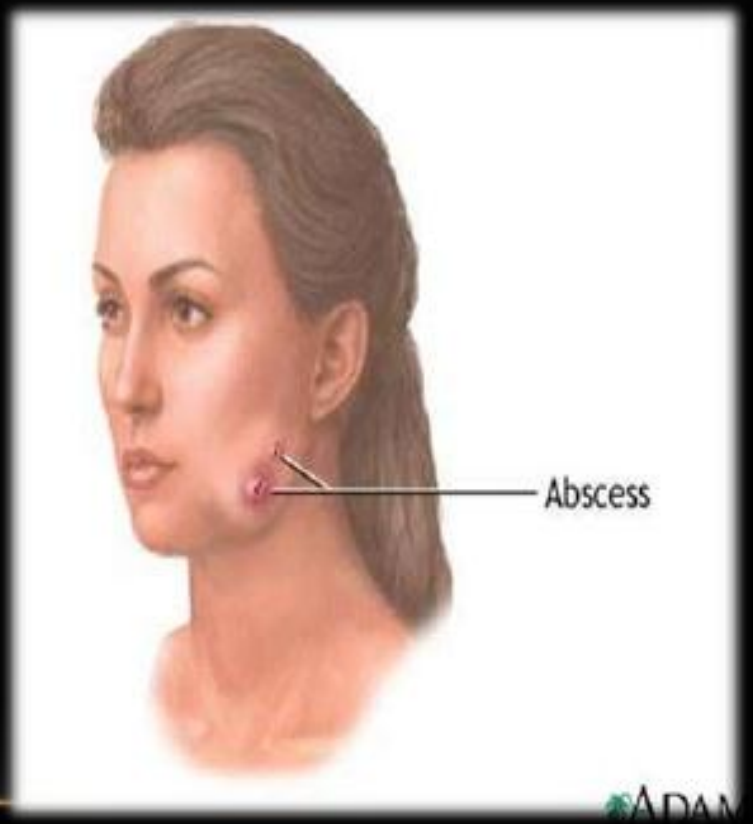


- Actinomyces species that cause human disease are not found in nature but are normal flora of the oropharynx, GI tract, and female genital tract. This is not an exogenous infection; therefore, no person-to-person spread of the pathogen occurs

ACTINOMYCOSIS

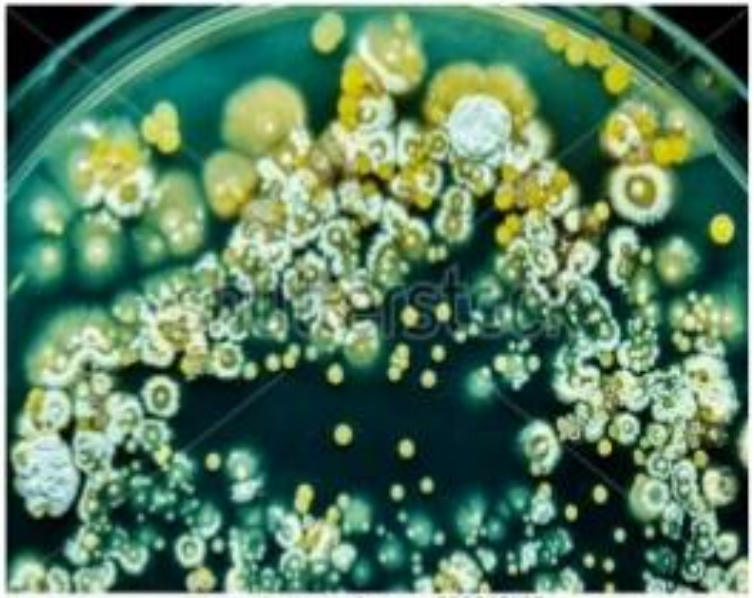
Not highly virulent (Opportunist)

- Component of Oral Flora
  - Periodontal pockets
  - Dental plaque
  - Tonsilar crypts
- Take advantage of injury to penetrate mucosal barriers
  - Coincident infection
  - Trauma
  - Surgery



CULTURING OF ACTINOMYCES

- Actinomyces species grow well in enriched media with brain-heart infusion and may be aided in growth by an atmosphere of 6-10% ambient carbon dioxide. They grow best at 37°C. Colonies can appear at 3-7 days, but, to ensure that no growth is missed, observe cultures for 21 days.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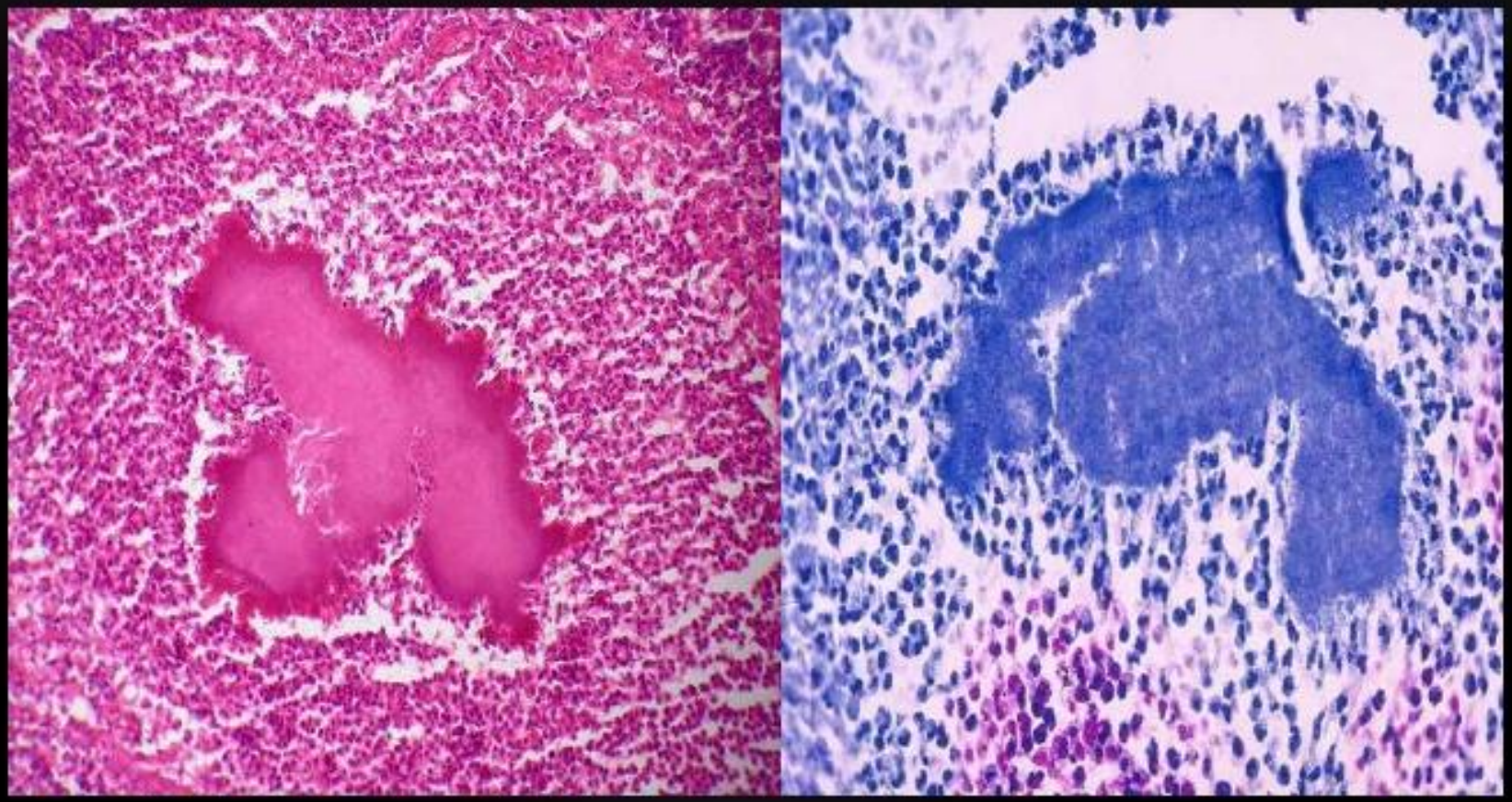
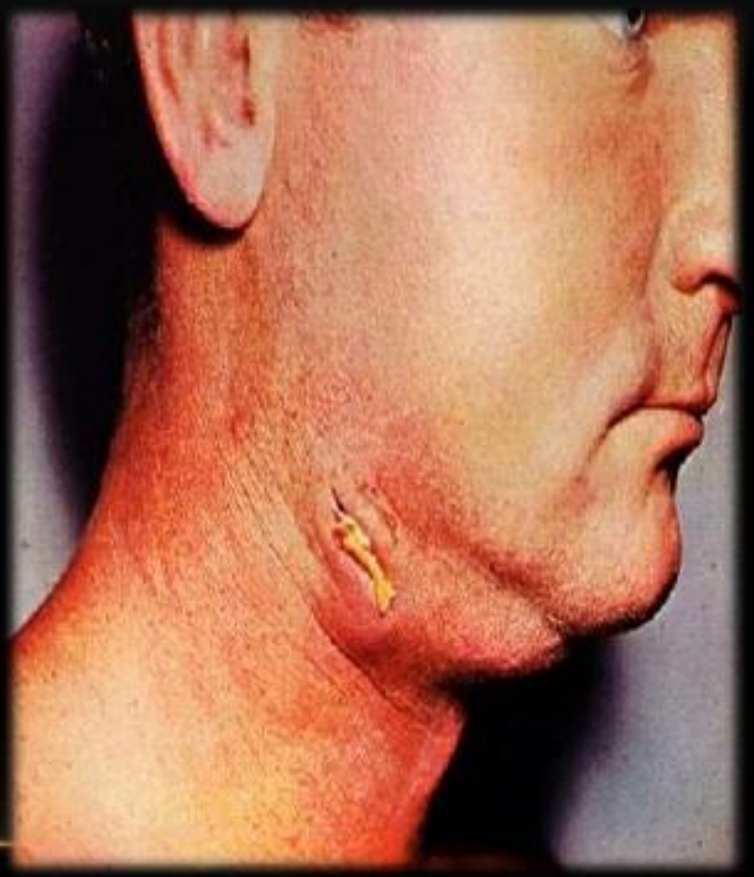
TYPICAL APPEARANCE OF HISTOPATHOLOGICAL EXAMINATION WITH SPECIAL STAINS



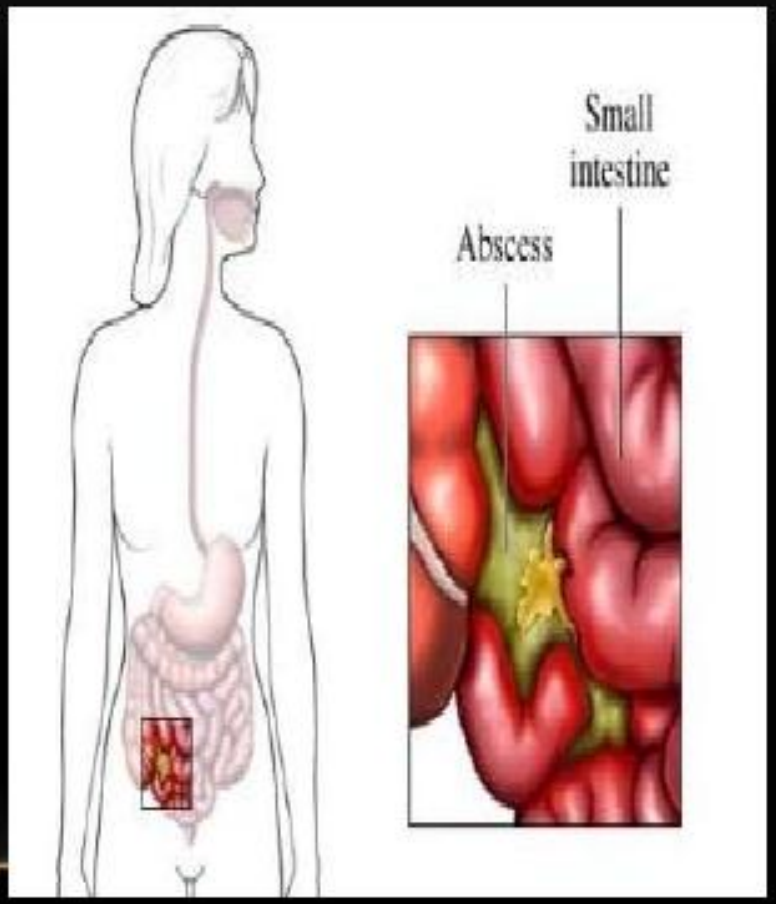
CERVICOFACIAL ACTINOMYCOSIS

- This is the most common and recognized presentation of the disease.
- Actinomyces species are commonly present in high concentrations in **tonsillar crypts and gingivodental crevices**. Many patients have a history of poor dentition, oral surgery or dental procedures, or trauma to the oral cavity.
- Chronic tonsillitis, mastoiditis, and otitis are also important risk factors for actinomycosis.



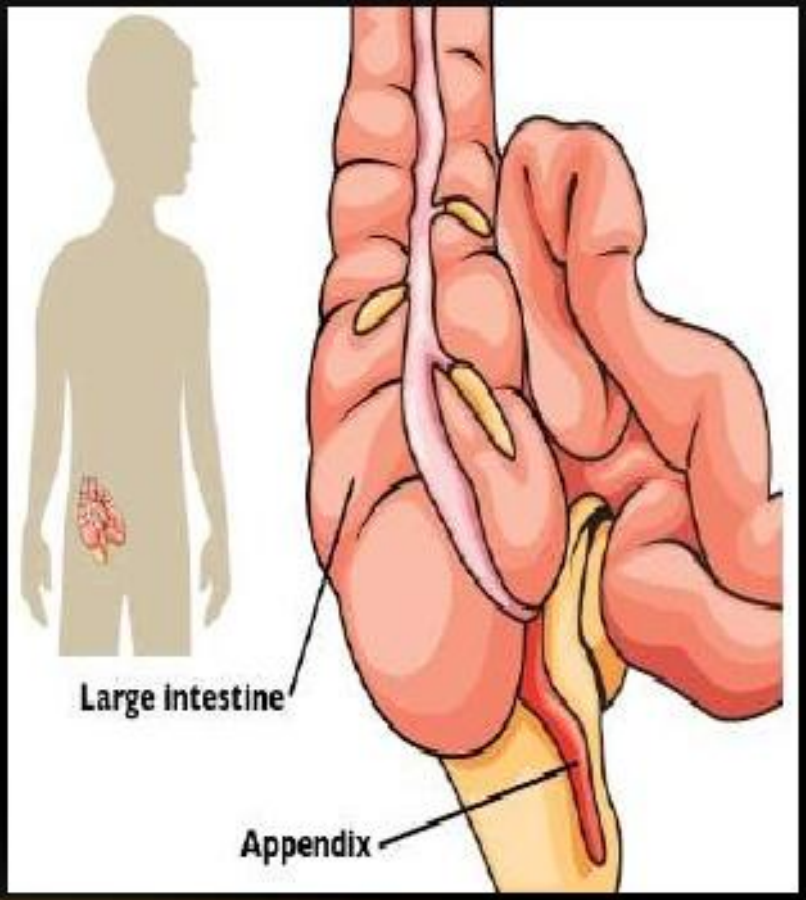
INTESTINAL ACTINOMYCOSIS

- The infection usually develops after GI mucosal integrity is broken from surgical procedures or trauma, although, on many occasions, the inciting conditions may not be apparent.



ABDOMINAL ACTINOMYCOSIS

- Appendicitis with perforation is the most common predisposing event, and, as a result, right-sided abdominal infection is far more common than left-sided abdominal infection. The inciting event can precede the diagnosis by months to years.



DIAGNOSIS:

- Gram stain.
- Culture. (poor growth in culture only in less than 50% of cases.) Sulphur granules (yellowish myecelial masses)
- **Specimens – open biopsy, aspiration material**
- **The discharge should mix with sterile saline in a universal bottle and allow to stand, particles will separate out.**
- **Place between 2 slides**
- **Crush and gram stain**
- **Observe for Gram positive branching filaments**

EXAMINATION OF DISCHARGES WILL HELP IN DIAGNOSIS

- Examination of drained fluid under a microscope shows "sulphur granules" in the fluid. They are yellowish granules made of clumped organisms

Taxonomy

Order: Spirochaetales

Family: *Spirochaetaceae*
Genus: *Trepanoma*
Borrelia

Family: *Leptospiraceae*
Genus: *Leptospira*

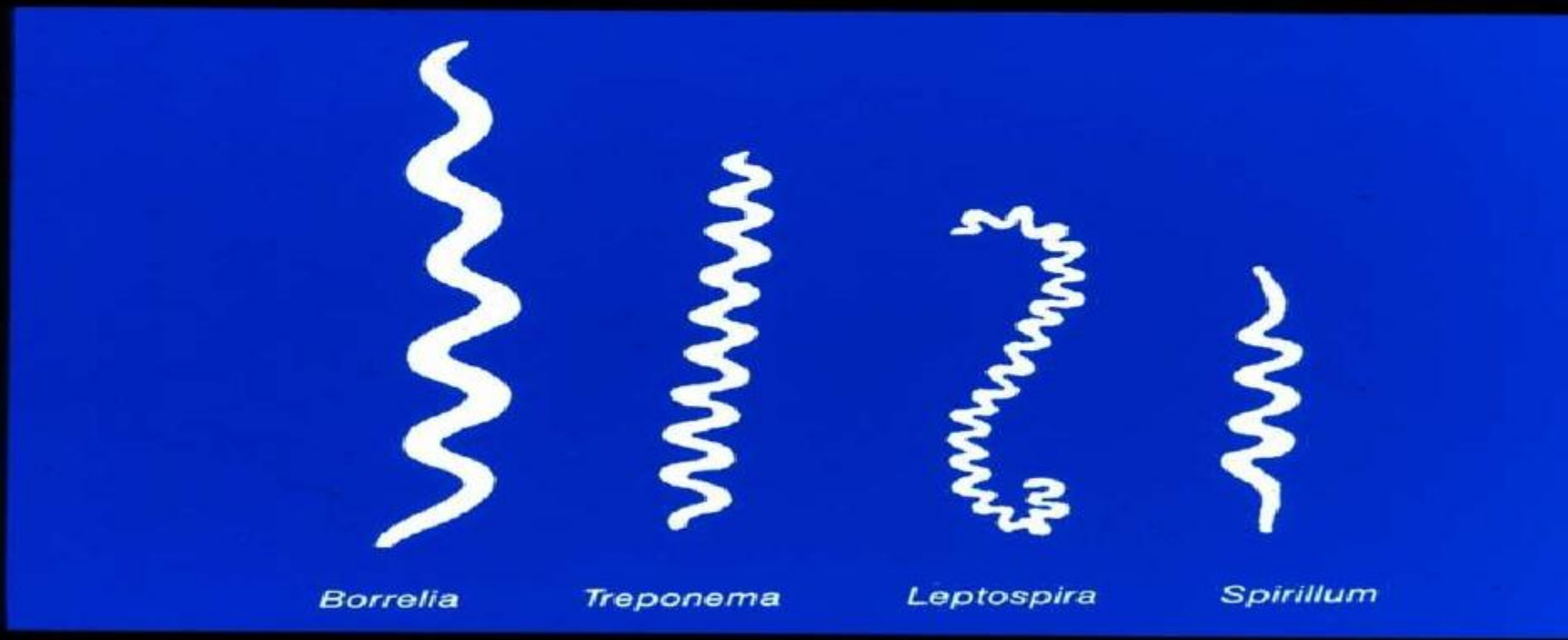
Human pathogen

A. Genera Trepanoma

B. Borreilia

C. Leptospira

How they appear



General Overview of Spirochaetales

A. Gram-negative spirochetes
B. Spirochete from Greek for "coiled hair"
C. Extremely thin and can be very long
D. Tightly coiled helical cells with tapered ends
E. Motile by Periplasmic flagella (a.k.a., axial fibrils or endoflegalla)

Spirochaetales *Associated Human Diseases*

Genus	Species	Disease
Treponema	*pallidum* ssp. *pallidum* *pallidum* ssp. *endemicum* *pallidum ssp. pertenue* *carateum*	Syphilis Bejel Yaws Pinta
Borrelia	*burgdorferi* *recurrentis* Many species	Lyme disease (borreliosis) Epidemic relapsing fever Endemic relapsing fever
Leptospira	*interrogans*	Leptospirosis (Weil's Disease)

What are Trepanoma

Trepos – Turn

Nema Meaning thread

Relatively short and slender

With fine spirals pointed and round ends

May be pathogenic or commensals in the mouth

Trepanoma pallidum

Greek words trepo "turning" & nema "head"

A. Morphology

1. Motile, sluggish in viscous environments
2. Size: 5 to 20 µm in length & 0.09 to 0.5 µm in diameter, with tapered ends
3. Structure
   - Multilayer cytoplasmic membrane
   - Flagella-like fibrils
   - Cell wall
   - Outer sheath (outer cell envelope)
   - Capsule-like outer coat

Treponema pallidum.

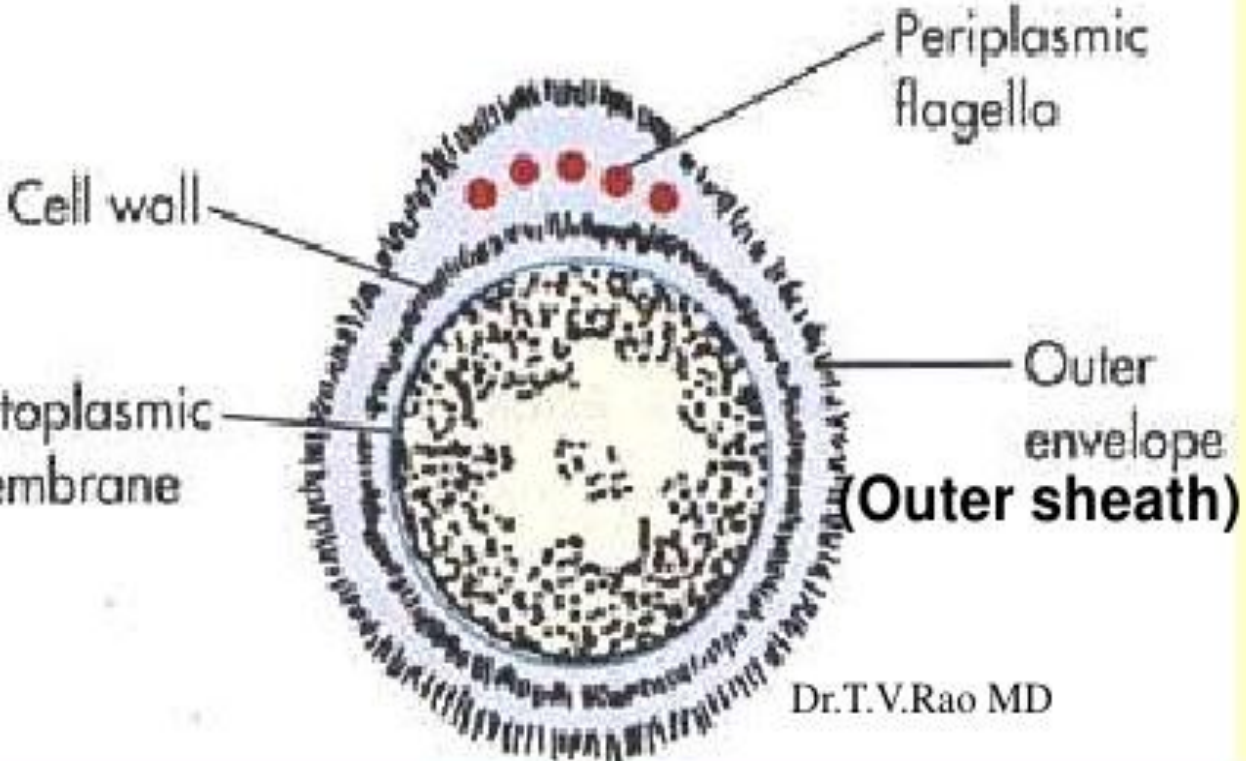
A. Spiral spirochete that is mobile of spirals varies from 4 to 14 Length 5 to 20 microns and very thin 0.1 to o.5 microns. Can be seen on fresh primary or secondary lesions by **dark field microscopy or fluorescent antibody techniques**

Trepanoma palladium

B. Physiology

- Difficult to culture
  - **Maintained in anaerobic** medium with albumin, sodium bicarbonate, pyruvate, cysteine
  - Microaerophilic

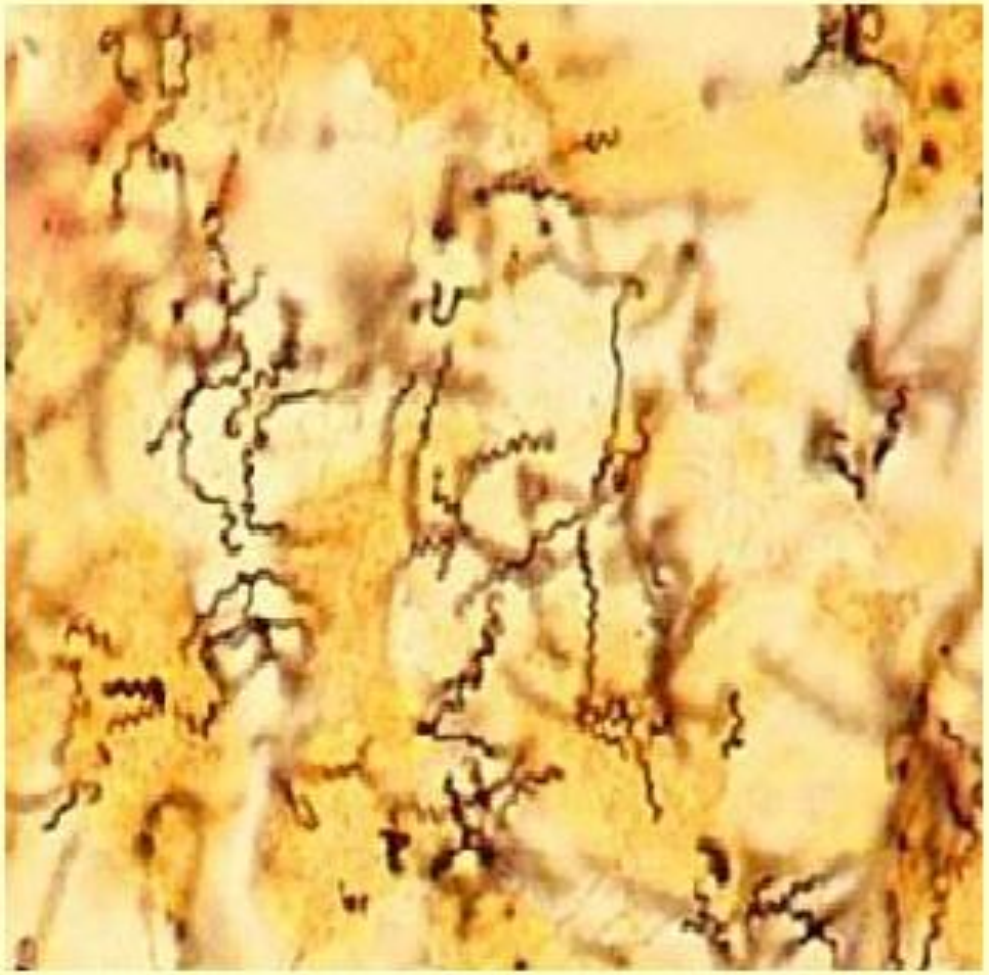
# of Spirochete with Periplasmic Flagella



NOTE: a.k.a., **endoflegalla**, **axial fibrils** or **axial filaments.**

Staining with special stains

Staining by Giemsa and Fontana



Antigenic structure

A. The Antigens are complex
B. Infection with Treponema will induce 3 types of Antigens
C. **Reagin Antibodies – STS**
D. Detected by **Standard tests for Syphilis**
E. 1 Wasserman Test, 2 Kahn Test
F. VDRL Test

Beef Heart Extracts - Antigen

Lipid Hapten – Cardiolipin

Chemically Dipphostidyl glycerol

Cardiolipin present in the Trenonems ?

Or a product of tissue Damage ?

Second Group Antigen
T.pallidum

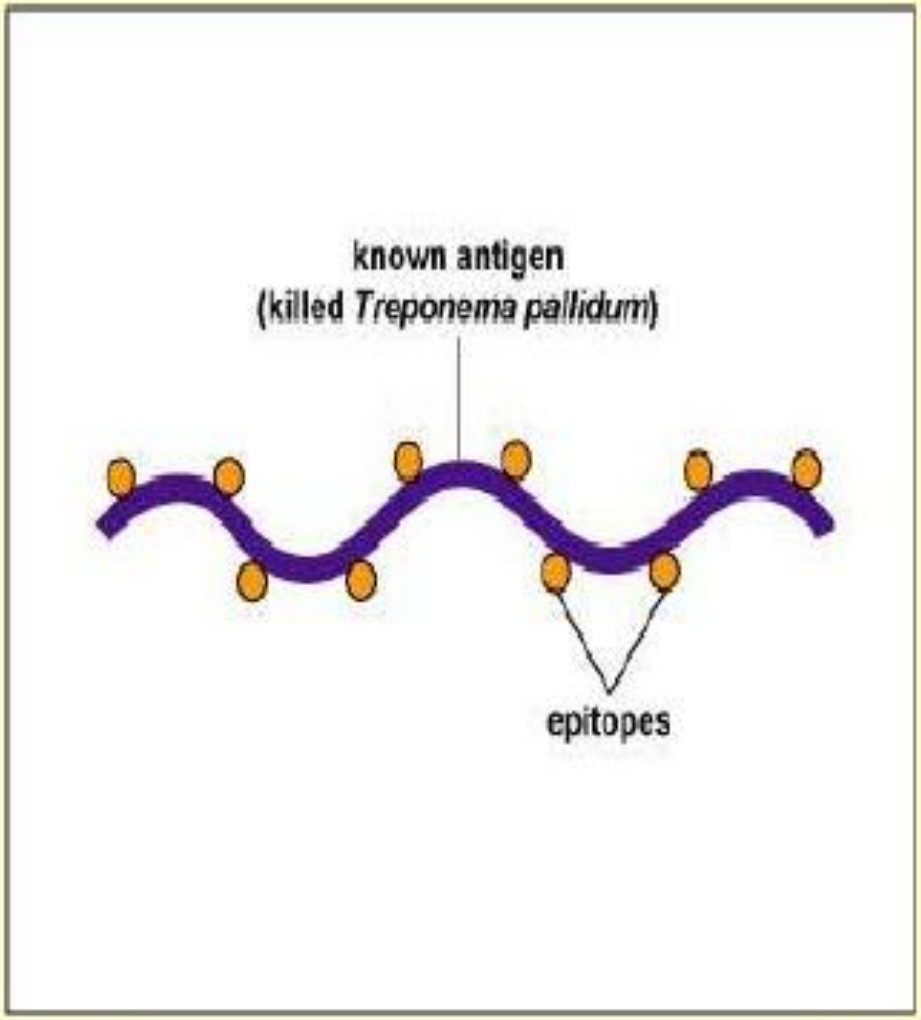
A. Present in T.pallidum and Non pathogenic cultivable treponemes

B. Reiter's Trenonems

Third Antigen

Polysaccharide species specific

Positive only in sera of patients infected with pathogenic Treponema



Venereal Syphilis

Venereal Syphilis caused by

T.pallidum Endemic syphilis

T. pallidum

Yaws T.pertune

Pinta T.carateum

STAGES OF SYPHILIS

1. Primary
2. Secondary
3. Latent
    i. Early latent
    ii. Late latent
4. Late or tertiary
    i. May involve any organ, but main parts are:
        - **Neurosyphilis**
        - **Cardiovascular syphilis**
        - **Late benign (gumma)**

Trepanoma pallidum

D. Clinical Infection: Syphilis

3. Clinical Manifestations

i. Primary Disease

- **Chancre: single lesion, non-tender & firm with a clean surface, raised border & reddish color**
- **Usually on the cervix, vaginal wall, anal canal**
- **Draining lymph nodes enlarged & non-tender**

Primary syphilis

a) One or more painless chancres (indurated raise edges & clear bases) that erupt in the genitalia, anus, nipples, tonsils or eyelids.

b) Starts as papule and then erode

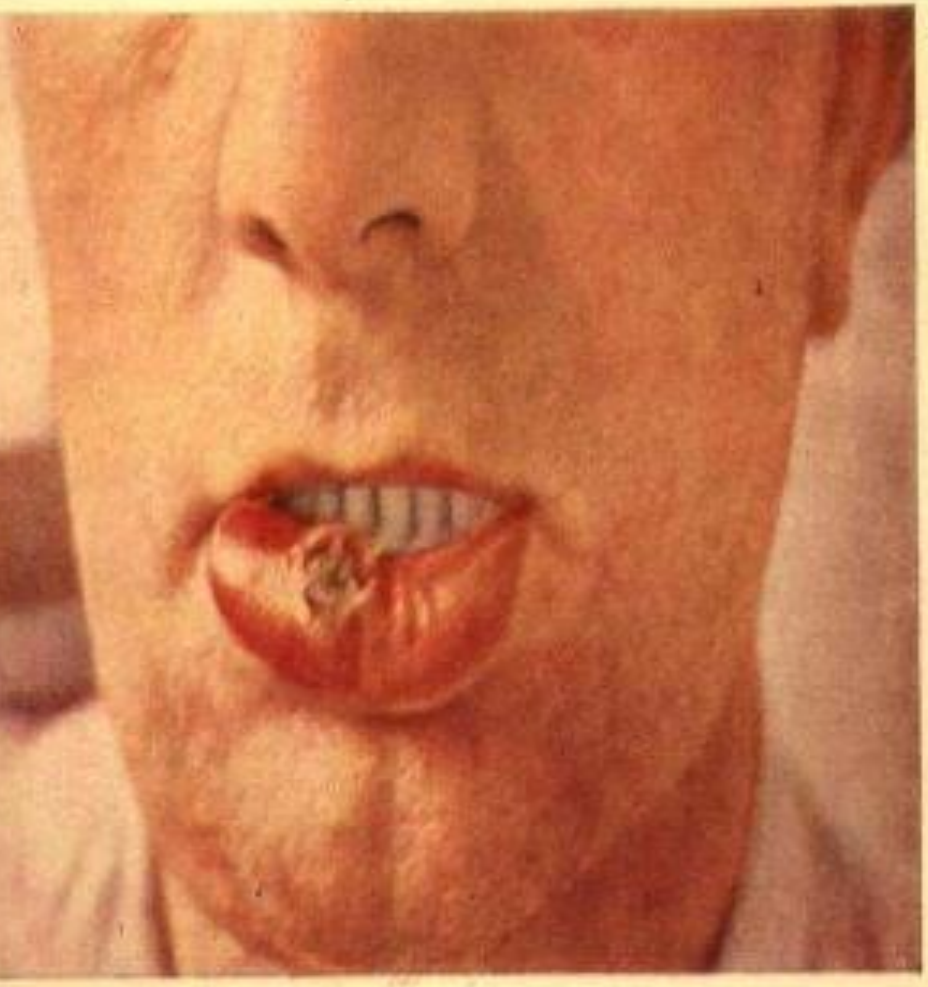
c) Disappear after three to six weeks even without treatment.

d) Lymphadenopathy that is either unilateral or bilateral

Chancre

The chancre usually heals spontaneously within 3-6 weeks, and **2-12 weeks later the symptoms of secondary syphilis develop.** These are highly variable and widespread but most commonly involve the skin where macular or pustular lesions develop, particularly on the trunk and extremities. The lesions of secondary syphilis are highly infectious.

Primary Syphilis - *Chanc*



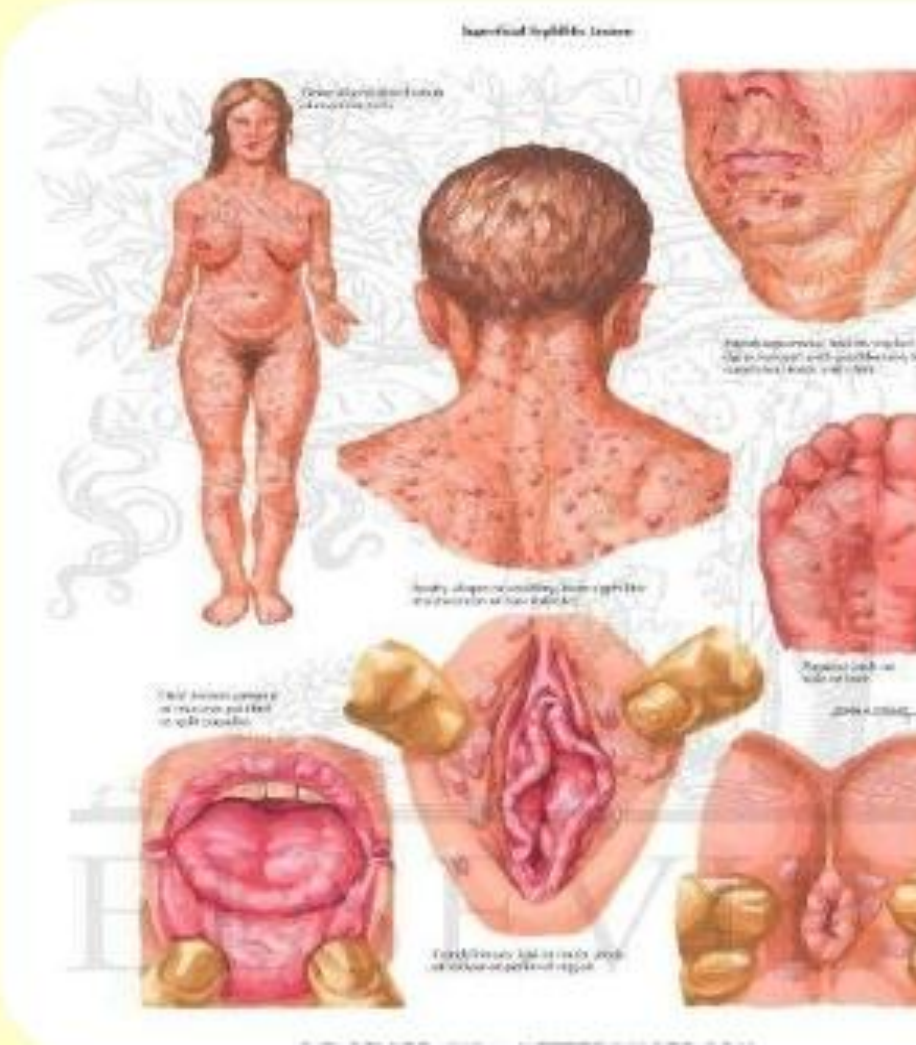
ig. 171. **Primary Syphilis of the Lower Lip.** A chancre appearing on the lower lip the same clinical appearance as one appearing on the genital mucosa. This lesion simulate squamous cell carcinoma.

Dr.T.V. Rao MD

Primary Syphilis

Secondary Syphilis

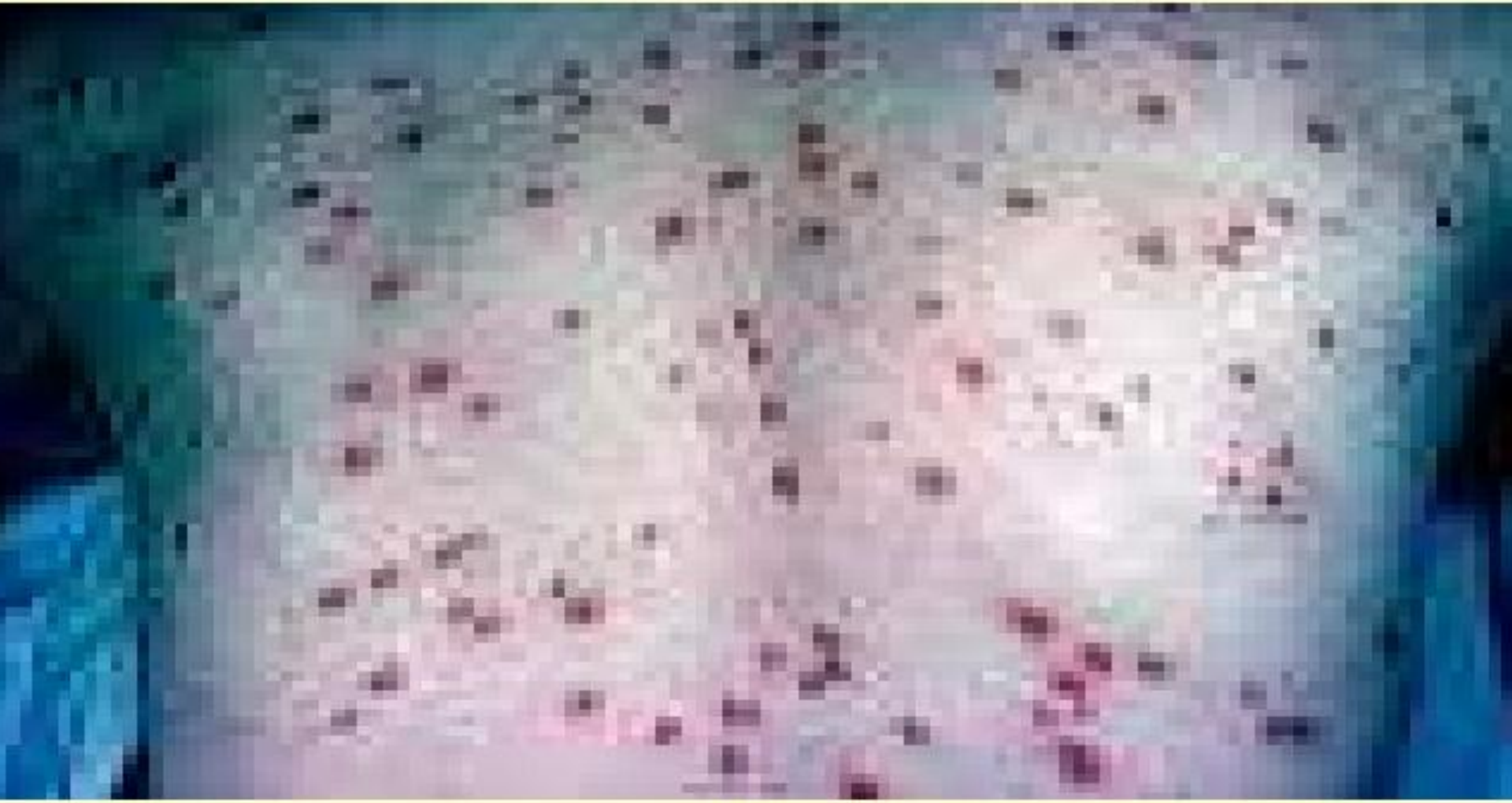
A. Secondary syphilis at 6-8 weeks – diffuse symptoms:
   1. Fever
   2. Headache
   3. Skin pustules
B. Usually disappears even without



Secondary Syphilis

Secondary Syphilis

Secondary Syphilis



Secondary syphilis

Tertiary Syphilis

A. Affects 2/3 of untreated cases
   1. Gummata: rubbery tumors
   2. Bone deformities
   3. Blindness
   4. Loss of coordination
   5. Paralysis
   6. Insanity

Tertiary Syphilis

Tertiary syphilis characterized by **localized granulomatous dermal lesions (gummas)** in which few organisms are present

- Granulomas reflect containment by the immunologic reaction of the host to chronic infection

Pathogenesis of T. pallidum *(cont.)*

Latent Stage Syphilis

- Following secondary disease, host enters latent period
  - First 4 years = **early latent**
  - Subsequent period = **late latent**
- **About 40% of late latent patients progress to late tertiary syphilitic**

Latent Syphilis

A. Latent syphilis

a) Reactive serologic test

b) Asymptomatic until death

A. Late syphilis

Three subtypes of Late syphilis

1. Late, benign syphilis

*Develops between 1 to 10 years after the infection

*Presence of gumma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Mother to Child Transmission

Infection in utero may have serious consequences for the fetus. Rarely, syphilis has been acquired by transfusion of infected fresh human blood.

Pathogenesis of T. pallidum *(cont.*

Congenital Syphilis

- Congenital syphilis results from **trans placental infection**
- *T. pallidum* **septicemia in the developing fetus and widespread dissemination**
- **Abortion, neonatal mortality, and late mental or physical problems** resulting from scars from the active disease and progressio of the active disease state

Congenital Syphilis

A. Passed from mother to fetus during pregnancy
   1. Abnormally shaped teeth
   2. Nasal septum collapses
   3. Skeletal abnormalities

DIAGNOSIS OF SYPHILIS

A. 1. History and clinical examination.

B. 2. Dark-field microscopy: special technique use to demonstrate the spirochete as shiny motile spiral structures with a dark background.

C. The specimen includes oozing from the lesion or sometimes L.N. aspirate. It is usually positive in the primary and secondary stages and it is most useful in the primary stage when the serological tests are still negative.

Diagnosis of syphilis

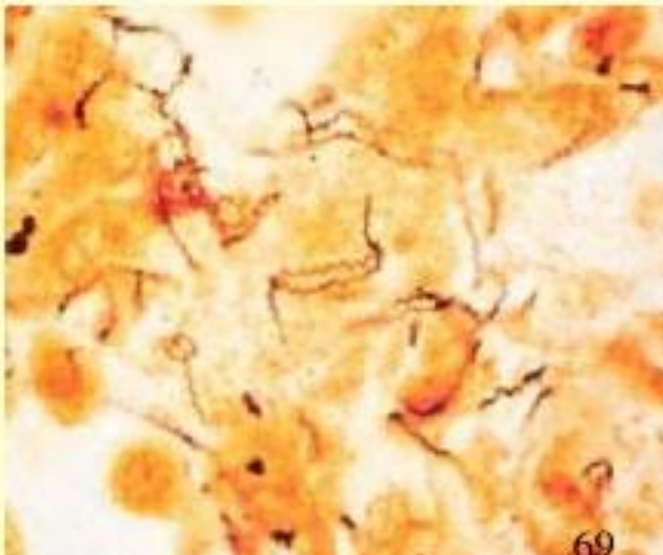
Direct detection of spirochetes :

Darkfield microscopy (motile bugs + experience + prompt examination)

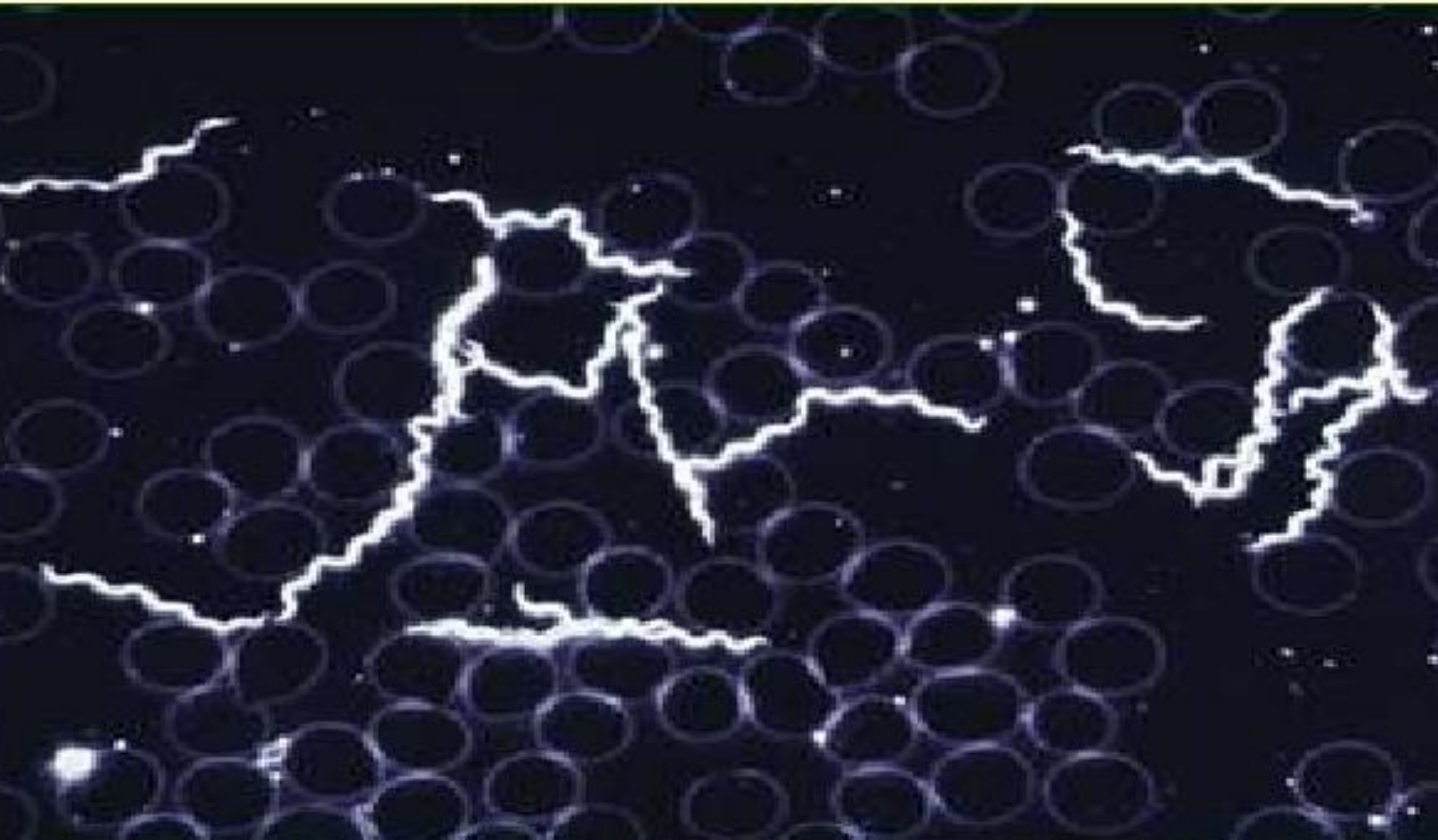
Silver stain

Culture : not used

Serology: non-specific and specific tests



Dark field Microscopy



Treponema cannot be cultivated in Culture Media

. The inability to grow most pathogenic Treponema in vitro, coupled with the transitory nature of many of the lesions, makes diagnosis of Treponema infection impossible by routine bacteriological methods

Cultivation of .. ?

Although the Treponemes are distantly related to Gram-negative bacteria, they do not stain by Gram's method, and modified staining procedures are used. Moreover, the pathogenic **Treponemes cannot be cultivated in laboratory media and are maintained by subculture in susceptible animals**.

Serologic Tests

A. Reveal patients immune status *not* whether they are currently infected
B. Use lipoidal antigens rather than *T. pallidum* or components of it; *non-treponemal antigen tests*
C. RPR; rapid plasma reagin
D. VDRL; Venereal Disease Research Laboratory

Treponema pallidum

F. Laboratory diagnosis

1. Serologic testing
   i. Nontreponemal Tests (uses Cardiolipin-lecithin as antigen)
      a. Complement-fixation tests (Wasserman & Kolmer test)
      b. Flocculation tests (Venereal Disease Research Laboratory, (VDRL), Hinton & rapid reagin tests)

Serologic Tests

A. Positive within 5 to 6 weeks after infection
B. Strongly positive in secondary phase
C. Strength of reaction is stated in dilutions
D. May become negative with treatment or over decades

Non-treponemal tests

A. Antigen: **cardiolipin** (beef heart) + lecithin + cholesterol

B. Detect nonspecific antibody **(Reagin)**: a mixture of IgM & IgG direct against some normal tissue antigens

C. VDRL (Venereal Disease Research Laboratory) test for serum and CSF samples

Venereal Disease Research Laboratory - VDRL

Flocculation test, antigen consists of very fine particles that precipitate out in the presence of reagin.

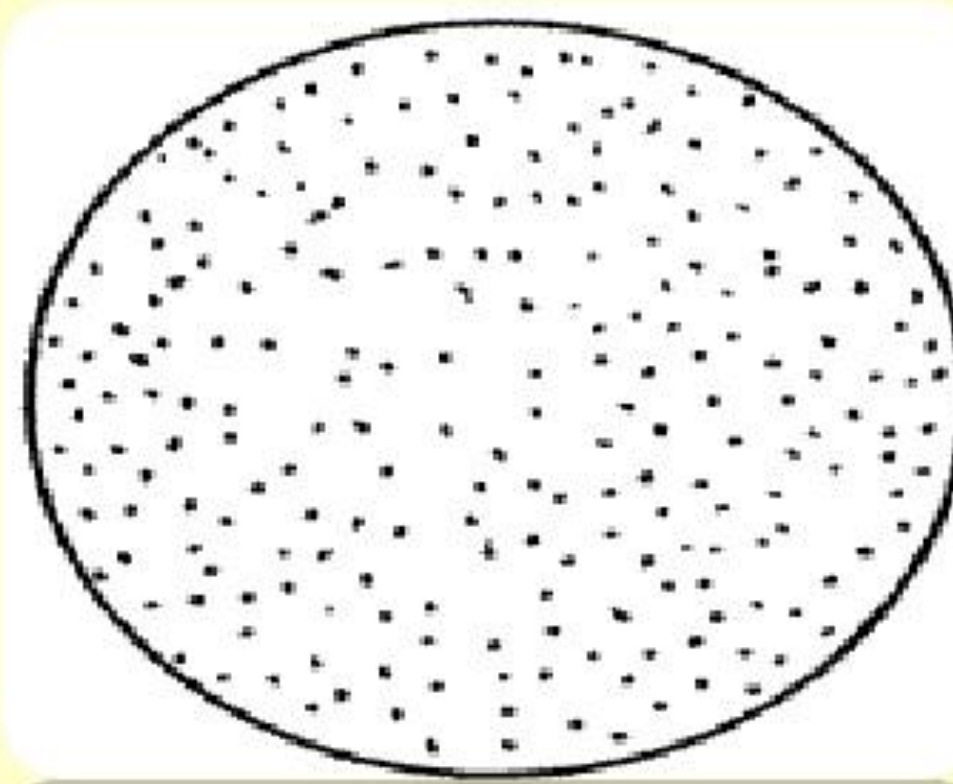
Utilizes an antigen which consists of ***cardiolipin, cholesterol and lecithin***.

1. Antigen very technique dependent.
2. Must be made up fresh daily.

Serum must be heated to 56 C for 30 minutes to remove anti-complementary activity which may cause false positive, if serum is not tested **within 4 hours** must be **reheated for 10 minutes**.

Calibrated syringe utilized to dispense antigen **must deliver 60 drops/ml +/- 2**drops

VDRL



A. Each preparation of antigen suspension should first be examined by testing with known positive or negative serum controls.

B. The antigen particles appear as short rod forms at magnification of about 100x. Aggregation of these particles into large or small clumps is interpreted as degrees of positivity

C. Reactive on left, non-reactive on right

Rapid Plasma Reagin Test - RPR

A. General screening test, can be adapted to automation.

B. CANNOT be performed on CSF.

C. Antigen
   1. VDRL cardiolipin antigen is **modified with cholin chloride** to make it more stable
   2. attached to charcoal particles to allow macroscopi reading
   3. antigen comes prepared and is very stable.

D. Serum or plasma may be used for testing, serum is **not** heated.

Specific serological tests of syphilis

A. A. Reiter protein complement fixation test.

B. B. **Fluorescent Treponemal antibody/absorption test, FTA/ABS. the most specific and most sensitive** .

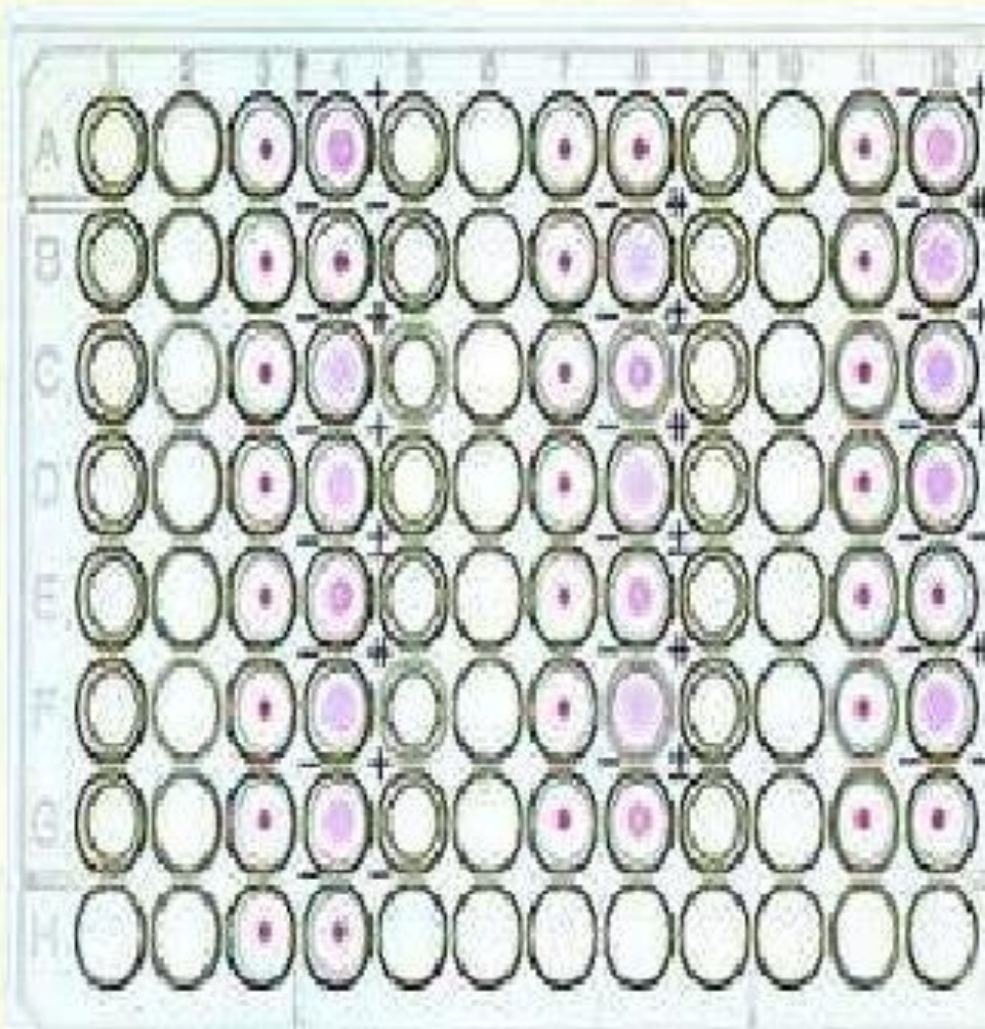
C. C. **Treponema pallidum haemagglutination test- TPHA-** D. Treponema pallidum immobilization test-

Treponema pallidum haemagglutination (TPHA)

A. Adapted to micro techniques (MHA-TP)

B. Tanned sheep RBCs are coated with T. pallidum antigen from Nichol's strain.

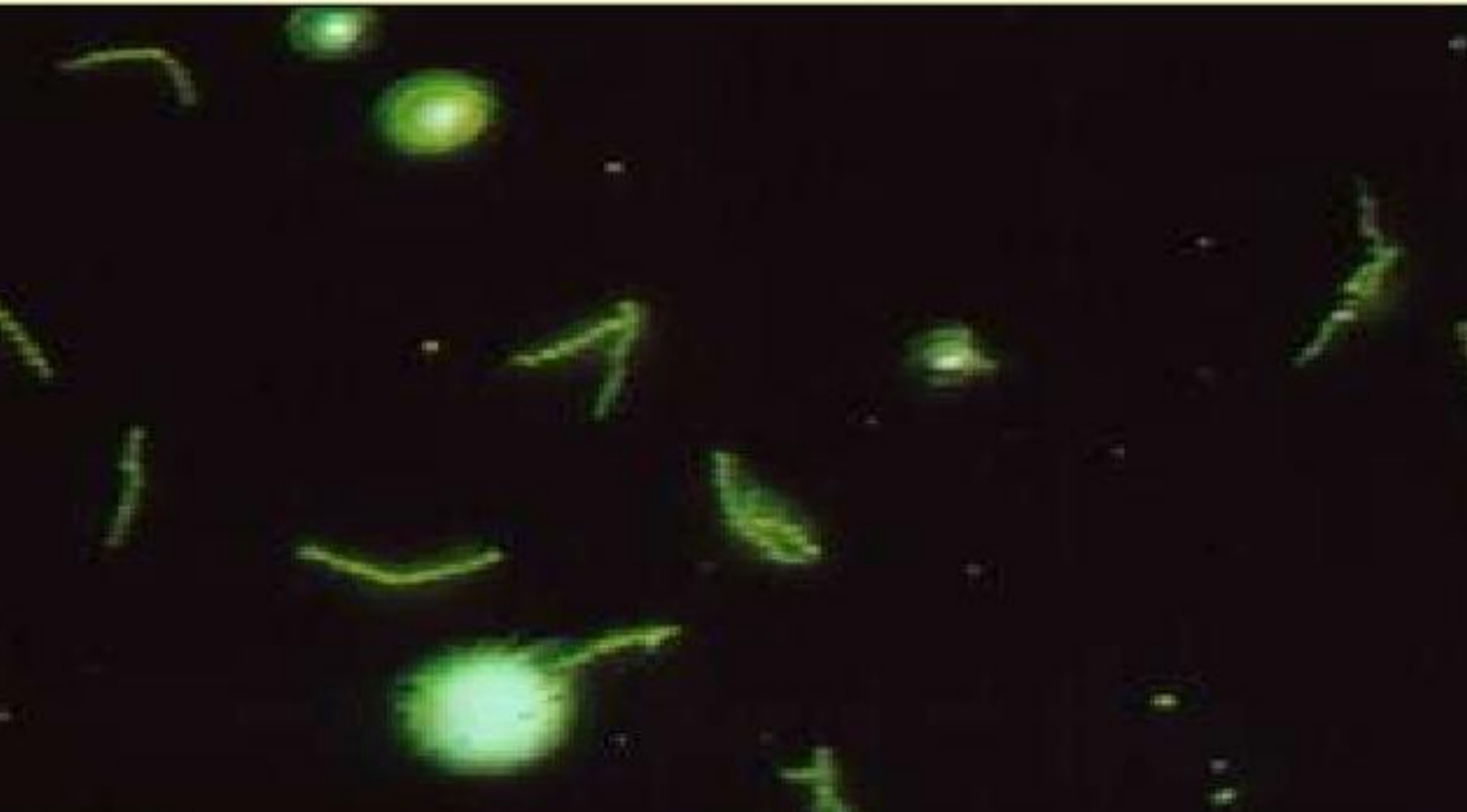
C. Agglutination of the RBCs is a positive result.



Fluorescent Treponemal Antibody Absorption Test (FTA-ABS)

A. Diluted, heat inactivated serum added to Reiter's strain of *T. pallidum* to remove cross reactivity due to other *Treponemes*.
B. Slides are coated with Nichol's strain of *T. pallidum* and add absorbed patient serum.
C. Slides are washed, and incubated with antibody bound to a fluorescent tag.
D. After washing the slides are examined for fluorescence.
E. Requires experienced personnel to read.
F. Highly sensitive and specific, but time

Positive FTA Test for Syphilis Viewed with a Fluorescent Microscope



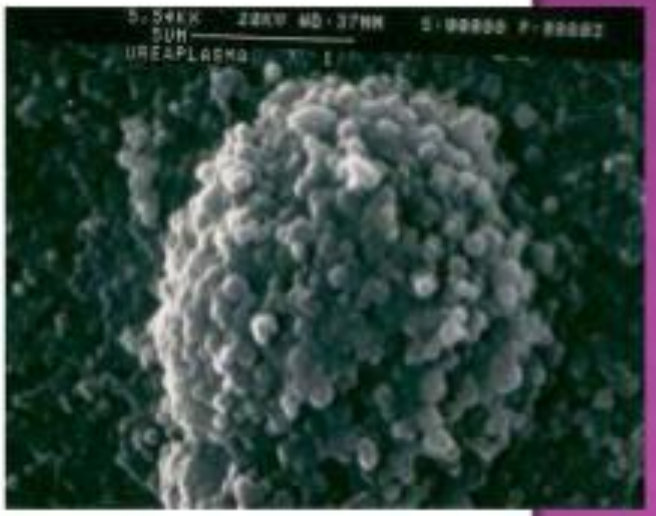
Mycoplasma

- ***Mycoplasma*** species are the smallest free-living organisms. These organisms are unique among prokaryotes in that they lack a cell wall.

- Mollicutes

- Familia *Mycoplasmataceae*

- *Mycoplasma pneumoneae.*
- *Mycoplasma hominis*
- *Mycoplasma genitalium*
- *Ureaplasma urealyticum*



Urogenital mycoplasmas

Table 3.1: Disease associations of urogenital mycoplasmas

Species	Disease associations[a]						
	Urethritis	Cervicitis	Bacterial vaginosis	Endometritis and/or PID	Preterm birth	Infertility (Women)	HIV transmission
M. genitalium	++++	+++	–	+++	+/–	+	+
M. hominis	–	–	++++	+/–	+/–	–	ND
Ureaplasmas (undifferentiated)	+/–	–	+++	ND	+	+/–	ND
U. urealyticum	+	ND	ND	ND	ND	ND	ND
U. parvum	–	ND	ND	ND	ND	ND	ND

ND, not determined; PID, pelvic inflammatory disease.

[a] ++++ strong association, +++ association in most studies, + association only from a few studies, +/– conflicting results.

Jensen and Unemo. WHO Manual. 2013

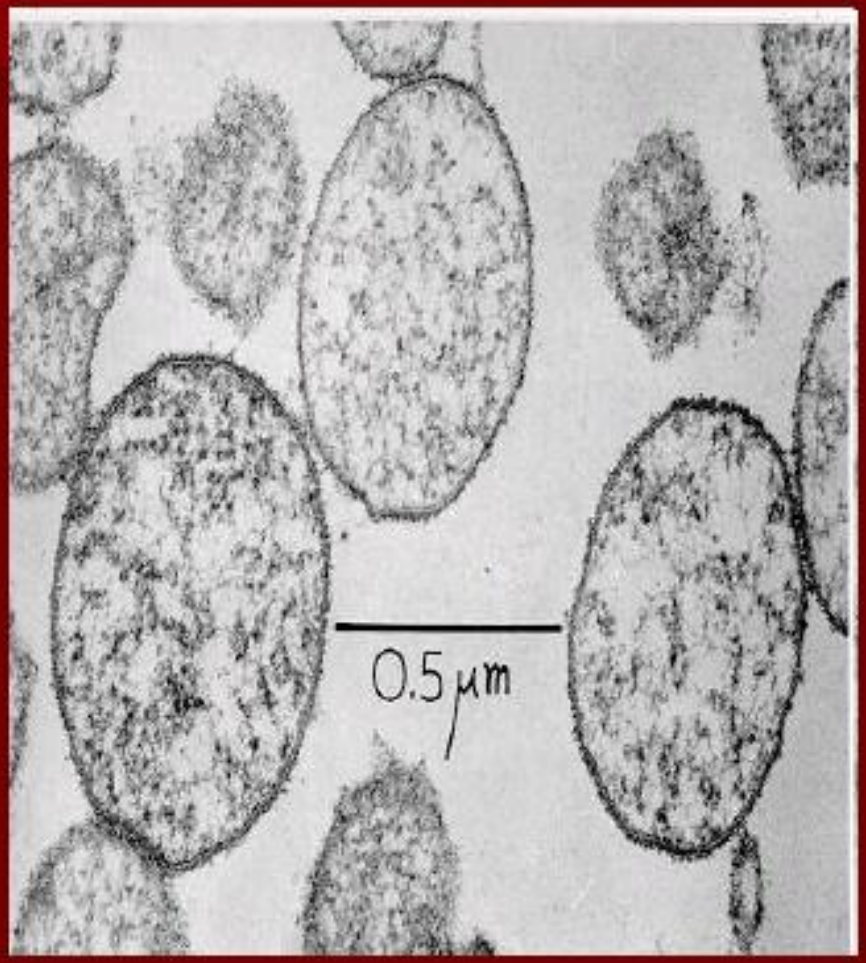
Urogenital mycoplasmas

- *M.hominis, U.urealyticum, U.parvum*- commonly detected in healthy individuals
- Their association with urogenital infection in either men or women remains to be conclusively proven

Basic Characters of Mycoplasma

- Prokaryotic microbes
- Size of 150-250 nm
- Lack of a cell wall
- Sterol-containing cell membrane
- Fastidious growth requirements
- Fried-egg or mulberry colonies on agar

Mycoplasma are cell wall deficient microorganisms



- Cross-section of **Mycoplasma** bacteria, a common cause of atypical pneumonia. This bacteria is unusual in that it lacks a cell wall.

Culturing Mycoplasma

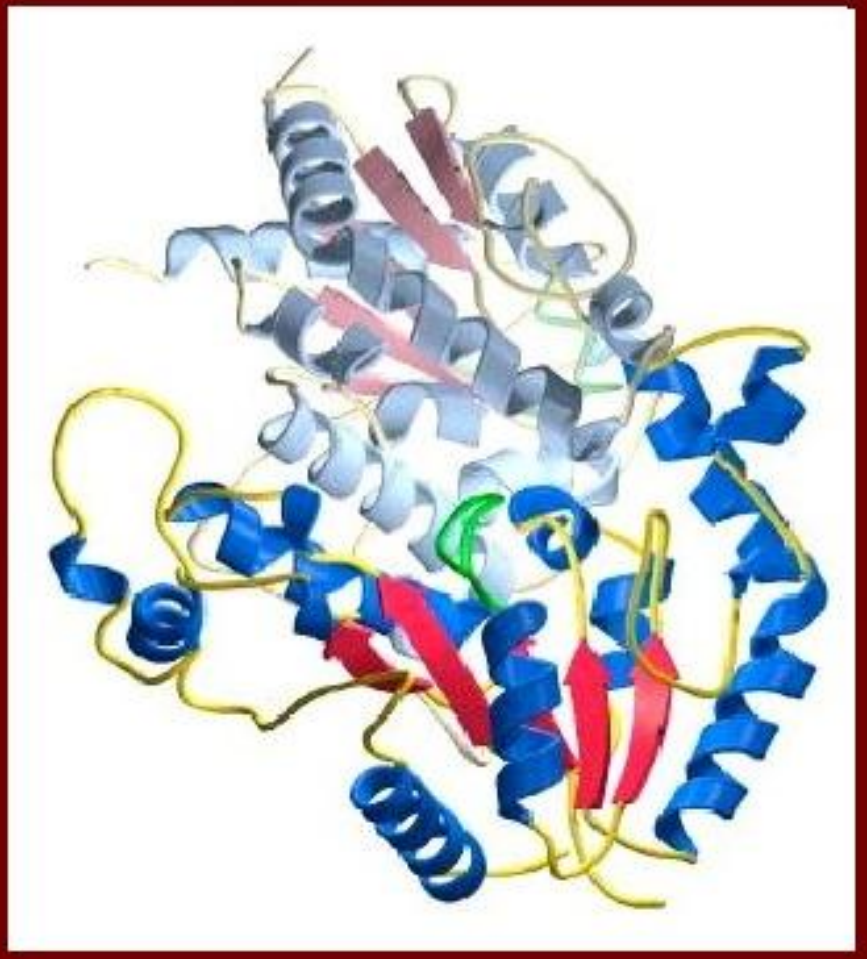
- Mycoplasma can be cultured on liquid or solid medium
- Growths optimally at 35 to 37^{0}c
- Medium of growth should be enriched with 20% horse or human serum.
- The colonies appears as fried egg appearance

Characters of Mycoplasma

- They are prokaryotes but lack a cell wall. However, they have a unique cell membrane that contains **sterols,** which are not present in either bacteria or viruses. *Mycoplasma* organisms are small (150-250 nm) and have deformable membranes. The name *Mycoplasma* refers to the plasticity of the bacterial forms resembling fungal elements.

Antigenic properties

- The surface antigens are glycolipids and protiens
- Glycolipids are identified by complement fixation.
- Proteins antigens detected by ELISA method.



Resistance

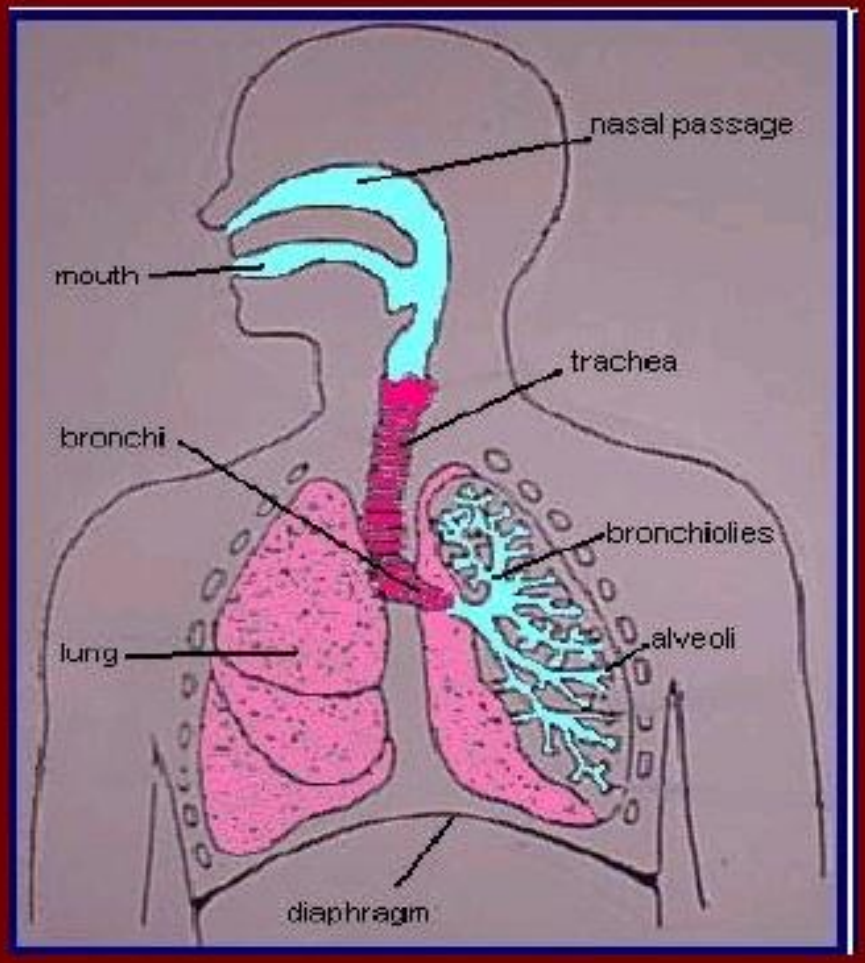
- They are normally destroyed by heat at 45^{0}c in 15 minutes.
- They are relatively resistant to pencillins, and Cephalosporins
- Sensitive to Tetracyclnes, and several other antibiotics

Clinical Manifestations

- Generalized aches and pains
- Fever (usually 102°F)
- Cough - Usually non-productive
- Sore throat (nonexudative Pharyngitis)
- Headache/ myalgias
- Chills but not rigors
- Nasal congestion with coryza
- Earache
- General malaise

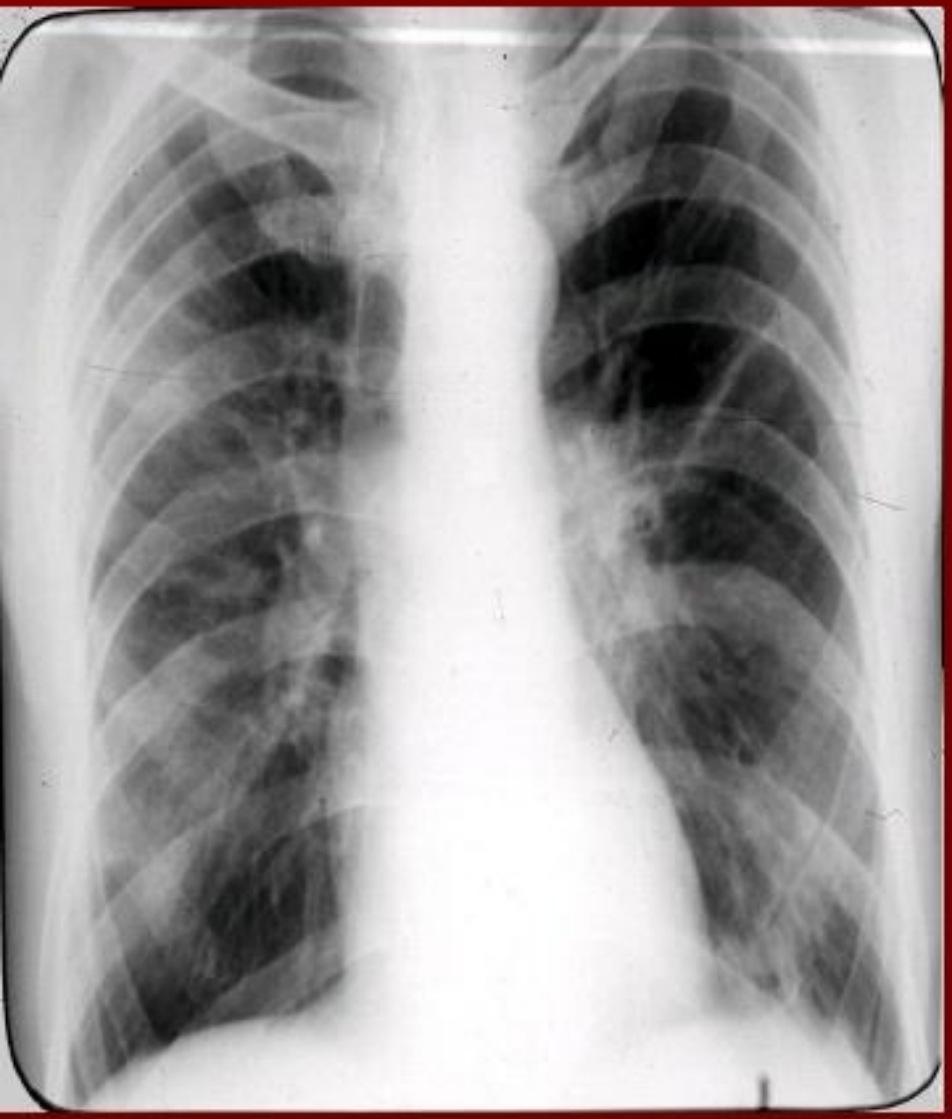
Respiratory spread

- Infection moves easily among people in close contact because it is spread primarily when infected droplets from the respiratory system circulate in the air due to coughing, spitting, or sneezing



Pneumonia leading Manifestation in Mycoplasma infections

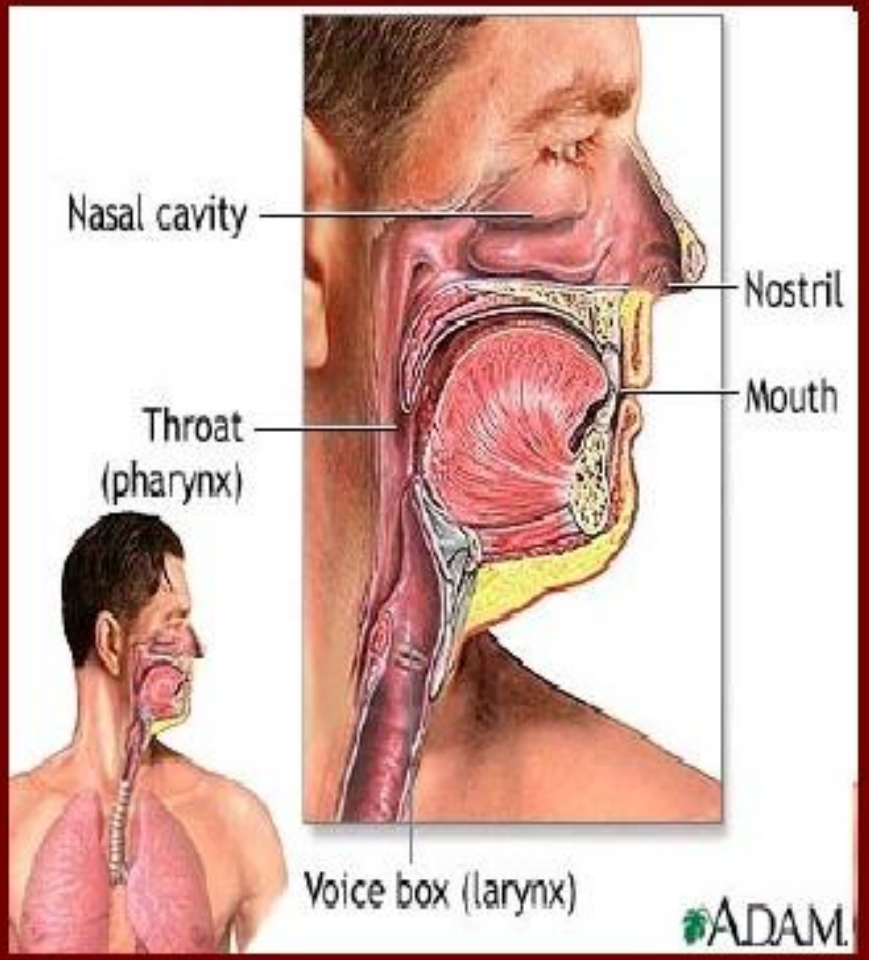
Pneumonia



- Pneumonia caused by Mycoplasma is also called atypical pneumonia, walking pneumonia, or community-acquired pneumonia.

Mycoplasma presents as non specific Respiratory infections

- Infections commonly involve the oropharynx, trachea, bronchi, and lungs, usually causing unilateral pneumonia of the lower lobe.
  The radiographic appearance cannot be distinguished from that of other nonbacterial pneumonias.



Radiological presentation

- The radiological picture is extremely variable, but one or both lower lobes are usually involved. The opacities usually start as partly mottled, partly node-like peribronchial opacities, which may gradually develop to involve whole segments or lobes

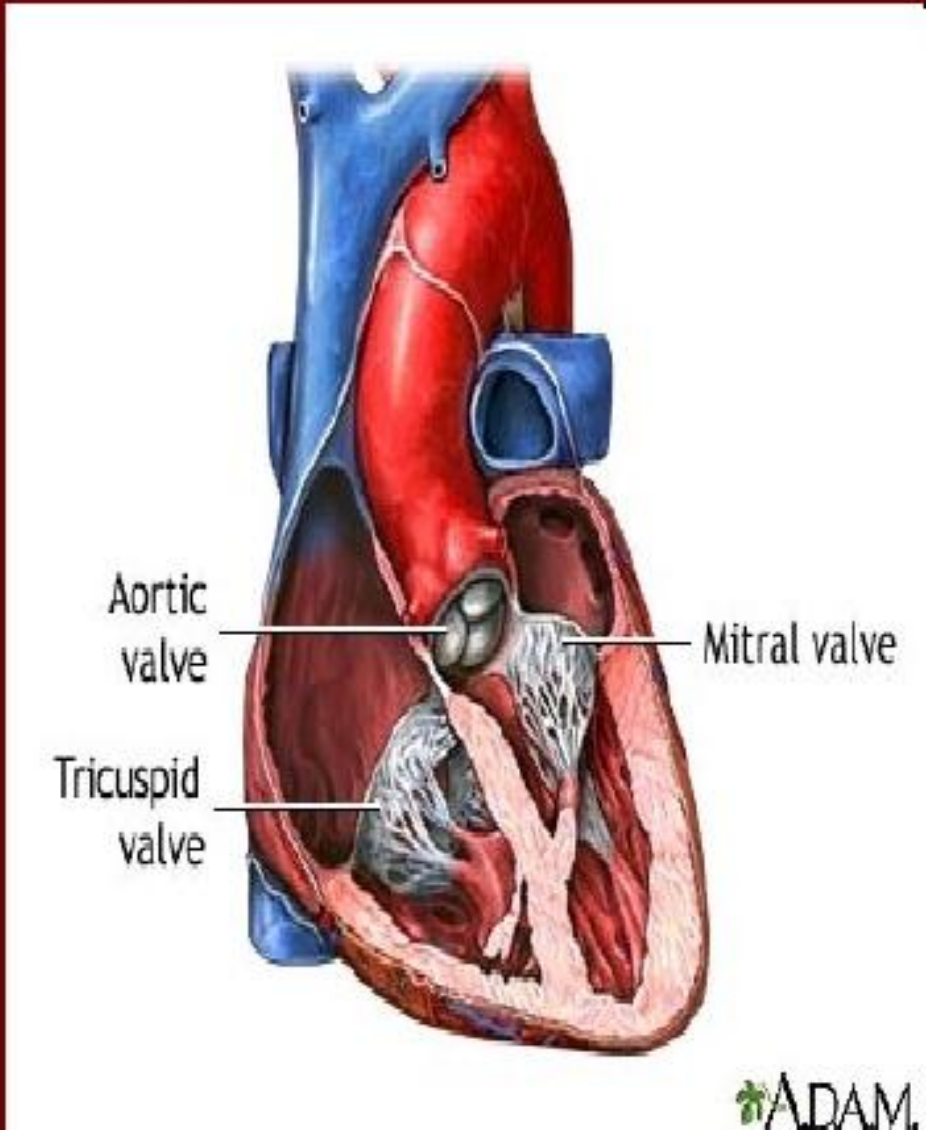
Mycoplasma in New born

- Colonization of infants by genital *Mycoplasma* species usually occurs during passage through an infected birth canal, and genital mycoplasmal organisms have been isolated from the upper respiratory tract in 15% of infants. Colonization usually does not persist beyond 2 years.

Cardiac Manifestations

- Arrhythmia and/or ECG abnormalities (conduction defects)
- Congestive failure
- Pericarditis
- Myocarditis
- Endocarditis

- Dr.T.V.Rao MD



Laboratory Diagnosis

- **Specimens** – throat swabs, respiratory secretions.

- **Microscopy** –

1. Highly pleomorphic, varying from small spherical shapes to longer branching filaments.
2. Gram negative, but better stained with Giemsa.

Laboratory Diagnosis

* Isolation of Mycoplasma (Culture) –

1. Semi solid enriched medium containing 20% horse or human serum, yeast extract & DNA. Penicillium & Thallium acetate are selective agents.
(serum – source of cholesterol & other lipids)

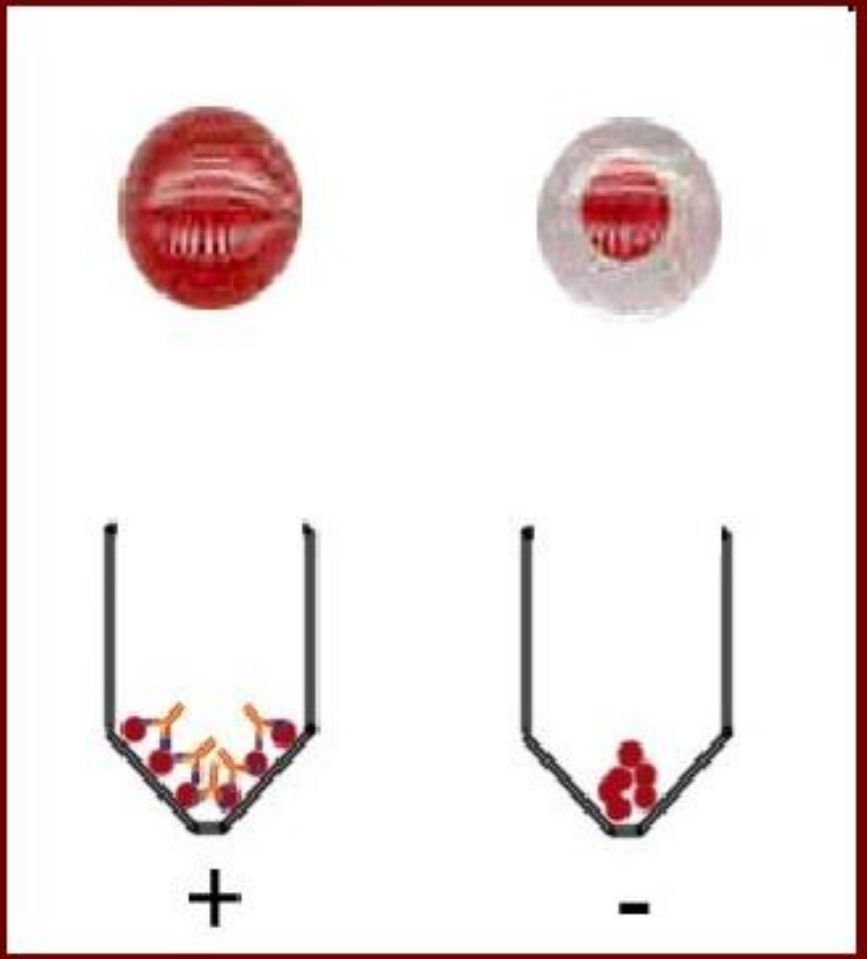
2. Incubate aerobically for 7 -12 days with 5-10% CO_2 at 35-37°C. (temp range 22- 41°C, parasites 35- 37°C, saprophytes – lower temp)

Laboratory Diagnosis

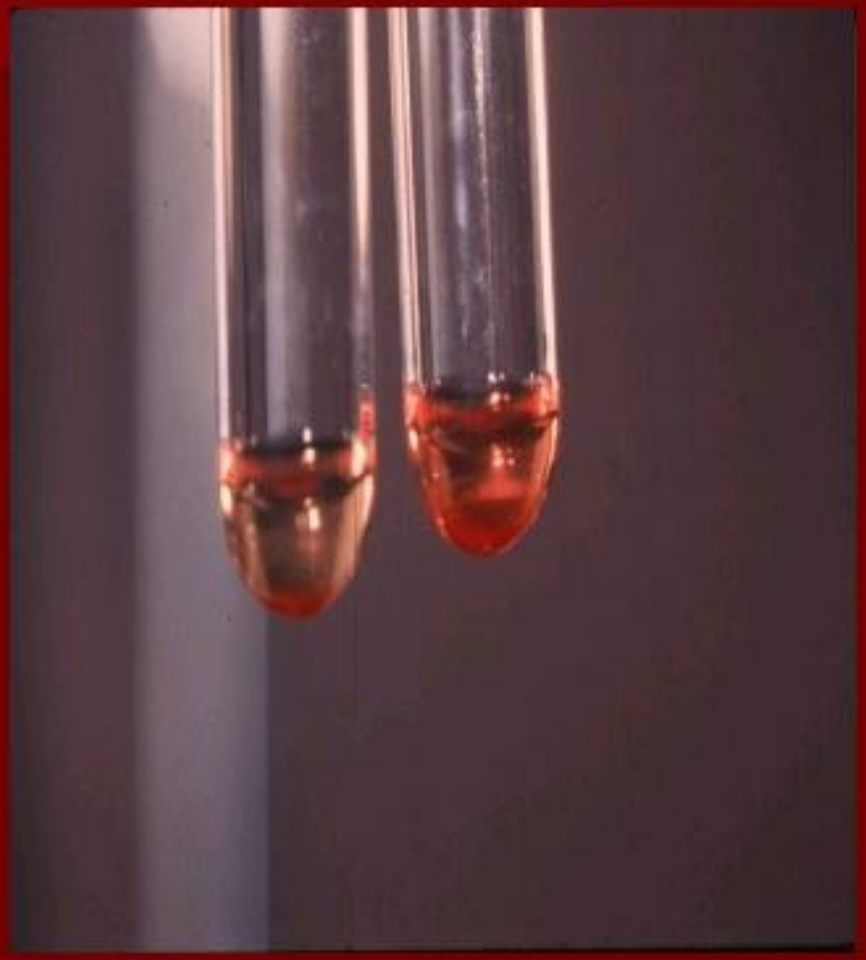
3. Typical "fried egg" appearance of colonies - Central opaque granular area of growth extending into the depth of the medium, surrounded by a flat, translucent peripheral zone.
4. Colonies best seen with a hand lens after staining with Diene's method.
5. Produce beta hemolytic colonies, can agglutinate guinea pig erythrocytes.

Cold Agglutination Test

- Cold Agglutination test is associated with macroglobulin antibodies that agglutinate human o RBC at low temperature

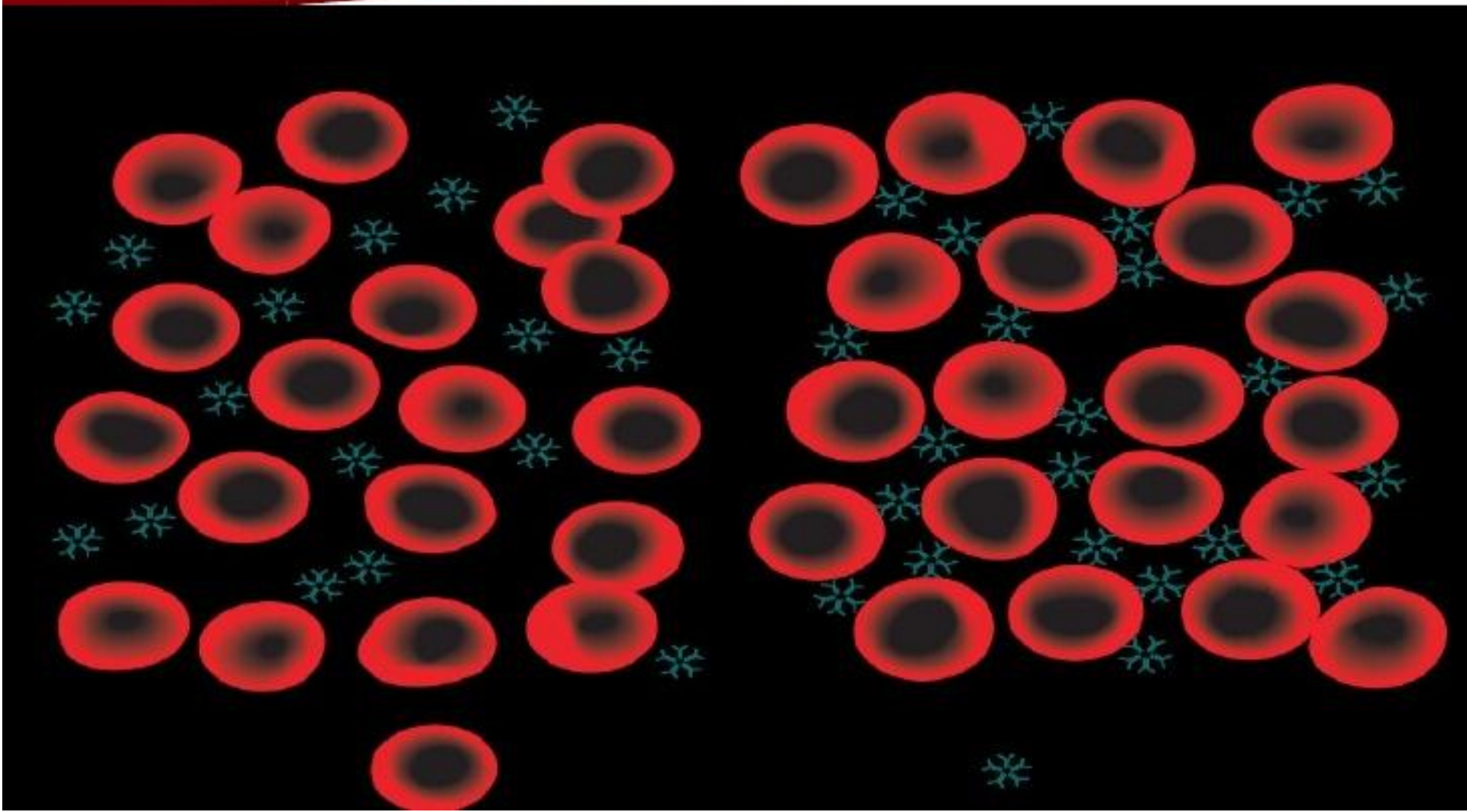


Cold Agglutination test procedure



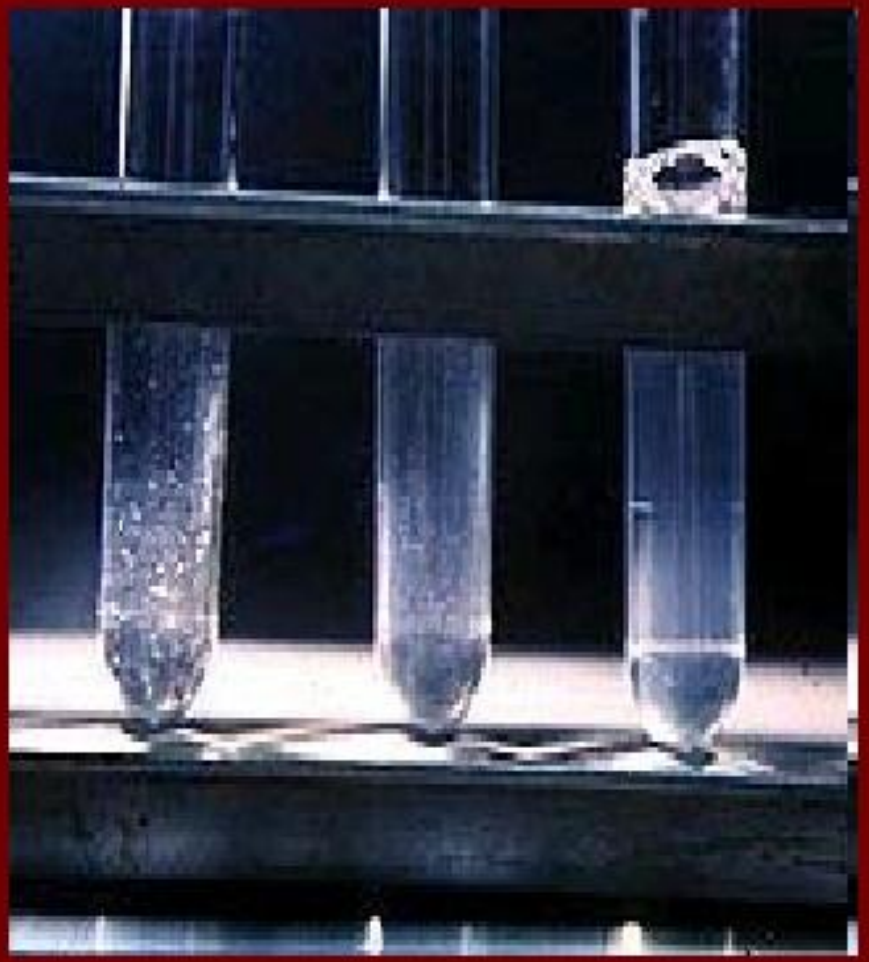
- The serial dilutions of patients serum are mixed with an equal volume of 0.2% washed human O group erythrocytes at low temperature
- The clumping is observed at 4^0c overnight.
- However the clumping is dissociated at 37^0c
- A titer of 1:32 or > is suggestive.
- A raised titer in paired serum sample is more suggestive of infection.

RBC showing non agglutinating and agglutinating RBC



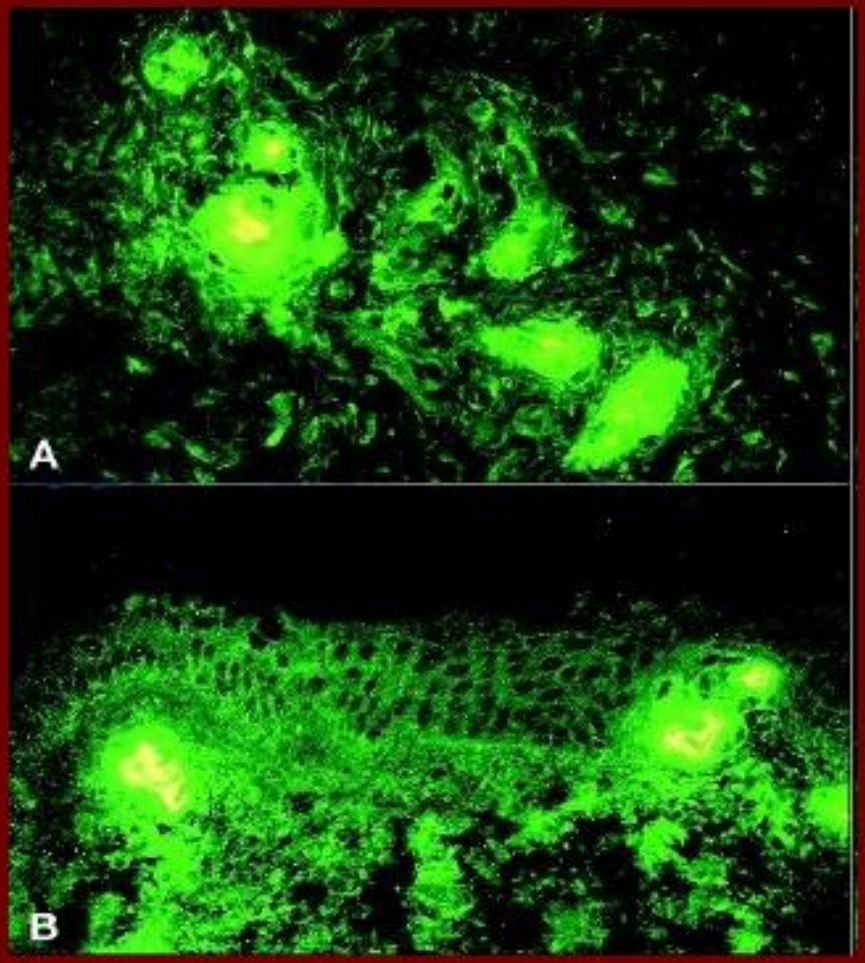
Streptococcal MG test

- The test is performed by mixing serial dilutions of patients serum with heat killed suspension of Streptococcus MG.
- The sample is incubated at 37^{0}c
- The agglutination titer of 1:20 or > is suggestive.

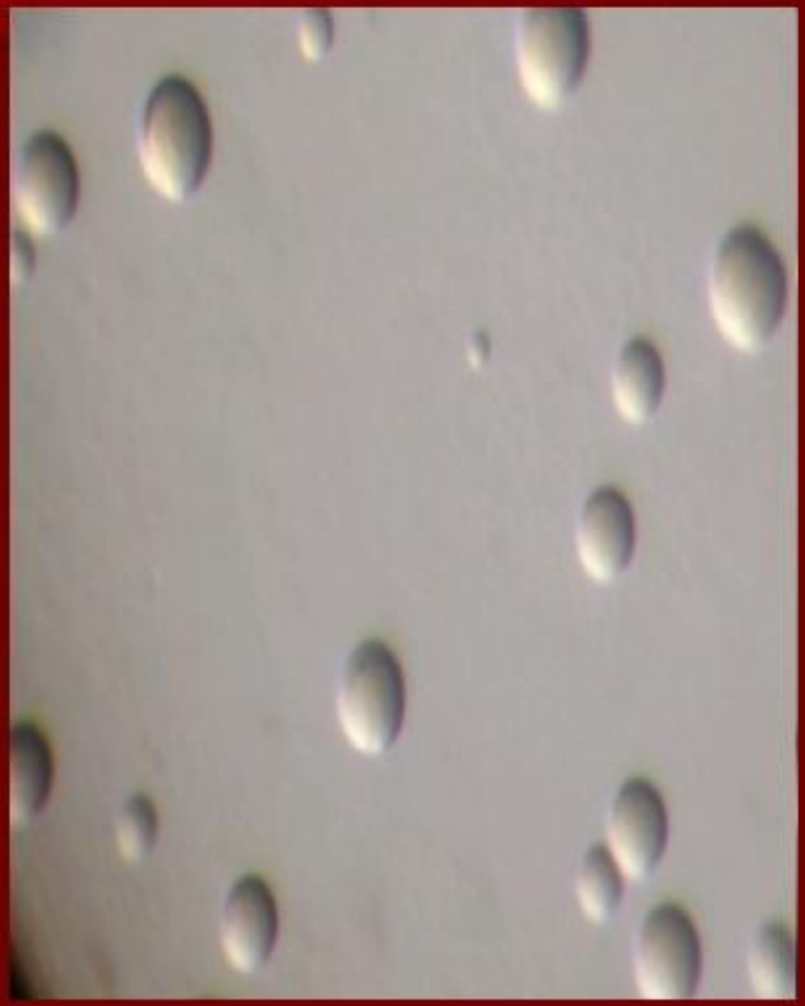


Other Serological Tests

- Immunofluorescence
- Hemagglutination inhibition test
- Complement fixation test *less sensitivie*.



Growth of Bacteriological Medium

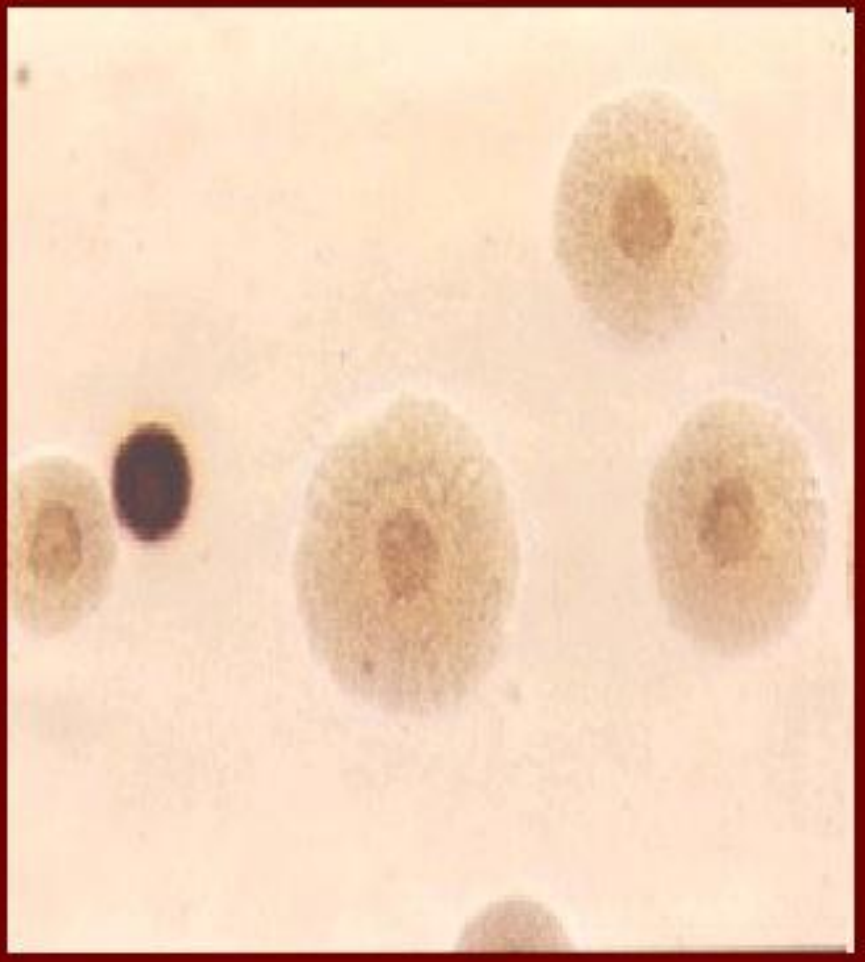


- For isolation swabs from throat or respiratory secretions inoculated not Mycoplasma medium
- The growth is slow and takes 1 – 3 weeks
- The colonies appear as fried egg, with central opaque granular area surrounded by flat translucent peripheral zone

Mycoplasma on PPLO agar

Typical Mycoplasma colonies on enriched medium

- The colonies showing typical fried egg appearance.
- The colonies appear 2-6 days of incubation.
- The size of the colonies can be from 10 – 600 microns in size.



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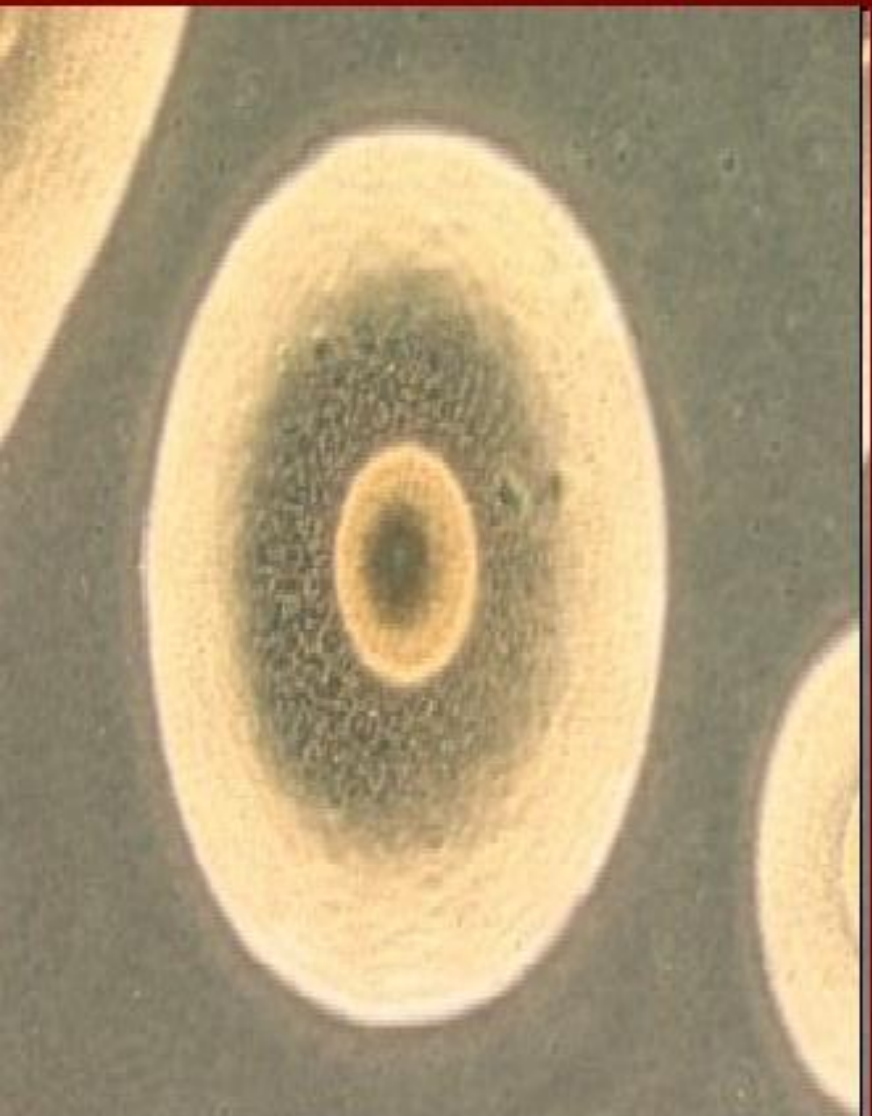
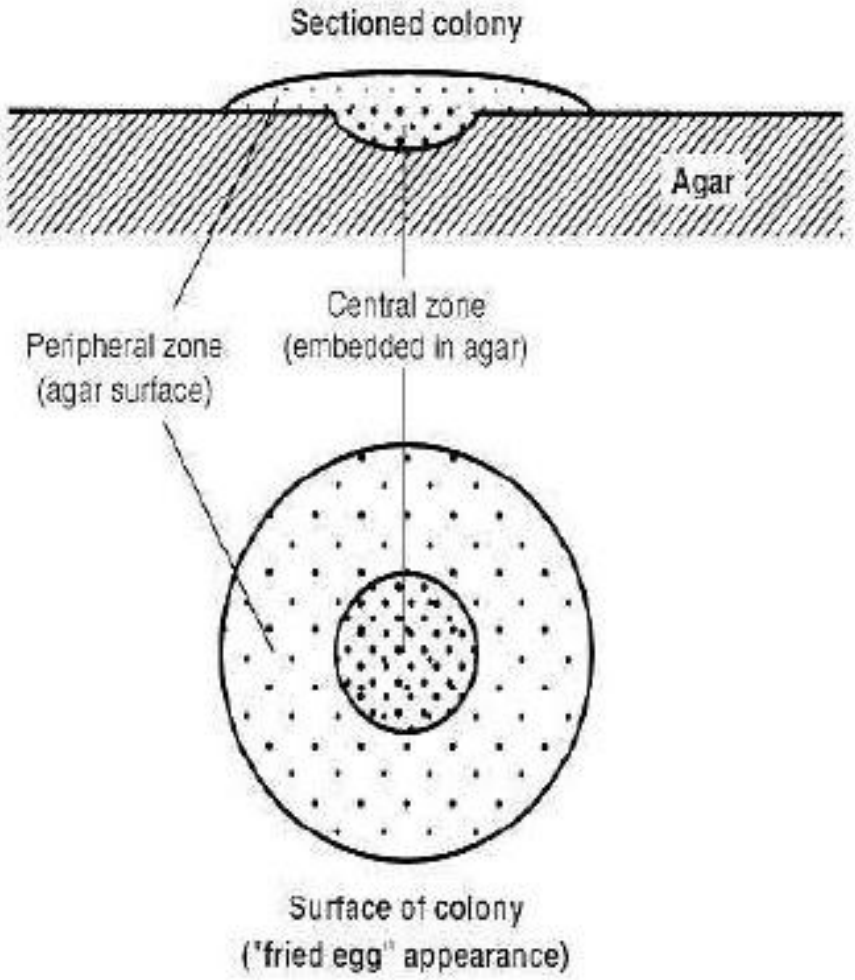
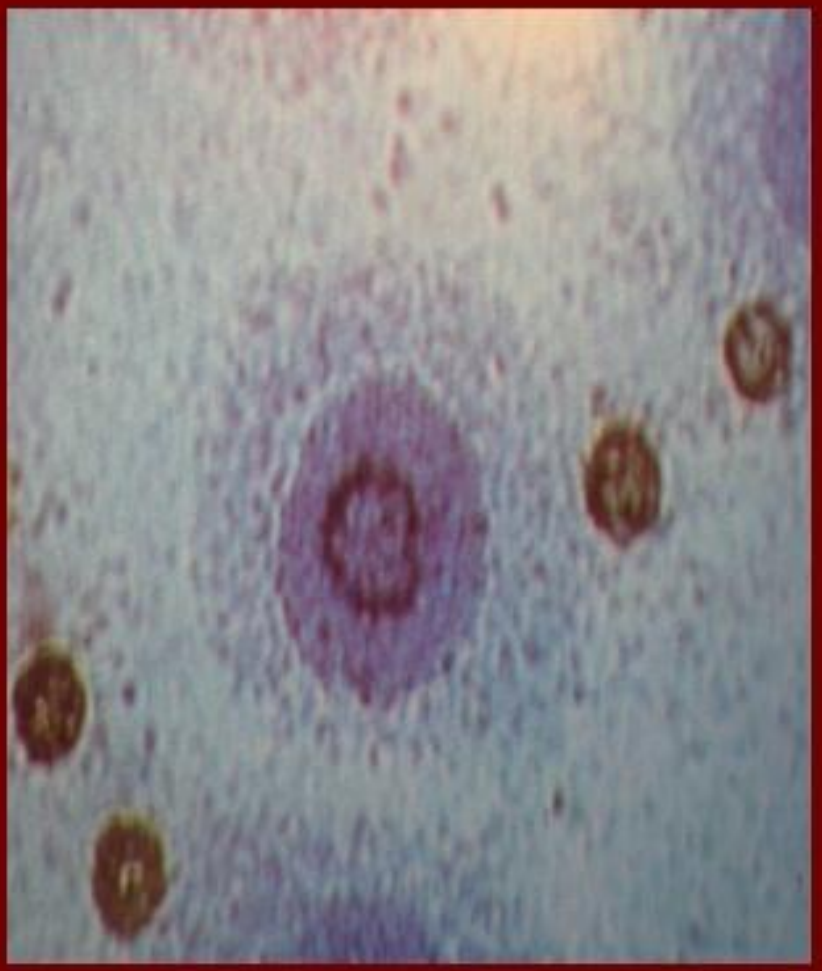
Colony characters of Mycoplasma isolates



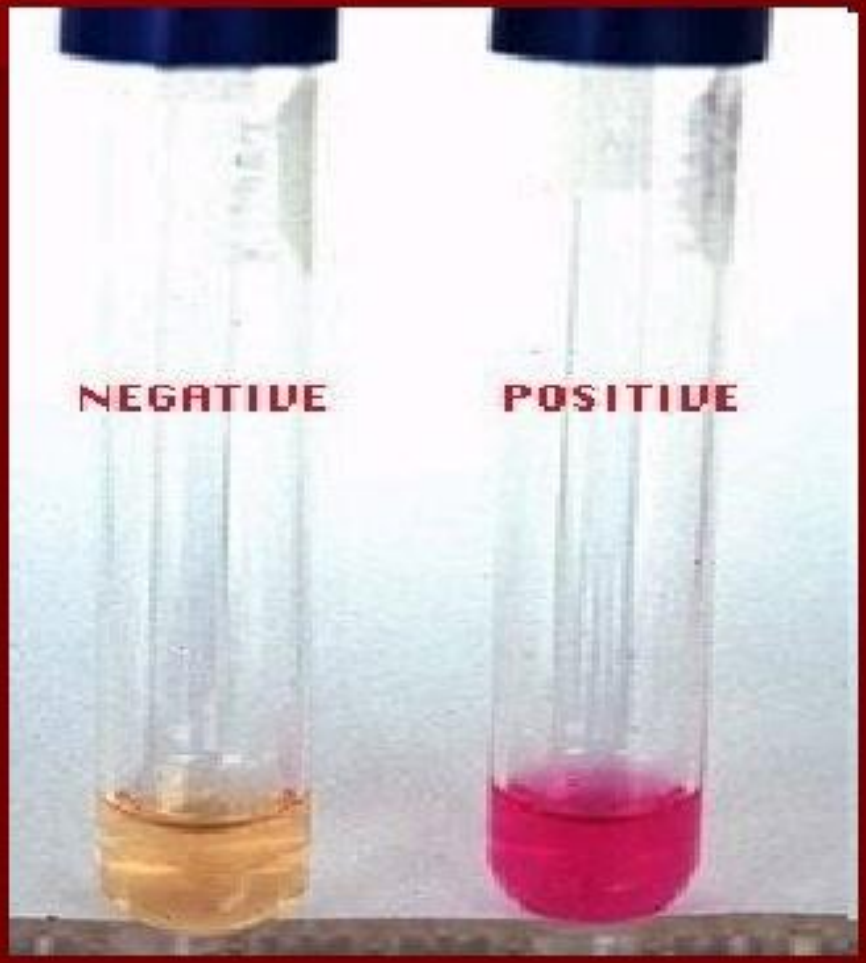


M. pneumoniae colonies demonostrated in Dienes method

- The colonies can be demonostrated by Dienes method.
- In which a block of agar containing the colony is cut and placed on a slide, covered with a cover slip on which has been dried in alcoholic solution of methylene blue and azure.



Biochemical Characters of Mycoplasma



- The metabolism of Mycoplasma are fermentative
- Most species utilize glucose or arginine
- Urea is hydrolyzed by ***Ureaplasma only***

Diagnosis of Urogenital Infections

- Material from urethra, cervical, or vaginal or centrifuged deposit of urine is added to separate vials with liquid mycoplasmal medium containing phenol red and 0.1% glucose, arginine or urea
- The Ureaplasmal urease also breaks down urea to ammonia

Newer methods in Diagnosis

- Phylogeny based rapid identification of urogenital Mycoplasmas and ureaplasmas based on amplification of part of 165rRNA gene by PCR is available

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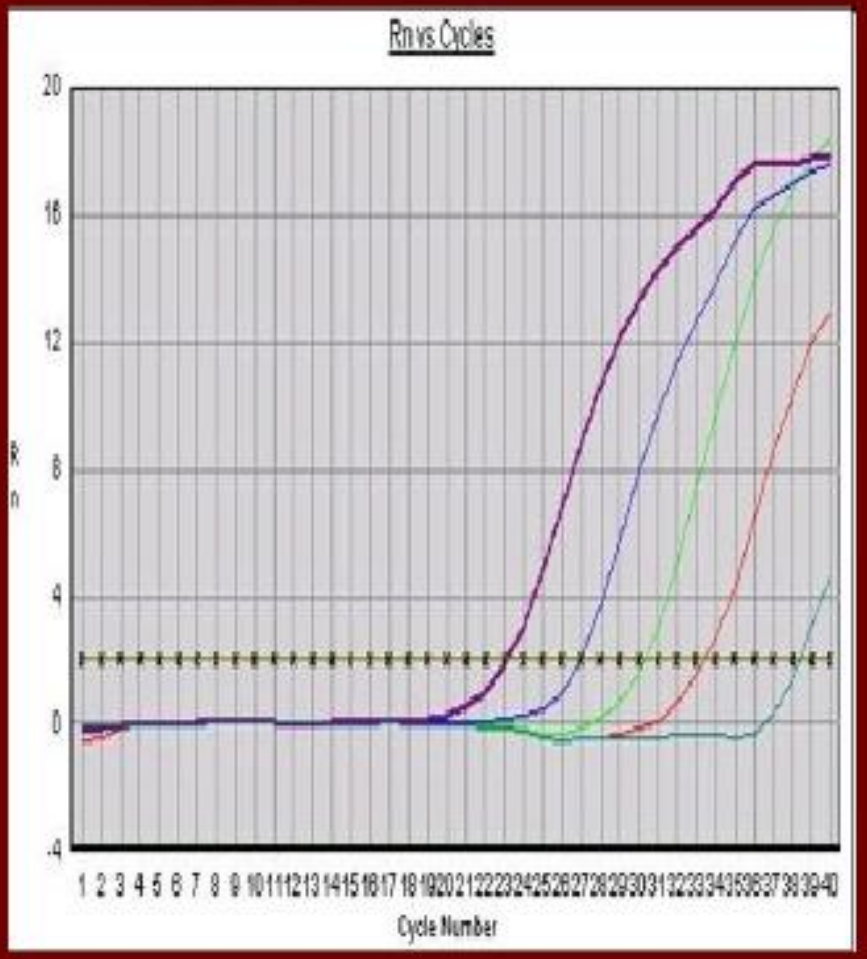
Advantages of PCR methods

- PCR methods are proving to be rapid, sensitive, and specific



Important species in Ureaplasma

- The *Ureaplasma* genus now is subdivided into 2 species: *U urealyticum* and *U parvum.* For clinical purposes, separating infections caused by the different 2 species is not possible or necessary. In both the clinical setting and in the diagnostic laboratory, they are considered *Ureaplasma* species.

Ureaplasma differs from Mycoplasma

- The Ureaplasma are the only non fermentative mollicutes i.e., they do not ferment the growth substrates such as carbohydrates and amino acids like other mollicutes but they depend on the hydrolysis of urea for their energy

Urease test differentiates Mycoplasma from Ureaplasma species