SHIGELLA


SHIGELLA

INTRODUCTION:

- *Shigella* is named after 'Shiga' who in (1896) isolated the first member of this genus from epidemic dysentery in Japan.

- Shigella is one of the most infectious bacteria and ingestion of few 100 to 200 organism can cause diease.

Shiga, Kiyoshi (1871–1957...

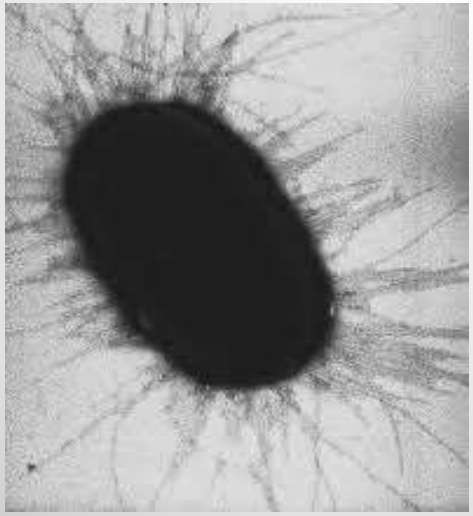
Kiyoshi Shiga (*Shiga Kiyoshi*, *February 7, 1871 – January 25, 1957* **Sendai, Miyagi**, Japan) was a Japanese **physician** and **bacteriologist**. In 1897, Shiga was credited with the discovery and identification of the ***Shigella dysenteriae*** microorganism which causes dysentery, and the **Shiga toxin** which is produced by the bacteria. He conducted research on other diseases such as tuberculosis and trypanosomiasis, and made many advancements in bacteriology and immunology.

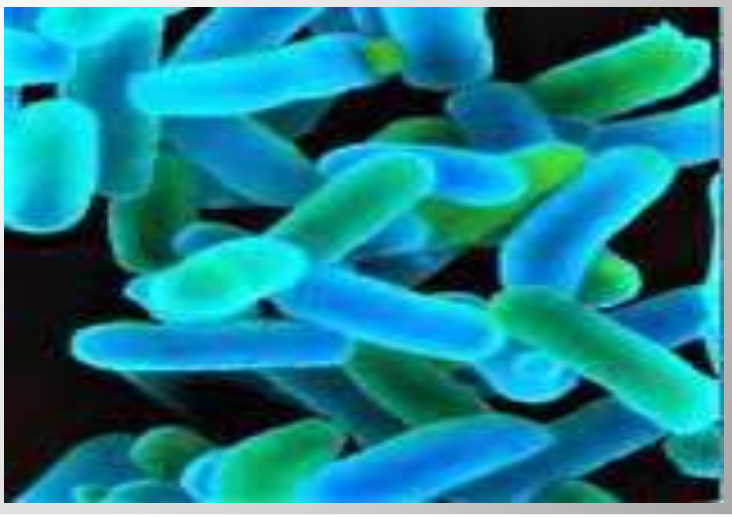
INRODUCTION (cont…)

- Most individuals are infect with shigellae when they ingest food or water contaminated with human fecal material.
- Shigella can survive upto 30 days in milk, eggs and cheese.
- The causative agent of bacillary dysentery (Disease characterized by severe abdominal cramps and the frequent painful passage of low volume stools containing blood and pus) belong to the genus *Shigella.*

MORPHOLOGY:

- *Shigella* are short. ***gram -ve rods***
- Non-sporing, non-motile
- Non-capsulated
- ***Fimbriae*** are present only in *S. flexneri*



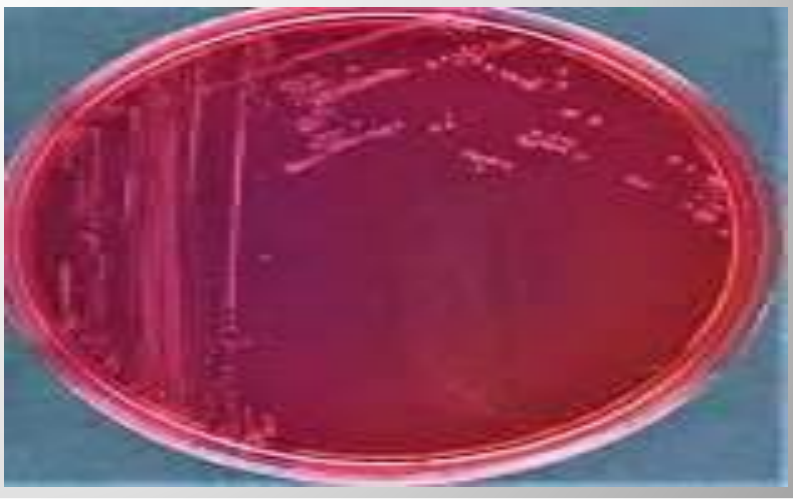


Cultural characteristics of Shigella:

- Aerobic and **facultative anaerobes.**
- Optimum temperature 37°C (Exception *S. sonnei* grow even at 10°C and 45°C).
- They grow on ordinary media however less readily than other Enterobacteria.

Nutrient agar and Blood agar:

On Nutrient agar and Blood agar, Colony are smooth, circular convex greyish or colorless, translucent often 2-3 mm diameter.
Those of *S. sonnei* are slightly larger and opaque than others.

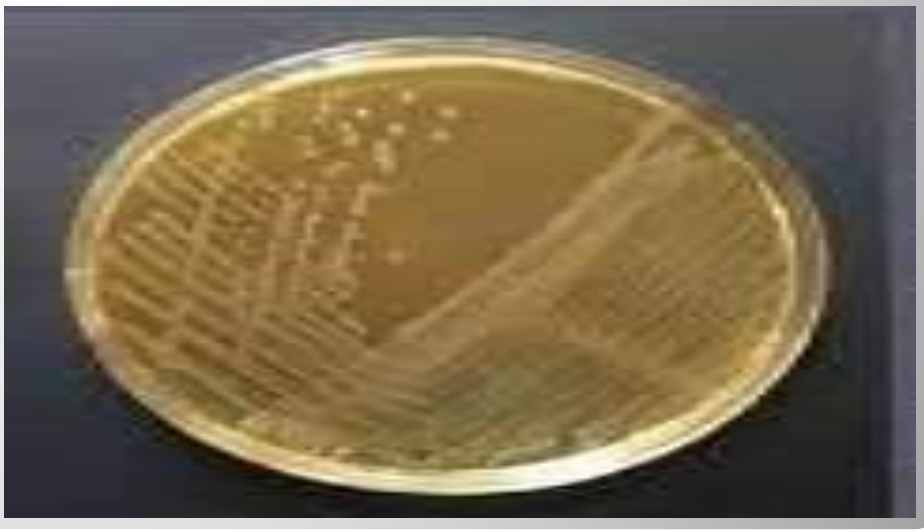


MacConkey agar (MA):

On MA, colonies are pale and yellowish (non-lactose fermenting). Exception *S. sonnei* being late lactose fermenting become pink when incubation period is prolonged.

Deoxycholate citrate agar (DCA):

On DCA, excellent selective medium for isolation of *Shigella* from faeces.
Colonies are pale and similar to though usually slightly smaller 1-1.5mm diameter and more translucent than those of *Salmonella.* They do not form black center.



Xylose lysine deoxycholate agar (XLD):

On XLD, probably the best selective media for *Shigella* being less inhibitory to *S. dysenteriae* and *S. flexneri* than DCA. Colonies are red and unlike those of most *Salmonella* without black centers.

Peptone water and nutrient broth:

Good growth with uniform turbidity on incubation over night at 37^{o}C.
In some cases, especially fimbriated form a surface pellicle on longer incubation.

Selenite F-broth:

Selenite F-broth enrich *S. sonnei* and *S. flexneri* but inhibitory to other *Shigella*.

Antigenic structure of Shigella:

Shigella are differentiated by their 'O' antigens into serotypes.

These are classified into 4 structures or subgroups based on a combination of biochemical and serological characteristics.

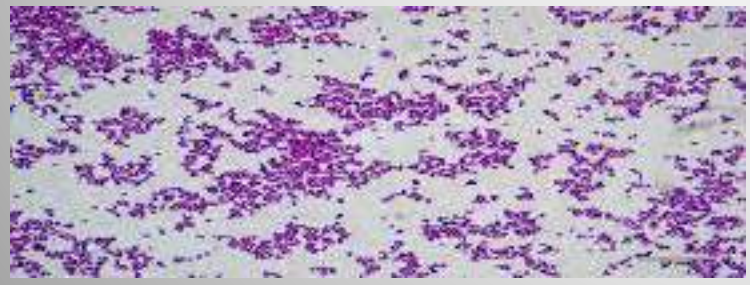
Shigella dysenteriae (Sub groupA):

1)These are mannitol non-fermenting, consists of 10 serotypes.

2)Shigella dysenteriae type-1 forms a toxin.

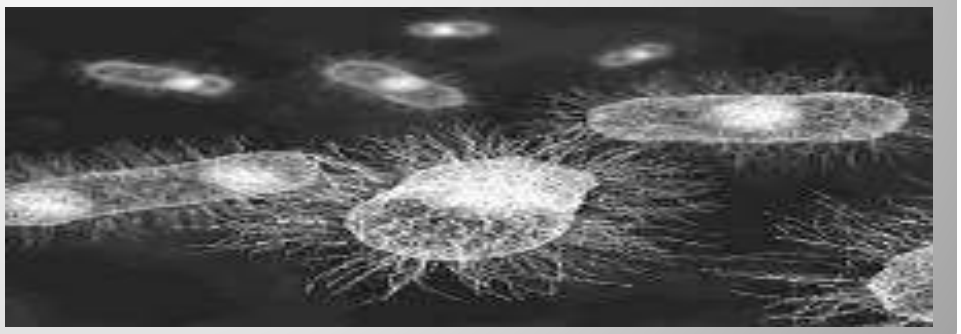
3)3 types of toxic activity have been demonstrated in *Shigella* culture filtrates.(**Neurotoxicity, enterotoxicity, and cytotoxicity**)

Shigella dysenteriae is **a species of the rod-shaped bacterial genus Shigella**. Shigella species can cause shigellosis (bacillary dysentery).



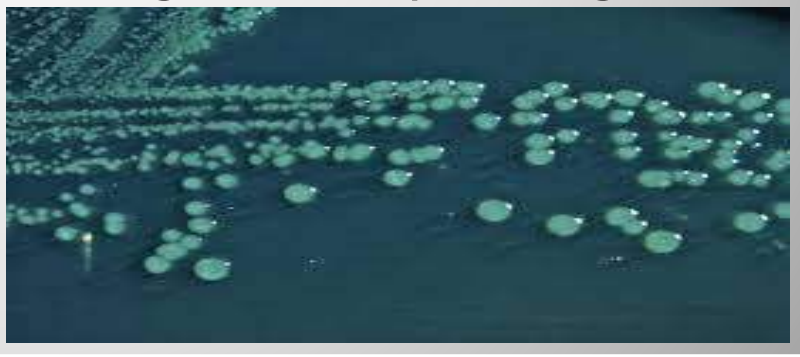
Shigella flexneri (Subgroup B):

Named after Flexner, who first time described first of the mannitol fermenting *Shigella* from Phillipines (1900). Based on type specific and group specific antigen, they have been classified into six serotypes (1-6) and several subtypes Shigella flexneri is a species of Gram-negative bacteria in the genus Shigella that can cause diarrhea in humans. Several different serogroups of Shigella are described; S. flexneri belongs to group B. S. flexneri infections can usually be treated with antibiotics, although some strains have become resistant.



Shigella boydii (Subgroup C):

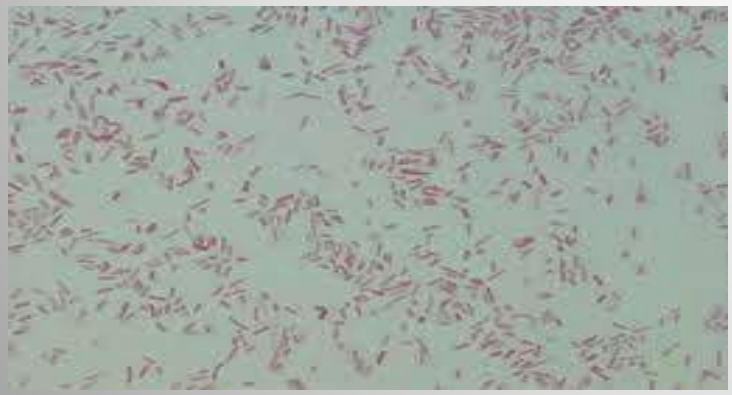
Consists of dysentery bacilli that resemble *S. flexneri* biochemically, but not antigenically.
After Boyd who first described this strain from India (1931).
S. boydii isolates least frequently.
15 serotypes have been identified.
Shigella boydii is a Gram-negative bacterium of the genus Shigella. Like other members of the genus, S. boydii is a nonmotile, nonsporeforming, rod-shaped bacterium which can cause dysentery in humans through fecal-oral contamination. S. boydii is the most genetically divergent species of the genus Shigella.

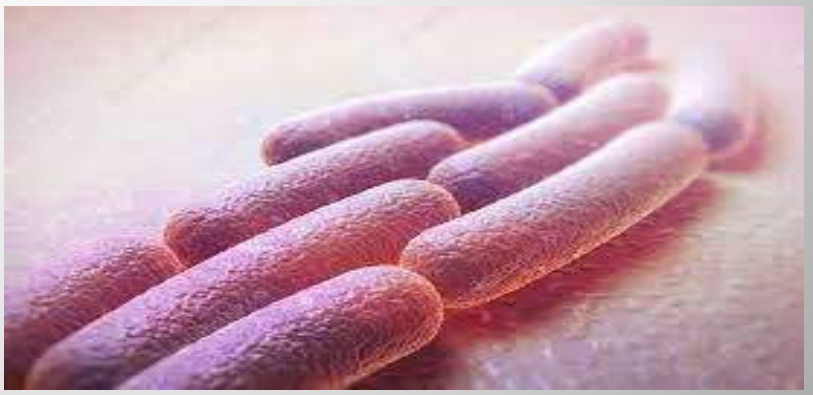


Shigella sonnei (Subgroup D):

1st time isolated by Sonne (1915) in Germany. Ferment lactose and sucrose late, indole negative. Causes mildest form of bacillary dysentery.

Shigella sonnei is a species of Shigella. Together with Shigella flexneri, it is responsible for 90% of shigellosis cases. Shigella sonnei is named for the Danish bacteriologist Carl Olaf Sonne.





Resistance:

1)Shigella are not specially resistant.
2)They are killed at 56°C in one hour and by 1% phenol in 30 minutes.
3)In ice they last for 1-6 months.
4)They remain viable in moist environment.
5) In faeces they die within few hours due acidity produced by growth of coliforms.

Biochemical tests of Shigella:

Carbohydrates utilization:

1)Most strains utilize sugar to produce acid but not gas though some strain *S. flexneri* and *S. boydii* form gas.
2)Glucose is fermented by almost all strains.
3)Lactose is not fermented within 24hrs.
4)However, *S. sonnei* and some strains of *S. dysenteriae* produce acid from lactose after prolonged incubation.
5)Mannitol fermentation is important characteristics. This differentiated Group A strain (which do not ferment mannitol) from group B, C and D, most strains of which ferment it.
6)Dulcitol is not fermented by most *Shigella.*
7)Sucrose is not fermented except *S. sonnei* andsomestrains of *S. flexneri.*
8)Adonitol and Inositol are also not fermented.
9)Xylose is not fermented except mannitol -ve biotype of *Shigella flexneri.*

Methyl red test: +ve
VP test: -ve
Reduce nitrate to nitrite
Catalase +ve
Indole -ve,
Citrate -ve
H_2S -ve
Urease -ve
KCN growth (-ve).
Gelatin not liquified.

Decarboxylation test:

Group A, B and C fail to decarboxylate lysine and ornithine.

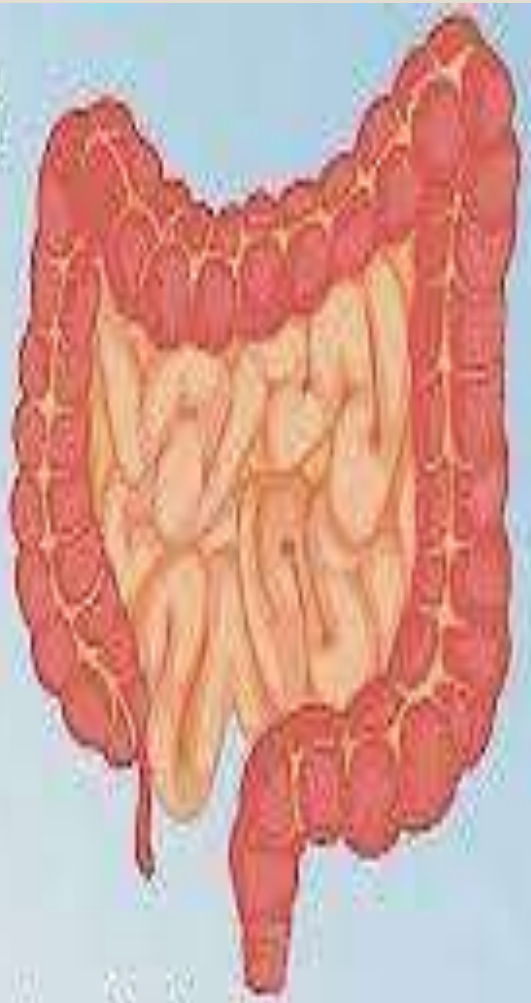
S. sonnei decarboxylate ornithine but not lysine

3-SYMPTOMS

- Symptoms of shigellosis typically start 1–2 days after exposure and include:
- Diarrhea (sometimes bloody)
- Fever
- Abdominal pain and cramping
- Tenesmus, a painful sensation of needing to move your bowels even when they are already empty
- In persons with healthy immune systems, symptoms usually last about 5 to 7 days, but you can suffer for up to 2 weeks and you can be contagious for that period of time as well.
- Persons with diarrhea usually recover completely, although it may be several months before their bowel habits are entirely normal again.

Shigella infection

AN INTESTINAL DISEASE CAUSED BY A FAMILY OF BACTERIA KNOWN AS SHIGELLA



SYMPTOMS

- Diarrhoea
- Abdominal pain or cramps
- Fever

COMPLICATIONS

- Dehydration
- Seizures
- Rectal prolapse
- Hemolytic uremic syndrome
- Toxic megacolon
- Reactive arthritis

1) SHIGELLOSIS:

Shigellosis is characterized by:

1)Abdominal cramps
2)Diarrhea
3)Fever
4)Bloody stools
5)The clinical signs and symptoms of the disease appear 1 to 3 days after the bacteria are ingested.
6)Shigella initially colonize the small intestine and begin to multiply within the first 12 hours.
7)The first sign of infection (profuse watery diarrhea without histologic evidence of mucosal invasion) is mediated by an enterotoxin.

8)However, the **cardinal feature of shigellosis is lower abdominal cramps and tenesmus** (straining to defecate), **with abundant pus and blood in the stool**. It results from the invasion of the colonic mucosa by the bacteria.

9)Abundant neutrophils, erythrocytes, and mucus are found in the stool.

10)Infection is generally self-limited, although antibiotic treatment is recommended to reduce the risk of secondary spread to family members and other contacts.

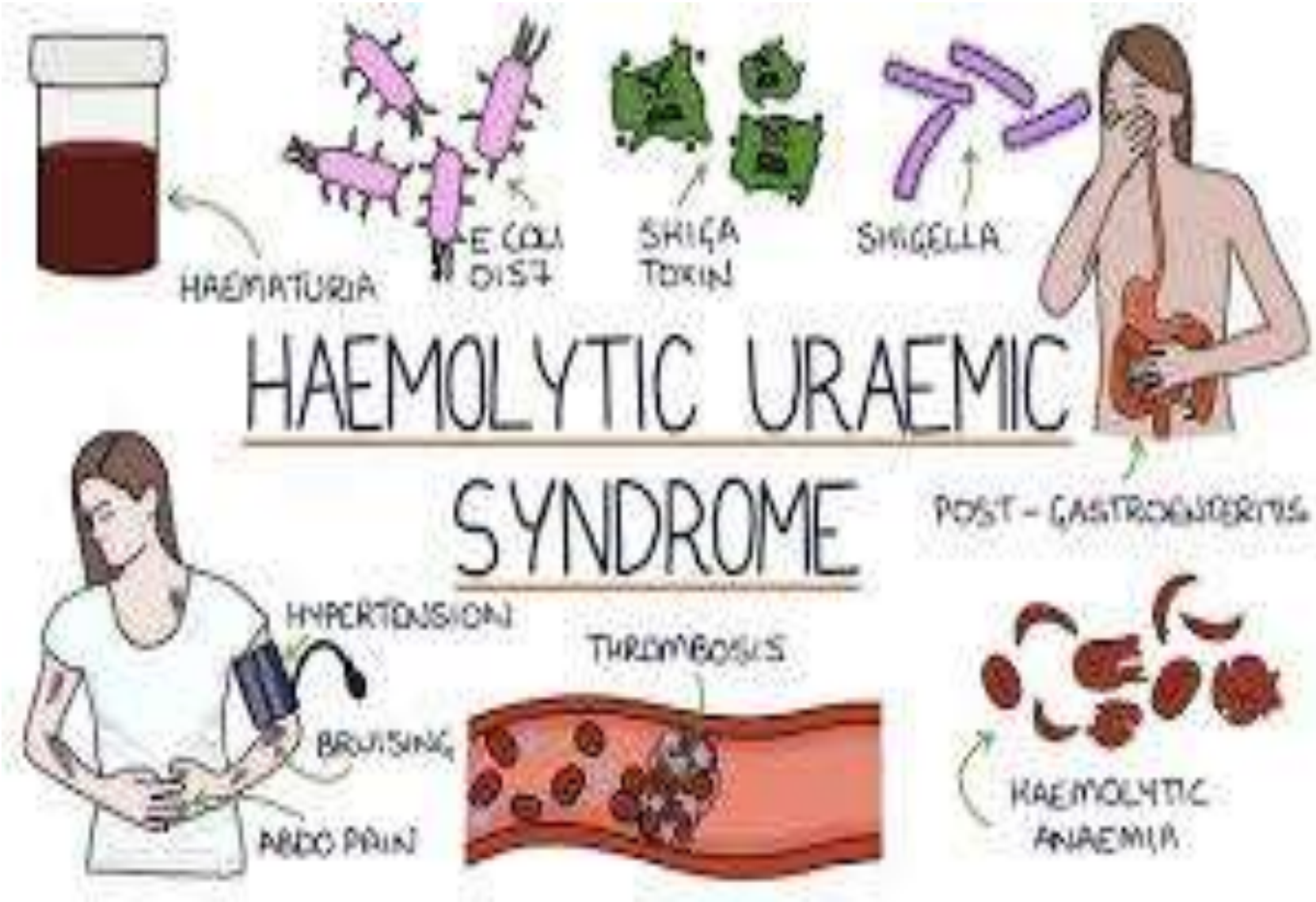
11)Asymptomatic colonization of the organism in the colon develops in a small number of patients and represents a persistent reservoir for infection.

The clinical features of Shigella dysenteriae type 1 infection includes:

1)toximea, sometimes bacteremia and severe dysentery leading to marked dehydration and protein loss
2)Inflammation and ulceration of the large intestine
3)Hemorrhage, abdominal pain and high fever
4)Death occur from circulatory collapse or kidney failure

2)Hemolytic Uremic Syndrome (HUS):

1)***Hemolytic-uremic syndrome (HUS)*** is a group of blood disorders characterized by low red blood cells, acute kidney failure, and low platelets.
2)HUS can occur after *S. dysenteriae* type 1 infection.
3)Convulsions may occur in children; the mechanism may be related to a rapid rate of temperature elevation or metabolic alterations and is associated with the production of the Shiga toxin.
4)It is usually complicated by severe dysentery, intravascular volume depletion, and cardiovascular collapse, and has a higher morbidity and mortality rate than HUS associated with *E. coli*.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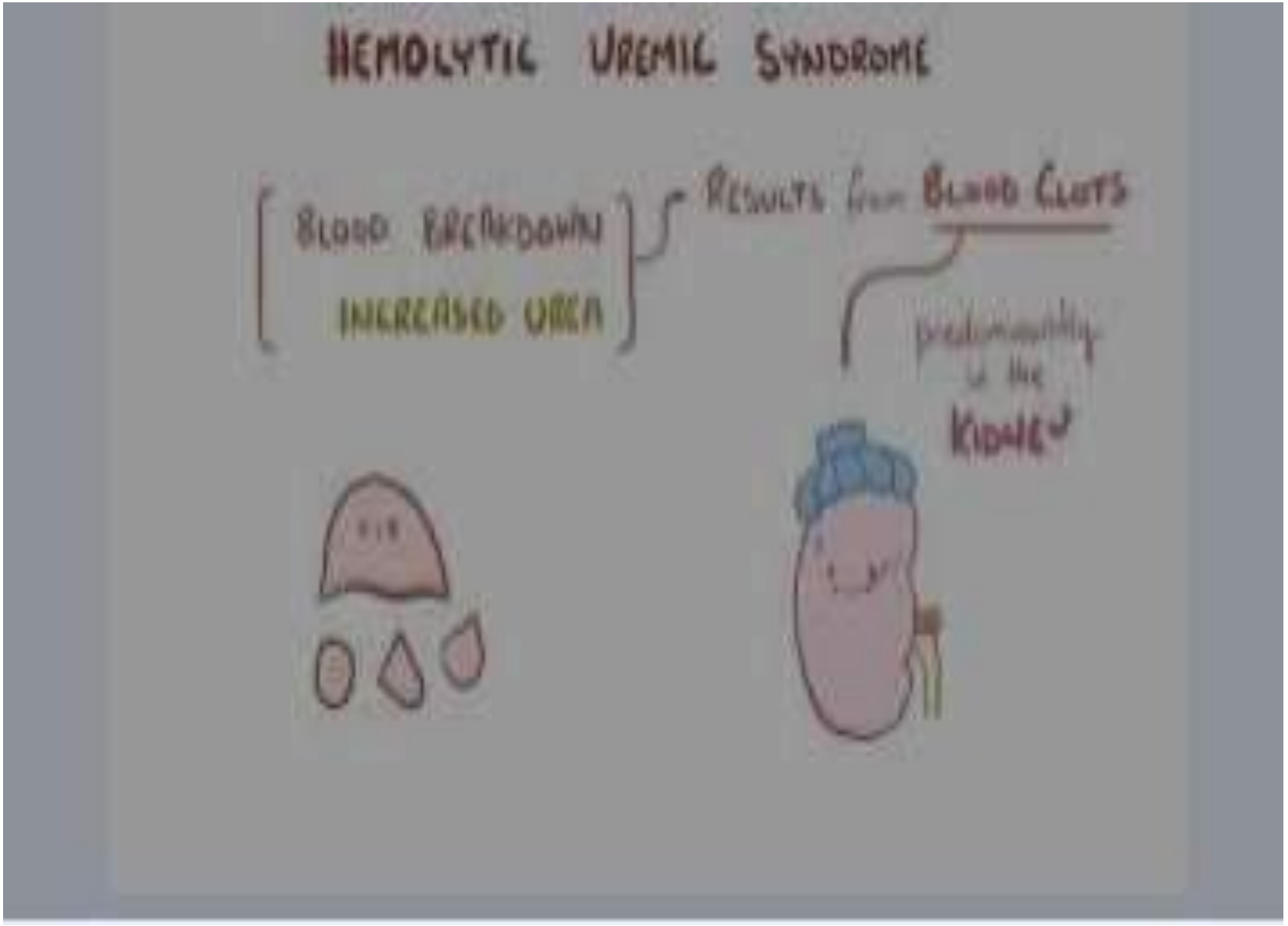

HEMOLYTIC UREMIC SYNDROME
BLOOD BREAKDOWN
INCREASED UREA
RESULTS FROM BLOOD CLOTS
predominantly in the
KIDNEY

Methods to Diagnose Shigellosis

- Shigellosis can be correctly diagnosed in most patients on the basis of fresh blood in the stool. Neutrophils in fecal smears is also a strongly suggestive sign. Nonetheless, watery, mucoid diarrhea may be the only symptom of many S sonnei infections, and any clinical diagnosis should be confirmed by cultivation of the etiologic agent from stools.

LAB DIAGNOSIS

- COLONIES ON MA/DCA : NLF PALE AND TRANSLUCENT
- COLONIES PICKED UP FOR THE FOLLOWING TESTS:
- HANGING DROP : NON MOTILE
- GRAM'S :GNB
- BIOCHEMICAL TESTS : IMVIC ++-- ANEROGENIC FERMENTERS
- SLIDE AGGLUTINATION WITH SPECIFIC HTS

Deoxycholate Citrate Agar (DCA)

Positive

Negative

Diagnostic Laboratory Tests

A. SPECIMENS

Fresh stool, mucus flecks, and rectal swabs for culture.

B. CULTURE

differential media (eg, MacConkey's or EMB agar) and selective media (Hektoen enteric agar or salmonella-shigella agar). Colorless (lactose-negative) colonies are seen on MacConkey's. These organisms fail to produce H2S, that produce acid but not gas in the butt and an alkaline slant in triple sugar iron agar medium, and that are nonmotile

Serologic diagnosis

- Serologic examination with polyvalent and monovalent anti-sera
- For serogrouping and serotyping.
- It is not used to diagnose *shigella* infection,it is for conformation.

5-TREATMENT

- Diarrhea caused by *Shigella* usually resolves without antibiotic treatment in 5 to 7 days.
- People with mild shigellosis may need only fluids and rest. Bismuth subsalicylate (e.g., Pepto-Bismol®) may be helpful, but medications that cause the gut to slow down, such as loperamide (e.g., Imodium®) or diphenoxylate with atropine (e.g., Lomotil®), should be avoided

Antibiotics

Ampicillin

Chloramphenicol

Tetracyclines

Nalidixic Acid

Nitrofurantoin

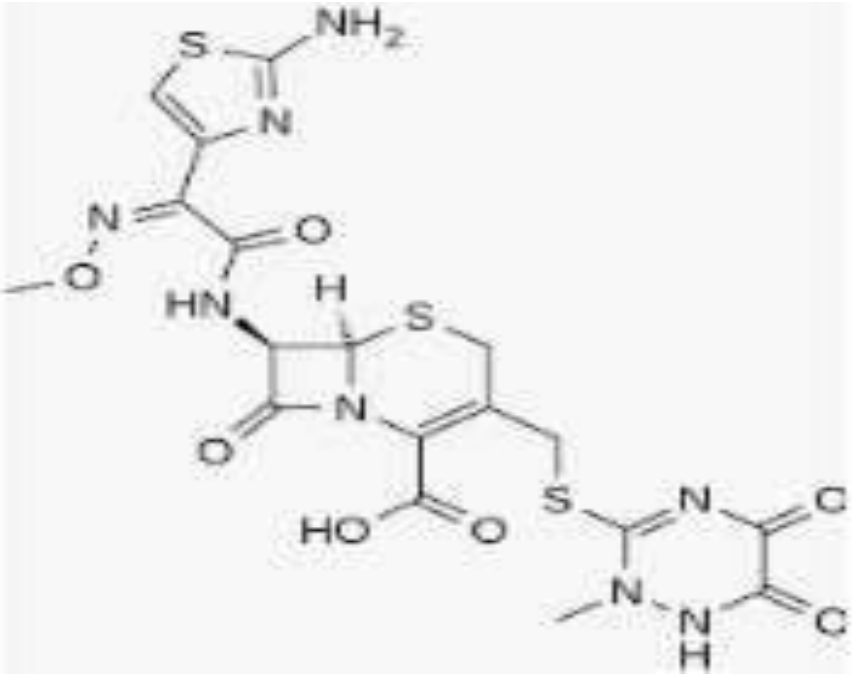
Oral aminoglycosides (gentamycin)

1st and 2nd generation cephalosporins

Amoxicillin

1. Antibiotics:. - ***Ceftriaxone (Rocephin):*** Third-generation cephalosporin with broad-spectrum, gram-negative activity; lower efficacy against gram-positive organisms; higher efficacy against resistant organisms. Bactericidal activity results from inhibiting cell wall synthesis by binding to one or more penicillin binding proteins. Exerts antimicrobial effect by interfering with synthesis of peptidoglycan, a major structural component of bacterial cell wall. Bacteria eventually lyse due to the ongoing activity of cell wall autolytic enzymes while cell wall assembly is arrested. Highly stable in presence of beta-lactamases, both penicillinase and cephalosporinase, of gram-negative and gram-positive bacteria. Approximately 33-67% of dose excreted unchanged in urine, and remainder secreted in bile and ultimately in feces as microbiologically inactive compounds. Reversibly binds to human plasma proteins, and binding have been reported to decrease from 95% bound at plasma concentrations < 25 mcg/mL to 85% bound at 300 mcg/mL. (1-2 g IV/IM qDay or divided BID for 4-14 days depending on type and severity of infection)


Rocephin®
Ceftriaxone
1 g i.v.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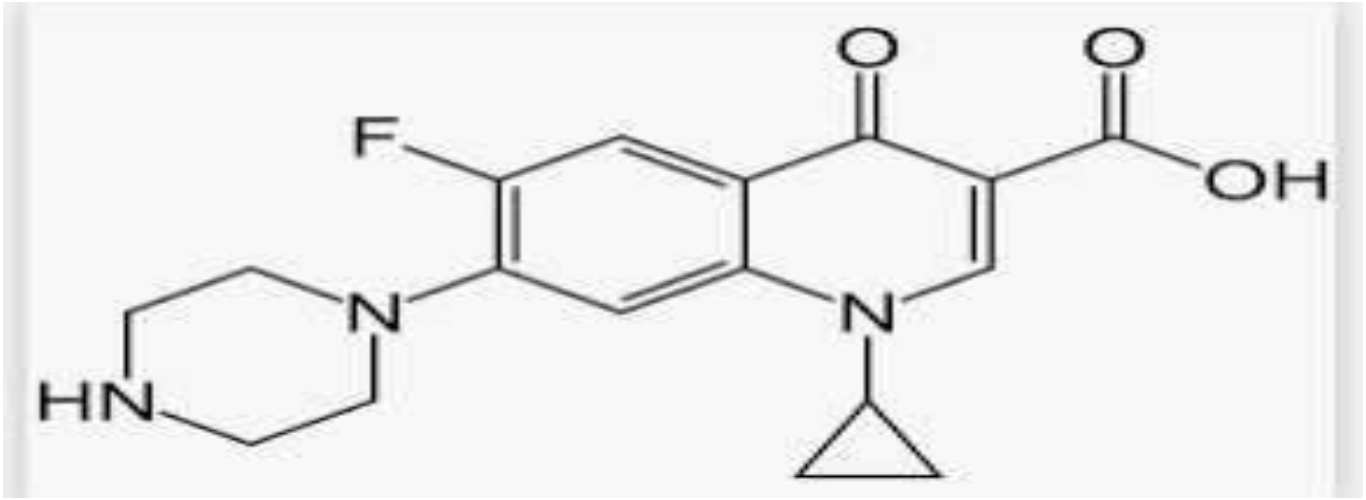
New Global Enterprises
Cefuroxime Axetil Tablets IP 500 mg
Pulmocef-500

Ciprofloxacin (Cipro):

Fluoroquinolone that inhibits bacterial DNA synthesis and, consequently, growth. (Mild/Moderate/Severe: 500 mg PO q12hr for 5-7 days)□ - Trimethoprim-sulfamethoxazole (Bactrim, Septra, Bactrim DS, Cotrim): Inhibits bacterial growth by inhibiting synthesis of dihydrofolic acid. Reasonable DOC in the United States due to few resistant strains. Dosing may be based on TMP component. (Indicated for treatment of enteritis caused by susceptible strains of Shigella flexneri and Shigella sonnei DS tablet: 1 tab PO q12hr for 5 days 8-10 mg TMP/kg/day IV divided q6-12hr for up to 5 days)


Ciprofloxacin Tablets IP


Ciprofloxacin for
Oral Suspension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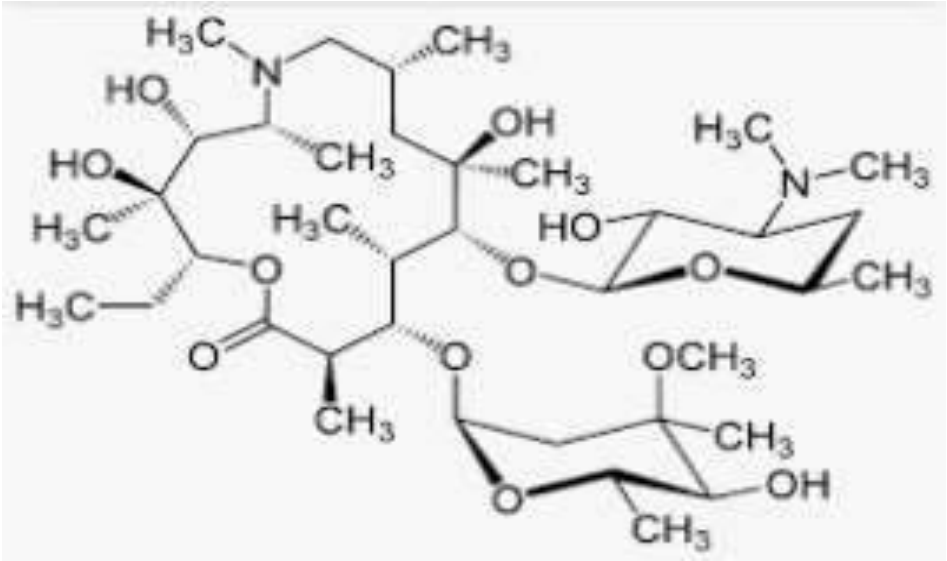
Azithromycin (Zithromax):

_Acts by binding to 50S ribosomal subunit of susceptible microorganisms and blocks dissociation of peptidyl tRNA from ribosomes, causing RNA-dependent protein synthesis to arrest. Nucleic acid synthesis is not affected. Concentrates in phagocytes and fibroblasts as demonstrated by in vitro incubation techniques. In vivo studies suggest that concentration in phagocytes may contribute to drug distribution to inflamed tissues. Treats mild-to-moderate microbial infections. Plasma concentrations are very low, but tissue concentrations are much higher, giving it value in treating intracellular organisms. Has a long tissue half- life.


NDC 68180-160-11
Azithromycin
Tablets USP
250 mg
LUPIN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Zithromax Sd Pfizer
NDC 63323-398-10 309810
AZITHROMYCIN
FOR INJECTION
equivalent to
500 mg*
of azithromycin
For IV infusion only
500 mg/vial
Rx only

PREVNTION AND CONTROL:

A vaccine for shigellosis is not currently available. Until a vaccine is available, the following measures can help prevent the dissemination of shigellosis:

1) Use of safe drinking water
2) Chlorination of unreliable water source
3)Strict handwashing
4)Refrigeration and proper preparation and cooking of food
5)Foodhandlers must be treated with antibiotics and should not be involved in food preparation as long as stool cultures are positive forShigella infection.
6)At least 48 hours of antibiotic treatment are usually required.

Postinfection carriage is generally less than 3-4 weeks. Mild cramps and diarrhea may continue for many days to weeks after treatment of shigellosis.

PREVENTION:(Cont...)


Thank you

MEDICAL BACTERIOLOY

CHLAMYDIAE

CHLAMYDIAE

INTRODUCTION:

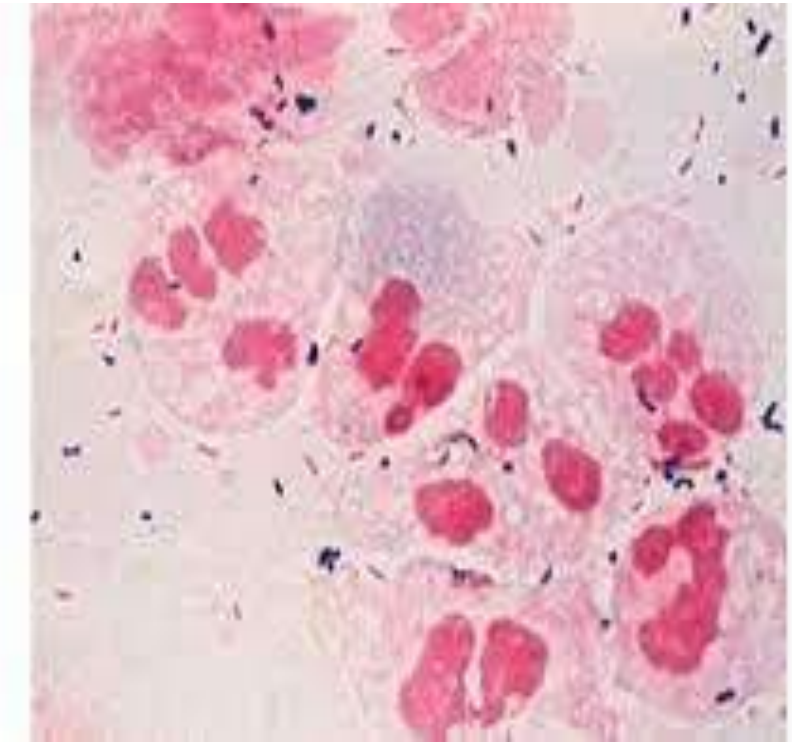
Chlamydiae are:

- *Aerobic,*
- *Chlamydia is extremely tiny.*
- *Obligate intracellular parasites of eukaryotic cells.*
- *They are small Gram-negative*
- *coccoid or rod shaped,*
- *Non-motile bacteria.*

- It does not have a peptidoglycan layer and has no muramic acid.
- Chlamydiae exhibit characteristics intermediate between bacteria and viruses.
- They are widespread in the natural world, being parasites of people, animals and birds with tropism for squamous epithelial cells and macrophages of the respiratory and gastrointestinal tract.

- Chlamydia is especially fond of columnar epithelial cells that line mucous membranes.
- This correlates well with the types of infection that Chlamydia causes, including:
  - ✓ conjunctivitis,
  - ✓ cervicitis, and
  - ✓ pneumonia.


alamy stock photo



They are recognized as bacteria as

- They have both DNA and RNA.
- They have cell wall (that resembles that of GNB) and ribosomes
- Replicate by binary fission
- Susceptible to antibiotics

SPECIES

There are three species

- C. trachomatis,
- Chlamydophila psittaci,
- Chlamydophila pneumoniaae

CELL STRUCTURE

- Chlamydiae have a cytoplasmic membrane and an outer membrane similar to Gram–negative bacteria but lack a peptidoglycan cell wall.
- Chlamydiae cannot synthesize their own ATP and require intracellular abode to remain viable.
- Chlamydiae exist in two forms:
  - **The elementary body and**
  - **The reticulate body.**

ELEMENTARY BODY (EB)

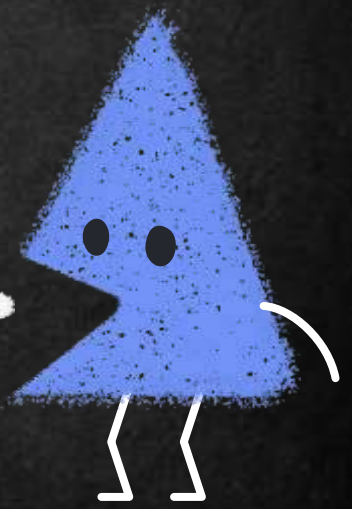
- The elementary body is the dispersal form, which is analogous to a spore.
- This dispersal form is about 0.3 µm or 200-300 nm in diameter.
- It is the extracellular infective form.
- It induces its own endocytosis upon exposure to target cells.

RETICULATE BODY (RB)

- Reticulate body is the intracellular, multiplicative form.
- It represents the noninfectious growing form.

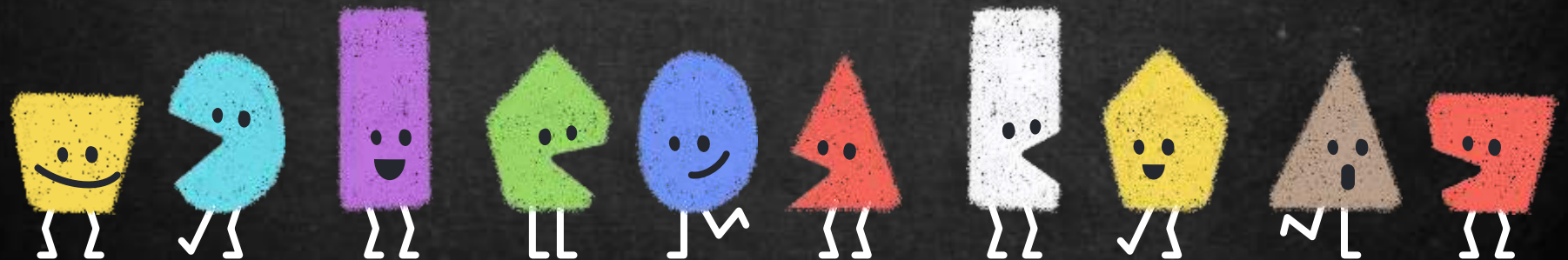
LIFE CYCLE

- The life cycle of Chlamydia trachomatis consists of two stages: elementary body and reticulate body.
- Upon endocytosis into the host cell EB prevents phagolysosomal fusion enabling intracellular survival of the bacteria.
- Once inside the endosome, the elementary body transforms into the larger reticulate body (500 – 1000 nm) as a result of the glycogen that is produced.



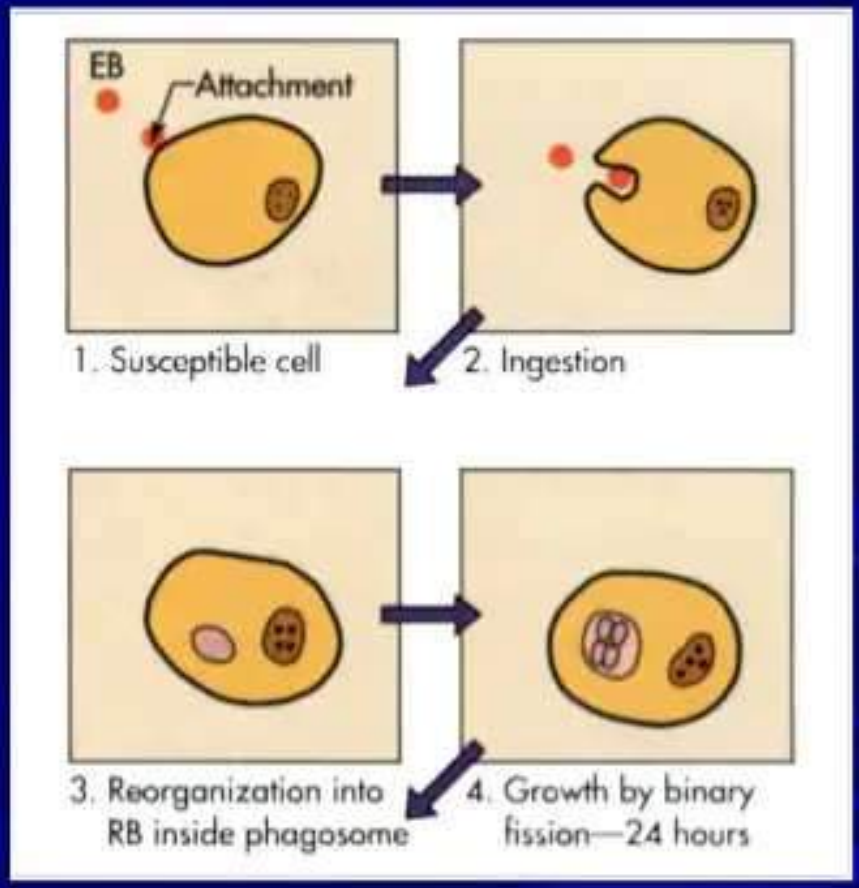
- The reticulate body is the reproductive form.
- It divides through binary fission at approximately 2-3 hours per generation.
- It contains no cell wall and is detected as an inclusion in the cell arranged as a mantle around the nucleus.
- The inclusion bodies are basophilic.

- They can also be stained by Lugol's iodine because of the presence of glycogen matrix.
- After division, the reticulate body transforms back to the elementary form and is released by the cell by exocytosis.
- One phagolysosome usually produces 100-1000 elementary bodies.
- The entire process takes 24 – 48 hours.
- The EB may infect new cells and the cycle continues



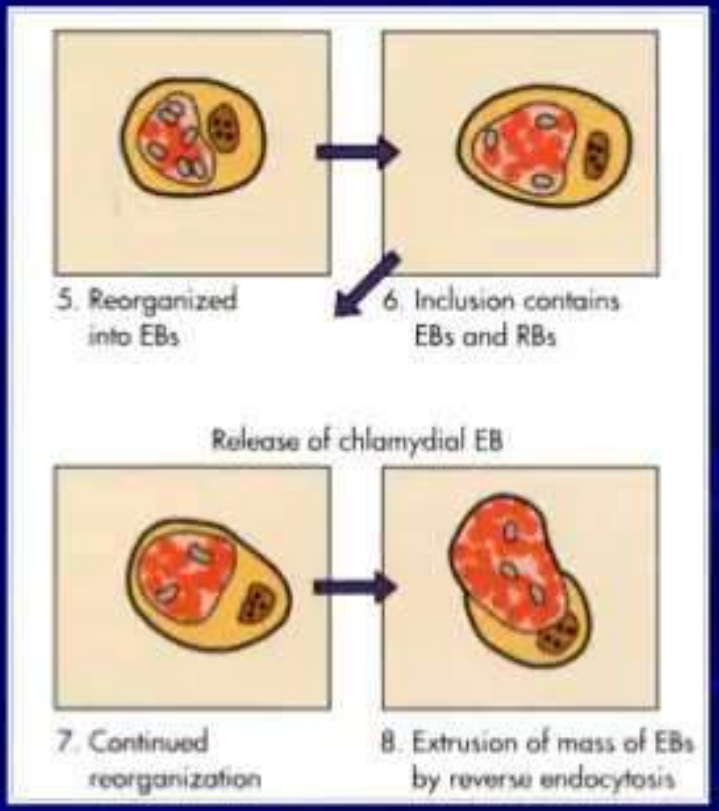
Developmental Cycle of Chlamydia

- EB bind to host cells
  - **Epithelial cell**
  - **Macrophage**
- Internalization
  - **Endocytosis**
  - **Phagocytosis**
- Inhibition of phagosome-lysosome fusion
- Reorganization into RB
- Growth of RB by binary fission



Developmental Cycle of Chlamydia

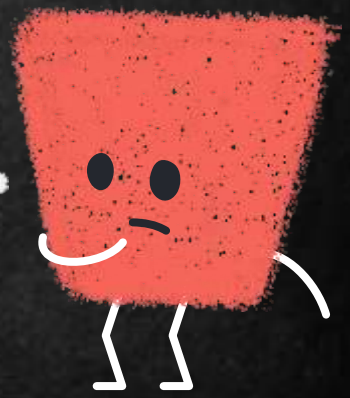
- Reorganization into EB
- Inclusion bodies
- Release of EB
  - Lysis -*C. psittaci*
  - Extrusion - *C. trachoma* and *C. pneumoniae*



ANTIGENIC STRUCTURE

Chlamydia antigens consist of 3 groups:

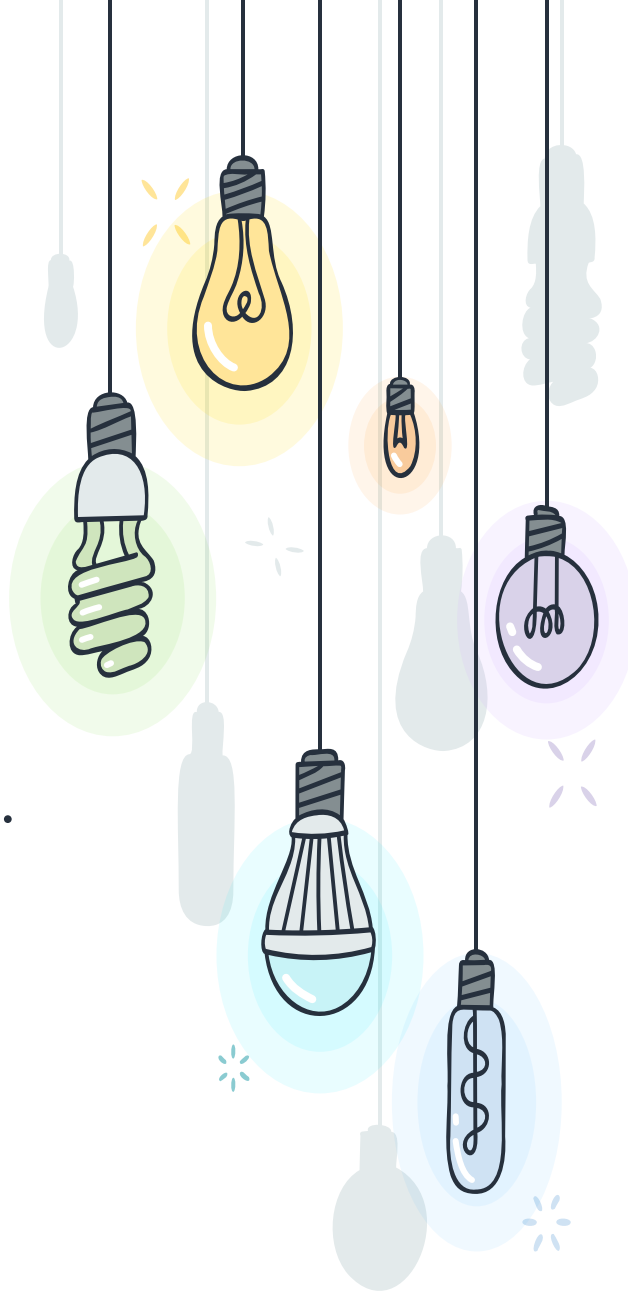
1. genus-specific antigen,
2. Species-specific protein antigen,
3. serotype-specific.
    - The sero type-specific antigens are located on MOMP and on the basis of this chlamydiae have been divided into many serovars or serotypes.



CULTURE

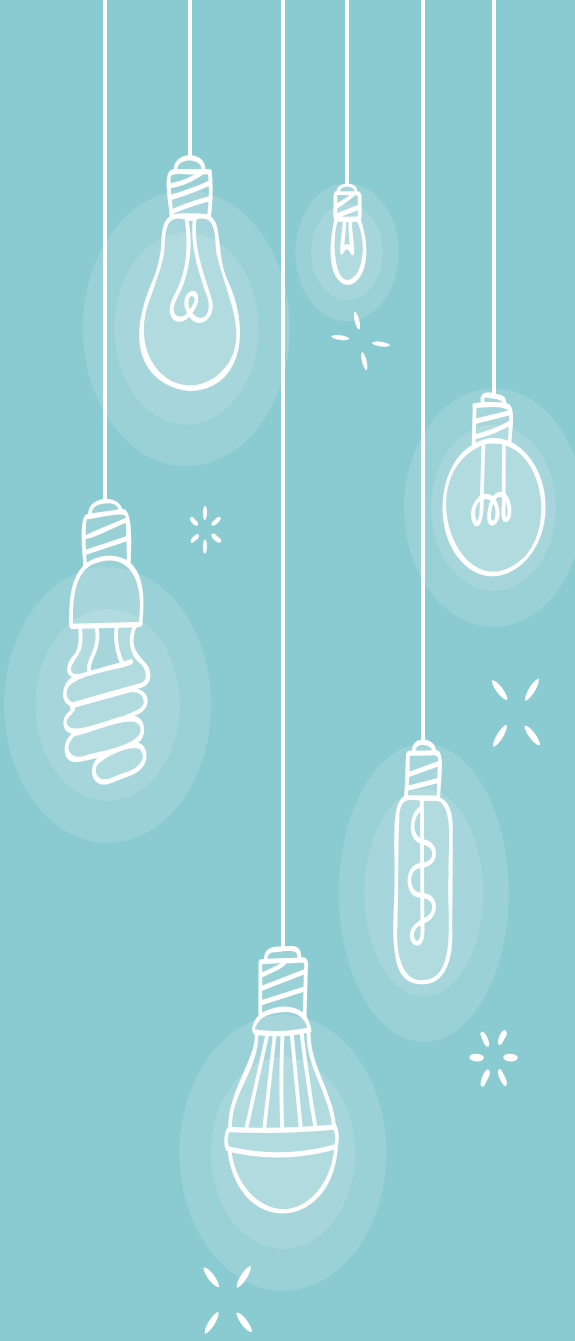
Chlamydiae can be isolated by the following methods:

(a) Animal inoculation: Mice can be inoculated through intranasal, intraperitoneal or intracerebral route. Mice die within 10 days. Smears made from lung, spleen, brain or peritoneal exudate demonstrate elementary bodies.



(b) Egg inoculation: Organisms can be isolated by egg yolk inoculation of the specimen. Impression smears can be stained by Giemsa or Gimenez.

(c) Tissue culture: McCoy cells treated with cycloheximide are the most commonly used cell lines. Irradiated or metabolically inhibited cell lines can also be used for isolation of chlamydia. Inclusion bodies can be visualized by staining the cell lines.

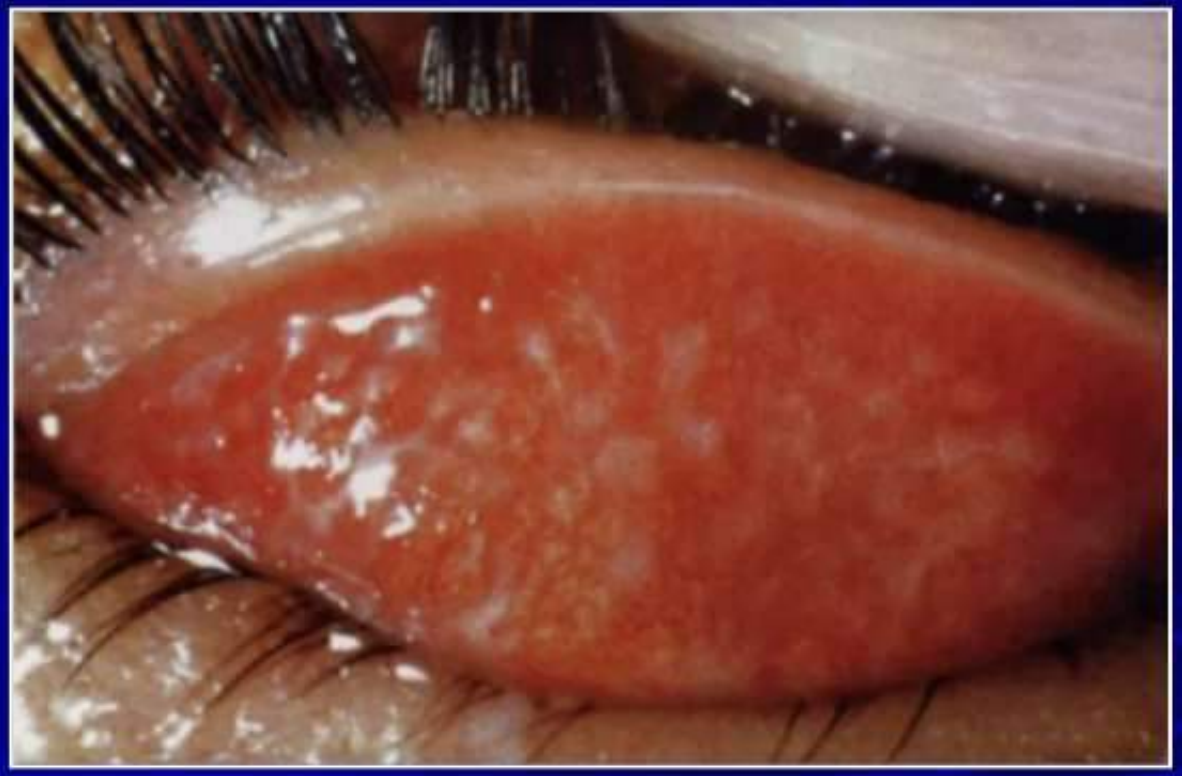


DISEASES PRODUCED BY CHLAMYDIA

- ***Ocular infections:*** *C.* trachomatis serotype A,B,Ba,C- is the leading cause of preventable blindness (caused by a chlamydia infection called trachoma) in the world. Other diseases produced are inclusion conjuctivitis (serotype D to K) and ophthalmia neonatorum.

- ***Respiratory infections:*** *C.* pneumoniae causes pneumonia. *C.* psittaci causes psittacosis.

Trachoma



Respiratory infections:

- *C.* pneumoniae causes pneumonia.
- *C.* psittaci causes psittacosis.

Pathogenesis - *C. psittaci*

- Inhalation of organisms in bird droppings
  - Person to person transmission is rare
- Hematogenous spread to spleen and liver
  - Local necrosis of tissue
- Hematogenous spread to lungs and other organs
- Lymphocytic inflammatory response
  - Edema, infiltration of macrophages, necrosis and occasionally hemorrhage
  - Mucus plugs may develop in alveoli
- Cyanosis and anoxia

- ***Genital infections:*** C. trachomatis is also the leading cause of sexually transmitted disease worldwide. It is associated with non-gonococcal urethritis and lymphogranuloma venereum (serotype L1, L2, L3). C. trachomatis is one of the major causes of pelvic inflammatory disease (PID) and infertility in women.

LABORATORY DIAGNOSIS

- ***Specimen collection***: Specimen should be collected by scraping the mucosa. Discharge should not be collected. Depending on the site of infection, ocular, urethral, cervical, sputum, respiratory secretions can be collected. In suspected Psittacosis, blood and sputum are collected for microscopy and culture and serum for serology.

Direct detection of antigen: Antigen detection is a rapid method of diagnosing chlamydial infection.

1. Light Microscopy: Inclusion bodies of C. trachomatis can be detected by staining with Lugol's iodine. Iodine can be used because inclusion bodies contain a glycogen matrix. Giemsa, Castaneda, Machiavello and Giminez methods are better and can be used to stain ocular, cervical or urethral specimen.

2. Immunofluoresence: Direct fluorescent antibody test detects major outer membrane proteins. It is now considered by many the test of choice for diagnosis.

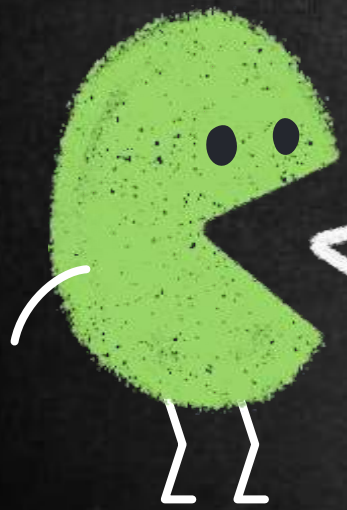
ELISA: Antigen and antibodies can be detected by ELISA. Antigen detection is more specific than antibody detection.

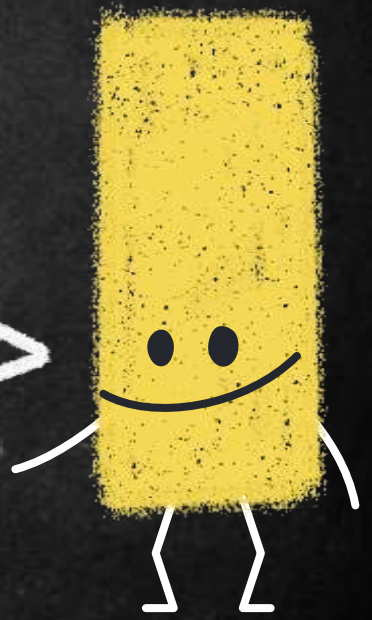
Isolation: Mice, fertilized hen's egg and tissue cultures can be used for isolation of chlamydia. The clinical specimen can be inoculated into the yolk sac of 6 to 8 day old eggs. Irradiated or cycloheximide treated McCoy cell culture is the preferred isolation method.

Molecular tools: Polymerase chain reaction, ligase chain reaction can be used for detection of chlamydia

TREATMENT

Sulphonamides and tetracycline are the drugs of choice. Single dose azithromycin is the drug of choice for non-gonoccocal urethritis





“

THANK YOU

TREPONEMA PALLADIUM

CONTENTS

- History
- Morphology
- Cultural characters
- Pathogenesis
- Stages of syphilis
- Lab diagnosis
- Treatment
- Prevention

INTRODUCTION

★ Treponema pallidum is a spirochete bacterium with various subspecies that cause the disease syphilis,bejels and yaws.

★ It is transmitted only among humans.

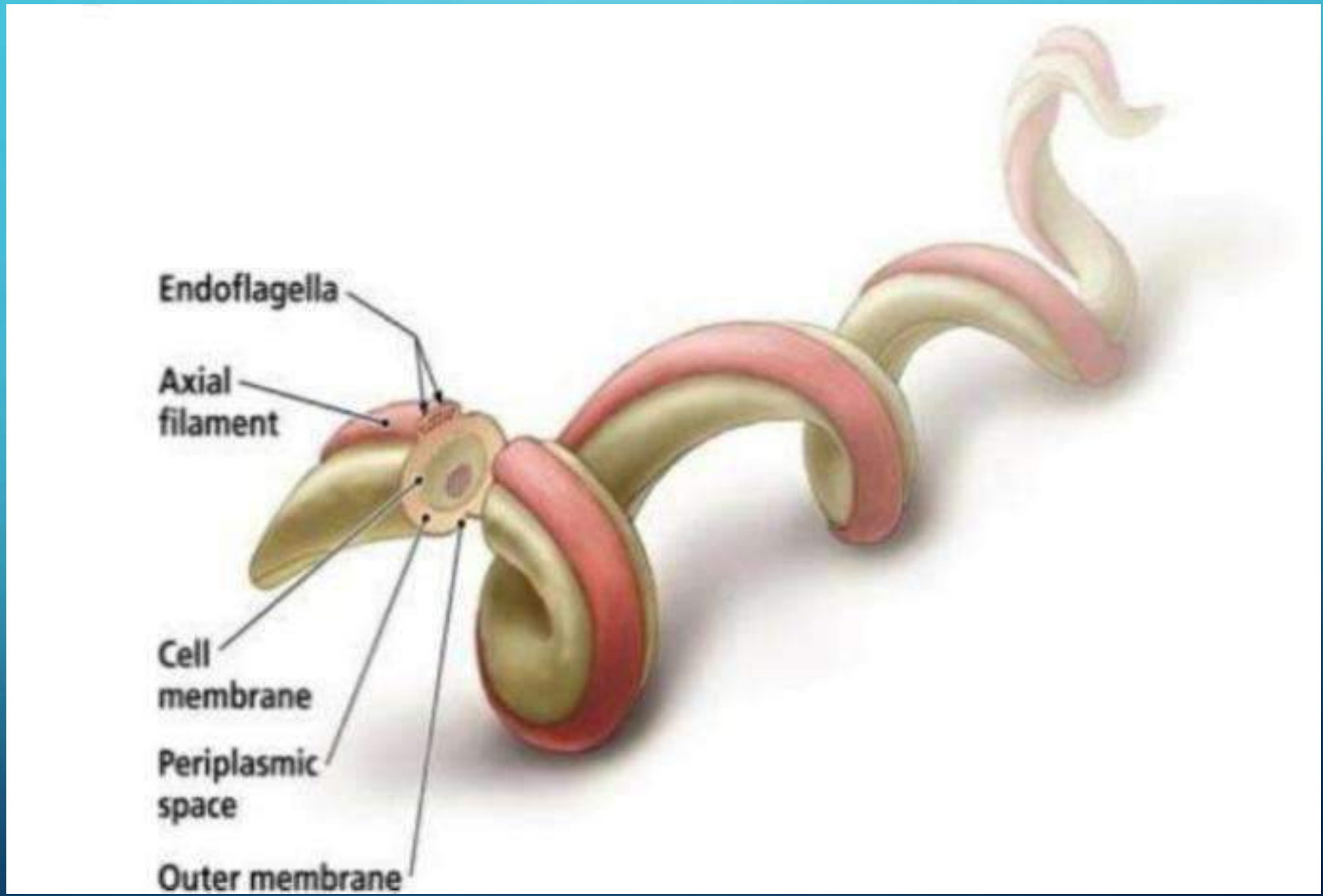
★ syphilis also called as STD's
(Sexually transmitted disease)

History

- Fritz Schaudinn (1871-1906) and Paul E. Hoffmann (1868-1959) discovered *Treponema pallidum* in serum in 1905.

Morphology

- Gram negative organism
- They are thin and long 6-14mu m in length and 0.2mu m in width
- Helically coiled shaped
- Actively motile organism
- It contains endoflagella
- Spirochaete organism


Endoflagella
Axial filament
Cell membrane
Periplasmic space
Outer membrane

CULTURAL CHARACTERISTICS

* Treponema pallidum has not been cultured in any artificial medias.

* Fastidious organism that exhibits narrow optimal ranges of:

- PH (7.2 to 7.4)
- Temperature (30 to 37°C)

RESISTANCE

* Delicate and inactivated by heat, soap ,cold ,antibiotics ,some chemicals ,UV radiation and glycerol.
    * Heat (41-42° c at 1hr)
    * Cold (0-4° C in 1-3 days)

Pathogenesis

Organism entry(Sexual contact)

↓

by penetrating the intact mucous membrane or entering through breaks in the skin

↓

Invade the blood stream and spreads to other body sites

↓

endarteritis

↓

Progressive tissue destruction

syphilis

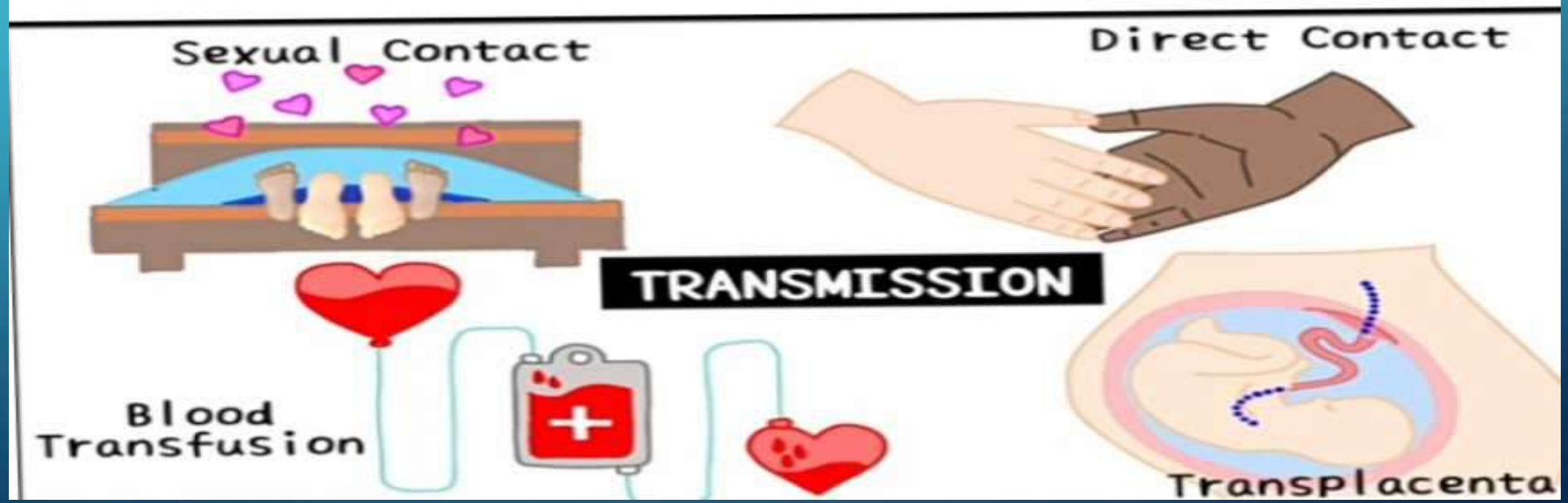
* Syphilis is a chronic bacterial infection caused by treponema pallidum.

* It is mainly transmitted by direct sexual intercourse.

* syphilis develops in stages and symptoms vary with each stage.

Mode of Transmission

- Direct sexual contact (90 – 96%)
- Blood transfusion
- Via placenta from infected pregnant mother ⇒ faetus ⇒ causes congenital syphilis.



- So that it is divided into,

1. ACQUIRED SYPHILLIS
2. CONGENITAL SYPHILLIS

1.Acquired syphilis

- 1. primary syphilis or primary stage
- 2. secondary syphilis or secondary stage
- 3. latent syphilis or latent stage
- 4. tertiary syphilis or tertiary stage

2.Congenital syphilis

PRIMARY SYPHILIS OR PRIMARY STAGE

- In this stage the papule appears in the genital area that ulcerates and forming a primary lesion called “chancre” or “hard chancre”.
- It is characterized by red, flat, painless and indurated ulcer with 1-2 cm in diameter.

- This lesion is also called as hunterian chancre, which is most frequently appears in the external genitalia(penis,labia,vagina)
- Occasionally the chancre may appear on mouth, lips, cheeks and nipples.

Primary Syphilis - *Chancre*

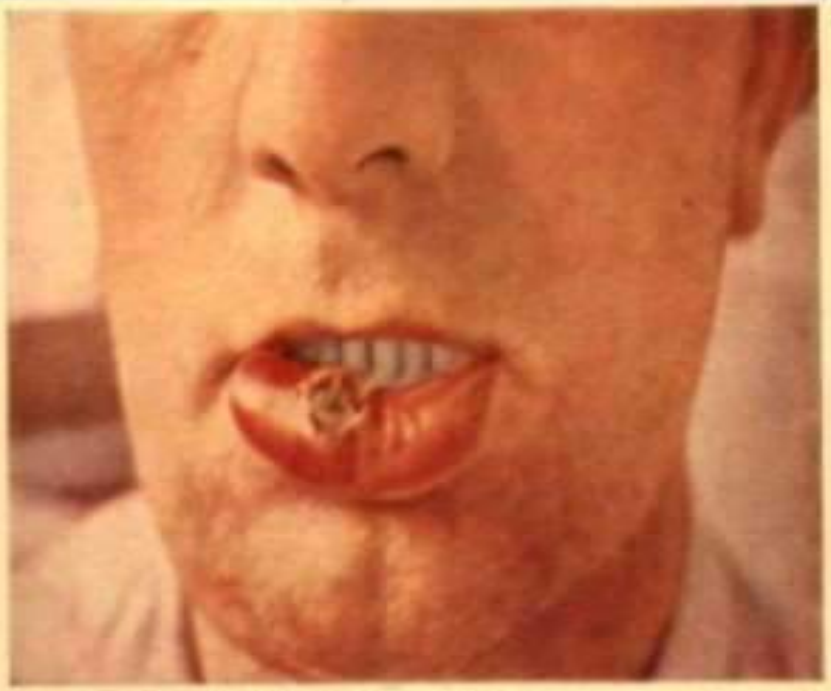
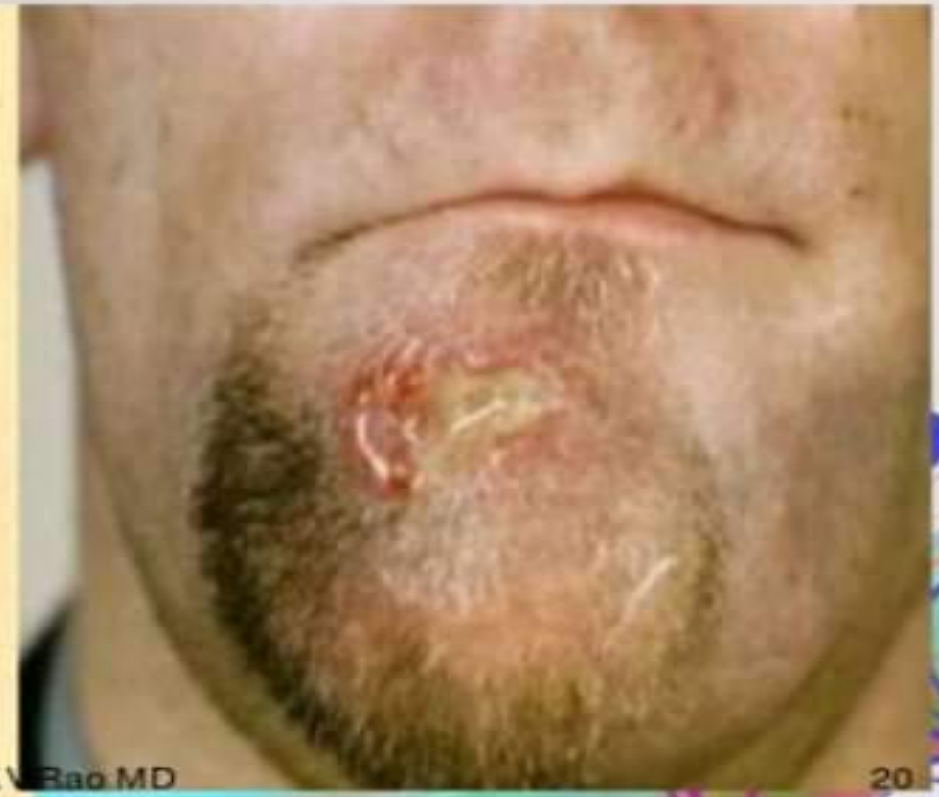


Fig. 171. **Primary Syphilis of the Lower Lip.** A chancre appearing on the lower lip has the same clinical appearance as one appearing on the genital mucosa. This lesion may simulate squamous cell carcinoma.



Secondary Syphilis

- Secondary lesions occur 3 to 6 weeks after the primary chancre appears
- may persist for weeks to months
- Primary and secondary stages may overlap
- Mucocutaneous lesions most common

- **Symptoms:**
  - fever
  - swollen lymph glands
  - sore throat
  - patchy hair loss
  - headaches
  - weight loss
  - muscle aches
  - fatigue

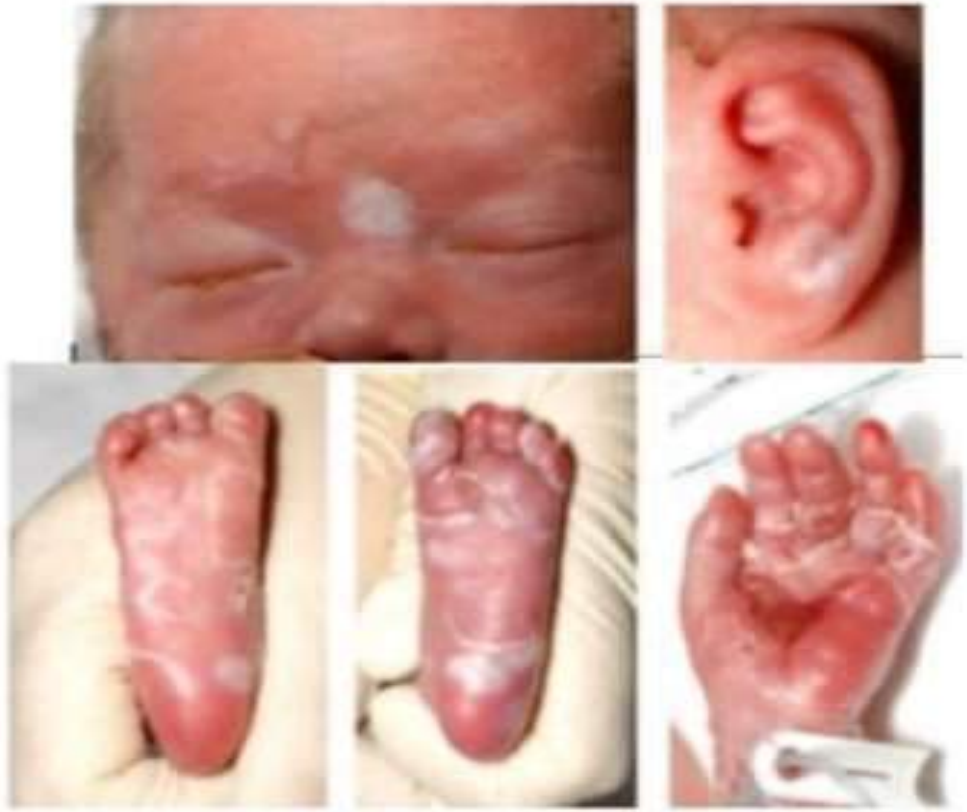
Secondary syphilis

Latent Syphilis

- Host suppresses infection-no lesions are clinically apparent
- Only evidence is positive serologic test
- May occur between
  - primary and secondary stages
  - secondary relapses
  - after secondary stage
- Categories:
  - Early latent: <1 year duration
  - Late latent: ≥1 year duration

Congenital Syphilis

- Congenital syphilis usually occurs following vertical transmission of T. pallidum from the infected mother to the fetus in utero, but neonates may also be infected during passage through the infected birth canal at delivery.



Laboratory Diagnosis

- Identification of *Treponema pallidum* in lesions
  - Darkfield microscopy
  - Direct fluorescent antibody - *T. pallidum* (DFA-TP)
  - PCR
- Serologic tests
  - Nontreponemal test
  - Treponemal tests

Direct fluorescent antibody staining for T.Pallidum(DFA-TP)

- The smears made from the exudates can be directly stained with fluorescein labelled antitrponemal antibody.
- This is also called as fluoroscein labelled antibody test.

- serology methods

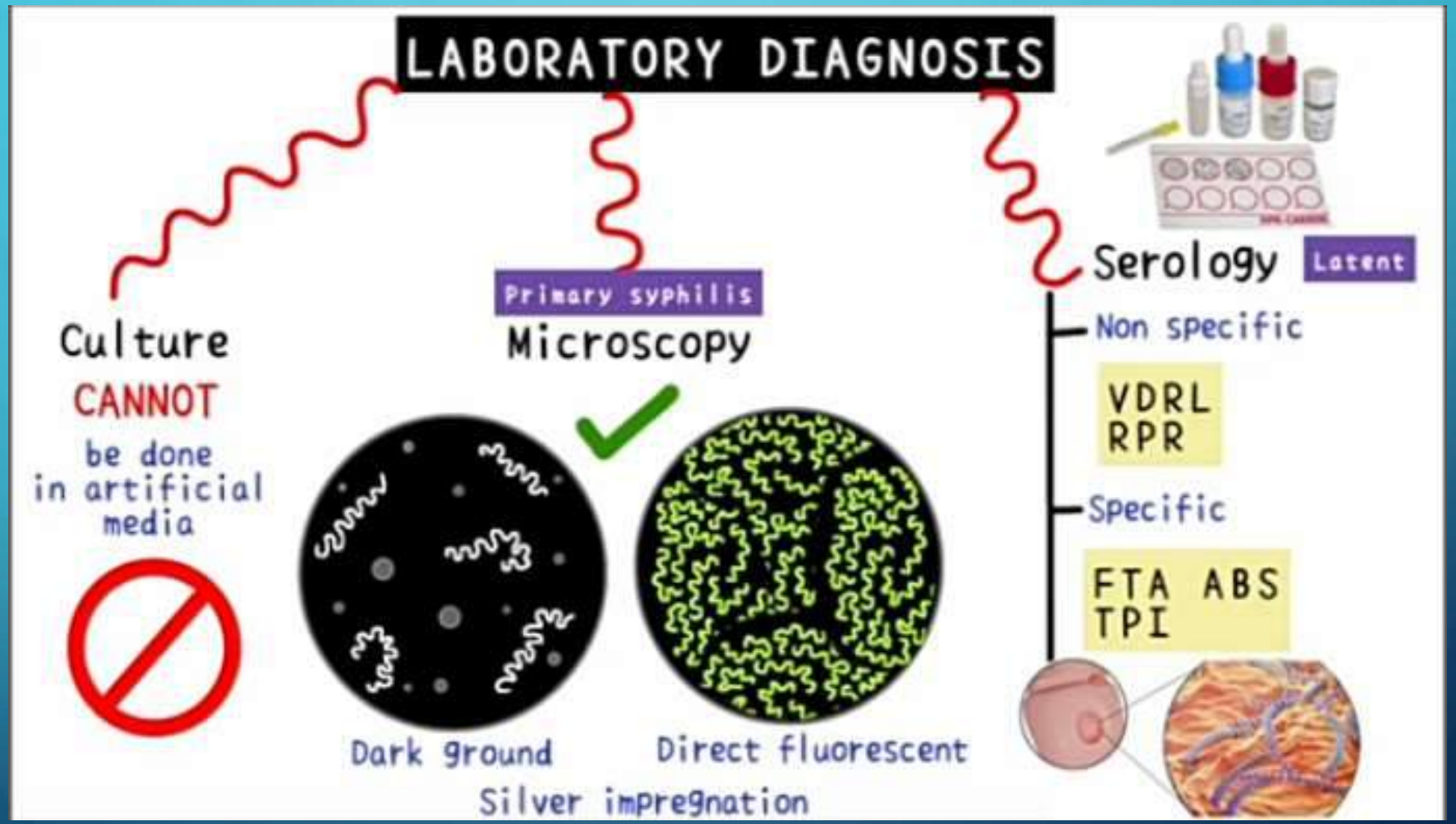
NON TREPONEMAL OR STANDARD TEST

- 1. flocculation test
  - VDRL test
  - RPR test
  - kahn test
- 2. Compliment fixation test

VDRL TEST

- It is a most widely used screening test which is rapid and economical.
- In VDRL test, 0.05 ml of serum which is heated at 56degree C for 30 min is taken.
- One drop of freshly prepared cardiolipin antigen is added over that.
- The slide is rotated at 180 rpm for 4 minutes.

- Positive: formation of clumps
- Negative: formation of uniformly disturbed crystals.


LABORATORY DIAGNOSIS
Culture
CANNOT
be done
in artificial
media
Primary syphilis
Microscopy
Dark ground
Direct fluorescent
Silver impregnation
Serology
Latent
Non specific
VDRL
RPR
Specific
FTA ABS
TPI

TREATMENT

- Early stages

  benzathine benzyl penicillin-single dose

  doxycycline- twice for 5 days

- Late stages

  benzathine benzyl penicillin- once in a week for 3 weeks.

THANK YOU

Staphylococcus aureus:

Morphology:

- Gram +ve cocci
- Spherical shape
- Size - 4 μm in diameter
- Grape like cluster due to cell division occurs in 3 planes
- Non motile
- Non spore forming
- Few strains have capsule at younger stage.

Cultural characteristics:

- Grow on ordinary medium. (Nutrient agar medium)
- Temp (10-42°C)
- Optimum temp 32°C
- pH (7.4 - 7.6)
- Aerobes
- Facultative anaerobes

Nutrient agar:

- Large colonies (2-4 mm in diameter)
- Circular
- Convex

Smooth shiny & it can easily emulsifiable. (soluble in liquid media)

- Golden yellow pigment is produced when incubating at 22°C.
- Supplement (Pigment production is enhance by 1% glycerol monoacetate
- Oily paint appearance.

Blood agar:

- Similary to nutrient agar (morphology).
- Hemolytic at 20-25% of CO_2 supplement
- Hemolysis mark on rabbit & sheep blood.
- Weak on horse blood agar.

Macconkey agar:

- Produce pink colour colonies due to lactose fermentation

Liquid media:

- Uniform turbidity

Selective medias:

- Salt Milk agar
- Salt broth (Rudlam's medium)

Bio chemical Test:

- Catalase positive.
- Hydrolyse Urea
- Reduce nitrates to nitrite
- MR-VP positive
- Indole negative

Staph. aureus

Pathogenecity:

- Infection
- Intoxications } Two types it causes

↓

Produce toxin

Infection

Cocci gains access to damaged skin →

↓

Mucosal / tissue sites.

↓

Colonise by adhering

↓

Escape Host defense (WBC cells)

↓

Causes disease

Communications:

Disease by bacterial toxins

Virulence factor

Capsular polysaccharides

↓

Maintains structurally integrity.

Teichoic acid:

→ Cell wall.

→ Protect opsonisation. (cell signal)

Peptidoglycan:

→ Protect phagocytosis / inhibit

Protein A:

→ Antiphagocytosis

Enzymes:

→ Coagulase

→ Enzyme boring clotting of blood in human.

Lipid hydrolases:

→ It helps in infecting skin & subcutaneous tissues.

Hyaluronidase:

It breaks down connective tissue

Proteases: Helps in initiation & spread of infection.

Toxins:

Cytolytic toxins

↙ ↘

4 haemolysins — Leucocidin

- Alpha
- Beta
- Gamma
- Delta

Alpha hemolysins:

Inhibits phagocytosis.

Toxic to macrophages, lysozymes, muscle tissues, renal cortex & circulatory system.

In-active at 70°C, reactive at 100°C. It lysis rabbit erythrocytes.

Beta hemolysins:

Hemolytic for sheep cells. Hemolysis not seen in human or rabbit. Hemolysin at 37°C.

Gamma hemolysins:

Hemolytic activity seen in sheep blood

Delta hemolysins:

Has detergent like effect on cell membranes of erythrocytes, Leukocytes, macrophages & platelets.

Leucocidin

It is also called as Panton-Valentine Leucocidin ~~Toxin~~ (or) PVL

i) Enterotoxins: Responsible for food poisoning, Nausea, Vomitting & diarrhoea within 2-6 hrs of production. Toxins are heat stable at 100°C upto 10-40 mins

Source of infection:

By a food handler.

8 - ag are produced

(A, B, C_1, C_2, C_3, D1, E & H)

Based on serological it can be identified (food poisoning) Latex agglutination test & ELISA

Toxic shock syndrome :-

Fever, hypotension, vomitting, diarrhoea, erythematous (lysis erythrocyte

It is associated with infection of mucosal sites by toxin shock syndrome tonin (TSST)

Exfoliative toxin.

Responsible for staphylococcal scalded skin syndrome. (SSSS)

Severe form → Ritter's disease.

Common diseases:

→ Skin & soft tissue.

→ Folliculitis

→ Wound infection

→ Abscess (boils in tissues)

→ Carbuncle

Respiratory disease

→ Pharyngitis sinos

Endovascular disease

Bacteriemia, septicemia

Epidemiology

Primary parasite to human & animals.

Colonising on skin & mucos membrane.

Source of infection:

Human patients & animals

10-30% healthy persons carries this disease via nose.

Vaginal carriage 5 – 10%.

Mode of transport

Direct contact

Air borne droplets.

Lab diagnosis :

→ Specimens : Sputum, pus

- Food poisoning : Feaces

- Microscopy :

Gram staining.

- Culture methods :

Blood agar & selective media

- Biochemical test :

- Serological test :

Anti α-lysin test

Treatment :

Vancomycin, benzyl penicillin & Methycilin.

Streptococcus pyogens :

→ Flesh eating bacteria.

→ Gram +ve cocci in chains / pairs.

Morphology :

- Cocci / spherical / oval in shape.
- 0.5 - 1.0 μm / diameter
- Cocci in chains in liquid media, than in the solid media.
- Chain formation due to cell division in 1 plane.
- Non motile
- Nonsporing
- Some have capsules.
- Composed of Hyaluronic acid

Cultural Characteristics :

- Aerobes / Facultative anaerobes.
- Temp range = 22 - 40°C
- Op temp = 37°
- Growth occur only in the presence of carbohydrates in the media (or) blood enrichment.

Blood Agar:

- After 24 hrs colonies are small, circular, semitransparent & convex. Clear hemolysis seen. Promoted by 5-10% CO_2.
- Virulent stain produce Matt colony.
- Avirulent stain produce Glossy colonies

Liquid media:

Glucose (or) serum broth - Growth occurs as granular turbidity with a powdery deposit.

Bio chemical test:

Catalase negative

Ferment Sugar

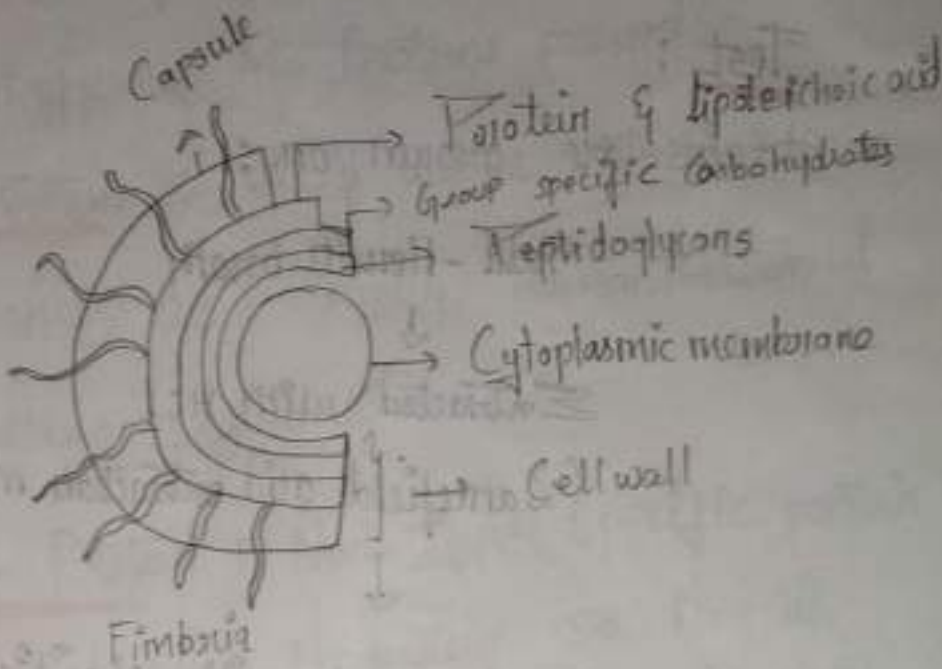
Produce Acids but no gas

PYR - Test [Pyrrolidonyl - beta - naphthyl - amide]

(It contains ribose)

fail to ferment ribose - This test helps to differentiate streptococcus pyrogens from others

Antigenic Structure of S. Pyogenes



Capsule : Inhibits phagocytosis.

Cell wall : Contains outer layer of ptn & lipoteichoic acid. Middle layer contain Group specific carbohydrates & Inner layer contains peptidoglycans

Peptidoglycon : Have pyrogenic & thrombolytic activity

Carbohydrate Ag : On the basis of Carbohydrates Ag, it is classified under Lancefield group A. This Ag shows cross reactive with some human tissues

It is an integral part of cell wall extraction by precipitation with group specific Antisera

Test :

Strains are grown on ;

Todd - Hewitt broth

↓

Extracted with HCl

(Lancefield acid extraction method)

↓

The extract react with specific antisera

↓

Precipitation within 5 mins

↓

this is because to do grouping b/w streptococcus.

Protein Ag :

- M- Protein
- T- Protein
- R- Protein

M - Protein : Important protein for typing as well as for virulence. Inhibits phagocytosis

It is antigenic. Heat & acid stable. Susceptable to tryptic digestion. Extracted by lancefield acid extraction method).

80 - types of M- Proteins present

T- Protein : Acid labile. Trypsin resistance Ag. Usually demonstrated by slide agglutination test

R- Protein : Non-type specific protein associated with M- protein so it is called as M- associated protein (MAP) This proteins are non-virulence.

Hair like pili : [Fimbria]

Project through capsule of group A streptococci. Pili consist of protein M-Ptn & covered with lipoteichoic acid. It is important for attachment to epithelial cells

Antigenic cross reaction :

Some streptococcus pyogens exhibits antigenic cross reaction. Antigenic relationship demonstrated b/w Capsular

(1) Hyaluronic acid with human synovial fluid (2) cell wall ptn react with Myocardium

~~Cell wall ptn & myocardium:~~

(3) Group A carbohydrates & Cardiac walls:

These cross reaction leads to rheumatic fever & tissue damage

Toxins & Enzymes:

Exotoxins responsible for virulence

Hemolysins:

1. Hemolysin O
2. Hemolysin S

(1) Hemolysin O: Inactive in oxidized form, reactive at 37°C by treating reducing agents. On blood agar activity seen only in pour plate, not in surface cultures. Heat labile. Contribute to virulence factor. Reducing agent used are Sodium dihydrosulphate (To convert them in active form)

(2) Hemolysin S: O_2 stable. On blood agar activity seen in spread plate method (Surface of blood agar) Soluable in serum. Non antigentic Inhibited by non-specific serum lipoproteins.

Pathogenicity :

Disease caused by Streptococcus pyogenes can be suppurative & non suppurative

Supporal

Suppurative :

① Respiratory infection.

Sore throat because primary site of invasion of human body is throat.

Common infections

Fever, Headache, rarely leads to meningities. From throat bacteria spread to the surrounding tissues leading to complications such as OTITIS media. (ear)

Ludwig's Angina : (connective tissue)

② Skin & Soft tissue infection

Infection of skin including wounds or burns :

Produce lymphangitis &

Cellulitis

Two typical skin infections:

① Erysipelas

② Impetigo

Erysipelas: Infection on superficial lymphatic vessels.

Skin redness
Swollen

Impetigo: Caused by M-type antigen. Mainly affects in children

glomerulonephritis

Non Suppurative

Streptococcus p infection lead to 2 important disease

① Acute Rheumatic fever (Problem in joints of synovial)

② Acute glomerulonephritis:

The essential lesion in RF

is carditis including connective tissue degeneration. & inflammatory myocardial lesions. - Aschoff Nodules.

Epidemiology:

Main source of S. pyogenes is Human Resp. tract -> Throat → Nasopharynx → Nose

Lab diagnosis:

- Tissue sample
- Sputum
- Lesions
- Serum

Microscopy:

Gram staining (Gram +ve cocci (chains)

Culture:

Blood agar

Maconkey agar: No growth

Serology : Antistreptolysin 'O' titration
(ASO test)

Anti DNA'S B test
↓
Useful for diagnosis of streptococcal
biodorma.

Streptozyme test :
Slide hemeagglutination test

Treatment
Penicillin G , Erythromycin
(or) Cephalexin (when the
above both creates allergic reaction
cephalexin is given).

Streptococcus pneumoniae:

Autolysis ↓ self action

Gram +Ve

Diplococci referred as pneumococci. Differs from other streptococci by its morphology bile solubility & optochin sensitivity & ~~s~~pecific polysaccharide capsules.

Mor Discovered by pasture & Sternberg noticed in (1881).

Fraenkel & Weichselbaum established relationship with other organisms.

It is the normal flora of respiratory tract.

Morphology:

Small, slightly elongated cocci with one end broad & other

end pointed. Flame shape appearance or lanceolate appearance.

Capsulated at early stage, Non motile, Non-sporing, readily stained with alanine dyes.

Cultural Characters

(1) Blood agar: In anaerobic condition β-hemolysis due to O_2 labile hemolysin O.

(2) Liquid media: Glucose broth Uniform turbidity. Cocci undergoes autolysis due to intracellular enzymes. Autolysis enhanced by bile salts & sodium lauryl sulphate. Heat killed cultures doesnot show autolysis.

Bio chemical test

Catalase, Oxidase - Negative.

Ferment several sugars & produce acids. HISS's serum sugar to

test for fermentation. Fermentation of inulin is a useful test for differentiating from other St. cocci

Bile solubility test : ① 10% Na (sod
Sodium deoxycholate → Add 1 ml →
broth culture ↓ → Culture clears
due to lysis of cocci

② 10% deoxy cholate on blood agar colony
→ Lysis in few mins (5 mins)

Principle : Autolytic amidase cleaves
bond b/w alanine & Muramic acid
present in the peptidoglycan

Optochin Sensitivity Test :

Optochin disc on blood agar leads to white zone of inhibition

Antigenic Properties

Capsules are most important for type specific Ag properties

Capsular polysaccharide helps to classify as different serotypes

Serotyping Test :-

Based on capsular Ag.

Agglutination test : done by using type specific anti-serum.

Precipitation test : Capsule swelling or quelling reaction

↓

① Suspension of Streptococcus pneumoniae

↓

Slide

↓ With type - Specific antiserum

+

Methylene blue

In the presence of homologus

anti-serum

↓

Capsules - Swollen sharply

↓

Delineated & refractile

PCR based test

Polymerase Chain Reaction Test

① Test have shown higher sensitivity in detection of infections.

② It is useful to detect small no. of specific DNA sequence of bacteria

Variations:

Repeated subculture leads to smooth to rough variations. (S-R)

R-type colonies eliminates phagocytosis.

Toxins & Other virulence factors:

① Capsular polysaccharide : It protect from phagocytosis.

② Pneumolysin : Membrane damage toxins & has cytotoxic & complement activating property.

③ Autolysins : Contribute to Virulence.

This strain produce Oe labile hemolysin & leucocidin

Pathogenicity: Strains colonise the human nasopharynx - Cause infection in the middle ear, paranasal sinus & respiratory tract by direct spread.

↓

Pneumococcal bacteriemia

↓

Leads to infection in heart, peritoneum or joints.

Infection is commonly endogenous but exogenous infection may also occur when virulent strains are present.

Symptoms: Pneumonia, Broncho pneumonia, acute tracheobronchitis, Bacteriemia, toxemia. ↓ damage respiratory epithelium. meningitis, OTITIS media, Conjunctivitis, Suppurative arthritis, Cavitis.

Epidemiology:

Source of infection - respi. tract.

Transmitted by - Contaminated droplets

Infection leads to pharyngeal carriage

↓

Disease results only when Host resistance is lower.

Lab diagnosis:

① Specimen : Sputum, Blood; Urine.

② Microscopy : Gram staining.
(Gram +ve diplococci)

③ Culture : Blood agar: Anaerobes
β-hemolysis due to O2 labile
hemolysin O.

④ Isolation from respiratory
Secretion facilitated using
blood agar with 5 μg/ml gentamycin (isolate only S. pneumonia)

⑤ Mouse inoculation : If specimen
contains S. pneumonia in scanty.
Isolation may be obtained by
intraperitorial inoculation in
mice & wait for 1-3 days. If
any inflamation observed the
test is +ve.

⑥ Molecular method.

CRP testing, PCR.

⑦ Treatment:

: Penicillin, Amonycilin, Penicillin resistance strains are subjected to Cephalosporin & Vancomycin.

NEISSERIA:

Neisseria meningitidis

Gram -Ve

Oval / Spherical in Shape

0.6 — 0.8 µm Size

Occur in pairs (cocci)

Non motile

Capsulated

Cultural Characters

Enriched media is required - blood, serum promotes the growth of bacteria by neutralizing certain inhibiting substance in the culture media. (Fastidious Org). Aerobes.

Optimum temp = 35 - 36°C, pH - 7.4 - 7.6.

i) Solid media :. 24 hrs Colonies are small, round & convex. It is easily emulsifiable

ii) Blood agar : Weak hemolysis, smooth & rough type colonies.

iii) Liquid media : Granular turbidity with little (or) no surface growth

iv) Chocolate agar

v) Starch casein

vi) Hydrolysis agar

vii) Selective media - Thayer - Martin

Biochem

Cat, Oxi - +ve

I, Hydrogen sulphate Not produced

Nitrates not reduced

Glucose & maltose utilize & produce acid, no gas.

Acid formation is weak

Antigenic Property:

Serogroups: Based on capsulated polysaccharide. It is classified into 13 serogroups. A, B, C, X, Y, W, (W_1, W_3, W_5)

Group: A = Associated with epidermic.

Group: C = Localized out break Endemic

Group: B = Both epidermic & localized out break

Serotypes:

S further classified into serotypes & subtypes

based on outer membrane ptn. 20 serotypes identified

Pathogenicity:

Disease present in cerebrospinal leads cs meningitis & Meningococcal septicemia. &

A Meningiococci strict human parasite inhabiting nasopharynx.

Infections usually Asymptomatic

Asymptomatic.

Rhinitis & pharyngitis

Meningitis.

cocci

↓ spread

Nasopharynx to meninges

↓ by travelling directly

Along perineural sheath of olfactory nerve

↓ through

Cribriform plate to subarachnoid space

↓ through

Blood stream

↓

On reaching CNS - Suppurative Lesions of meningis starts

↓

cocci found in spinal fluid

↓

Untreated cases leads to blindness & deafness

↓

Some cases develop chronic meningitis

Symptoms :

① Meningio coccemia - leads to ~~at au~~ acute fever with chills ② malaise & Stupor (sleepy) ③ Petechial rash occurs ④ Waterhouse syndrome - Shock disseminated intravascular coagulation &

multisystem failure

(5) Meningococcal disease favoured by the deficiency of complement components (C5 - C9)

Pathogenesis:

Occured due to endotoxin, lipopolysaccharide released by autolysis.

- Vascular endothelium is sensitive to endotoxin. All major inflammatory cascade system as well as cytokines are triggered & up regulated. In fulminant case - Adrenal Hemorrhage prefacdim shock

Epidemiology: Human nasopharynx is the only reservoir. Transmission is essentially by air borne droplets. 5-10% carrier rate during inter epidemic periods. In India serogroup A most

Common cause of epidemic & endamic

Lab

Specimen - CSF, blood, Nasopharyngeal swab.

Blood culture - Culture should be incubated at 4-7 day with daily subculture.

Meningococcal ag found in blood in active disease

Nasopharangial Swab - Used for detection of carriers

Sampling : Done without contamination.

Petechial lesion : (Red patches).

sometimes meningococci demonstrated in petechial lesion by microscopy & culture

~~Ato~~ within 12 hrs

Autopsy : Specimen collected from meninges surface of brain & spinal cord

Serology : Based on Ag Ab reaction. bacteria was identified

Molecular diagnosis : DNA sequence in CSF or blood by PCR

Treatment :

Sulphonamides if resistance

Intravenous penicillin G.

Chloramphenicol.

Third generation cephalosporin

Neisseria gonorrhoeae :

Morphology : G^{-ve} diplococci, kidney shape. It has pili - It helps the adhesion to mucosal surface & promote virulence by inhibiting phagocytosis.

C.C : Aerobic, Sometime anaerobic, difficult to grow, pH 7.2 - 7.6, Temp 35-36°. 5-10% CO_2 is essential for growth. Grow well on chocolate & mullen hinton agar.

Selective media - Thayer & Martin. [contains vancomycin, colistin & Nystatin which inhibits the contamination of Organisms.]

Motility - Twitching motility.

Colonies - Small, round, translucent, convex. It produce 4 - types of Colonies [T_1 - T_4]

T_1 & T_2 = Small colonies [brown colour]

T_3 & T_4 = Large colonies [colourless]

Symptoms : Venereal diseases [STD]

SAMPLE : Vaginal swab

25 Corynebacterium

INTRODUCTION

Corynebacteria are Gram-positive, non-acid fast, non-motile rods with irregularly stained segments, due to the presence of granules in some species. They frequently show club-shaped swellings—hence, the name *Corynebacterium* (from *coryne*, meaning club).

The most important member of the genus is *C.diphtheriae*, the causative agent of diphtheria (*case*). The disease was first recognised as a clinical entity by Bretonneau (1826) who called it 'diphtherite' (from *diphtheros*, meaning leather). The name is derived from the tough, leathery pseudomembrane formed in the disease.

The diphtheria bacillus was first observed and described by Klebs (1883) but was first cultivated by Loeffler (1884). It is hence known as the Klebs–Loeffler bacillus or KLB. Loeffler studied the effect of the bacillus in experimental animals and concluded that the disease was due to some diffusible product of the bacillus. Roux and Yersin (1888) discovered the diphtheria exotoxin and established its pathogenic effect. The antitoxin was described by von Behring (1890).

CORYNEBACTERIUM DIPHTHERIAE

Morphology

The diphtheria bacillus is a slender rod with a tendency to clubbing at one or both ends. The bacilli are pleomorphic, measuring approximately 3–6 μm × 0.6–0.8 μm. They are non-sporing, non-capsulated and non-motile. They are Gram-positive but tend to be decolourised easily. They possess polymetaphosphate granules that serve as storage granules and are called **volutin** or **Babes–Ernst granules or metachromatic granules**. These give the bacilli a beaded appearance when stained with aniline dyes. The bacilli are arranged in characteristic pairs, palisades (resembling the stakes of a fence) or small groups. They often appear at various angles to each other, resembling the letters V or L. This has been called the **Chinese letter** or **cuneiform** arrangement, due to incomplete separation of the daughter cells after binary fission.

Corynebacterium diphtheriae

Clinical Case A five-year-old child presented to the pediatrics outpatient department with a history of pain in the throat and difficulty in swallowing. He had had low-grade fever for the past two days. On examination, he was found to have

Antigenic structure

Diphtheria bacilli are antigenically heterogeneous. Based on colonial and other characteristics, *C.diphtheriae* have been typed as gravis, intermedius and mitis. By agglutination, gravis strains have been classified into 13 antigenic types, intermedius into 4 and mitis into 40 types. Gravis strains of types I and III have been reported to be common in Great Britain, type II worldwide, type IV mainly in Egypt and type V in the USA. No connection has been established between type-specificity and other characters.

Pathogenicity

Toxin

Virulent strains of diphtheria bacilli produce a very powerful exotoxin (Table 25.1). The pathogenic effects of the bacillus are due to the toxin. Almost all strains of gravis and intermedius (about 95–99 per cent) are toxigenic, while only about 80–85 per cent of mitis strains are so.

The proportions vary with the origin of the cultures tested. Strains of all three types are invariably virulent when isolated from acute cases. Avirulent strains are common among convalescents, contacts and carriers, particularly in those with extrafaucial infection.

There is considerable variation in the amount of toxin produced by the different strains, some producing it abundantly and others only poorly, but the toxins produced by all strains of the diphtheria bacilli are qualitatively similar. The standard strain almost universally used for toxin production is the **Park–Williams 8 strain**, which has been variously described as a mitis (Topley and Wilson) and intermedius (Cruickshank).

Properties: The diphtheria toxin is a protein and has been crystallised. It has a molecular weight of about 62,000. It is extremely potent and the lethal dose for a 250 g guinea pig is 0.0001 mg. It consists of two fragments, **A and B**, of MW 24,000 and 38,000, respectively. Both fragments are necessary for the toxic effect. When released by the bacterium, the toxin is inactive because the active site on fragment A is masked. Activation is probably accomplished by proteases present in the culture medium and infected tissues (Table 25.1). All the enzymatic activity of the toxin is present in fragment A. Fragment B is responsible for binding the toxin to the cells. The antibody to fragment B protects by preventing the binding of the toxin to the cells. The toxin is labile. Prolonged storage, incubation at 37°C for 4–6 weeks, treatment with 0.2–0.4% formalin or acid pH converts it to toxoid. **Toxoid is a toxin that has lost its toxicity but not its antigenicity.** It is capable of inducing antitoxin antibodies and is used as a vaccine candidate.

The **factors affecting toxin production** are as follows:

- The toxigenicity of the diphtheria bacillus depends on the intracellular presence of **corynephage (tox+)**, which act as the genetic determinant controlling toxin production. Non-toxigenic strains may be rendered toxigenic by infecting them with **beta** or some other phage. This is known as **lysogenic** or **phage conversion**. The toxigenicity remains only as long as the bacillus is lysogenic. When the bacillus is cured of its phage, as by growing it in the presence of antiphage serum, it loses its toxigenic capacity.
- Toxin production is also influenced by the concentration of **iron** in the medium. The optimum level of iron for toxin production is 0.1 mg/l, while a concentration of 0.5 mg/l inhibits the formation of toxin.

Mechanism of action: The diphtheria toxin acts by inhibiting protein synthesis. Specifically, fragment B helps in binding and fragment A inhibits polypeptide chain elongation in the presence of nicotinamide adenine dinucleotide by inactivating the elongation factor EF-2. It has a special affinity for certain tissues such as the myocardium, adrenals and nerve endings.

Clinical features

The incubation period in diphtheria is commonly 3–4 days but may on occasion be as short as one day. In carriers, the incubation period may be very prolonged. The site of infection may be:

Table 25.1 *Characteristics of diphtheria toxin*

Lethal dose	0.1 µg/kg
Structure	2 subunits a) A-Active domain—responsible for action b) B-Binding domain—trigger entry host cell
Host cell receptor	CD9 and HBEGF-like precursor
Mechanism	Entry by receptor method endocytosis Action—inhibits protein synthesis by inactivating EF2 (Similar action demonstrated by exo toxin of *Psuedomonas aeruginosa*)

- Otitic
- Nasal
- Genital: vulval, vaginal or prepucial
- Pharyngeal (most common)
- Laryngeal
- Conjunctival
- Cutaneous

Pharyngeal diphtheria is the most common type and may vary from mild catarrhal inflammation to very widespread involvement (*Case*). According to the **clinical severity**, diphtheria may be classified as:

- **Malignant** or **hypertoxic** in which there is severe toxemia with marked adenitis (bull neck). Death is due to circulatory failure. There is a high incidence of paralytic sequelae in those who recover.
- **Septic**, which leads to ulceration, cellulitis and even gangrene around the pseudomembrane.
- **Hemorrhagic**, which is characterised by bleeding from the edge of the membrane, epistaxis, conjunctival hemorrhage, purpura and generalised bleeding tendency.

Common complications are:

- **Asphyxia** due to mechanical obstruction of the respiratory passage by the pseudomembrane, for which an emergency tracheostomy may become necessary.
- **Acute circulatory failure**, which may be peripheral or cardiac.
- **Post-diphtheritic paralysis**, which typically occurs in the third or fourth week of the disease; palatine and ciliary, but not papillary, paralysis is characteristic, and spontaneous recovery is the rule.
- **Toxemia**, in which the bacilli remain confined to the site of entry, where they multiply and form the toxin, which is absorbed and produce toxic damage to the heart (myocarditis), kidney (tubular necrosis), liver and adrenal glands.
- **Local necrotic changes**, leading to fibrinous exudates; these, together with the disintegrating epithelial cells, leucocytes, erythrocytes and bacteria, constitute the pseudomembrane, which is characteristic of diphtheritic infection.
- **Mechanical**, caused by the membrane.
- **Non-toxigenic strains**, which may cause infection even in immunised individuals, as immunity with the toxoid does not confer antibacterial immunity. Such infection is mild, though pseudomembrane formation may sometimes occur.

Cutaneous diphtheria: In the tropics, diphtheria bacilli infect the skin more often than the respiratory tract. Toxigenic diphtheria bacilli may persist in the skin for over three years. Cutaneous infections may stimulate natural immunity to diphtheria but may also lead to **pharyngeal diphtheria** in non-immune contacts. Cutaneous infections are usually secondary to pre-existing skin lesions. Sometimes, diphtheritic whitlow or ulcer may occur. Cutaneous infections are commonly caused by non-toxigenic strains of the diphtheria bacilli. Fomites do not seem to play an important role, though in special situations, toys and pencils may act as vehicles of infection.

Typing

Typing methods are used to determine the transcontinental spread. This has become important because infection can be introduced from the countries where outbreaks continue to occur in children into countries that have been able to contain the infection, but adolescent and adult population are still susceptible to the infections.

Typing methods

- **Biotyping:** This is done based on biochemical tests, mostly using automated systems.
- **Ribotyping:** This is presently considered to be the most useful method to type the strains of *C.diphtheriae*.
- **Molecular methods** like **Pulse Field Gel Electrophoresis (PFGE), Random Amplification of Polymorphic DNA (RAPD)** or **Amplified Fragment Length Polymorphism (AFLP)** are the other molecular methods used for typing.
- **Bacteriophage typing:** About 15 bacteriophage types have been described. Types I and III are mitis, IV and VI intermedius, VII avirulent gravis and the remainder virulent gravis. The phage types are apparently stable. A system of bacteriocin (diphthericin) typing has also been described. Other methods of typing include bacterial polypeptide analysis, DNA restriction patterns and hybridisation with DNA probes. However, due to poor discriminatory power, this is not used any longer.

Laboratory diagnosis

Laboratory confirmation of diphtheria is necessary for the initiation of control measures and for epidemiological purposes but not for the treatment of individual cases. Specific treatment should be instituted **immediately** on suspicion of diphtheria without waiting for laboratory tests. Any delay may be fatal.

1. Specimen

Preferably, two dacron swabs from the lesions are collected under vision, using a tongue depressor from the lesion. The area under the visible membrane should be sampled.

2. Microscopy

Stained smear examination: Smears are stained with methylene blue or one of the special stains.

Neisser's or **Albert's stain:** This stain will show the bacilli with metachromatic granules and in the typical arrangement (Fig. 25.1). Albert's stain may show delicate green bacilli with purple-blue metachromatic granules. The granules are often situated at the poles of the bacilli and are called **polar bodies**.

By Gram stain, the granules are more strongly Gram-positive than the rest of the bacterial cell.

Loeffler's methylene blue: The granules take up a bluish-purple colour and are, hence, called **metachromatic granules.** However, the bacilli may not always be demonstrable in smears from the lesion; confident differentiation from some commensal corynebacteria normally found in the throat may also be difficult.

Gram- or Leishman-stained smear: This is done to rule out Vincent's spirochetes and fusiform bacilli, responsible for Vincent's angina which clinically resembles diphtheria.

Immunofluorescence: Diphtheria bacilli may be identified in smears by **direct immunofluorescence test**. This is more specific as the smear is stained with specific antibody.

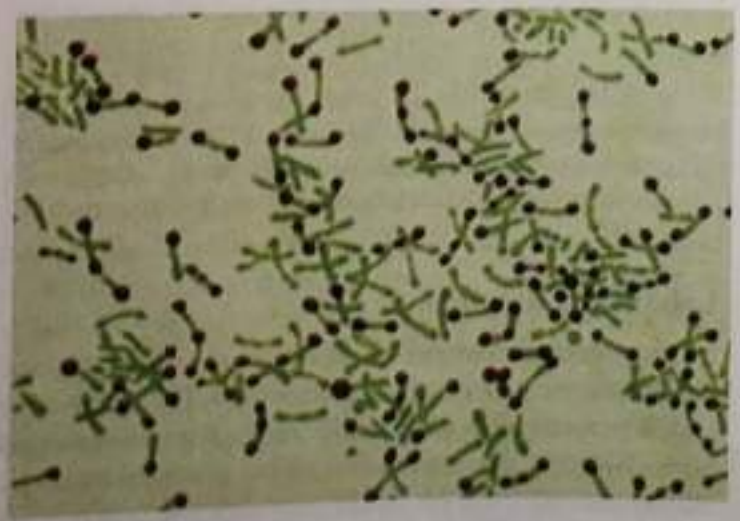


Fig. 25.1 Corynebacterium on Albert's stain

3. Isolation by culture

Enrichment with blood, serum or egg is necessary for good growth, as it is scanty on ordinary media. The optimum temperature for growth is 37°C (range 15–40°C) and the optimum pH is 7.2. *C.diphtheriae* is an aerobe and a facultative anaerobe.

The usual media employed for the cultivation of the diphtheria bacillus are:

Loeffler's serum slope: Growth is rapid on Loeffler's serum slope. The colonies can be visualised in 6–8 hours. Colonies appear at first as small, circular white opaque discs but enlarge on continued incubation and may acquire a distinct yellow tint.

Tellurite blood agar: Several modifications of tellurite blood agar have been utilised, such as **McLeod's** and **Hoyle's** media. Tellurite (0.04%) inhibits the growth of most other bacteria, acting as a selective agent. Diphtheria bacilli reduce tellurite to metallic tellurium which is incorporated in the colonies, giving them a grey or black colour. The growth of diphtheria bacilli may be delayed on the tellurite medium and colonies may take two days to appear. **Plates will have to be incubated for at least two days before being considered negative, as growth may sometimes be delayed.** The tellurite medium is particularly important in the isolation of diphtheria bacilli from convalescents, contacts and carriers, as in these cases they may be outnumbered by other bacteria.

Based on colonial morphology on the tellurite medium and other properties, McLeod classified diphtheria bacilli into three types: **gravis, intermedius** and **mitis.** Table 25.2 lists the characteristics of the three types.

Sheep blood agar: This is required to differentiate colonies from streptococcal or staphylococcal pharyngitis, which may simulate diphtheria. If the swab cannot be inoculated promptly, it should be kept moistened with sterile serum so that the bacilli remain viable. The serum slope may show growth in 4–8 hours but, if negative, will have to be incubated for 24 hours.

4. Biochemical reactions

Hiss's serum sugars: Diphtheria bacilli ferment glucose, galactose, maltose and dextrin with the production of acid but without gas; they do not ferment lactose, mannitol or sucrose. Some strains of virulent diphtheria bacilli have been found to ferment sucrose. It is necessary to use Hiss's serum sugars for fermenta-

Table 25.2 *Type differentiation of diphtheria bacilli*

	Gravis	Intermedius	Mitis
Morphology	Usually short rods, with uniform staining, few or no granules. Some degree of pleomorphism, with irregularly barred, snow-shoe and tear-drop forms	Long barred forms with clubbed ends; poor granulation, very pleomorphic	Long, curved, pleomorphic rods with prominent granules
Colony on tellurite blood agar	In 18 hours, colony is 1–2 mm in size, with greyish-black centre, paler, semitranslucent periphery and commencing crenation of edge. In 2–3 days, 3–5 mm in size, flat colony with raised dark centre and crenated edge with radial striation—'**daisy head**' colony	18-hour colony, small, 1 mm in size, misty. Does not enlarge in 48 hours, dull granular centre with smoother, more glistening periphery and a lighter ring near the edge—'**frog's egg**' colony	Size variable, shiny black. In 2–3 days, colonies become flat, with a central elevation—'**poached egg**' colony
Consistency of colonies	Like 'cold margarine', brittle, moves as a whole on the plate, not easily picked out or emulsifiable	Intermediate between gravis and mitis	Soft, buttery, easily emulsifiable
Hemolysis	Variable	Non-hemolytic	Usually hemolytic
Growth in broth	Surface pellicle, granular deposit, little or no turbidity	Turbidity in 24 hours, clearing in 48 hours, with fine granular sediment	Diffuse turbidity with soft pellicle later
Glycogen and starch fermentation	Positive	Negative	Negative

tion tests. Proteolytic activity is absent. They do not hydrolyse urea or form phosphatase.

3. Demonstration of toxicity (virulence testing)

Any isolate of the diphtheria bacillus should be tested for toxigenicity for the bacteriological diagnosis to be complete. Virulence testing may be by the in vivo or in vitro methods.

In vivo tests

- **Subcutaneous test:** The growth from an overnight culture on Loeffler's slope is emulsified in 2–4 ml of broth and 0.8 ml of the emulsion injected subcutaneously into two guinea pigs, one of which has been protected with 500 units of the diphtheria antitoxin 18–24 hours before. If the strain is virulent, the unprotected animal will die within four days. The method is not usually employed as it results in death of the animals.
- **Intracutaneous test:** The broth emulsion of the culture is inoculated intracutaneously into two guinea pigs (or rabbits) so that each receives 0.1 ml in two different sites. One animal acts as the control and should have received 500 units of antitoxin the previous day. The other is given 50 units of antitoxin intraperitoneally four hours after the skin test, in order to prevent death. Toxigenicity is indicated by inflammatory reaction at the site of injection, progressing to necrosis in 48–72 hours in the test animal and no change in the control animal. An advantage in the intracutaneous test is that the animals do not die. As many as ten strains can be tested at a time on a rabbit.

In vitro tests

- **Elek's gel precipitation test:** A rectangular strip of filter paper impregnated with diphtheria antitoxin (1000 units/ml) is placed on the surface of a 20% normal horse serum agar in a petri dish while the medium is still fluid. If horse serum is not available, sheep or rabbit serum may be used. When the agar has set, the surface is dried and narrow streaks of the strains are made at right angles to the filter paper strip. **Positive and negative controls should be tested.** The plate is incubated at 37°C for 24–48 hours. Toxins produced by the bacterial growth will diffuse in the agar and, where it meets the antitoxin at optimum concentration, will produce a line of precipitation (Fig. 25.2). The presence of such arrowhead lines of precipitates indicates that the strain is toxigenic. No precipitate will form in the case of non-toxigenic strains. This test is very convenient and economical but some brands of peptone and some samples of serum do not give satisfactory results.

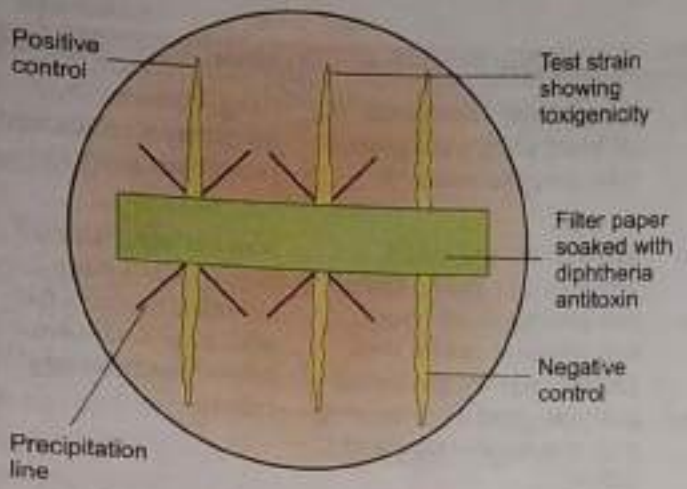


Fig. 25.2 Elek's gel precipitation test

Tissue culture test: The toxigenicity of diphtheria bacilli can be demonstrated by incorporating the strains in the agar overlay of cell culture monolayers. The toxin produced diffuses into the cells below and causes lysis of the cells.

PCR: Polymerase chain reaction for detection of Toxin gene *(tox)* has been developed to detect the presence of genes coding for the toxin, in clinical isolates.

ELISA: The test may be done to detect toxin from the patient's isolate using antitoxin and enzyme-substrate system.

Epidemiology

Faucial diphtheria was formerly an important pediatric disease all over the world but following the development of effective prophylactics and mass immunisation, the disease has been virtually eradicated from most advanced countries. In developing countries where childhood immunisation programmes have been implemented effectively, diphtheria has become rare but in others it continues to be a serious problem. In India, it is included in the universal immunisation programme for infants.

Gravis, intermedius and **mitis** were originally proposed to relate to the clinical severity of the disease produced by the three types—gravis, causing the most serious, and mitis the mildest variety, with intermedius being responsible for disease of intermediate severity. However, this association is not constant.

- The gravis and intermedius types are associated with high case fatality rates, while mitis infections are less lethal.
- Paralytic complications are most common in gravis, hemorrhagic complications in gravis and intermedius, and obstructive lesions in the air passage in mitis infections.
- In general, mitis is the predominant strain in endemic areas, while gravis and intermedius tend to be epidemic. The mitis type is abler than the more virulent types to establish a commensal relationship with the host.
- Wide variations have been noted in the frequency of the different types in different places at different times.

Evidence shows that the gravis and, to a lesser extent, the intermedius strains are able to spread more readily than mitis in populations naturally immune or artificially immunised. Table 25.2 lists the characteristics of the three types. The prolonged and extensive epidemic of diphtheria in parts of the erstwhile Soviet Union in the 1990s, involving several thousands, with a mortality rate of up to 20 per cent is a warning of what can befall countries that neglect immunisation and let living conditions deteriorate.

Age: In endemic areas, it is mainly a disease of childhood. It is rare in the first year of life due to the passive immunity obtained from the mother, reaches a peak between 2 and 5 years, falls slowly between 5 and 10 years, and rapidly between 10 and 15 years with only very low incidence afterwards because of active immunity acquired by repeated subclinical infections.

Asymptomatic carriers who outnumber cases by a hundredfold or more in endemic areas are the most important sources of infection. In the temperate regions, carriage is mainly in the nose and throat. Nasal carriers harbour the bacilli for longer periods than pharyngeal carriers.

In nature, diphtheria is virtually confined to human beings, though cows may on occasion be found to have diphtheritic infection of the udder. The infection in such cases is invariably transmitted by the milker. The infection may be spread through the milk of infected cows.

Prophylaxis

Protective immunity

Diptheria is a disease which is due to the toxin and not the invasion of the pathogen. Therefore, the protective immunity is dependent on the levels of antitoxin antibodies present in circulation. The objective of immunisation

is to increase protective levels of antitoxins in circulation. The susceptibility of an individual is tested by determining the antitoxic level using the following tests.

- **In vivo Schick**: This is a neutralisation-based skin test introduced in 1913. This test is no longer in use.
- **In vitro assays:** The circulating antitoxin level can also be determined by serological tests such as passive hemagglutination or by neutralisation in cell culture. **Antitoxin levels of more than 0.1 IU/per ml of blood is considered an index of protective antibody level.**

Vaccines

Diphtheria can be controlled by immunisation. Three methods of immunisation are available: active, passive and combined. Of these, only active immunisation can provide herd immunity and lead to eradication of the disease. Passive and combined immunisation can provide emergency protection to susceptible individuals exposed to risk.

Active immunisation: Diphtheria immunisation is done using a killed vaccine. Currently, two preparations are available for active immunisation:

- **Formol toxoid** (also known as fluid toxoid) is prepared by incubating the toxin with formalin.
- **Adsorbed toxoid** is purified toxoid adsorbed onto insoluble aluminium phosphate, less often the hydroxide. It is much more immunogenic than formol toxoid.

Dosage: Diphtheria toxoid is usually given in children as a trivalent preparation containing tetanus toxoid and pertussis vaccine, as the **DTP, DPT or triple vaccine**. For young children, diphtheria toxoid is given in a dose of 10–25 Lf (limit of flocculation) units to all recommended individuals.

Schedule: The schedule of primary immunisation of infants and children consists of three doses of DPT given at intervals of at least four weeks, preferably six weeks or more, followed by a fourth dose about a year afterwards. A further booster dose is given at school entry.

Adult immunisation: Smaller doses (1–2 Lf units) are used for older children and adults to minimise adverse reactions. In toxoid preparations, the lower dose of toxoid is indicated by the small letter 'd' and the full dose by capital 'D'. For example, the tetanus diphtheria vaccine for adults containing low-dose diphtheria toxoid is referred to as 'Td'.

Some **side effects** or adverse reactions which might occur after vaccination are injection site reactions (redness, warmth, swelling, tenderness, itching, pain, hives, and rash), fever, drowsiness, fretfulness, vomiting, anorexia, persistent crying (in infants), and rarely, convulsions.

Passive immunisation: This is an emergency measure to be used when susceptible persons are exposed to infection, as when a case of diphtheria is admitted to general pediatric wards. It consists of the subcutaneous administration of 500–1000 units of antitoxin (antidiphtheritic serum, ADS). As this is a horse serum, precaution against hypersensitivity should be observed.

Combined immunisation: This consists of administration of the first dose of adsorbed toxoid on one arm, while ADS is given on the other arm, to be continued for the full course of active immunisation. Ideally, all cases that receive ADS prophylactically should receive combined immunisation.

Chemoprophylaxis
This is sometimes given to the susceptible close contacts of a diphtheria patient, with erythromycin along with the booster dose of vaccine.

Treatment

Specific treatment of diphtheria consists of antitoxic and antibiotic therapy. **Antitoxin** should be given immediately when diphtheria is suspected, as the fatality rate increases with delay in starting antitoxic treatment. **The recommended dose is 20,000–100,000 units for serious cases, half the dose being given intravenously**. Antitoxin treatment is generally not indicated in cutaneous diphtheria as the causative strains are usually non-toxigenic.

C.diphtheriae is sensitive to **penicillin** and can be cleared from the throat within a few days by penicillin treatment. Diphtheria patients are given a course of penicillin though it only supplements and does not replace antitoxin therapy.

Erythromycin is more active than penicillin in the treatment of **carriers.**

OTHER PATHOGENIC CORYNEBACTERIA

These can be divided into lipophilic and non-lipophilic based on the improved growth on adding lipid to the medium.

Mycobacterium tuberculosis:

Gram +ve (gram staing)

Rod shape

Length 3 µm x 0.3 µm (diameter)

Pairs (or) clumps

Stained by Carbol fuchsin

AFB called as Ziehl Neelsen

Cultural Characteristics:

low generation

14-15 days incubation period

sometimes 8-weeks Colonies (2-8 weeks)

Temp: 35-40°C

Opt. temp: 37°C

Temp below 25°C ✗

Above 40°C ✗ (growth)

pH 6.4-7.0

It is an obligate aerobe

Growth enhance = 0.5%.

of glycerol is added

Selective medium = LJ

Lowenstein Jensen

Medium, It also grows in coagulated in hens egg, mineral salt solution.

Asparagin, Malachite green.

LJ Medium = Colonies are day, rough, creamy white, Sometimes yellowish colonies are seen.

Dubo's broth medium = Used for the growth Mycobacterium tuberculosis

Resistance

It can withstand 60°C, for 15-20 mins. It is sensitive when exposed to sunlight for 2hrs

Room temp = 6-8 months. If it is stored at -20°C it can survive for 2 yrs.

The Organisms are resistance to

5% phenol

15% of sulphuric acid

3% of Nitric acid

5% oxalic acid

4% Sodium hydroxide

Formaldehyde

Glutaraldehyde.

80% of ethanol for 10 mins

Bio chemical test:

Niacin Test - The Organism for Niacin when it is grown in egg medium ; 10% cynogin cynojen 4% alanine, 96% ethanol

Colonies are yellow colour.

Aryl sulphatase Test : 0.001 mo

- lority of tripotassium phenolphthalein disulphate, 2N NaOH

Pink colour media Change = +Ve

Red bind VIR / AV - bind x

Neutral red test →

Catalase test → Effervescence → +Ve presence

Peroxidase

Amidase test, Nitrate reduction test → red colour

Differentiate M.B

Antigenic property:

Lipid, Protein - Tuberculin

↓

It contains polypeptide & phosphatide Ag.

Host Range:

It causes natural infection in humans, dog & other animal

It is infectious to Guinea pigs Hamsters;

Pathogenesis

Pulmonary TB

↓

Inhalation

↓

Sputum formation

↓

Cough, Sneezing, speaking, releases mycobacterium they colonise

In the URT (upper respiratory tract)

↓

10,000 bacilli in 1ml of sputum

↓

In Lungs ~~are~~ M.B are ingested by alveolar macrophages

↓

No toxins are released

↓

Induces CD4 (cell of differentiation)

T - cells; cytokines, IFN, Macrophages

DHS (delayed hypersensitivity rxn) typ 4

↓

M.B Lesion → Tubercle

Primary TB

Post TB

↓

Primary TB :

① Causes Tuberculosis pneumonia

② 3-8 weeks incubation,
Forms hypersensitive nodules

③ Remain Latent

More than 10,000

Post / Secondary TB

① Due to reactivation of M.B

② It affects upper lobes in lungs

③ Causes Necrosis & Tissue destruction

④ They Causes lesion in lungs & Other Organs.

Pulmonary TB - Lab diagnosis :

Microscopy - Sputum Sample,
Acid F. Staining

~~First~~ carbo

(1) Sputum sample is collected & smear is prepared. & sterilized.

(2) Then carbol fuchsin is added & it is steamed for 8 mins.

(3) Then the slide is rinsed with running tap H_2O.

(4) then ethyl alcohol is added & allowed for 1 mins

(5) the slides are again washed & dried.

(6) finally methylene blue is added & allowed for 2-3 mins

(7) Washed in tap H_2O.

(1) Microscopic examination

AFB Test : Normal Mi

1 + = when there are 3-9 bacilli in the entire smear.

2 + = 10 (or) more "

3+ = 100 (or) more " (infective form)

Fluorescent microscope dye

1. Auramine phenol
   Auramine Rhodamine

Con. Methods: When the sputum is treated with anti Sodium Carbonate or hypochloride

Petroff's method: Sputum incubated with NaOH at 37°C for 20 mins & then centrifuged at 3000 RPM for 20 mins & then sediment is neutralized with Na & Nacl

Culture;

IUAT – LJ medium incubation 8 – 14 days

Animal Inoculation:

Sample is intramuscularly

injected into the thighs of Guinea pigs for 12 week (incubation) (15)

forms → Ulcer → Pus with lesion → (Enlargement of Lymph & Spleen) +ve for AFB

PCR = Amplification (known start)

PCR = Polymerase Chain Reaction

↓

Inserting / Joining

Tuberculin Test:

↓

Toxoid

This test is otherwise called as Mantoux

0.01 ml of sample injected intra dermally. It shows wheel flare in 48-72 hrs (+ve for M.tub) for humans

. Swelling = 10mm (1cm)

Treatment:

~~Rifampicin~~

Rifampicin (R)

Pyrazinamide (Z)

Isoniazid (H)

Streptomycin (HRZE)

Ethambutol (E)

Treatment (Antibiotics)

↓

Mycobacterium leprae :

(1)

Acid fast staining

Gram +ve rod

Morphology:

Rod shape with polar bodies

Gram +Ve

Intracellular elements stained - Carbol Fuchsin

Arranged as cigar bundles

~~Grown in 28 medium~~

Living cells needed - Foot pads of mice at 20°C

Causes granuloma in 1-6 months & produce lesions with lepromatous leprosy (Lymphatic nodes damage)

Leprosy:

Chronic granulomatous disease involving in the skin, peripheral nerves, nasal mucosa affecting tissues or organs

Types:

Lepromatous
Tuberculoid
Erythrophy
Indeterminate

(1)

Bacilli in large numbers & called as multibacillary disease with superficial nodular lesion with granulation tissues. Mononuclear are conveted as lepra cells. Nodules are ulcerated, infected & causes distoration, they are invade mucosa in nose, mouth, URT (upper respiratory Trad). They are more infective & shows -ve for lepromin test auto Ab's are commonly formed

(2)

Lesions are with macular anesthetic patches in hands & feet & ↑sed lesions are seen & this

stage is called pauci bacillary disease. +ve for lepromin test.

(4)

(3) Dimorphus :

-Ve for lepromin test.

(4) Indeter

It causes unstable tissue lesion. They are non-characteristic & healing spontaneous

Lepromin Test : special test for leprosy

Causes : delayed hypersensitivity reactions

lepromin

↓

Ag's are boiled & emulsified (min)

Ag → PLn (protein)

↓

Injected intradermally

↓

Gives a rxn in body

1st rxn contains erythema induration with 24-48 hrs

↓

2nd rxn late rxn starts after 1-2 weeks

↓

Rxn will be peak in 4 week

↓

Skin nodules ulcerated with lymphocytes, epithelial cells, giant cells.

↓

CMI is induced

↓

Hypersensitivity rxn are visualized.

↓

Lab diagnosis:

Lepromin test → feet / hand

lesions is collected → Lepromin

test → Microscopy → Acid F stain

→ Pinkrod, Pus cells blue colour.

→ Injected in mouse feet (24-48

hrs) swelling means +ve

Rifampicin - 600 mg / months

Dapsone - 100 mg for 6 months.

Clostridium botulinum

⇒ It causes botulism - A paralytic disease from food poisoning.

⇒ Named derived from sausage (. (latin word)

⇒ Van Ermengem Isolated in 1896

⇒ Saprophyte Organism.

⇒ Occurs in virgin soil, Vegetable silage.

Morphology: G+ve bacillus, Size = 5 x 1 μm, Non capsulated, Motile by peritrichous flagella. Spore producing bacteria - Oval shape spores bulging spores

Cultural Characteristics: Strict anaerobes, Temp: 35°C, Some strain grow at 1-5°C. Good growth in Ordinary media Colonies - Large, irregular, semi transparent. Spores produce when bacteria grown in alkaline glucose, gelatin media at the temp: 20-25°C

Resistance: Spores are heat & radiation resistant - Surviving

for several hrs at 100°C; upto 10mins at 120°C.

Classification:

Based on toxins production. Classified into eight types

[A, B, C1, C2, D, E, F & G].

Toxins are neutralized by homologous antiserum. All toxins shows neuro-toxic activity

Toxins: Powerful toxin is exotoxins. Toxins are produced intracellularly & appears in the medium only on cell death. Initially as non toxic. Trypsin & proteolytic enzymes activates to produce active toxins. 1-2 µg is lethal dosage for human. 0.33 µg lethal dose for mice. Toxins are inactivated at 80°C for 40 mins & 100°C for 10 mins. Food with toxins

- Pressure cooker for 20mins considered as safe. Small intestine readily observe this toxins. It acts by blocking the production of acetylcholine at the synapses neuromuscular junctions. Onsets (symptoms) diplopia - Double vision. dysphagia - difficult in swallowing dysarthria - speaking slowly. Sometimes death by respiratory paralysis.

Clinical use of toxins:

Used for the therapy of neuromuscular disease. Available as botox.

Pathogenicity

C. botulinum is non invasive, non infectious, Pathogenicity is due

to the action of toxins

Botulism;

Three types:

① Food borne botulisms

Caused by ingestion of toxins.

Type A, B & E Cause human disease. Type C & D for cattle & so fo.

Type G rarely causes disease in human & deads to sudden death.

Symptoms begins 12-36 hrs after ingestion of food.

→ No vomitting & diarrhoea.

→ Coma may be supreme.

→ Death due to respiratory failure

② Wound botulism

⇒ Very rare, Type A - responsible for wound botulism.

③ Infant botulism: Causes disease in infants below 6 months. Spores are ingested in food & get established in gut & produce toxins which leads to severe complications. (i,e) constipation, Poor feeding, lethargy (absence of energy), weakness, weak cry, loss of head control.

∠Δ

Sample - Sputum
stool or faeces

Microscopy: Gram staining
Culture: Anaerobic condition

Animal inoculation: Guinea pig

Typing done by passive protection with type specific antitoxins

Control is by proper canning & preservation.

when outbreak occurs, antitoxin should be given intramuscularly.

Active immunization:

2 injections [alluminium sulphate) absorbed toxin given at an interval of 10 weeks. followed by a booster dosage a yr later]. Polyvalent antisorum of type A, B & E are administrated

Clostridium perfringens:- (H_2S production)

It is cultivated by Achalme

(Gas gangrene also known as) Discovered = Welch & Nuttall

It was 1st isolated from blood & organs of cadaver → (dead bodies)

Causes gas gangrene, food poisoning & neurotoxicity.

Habitant of large inst. of human & animals

Found in feces, spores commonly seen in soil, dust & air

G+ve bacillus - straight & parallel with rounded heads

Size 4 - 6 μm x 1 μm

Occurs in chain or small bundles

Capsulated

Non - motile.

sporing.

Cultural cha

dnaerobes, microaerophillic, pH 5.5 - 8

Temp = 20 - 50°C, Opt temp = 37°C

Temp 45°C → 10 mins generations starts

Selective media - Robertson's cooked meat broth at 45°C for 4 - 6 hrs (incubation) the meat is turned pink but not digested.

Litmus milk :-

fermentation of lactose leads to formation of acid Litmus blue - red indicated by change in colour. (stormy fermentation) → Acid coagulates casein & clotted milk is disturbed due to vigorous gas production.

Blood agar = Hemolysis seen in rabbit, sheep or human blood

Target hemolysis = Narrow zone of complete hemolysis due to theta toxin & a much wider zone of incomplete hemolysis due to α - toxins.

Bio chemical test

Indole = -Ve

MR = +Ve

VP = -Ve

H_2S = Production

Reduce Nitrates — Nitrites

Ferment glucose, maltose, lactose sucrose. Produce Acid & gas

Resistance: Food poisoning strain type A & type C resistant for boiling upto 1-3 hrs. Spores are destroyed in autoclave (121°C - 15 mins)

Spores are resistant to antiseptic & disinfectants

Classification

5 type = Type A, B, C, D, E based on toxin production

Toxins : Produce 12 toxins & some enzymes.

Major toxins = α, β, Episilon, iota

Alpha toxins : Responsible for toxemia of gasgangrian, lethal dermonecrotic & hemolytic.

Phospholipidase : lecithin into phosphoral choline & diglyceride

↓

Reactions seen in opalescence in serum or egg yolk media

↓

Neutralized by antitoxins

Nagler's reaction :

C. perfringens grown on a medium containing 6% agar, 5% peptic digest of sheep blood & 20% human serum.

Antitoxin spread on one half of the plate & other half of the plate without antitoxin after incubation plate with antitoxin - No colonies (No zones) whereas the plate with noantitoxin have colonies.

Symptoms :

Gas gangrene caused by C. perfringens type A -> leads to wound contamination or anaerobic cellulitis

Food poisoning -> Type A & perfringens alpha & theta toxin responsible for food poisoning. Food poison is caused by C. perfringens type A present in the meat

Contaminated meat is cooked

↓

Spores in the intestine survive

↓

During storage or warming they germinate & multiply in anaerobic environment present in the cooked meat

↓

Large no. of clostridia

↓ Pass

Gastric acid - Due to high protein in the meal.

↓

Reach to the intestine

↓

Produce enterotoxine

↓

After 24 hrs abdominal pain, diarrhoea & vomitting

Gangrenous appendicitis:

Type A - responsible,

Necrotising enteritis = Type-C strain responsible

Heat resistance spore germinate in the intestine produce β-toxin which leads to mucosal necrosis.

Fig bel : Eating of Pork

Meningitis : Thoracic infection, urogenetal infection;

Lab diagnosis

Specimen : Muscules from wound, necrotic tissues.

Gram staining : Gram positive bacillus

Nagler reaction :

Selective media : Robertson cooked meat broth.

Different

inoculated & heated at 100°C.

↓ 5mins, 10mins, 15 & 20min

~~Anaerob.~~ Incubated

↓

Subcultured on blood agar

↓

If cobries are present the test is +ve.

Treatment

→ Surgery

→ Metronidazole.

→ Gentamycin

→ Amoxilin.

Clostridium tetani :-

[Muscle cramp] [Wound infection]

Causative Organisms of tetanus was

discovered by Hippocrates

Further explained by

Hippocrates & Arataeus / Carle & Rattone Nicolaier.

C. tetani present in soil & intestine of humans & animals.

Morphology :-

G+ve bacilli, 4-8 μm × 0.5 μm in size. Occurs in chains or single, drumstick appearance. Non capsulated, Motile Organisms

C.C :- Obligate anaerobe, Op: temp : 37°C, pH = 7.4, Ordinary media = Growth improve by blood & serum. [Fine translucent film of growth is produced that is practically invisible & it can be identified with filamentous advancing edge].

This property enable separation of C. tetani from mixed cultures.

Deep Agar Shake culture: Colonies are spherical fluffy balls with the diameter of 1-3 mm.

Gelatin stab culture: Inverted fir tree appearance with slope liquefaction.

Robertson cooked meat broth: Turbidity growth with gas formation.

Meat is not digested but turned into black colour.

Blood agar: α-hemolysis, Later def. develops into β-hemolysis due to tetanolysin.

Bio. Ch Indole = +ve, MR VP = -ve

H_2S = Not formed, Nitrates not produced,

Sugar utilization is absent.

On Macconkey agar = Greenish fluorescent growth.

Resistance: killed by boiling for 10-15 mins. Spores destroyed by 121°C for 20 mins. Spores survive in soil for yrs.

Resistance to antiseptics: 1% Iodine solution kills the spores within few hrs.

Classification:

10 Types: Type I to Type X

Based on flagella. Type VI = are non flagellated rest are flagellated strains. All types produce same toxins.

Toxins: Two distinct Toxins;

Hemolysins or Tetanolysin

Neurotoxin or Tetanospasmin

Tetanolysin: Heat labile, O2 labile, Not relavent in tetanus

Tetanospasmin: Responsible for tetanus. O2 stable, Heat labile, Inactivated at 65°C for 5 mins. Neutralized by antitoxin Amount of toxins produced depends on strain of bacillus & type of culture medium.

Pathogenicity

C. tetani spores germinate & produce toxins during favorable conditions.

→ Toxins produced observed by motor nerve endings

↓

Transport to CNS intraxonally

↓

Toxins fixed by gangliosides of grey matter of nervous tissues

↓

Toxins blocks synaptic inhibition in spinal cord

↓

Causes → Uncontrolled spread of impulses anywhere in CNS

↓

Results → Muscle rigidity & spasms

Tonicity of Tetano spasmin is influenced by root of administration when given orally destroyed by digestive enzymes. Subcutaneous Intramuscular &

Intravenous injections are effective

Symptoms:

Muscular spasms
Wound infection.

Lab diagnosis

Specimen = Wound tissue & Wound swab.

Microscopy = Gram stain (Drumstick bacilli is observed)

Culture = (1) Blood agar, swabs inoculated on one half of the blood agar & incubated anaerobically for 1-2 days

↓

Swarming growth detected opposite half of the plate.

(2) 3 Tubes of cooked meat broth

→ 1st tube at 80°C for 15 mins
→ 2nd tube 80°C for 5 mins
→ 3rd tube left untreated
→ Cooked meat tubes incubated at 37°C & subcultured on one half of

the blood agar upto 4 days. Look for hemolysis

(3) Animal model: 2-4 days old cooked meat culture (0.2 ml - inoculated in tail of mice)

→ 2nd mice with antitoxins

→ Symptoms: stiffness in tail, trunk & forelimbs. Animal dies within 2 days.

Treatment

Surgical removal of wounds.

Penicillin, Erythromycin 500mg for 5 days, Bacitracin, antitetanus serum for → intramuscular infection, Tetanus toxoid.

Bacillus anthracis :

Rod shaped, G +ve ; Motile Organism, Heat resistance spores are formed. (Bacillus)

Causes – Anthrax.

Morphology :

Rod shape, l = 3 - 10μm x 1 - 1.6μmb

Capsule producing Organism : Bamboo stick appearance. By adding 2% NaCl. we can see the spore formation. If we add CaCl2 it inhibit spore formation Spores are formed at the centre & it is oval in shape. It is non-acid fast (purple colour) bacilli. Stain - Sudan Black B stain. Sometimes polychrome methylene blue. (Black colour)

C. C

Aerobic, facultative anaerobes, Grows on the normal Ordinary media. (Irregular) & shows Medusa head appearance.

Contains virulence capsules with rough colonies. In the gelatin stab - Fir Tree appearance. Blood agar - Non hemolytic. Grows in PLET (Polymyxin, lysozyme, EDTA) → selective medium

B. Ch : +Ve for glu, Mal. Suc; Converts Nitrate to Nitrite. Catalase +Ve;

Pathogenicity

Cattles & sheep Causes Arthritis

↓

the infection is transfered from birds

Subcutaneous inoculation into guinea pigs It dies within 28-48 hrs by causing local haemorrhagic edema, enlarged dark reddish spleen.

2 factors for Causing Infection

Capsular Polypeptide

Toxin - Antitoxin.

Capsular polypeptide : It inhibits phagocytosis, Loss of plasmid in Organism. Leads to loss of virulence

Toxin- Antitoxin

Three fractions factors.

i) EF - Edema (blocking) factor
ii) Protective Ag Factor - PF
iii) Lethal (death) factor - LF

} Endotoxin

Protective Ag Factor : Goes & binds to the target cell & provides attachment site for EF & LF to entry of the cell.

EF Factor : It stimulates the production of adenine cyclase - Activated only inside the target cell leading to the accumulation of cyclin AMP

Lethal factor : Leads to death of cell