GRAM POSITIVE RODS
CORYNEBACTERIUM DIPHTHERIAE

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Learning objectives

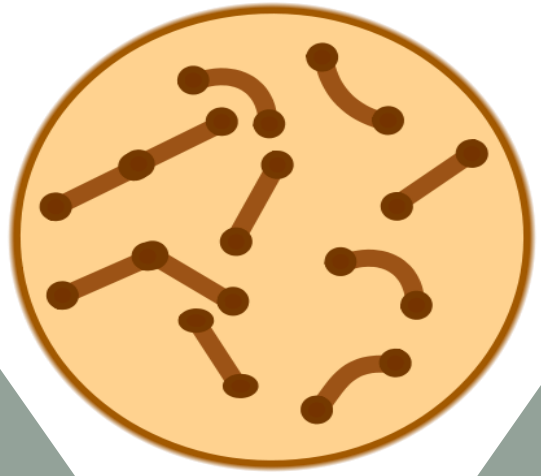
- To classify the non-spore forming gram positive rods

- To identify the special features of Corynebacterium and Listeria
- To explain the transmission, pathogenesis and clinical findings of diseases caused by Corynebacterium and Listeria
- To evaluate the lab diagnosis of Corynebacterium diphtheriae and Listeria monocytogenes

Non-Spore Forming, Non-filamentous Gram-Positive Rods

Organism	Type of Pathogenesis	Typical Disease	Predisposing Factor	Mode of Prevention
Cor. diphtheriae	Toxigenic	Diphtheria	Failure to immunize	Toxoid vaccine
L. monocytogenes	Pyogenic	Meningitis; sepsis	Neonate; immunosuppression	No vaccine; pasteurize milk products

Corynebacterium diphtheriae

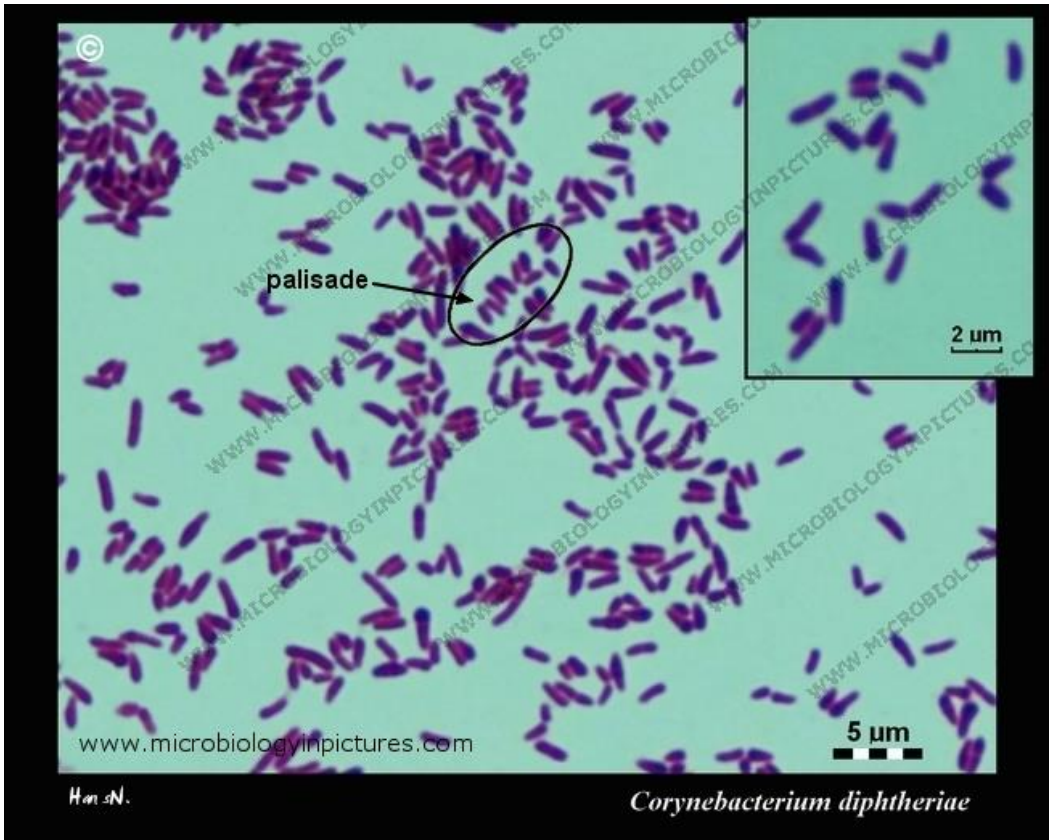


Corynebacterium diphtheriae causes **Diphtheria.**

Corynebacterium species (diphtheroids) cause **opportunistic infections.**

Corynebacterium diphtheriae

- **Gram-positive rods:** appear **club-shaped/chinese letter appearance/ Beaded** appearance.



Metachromatic or Volutin Granules

- Beads of Corynebacterium have granules

  intracytoplasmic (inside the cytoplasm of a cell) storage form of complexed inorganic polyphosphate.

- Their production used as identifying criteria when attempting to isolate *Corynebacterium diphtheriae* on Löffler's medium.

- Polyphosphate granules called metachromatic granules due to their display of metachromatic effect (they appear red when stained with methylene blue).

Transmission & Clinical presentation

- Humans only natural host.
- **Transmitted by:**
- **Airborne droplets. Respiratory diphtheria**

  or
- **Infect skin at site of a pre-existing skin lesion. Cutaneous diphtheria**

- Occurs primarily in tropics but can occur worldwide in indigent persons with poor skin hygiene.

Pathogenesis
Disease is exotoxin mediated

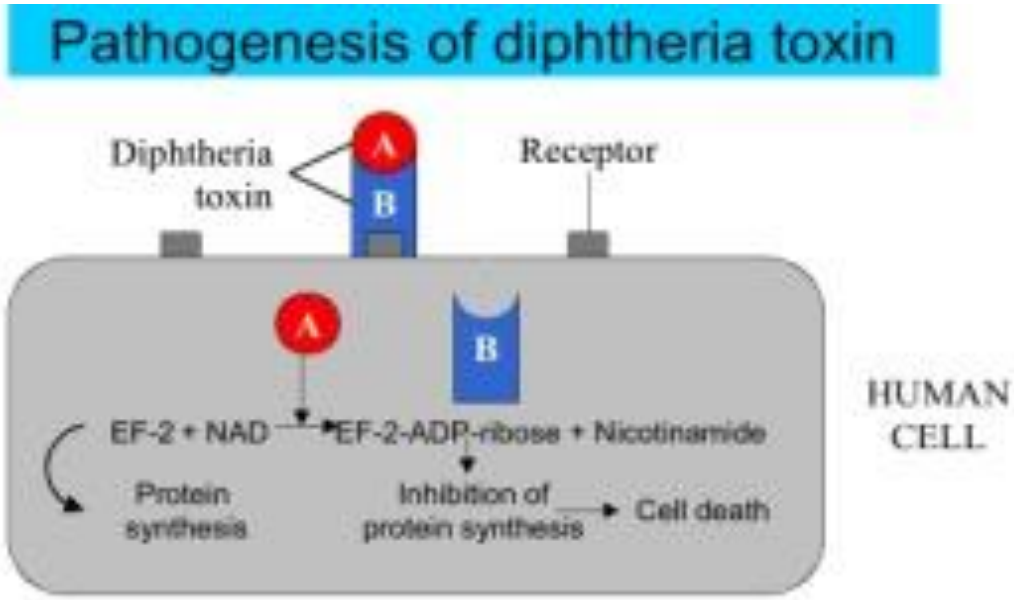
- Diphtheria toxin inhibits protein synthesis by **ADP-ribosylation of elongation factor 2.** (EF-2).

- Toxin affects all eukaryotic cells.

- Toxin has two functional domains.
- First domain (B subunit) mediates binding of toxin to glycoprotein receptors on cell membrane.

- Second domain (A subunit) possesses enzymatic activity that cleaves nicotinamide from nicotinamide adenine dinucleotide (NAD) & transfers remaining ADP-ribose to EF-2, thereby inactivating it.

Diphtheria Toxin:



ADP ribosyulation of EF-2 (elongation factor-2)

- Subunit B: Binding subunit - help attachment to cell receptors
- Subunit A: Active subunit- cleaves nicotinamide from NAD and transfers the remaining ADP-ribose to EF-2 (ADP-robosylation)
- Inactivates EF-2 and shuts of protein synthesis - cell death

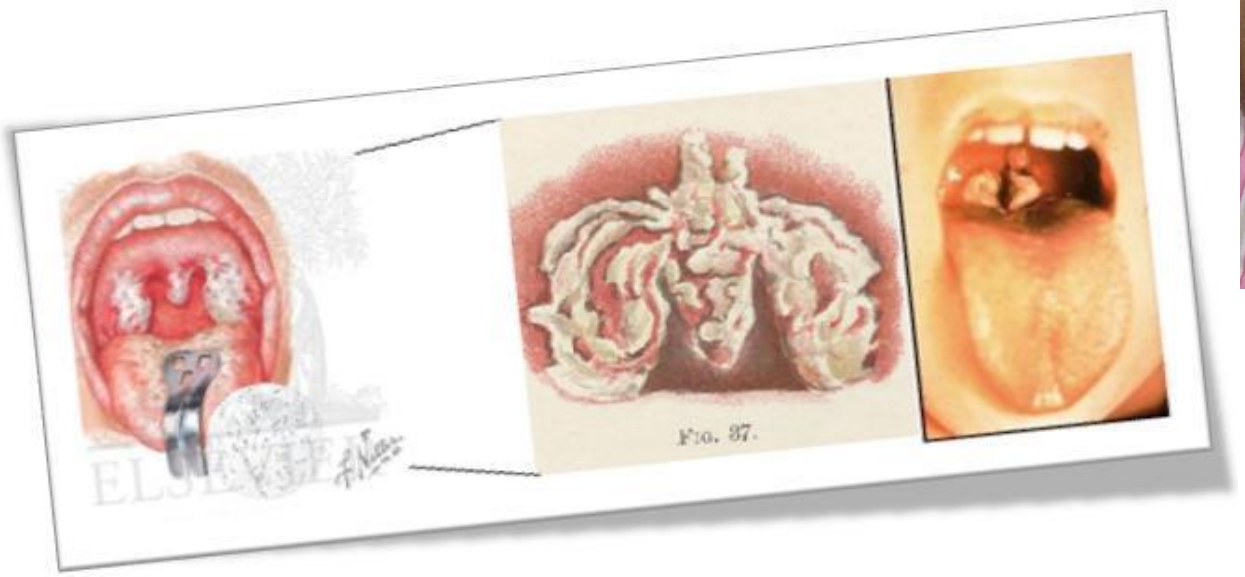
Pathology

- Local inflammation in throat, with a fibrinous exudate forming tough, adherent, gray **pseudomembranes.**

- **Schick's test: To assess i**mmune status of a person.
- Procedure: Intradermal injection of 0.1 ml of purified standardized toxin.
- Interpretation: The toxin will cause Inflammation at site 4 to 7 days later, if patient has no antitoxin.
- If no inflammation, antitoxin is present and the patient is immune.

Clinical findings

- Thick, gray, adherent **pseudomembrane** over the tonsils & throat.
- Shortness of Breath.
- Respiratory distress.
- **Nonspecific symptoms** :
- fever, sore throat & cervical adenopathy.



- **Respiratory Diphtheria:** Extension of membrane into larynx & trachea, causing airway obstruction.
- **Cutaneous diphtheria:**

  causes ulcerating

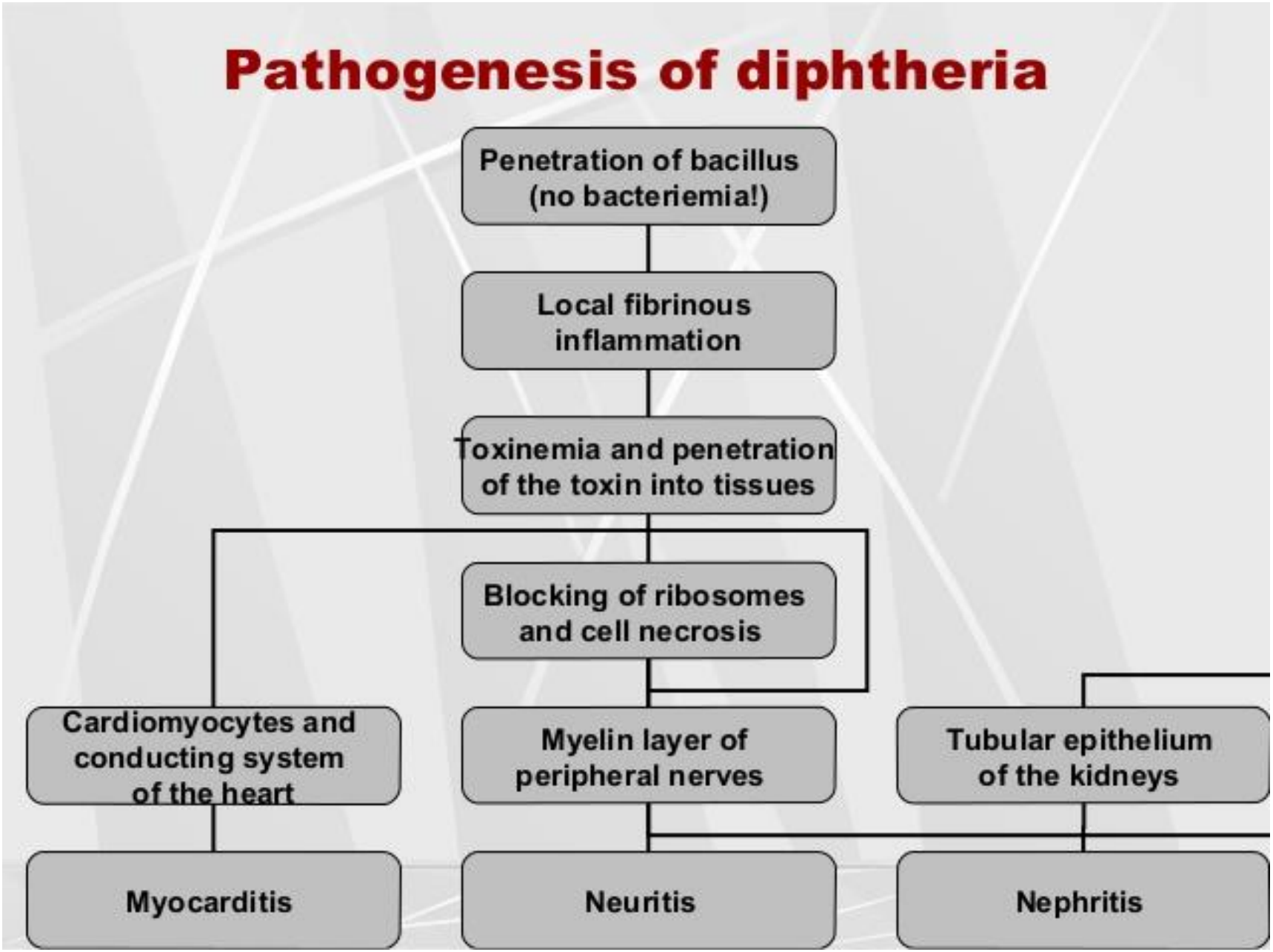
  skin lesions covered by

  a gray membrane.

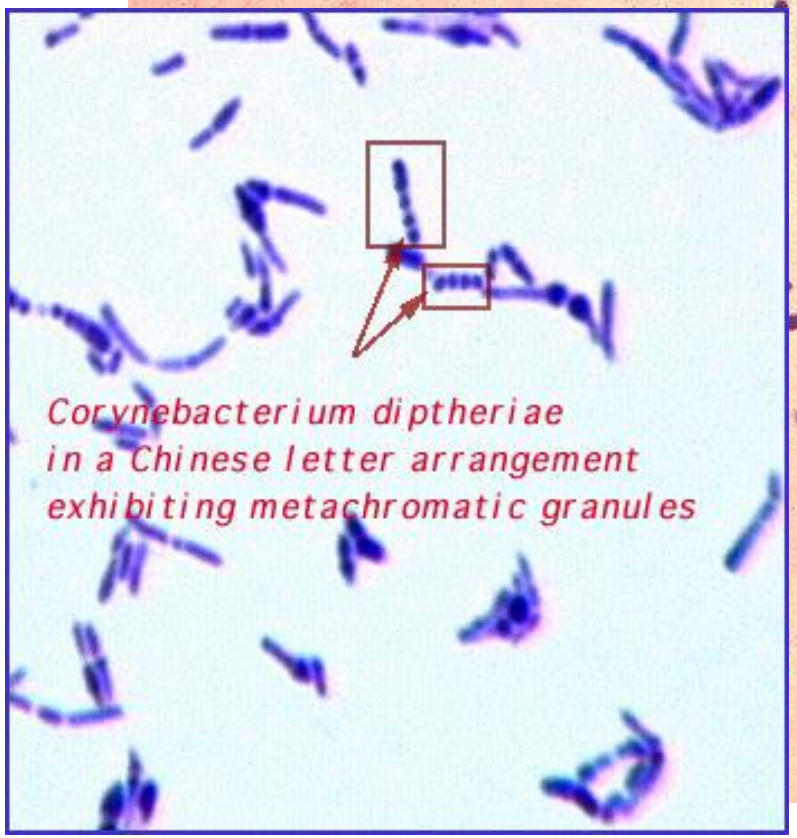
Complications:

- Myocarditis, arrhythmias & circulatory collapse.
- Nerve weakness or paralysis (cranial nerves).
- Paralysis of muscles of soft palate & pharynx causing regurgitation of fluids through nose.
- Peripheral neuritis affecting the muscles of extremities.

Pathogenesis of diphtheria

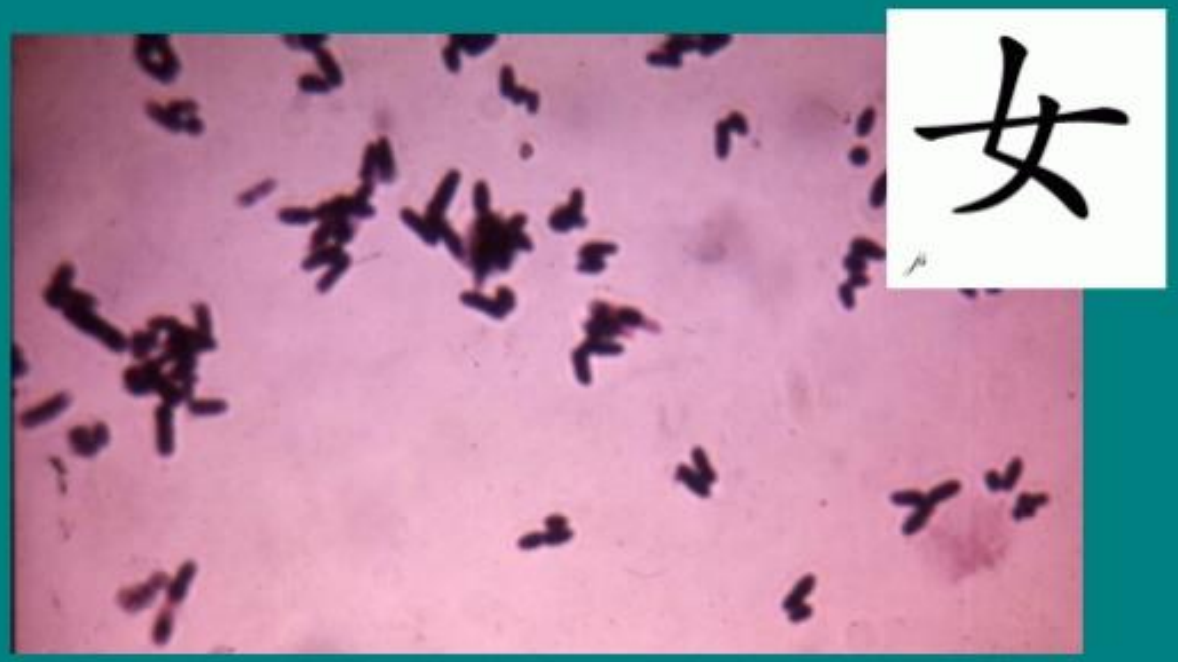


Gram positive rods having chinese letter apppearance or club shaped. They have metachromatic granules at one end. 0.5-1 um in diameter.



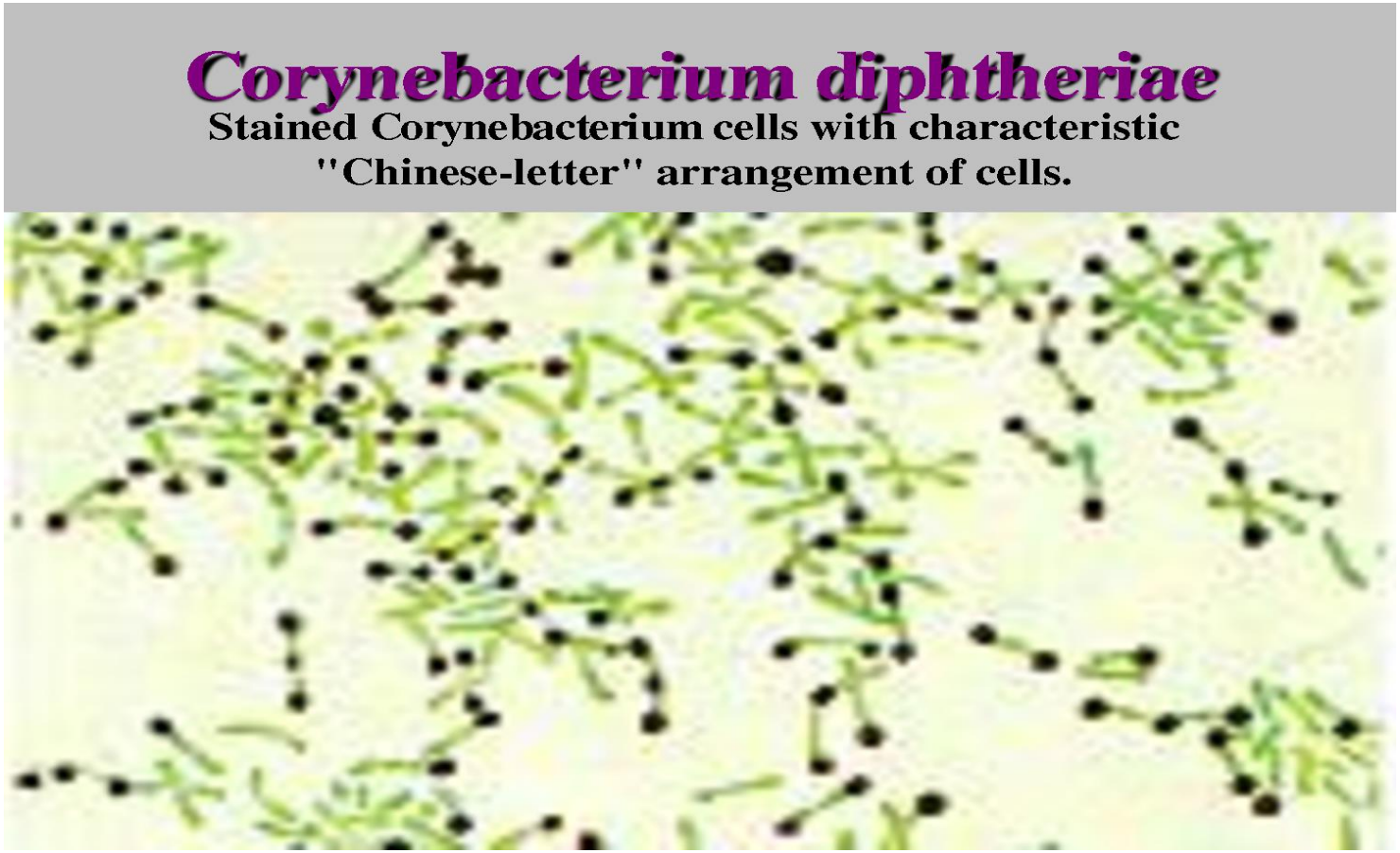
Gram stain

Diphtheroids (Corynebacteria)gram positive pleomorphic rods in Chinese letter forms 1.4 X 3 µm



Albert Staining & Methylene blue Staining

Granules stain metachromatically: Bacteria stained blue or green, Granules stained red.



Culture & Sensitivity

- **Specimen: Throat swab:** Can be cultured on, tellurite blood agar ,Loffler's serum, blood agar.
- **Tellurite/Tinsdale blood agar:** Gray black, brown to black colonies.
- **Blood agar**: Small, granular, gray, with irregular edges having small zones of hemolysis,

Biochemical tests

- **Motility tests:** Non-motile organisms.

- **Serological tests:** For detection of toxins:

1. **PCR assay**
2. **ELISA**
3. **Immunochromatographic strips**

Treatment

- **Antitoxin: S**hould be given immediately on basis of clinical impression, due to delay in laboratory diagnostic procedures.
- Toxin binds rapidly & irreversibly to cells & once bound, cannot be neutralized by antitoxin.
- Antitoxin neutralizes unbound toxin in blood.

- **Antibiotics:** Penicillin G or erythromycin.

- Antibiotics inhibit growth of organism, reduce toxin production & decrease incidence of chronic carriers.

Prevention

- Children immunized with **diphtheria toxoid** (combination of diphtheria toxoid, tetanus toxoid & pertussis vaccine (**DTaP**).
- Three doses given at 2, 4, and 6 months of age, with boosters at 1 & 6 years of age.
- Booster every 10 years

Listeria monocytogenes

Listeria monocytogenes
Epidemiology & Transmission

- Distributed worldwide in animals, plants & soil.

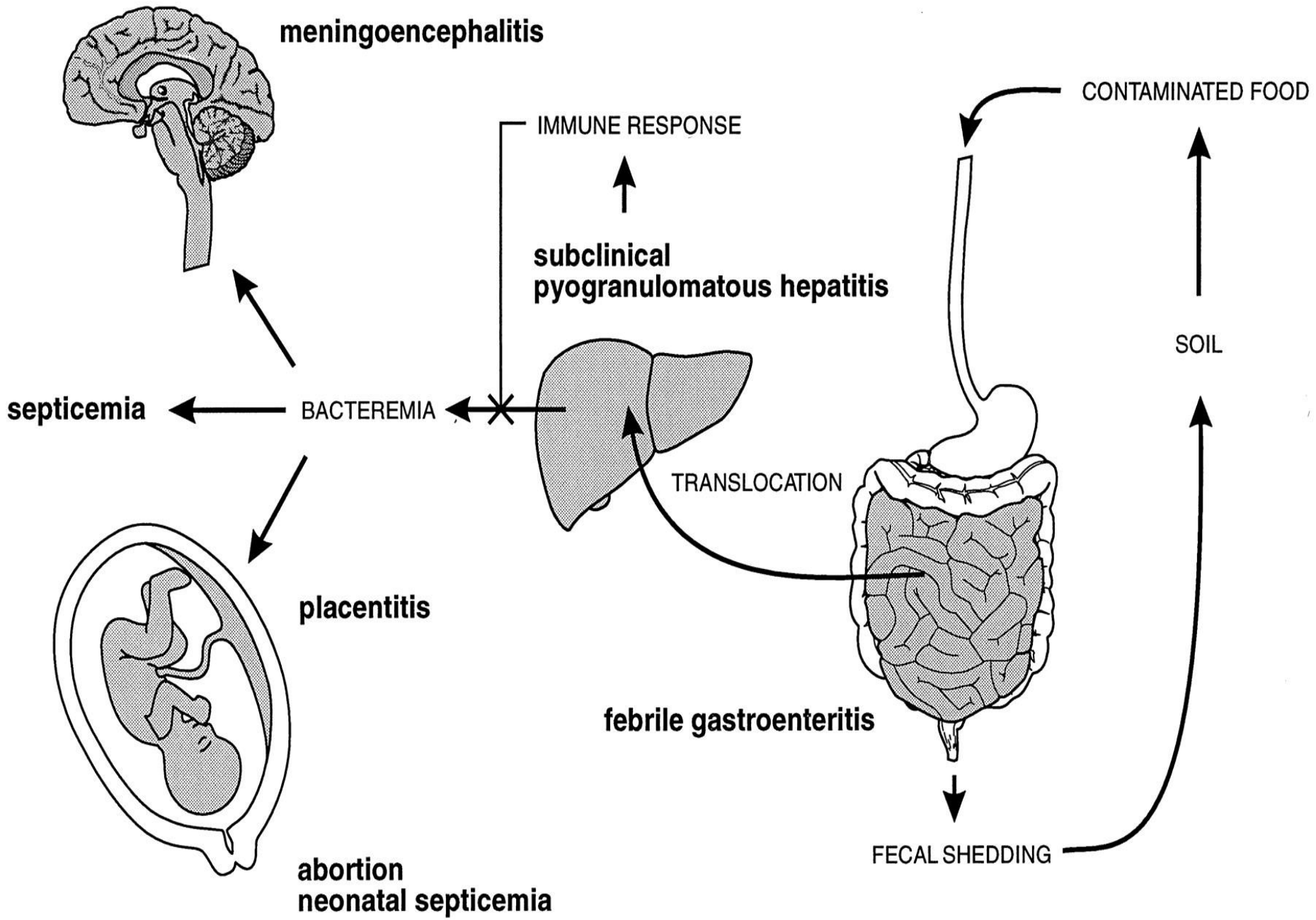
- Route Of Transmission: Transmitted to humans primarily by ingestion of unpasteurized milk products, undercooked meat, raw vegetables & contact with domestic farm animals.

- In United States, listeriosis primarily a foodborne disease associated with eating unpasteurized cheese.

Listeria infections occur in two clinical settings:

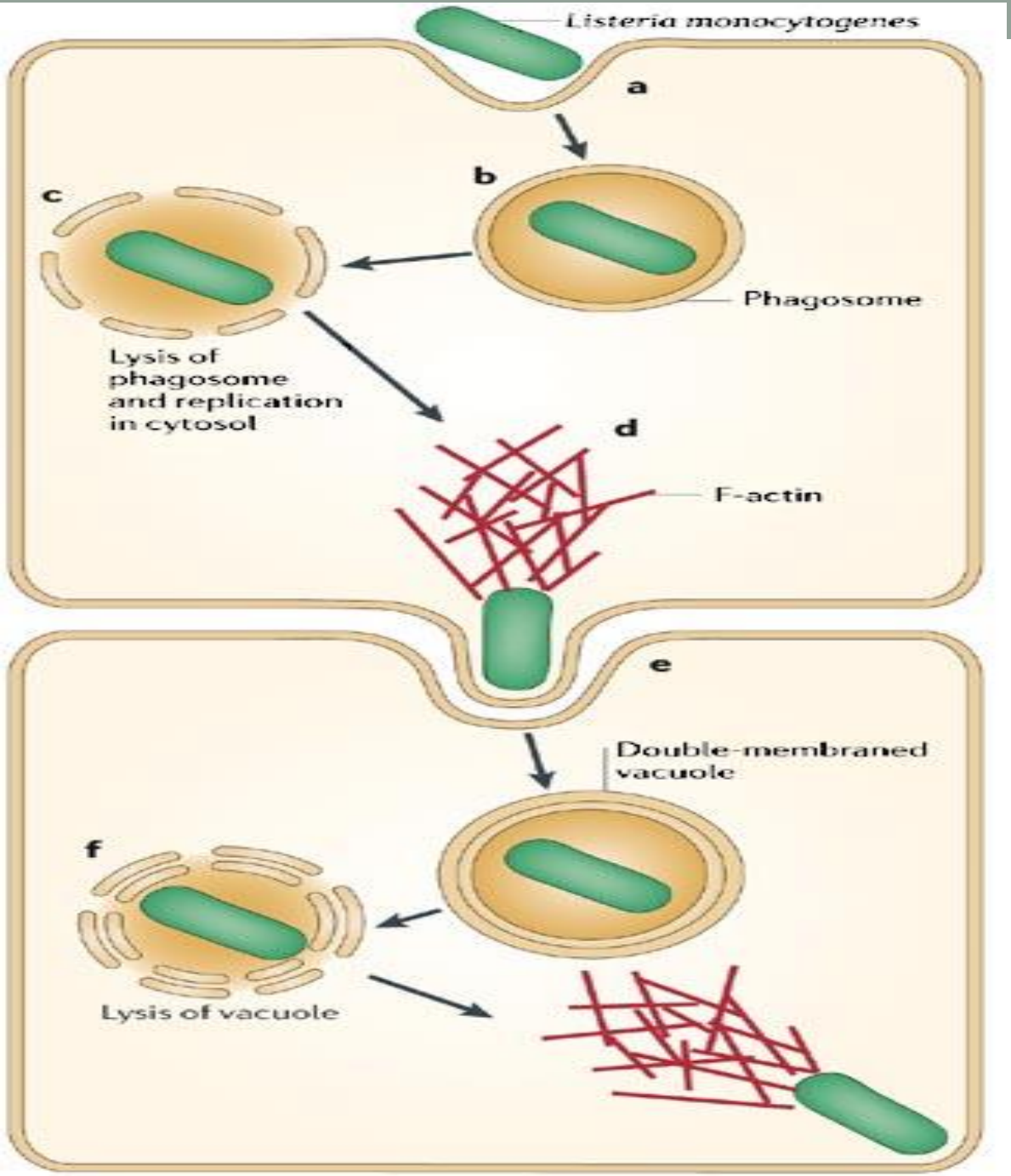
(1) In fetus or in newborn (transmission **across placenta** or **during delivery)**.

(2) In pregnant women & immunosuppressed adults. (especially renal transplant patients).


meningoencephalitis
IMMUNE RESPONSE
CONTAMINATED FOOD
subclinical
pyogranulomatous hepatitis
SOIL
septicemia
BACTEREMIA
TRANSLOCATION
placentitis
febrile gastroenteritis
FECAL SHEDDING
abortion
neonatal septicemia

Pathogenesis:

- Pathogenesis depends on organism's ability to invade & survive within cells.
- Invasion mediated by internalin made by *Listeria* and E-cadherin on surface of human cells.
- Ability of *Listeria* to pass placenta, enter meninges & invade GIT depends on interaction of internalin & E-cadherin on those tissues.
- On entering the cell, organism produces **listeriolysin,** which allows it to escape from phagosome into cytoplasm, thereby escaping destruction in phagosome.
- Because *Listeria* preferentially grows intracellularly, cell-mediated immunity is a more important host defense than humoral immunity.
- Suppression of **cell-mediated immunity** predisposes to *Listeria* infections.
- *L. monocytogenes* can move from cell to cell by means of **actin rockets—**filaments of actin polymerize and propel the bacteria through the membrane of one human cell and into another.


Listeria monocytogenes
a
b
Phagosome
c
Lysis of
phagosome
and replication
in cytosol
d
F-actin
e
Double-membraned
vacuole
f
Lysis of vacuole

Clinical Findings

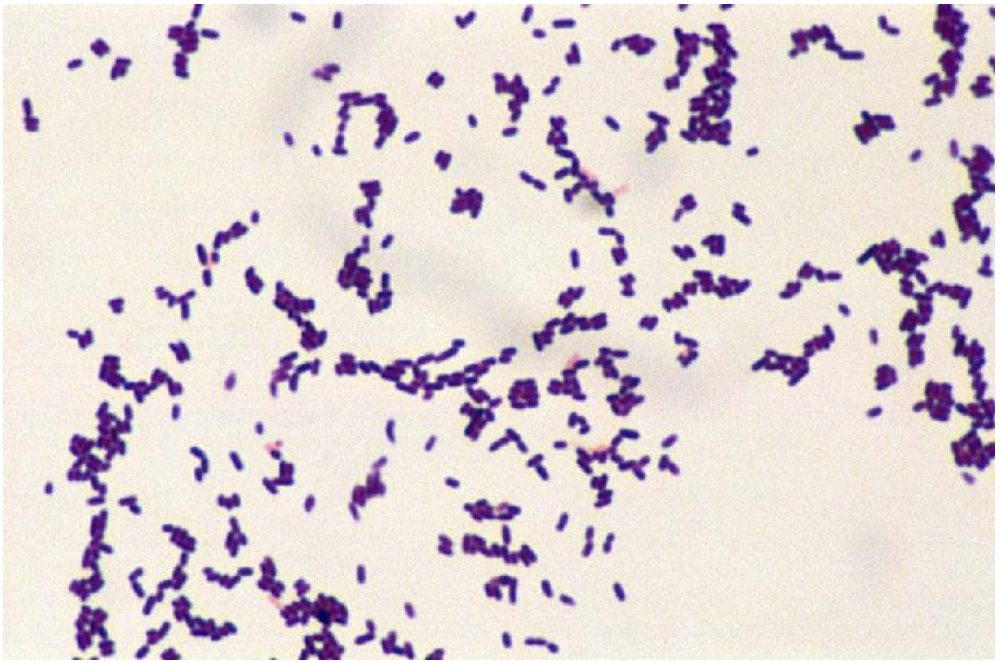
- **During pregnancy:** Abortion, premature delivery, or sepsis during peripartum period.
- **Newborns infected at time of delivery:** Acute meningitis 1 to 4 weeks later. Bacteria reach meninges via bloodstream (bacteremia).
- **Infected mother:** Asymptomatic or influenzalike illness.
- **In immunocompromised adults:** Either sepsis or meningitis.

- **Grows well at cold temperatures, so storage of contaminated food in refrigerator increase risk of gastroenteritis. (cold enhancement).**

- Gastroenteritis characterized by watery diarrhea, fever, headache, myalgias, and abdominal cramps but little vomiting. (Outbreaks usually caused by contaminated dairy products, undercooked meats like chicken & hot dogs).

Laboratory diagnosis

- **Gram stain:** Small **Gram-positive rods** arranged in V- or L-shaped forms similar to *Corynebacteria &* diphtheroids.



- **Motility Test: Tumbling** movement distinguishes it from *Corynebacteria* (non-motile).

- **Culture:** Formation of small, gray colonies with a narrow zone of β-hemolysis on a blood agar plate.

Blood agar

- **Sugar fermentation tests**: Used for identification of *L. monocytogenes*.

Treatment

- **Treatment of invasive disease: (**meningitis & sepsis): Trimethoprim-sulfamethoxazole, Combinations, ampicillin & gentamicin **or** ampicillin & trimethoprim-sulfamethoxazole.

- Resistant strains rare.

- *Listeria* gastroenteritis: Does not require treatment.

Prevention

- Difficult, no immunization.
- Limit exposure of pregnant women & immunosuppressed patients to farm animals, unpasteurized milk products & raw vegetables.
- Immunocompromised patients: Trimethoprim-sulfamethoxazole to prevent *Pneumocystis* pneumonia can also prevent listeriosis.