

Corynebacterium, Listeria, and Erysipelothrix

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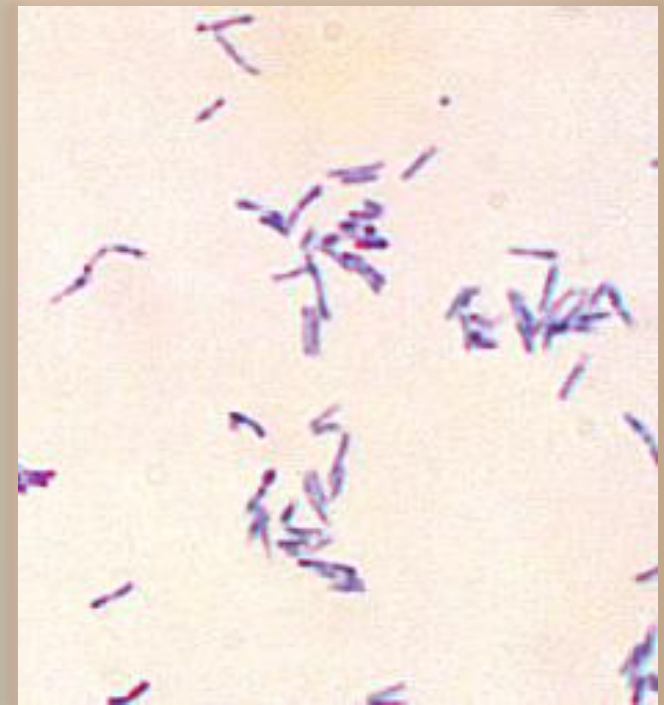
Corynebacteria

- ▶ Members of coryneform bacteria
- ▶ Coryneform bacteria;
 - ▶ Gram-positive rods
 - ▶ Non-spore forming, nonmotile, non-acid-fast
 - ▶ *Corynebacterium*
 - ▶ *Arcanobacterium*
 - ▶ ...



Corynebacteria

- ▶ Aerobic or facultatively anaerobic, nonmotile, and catalase positive
- ▶ Cell wall contains short-chain **mycolic acids**
- ▶ Gram stain: clumps and short chains (V or Y configurations) of irregularly shaped (club-shaped) rods
- ▶ **Metachromatic granules (+)**



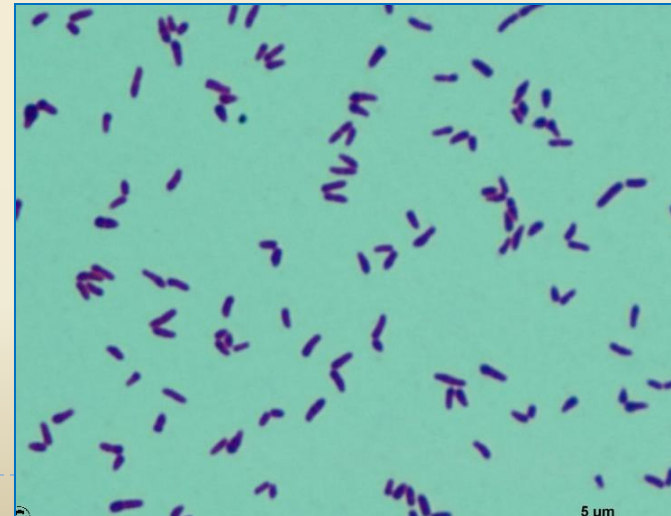
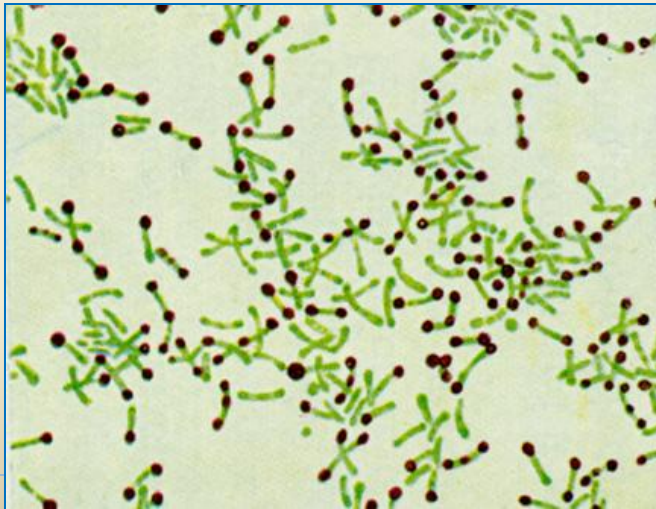
Corynebacteria

- ▶ Normally colonize the skin, upper respiratory tract, gastrointestinal tract, and urogenital tract in humans
- ▶ Can be opportunistic pathogens; a few are more commonly associated with human disease
- ▶ *Corynebacterium diphtheriae*: etiologic agent of diphtheria



Corynebacterium diphtheriae

- ▶ Specific stains: **Metachromatic granules**
- ▶ Smears: X or Y shaped bacilli
- ▶ Four biotypes of *C. diphtheriae*;
 - ▶ mitis, belfanti, gravis, intermedius,
 - ▶ **Biotype mitis:** most common



Corynebacterium diphtheriae

Pathogenesis

- ▶ Asymptomatic carriage in the oropharynx or on the skin
- ▶ Spread by respiratory droplets or skin contact
- ▶ The bacilli then grow on mucous membranes or in skin abrasions, and toxigenic strains start producing toxin



Corynebacterium diphtheriae

Pathogenesis – Exotoxin

- ▶ Diphtheria toxin: **Major virulence factor**
- ▶ **A-B exotoxin**
- ▶ **tox gene**: Introduced by a lysogenic phage (**β -phage**)
- ▶ Three functional regions on the toxin molecule:
 - ▶ A subunit: Catalytic region
 - ▶ B subunit: Receptor-binding region and translocation region
- ▶ The receptors for the toxin:
 - ▶ **CD-9**
 - ▶ **Heparin-binding epidermal growth factor (HB-EGF)**

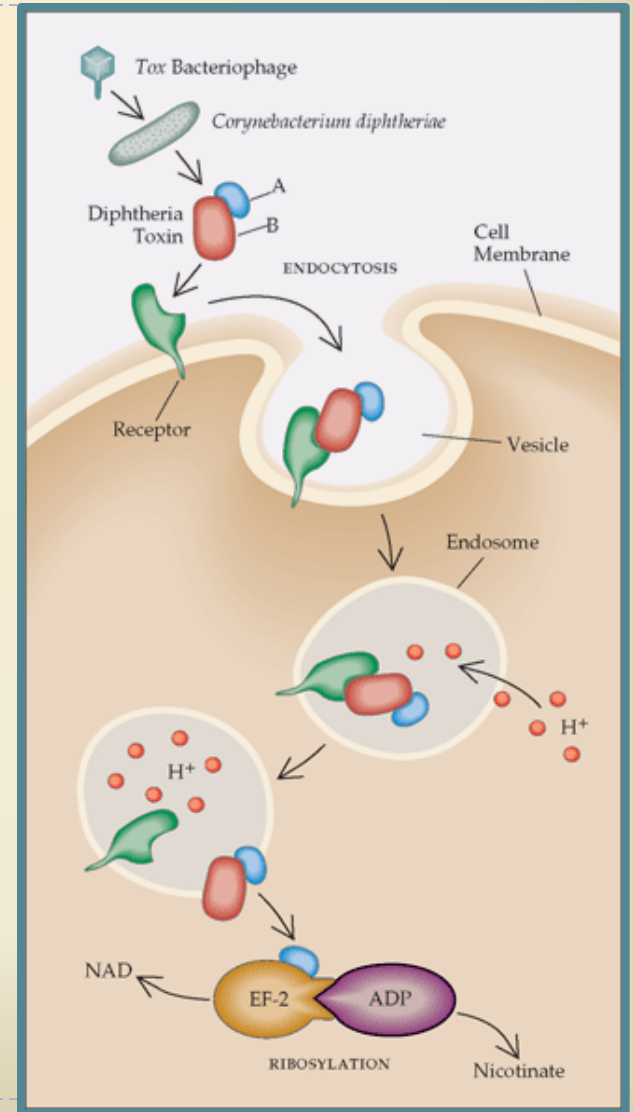


Corynebacterium diphtheriae

Pathogenesis – Exotoxin

Function of the toxin:

- ▶ A subunit terminates host cell **protein synthesis** by inactivating **elongation factor 2 (EF-2)**
- ▶ *EF-2* \longrightarrow translocation of polypeptidyl-transfer RNA from the acceptor to the donor site on the eukaryotic ribosome



Corynebacterium diphtheriae

Clinical diseases

- ▶ Respiratory diphtheria
- ▶ Cutaneous diphtheria



Corynebacterium diphtheriae

Clinical diseases – Respiratory diphtheria

- ▶ Bacteria —→ epithelial cells in the pharynx or adjacent surfaces
- ▶ Exotoxin —→ localized damage
- ▶ Sudden onset with malaise, sore throat, **exudative pharyngitis**, and a low-grade fever
- ▶ The exudate evolves into a thick grayish **pseudomembrane** (tonsils, pharynx, or larynx)
 - ▶ Composed of bacteria, lymphocytes, plasma cells, fibrin and dead cells



Corynebacterium diphtheriae

Clinical diseases – Respiratory diphtheria

- ▶ The pseudomembrane firmly adheres to the respiratory tissue
 - ▶ It is difficult to dislodge without making the underlying tissue bleed **(unique to diphtheria)**
- ▶ Enlarged regional lymph nodes in the neck and marked edema (bull neck)



Corynebacterium diphtheriae

Clinical diseases – Respiratory diphtheria

- ▶ Toxic damage in the heart muscle (**myocarditis**), liver, kidneys (tubular necrosis), and adrenal glands
- ▶ **Nerve damage** (demyelination) – paralysis of the soft palate, eye muscles, or extremities
- ▶ Complications in severe disease;
 - ▶ Breathing obstruction, cardiac arrhythmia, coma and death



Corynebacterium diphtheriae

Clinical diseases – Cutaneous diphtheria

- ▶ Skin contact with infected persons
- ▶ Skin colonization and entry into the subcutaneous tissue through breaks in the skin
- ▶ Papule evolves into **chronic, nonhealing ulcer**
- ▶ Systemic signs can occur as a result of the exotoxin effects



Corynebacterium diphtheriae

Clinical diseases

- ▶ Nontoxigenic strains of *C. diphtheriae*:
 - ▶ Do not produce classic diphtheria
 - ▶ Associated with other diseases
 - ▶ Pharyngitis, septicemia, endocarditis, septic arthritis, osteomyelitis, and abscess formation



Corynebacterium diphtheriae

Laboratory diagnosis

- ▶ Swabs and microscopy
- ▶ Culture
- ▶ Toxigenicity testing
 - ▶ Elek test
 - ▶ Molecular tests



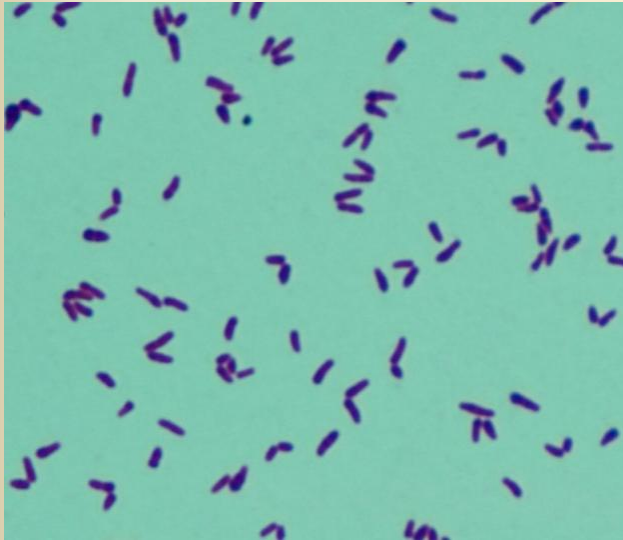
Corynebacterium diphtheriae

Laboratory diagnosis – Swabs and microscopy

- ▶ From the nose, throat, or other suspected lesions
- ▶ Should be placed in semi-solid transport media
- ▶ Microscopic examination is unreliable:
 - ▶ Metachromatic granules are not specific to *C. diphtheriae*

Corynebacterium diphtheriae

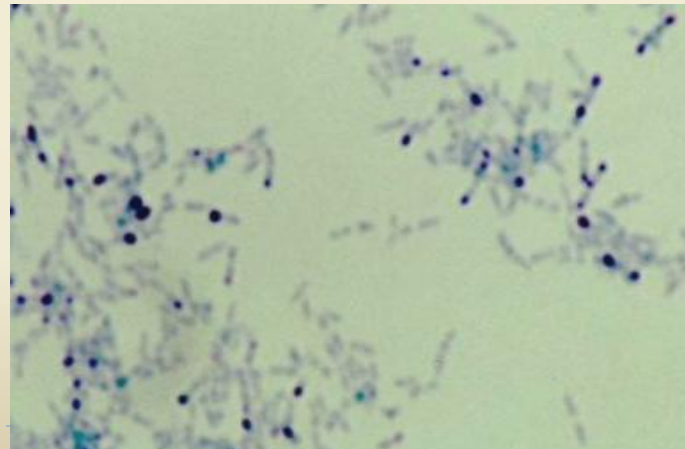
Laboratory diagnosis – Microscopy



C. diphtheriae
(Gram stain)



C. diphtheriae
metachromatic
granules
(Neisser stain)



C. diphtheriae
metachromatic
granules
**(Methylene
blue stain)**

Corynebacterium diphtheriae

Laboratory diagnosis – Culture

- ▶ Blood agar (to rule out hemolytic streptococci)
- ▶ Small colonies



Corynebacterium diphtheriae

Laboratory diagnosis – Culture

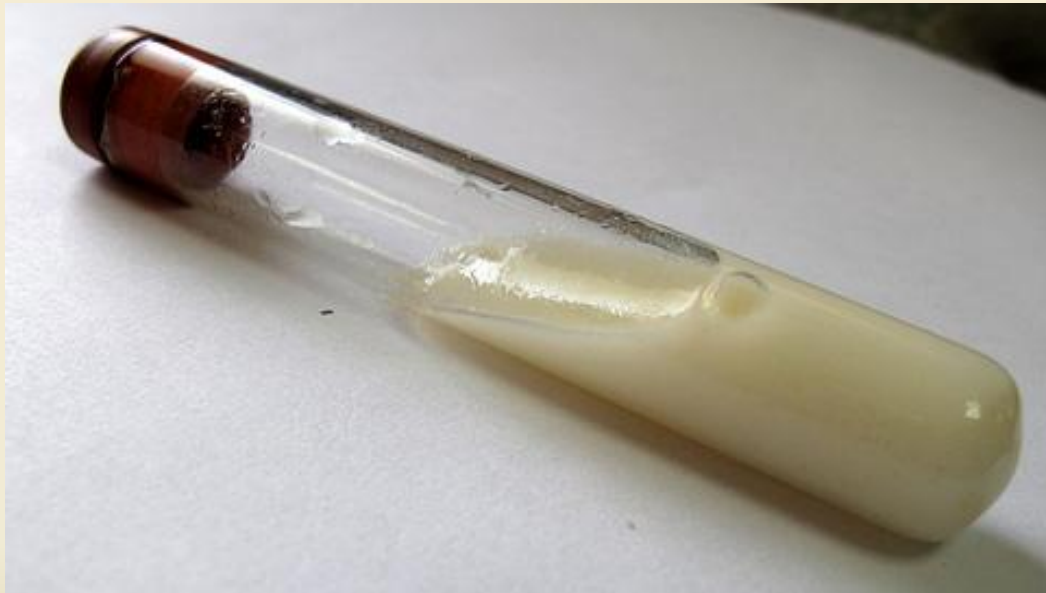
- ▶ Selective medium
(cysteine-tellurite blood agar – CTBA):
 - ▶ **Tellurite;**
 - ▶ Inhibits the growth of many other bacteria
 - ▶ Reduced by *C. diphtheriae* (produces **gray-black colonies**)
 - ▶ Degradation of **cysteine** by *C. diphtheriae* produces a **brown halo** around the colonies



Corynebacterium diphtheriae

Laboratory diagnosis – Culture

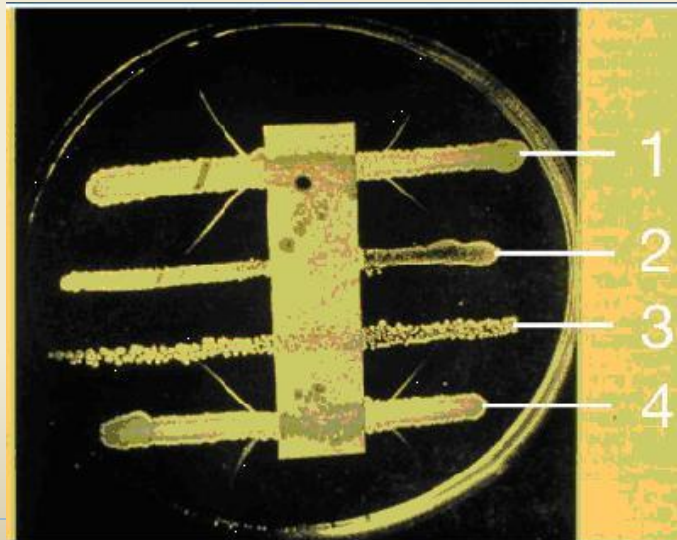
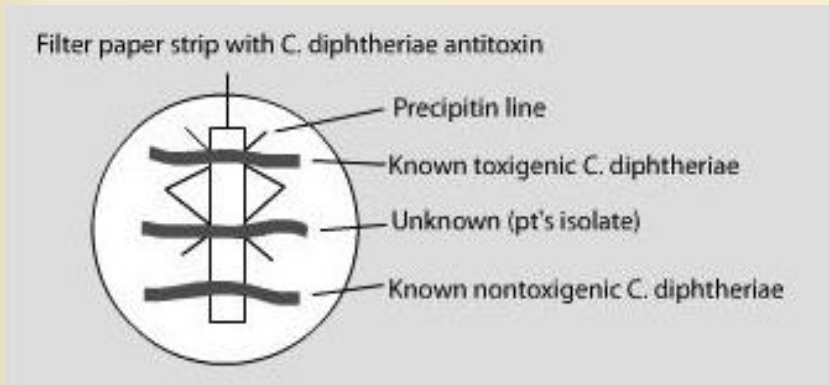
- ▶ **Löffler's medium:**
 - ▶ Enhances production of **metachromatic granules**



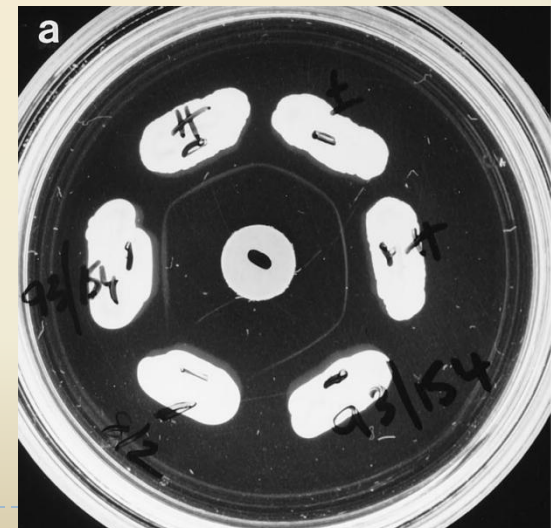
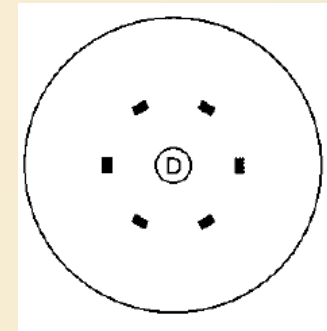
Corynebacterium diphtheriae

Laboratory diagnosis – Toxigenicity

Conventional Elek test:



Modified Elek test:



Corynebacterium diphtheriae

Treatment, Prevention and Control

- ▶ Early administration of **diphtheria antitoxin**
- ▶ Penicillin or erythromycin
 - ▶ Eliminate *C. diphtheriae* and terminate toxin production
- ▶ **Toxoid vaccine** (nontoxic, immunogenic toxoid)
 - ▶ Combined with tetanus toxoid (**Td**)
 - ▶ Combined with tetanus + pertussis vaccine (**DPT**)
 - ▶ Five injections of DPT (2, 4, 6, 15-18 months and 4-6 years), and booster vaccination of Td every 10 year



Other *Corynebacterium* species

- ▶ Part of the indigenous human flora
 - ▶ Mucous membranes of the skin, respiratory tract, urinary tract, and conjunctiva
 - ▶ Capable of causing disease
 - ▶ *C. jeikeium*
 - ▶ *C. urealyticum*
 - ▶ *C. amycolatum*
 - ▶ *C. ulcerans*
 - ▶ *C. pseudotuberculosis*
- Lipophilic corynebacteria
- Nonlipophilic corynebacteria

Corynebacterium jeikeium

- ▶ **Opportunistic pathogen**
- ▶ Septicemia, endocarditis, wound infections, foreign body (catheter, shunt, prosthesis) infections
- ▶ **Very resistant to antibiotics**

- ▶ *C. jeikeium*, *C. urealyticum*, and *C. amycolatum*:
 - ▶ Resistant to most antibiotics
 - ▶ Vancomycin must be given

Corynebacterium urealyticum

- ▶ Strong **urease** producer
- ▶ *C. urealyticum* is the **most common urease-producing** *Corynebacterium* species
- ▶ Makes the urine alkaline, leading to the formation of **renal stones**
- ▶ Urinary tract infections, septicemia, endocarditis, wound infections
- ▶ **Resistant to many antibiotics**



Corynebacterium amycolatum

- ▶ The **most commonly isolated** *Corynebacterium* species in clinical specimens
- ▶ **Opportunistic pathogen**
- ▶ Wound infections, foreign body infections, septicemia, urinary tract infections, respiratory tract infections
- ▶ **Resistant to many antibiotics**



Corynebacterium ulcerans and *Corynebacterium pseudotuberculosis*

- ▶ Closely related to *C. diphtheriae*
- ▶ Can carry the **diphtheria toxin gene**
- ▶ *C. ulcerans* and *C. pseudotuberculosis* (rare) can cause **respiratory diphtheria**
 - ▶ Same treatment with disease caused by *C. diphtheriae*



Other coryneform bacteria

Arcanobacterium

- ▶ Irregularly shaped, Gram-positive rods
- ▶ *Arcanobacterium haemolyticum* produces **β -hemolysis** on blood agar
- ▶ Catalase negative

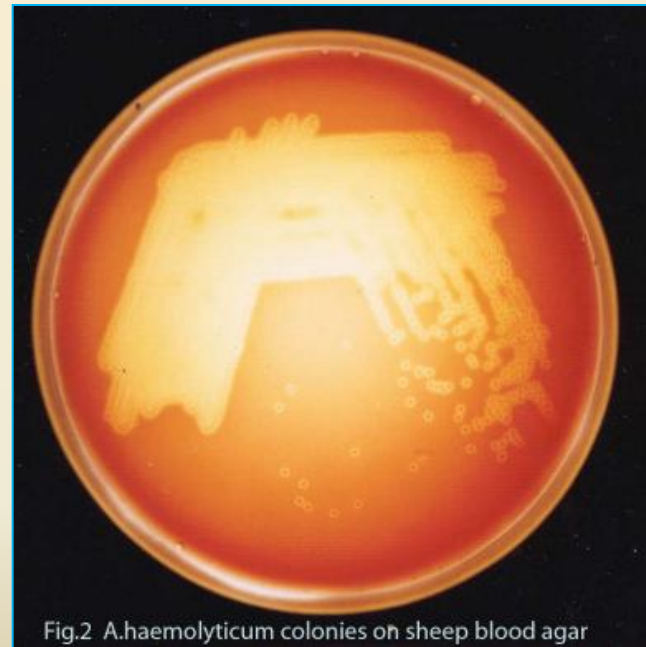
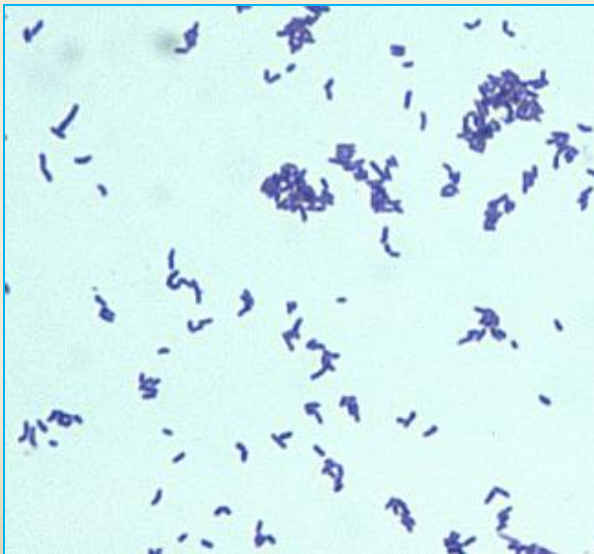


Fig.2 *A. haemolyticum* colonies on sheep blood agar

Other coryneform bacteria

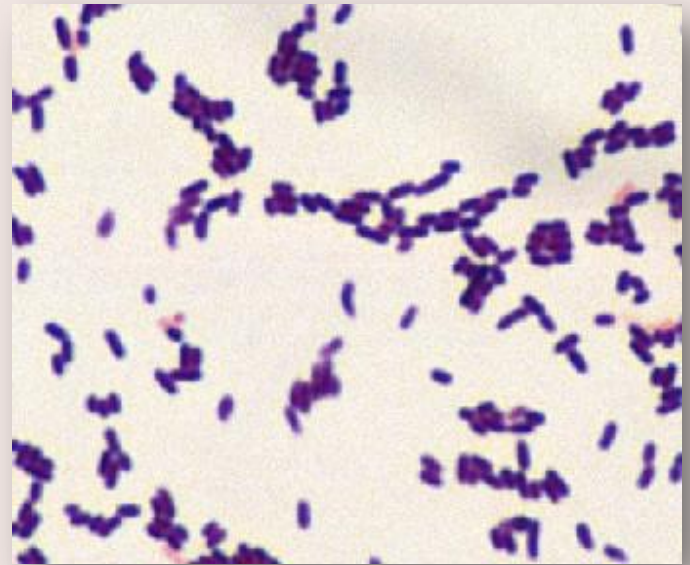
Arcanobacterium

- ▶ Clinical diseases:
 - ▶ **Pharyngitis with a scarlet fever-like rash**, polymicrobial wound infections, septicemia and endocarditis
- ▶ **Difference from Group A streptococci:**
 - ▶ Gram stain morphology and biochemical characteristics
- ▶ Treatment:
 - ▶ Penicillin or erythromycin



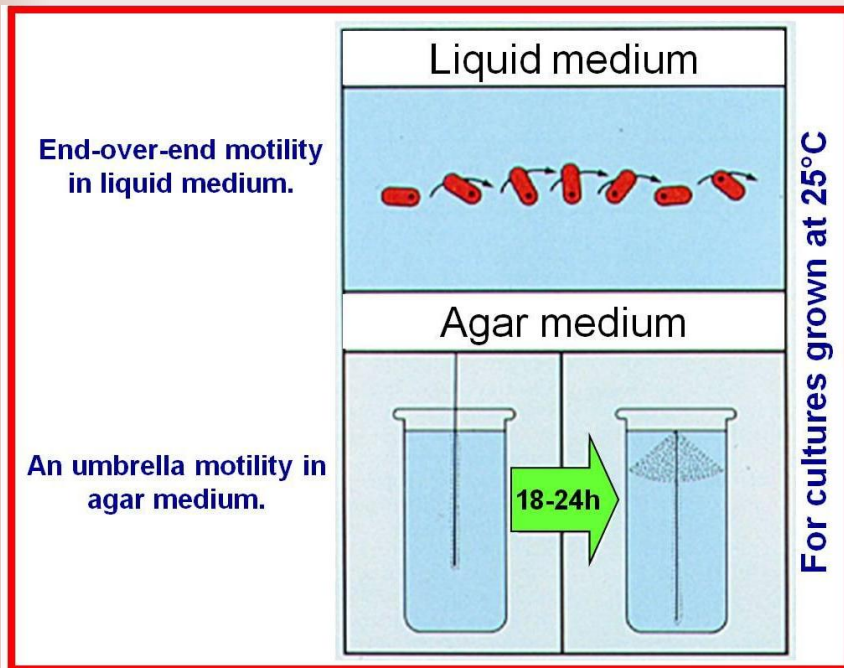
Listeria monocytogenes

- ▶ Short, nonbranching, Gram-positive, facultatively anaerobic rod
- ▶ Ability to grow;
 - ▶ At broad temperature range (1°C to 45°C)
 - ▶ In a wide pH range
 - ▶ In a high concentration of salt



Listeria monocytogenes

- ▶ **Motile at 22°C-28°C** (end-over-end tumbling motion); but not at 37°C



Listeria monocytogenes

Virulence

- ▶ **Surface proteins**

- ▶ Adhesins, Internalin A and B, Act A

- ▶ **Hemolysins**

- ▶ Listeriolysin O, phospholipase C

- ▶ **Siderophore production**

- ▶ Obtain iron from transferrin

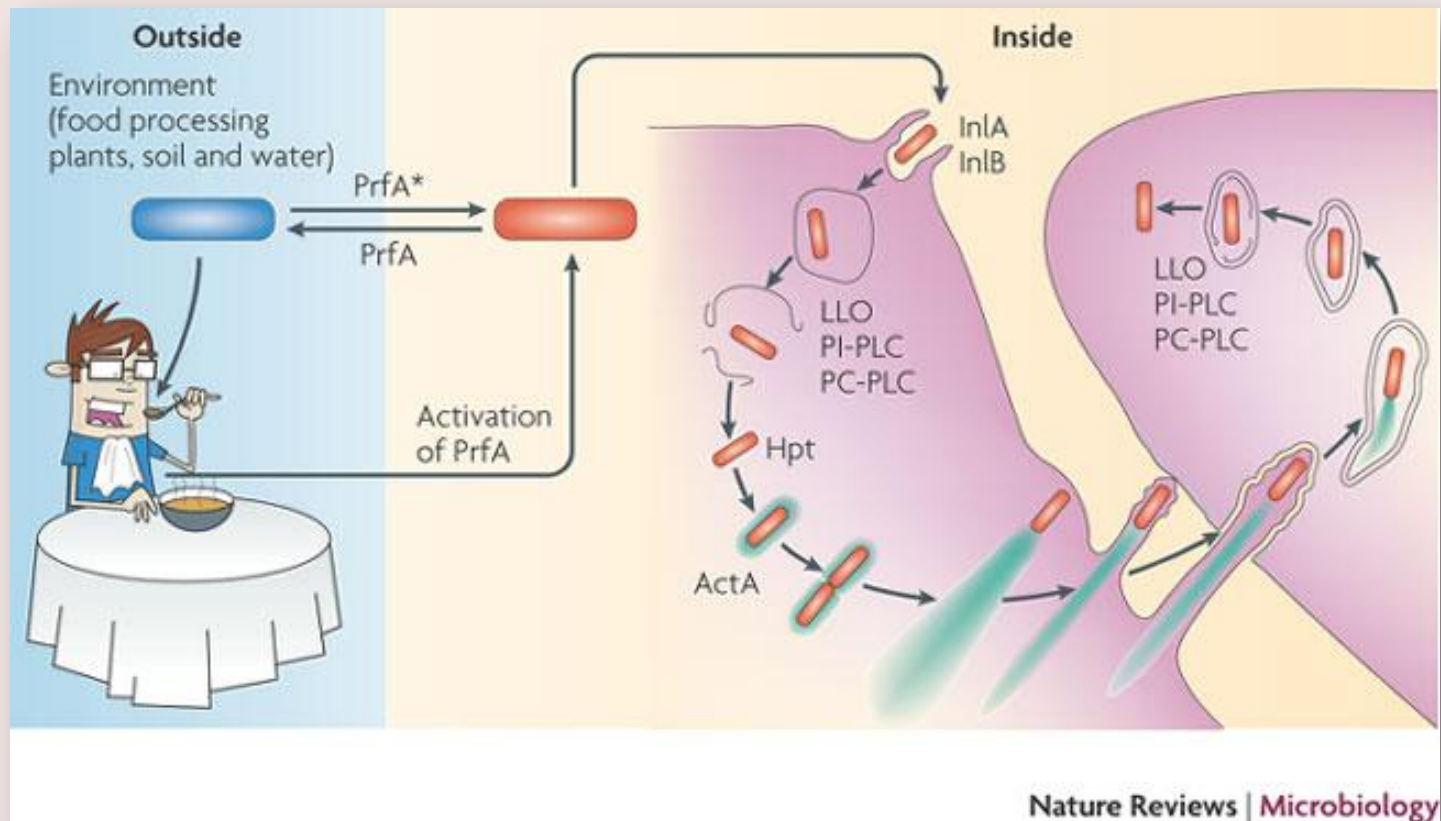
- ▶ **Facultative intracellular pathogen**

- ▶ Avoid antibody-mediated clearance
-



Listeria monocytogenes

Pathogenesis



Listeria monocytogenes

Pathogenesis

- ▶ Enters the body through the **gastrointestinal tract**
 - ▶ Cell wall surface proteins: **Internalin A (InlA)** and **Internalin B (InlB)**
 - ▶ Internalins interact with **E-cadherin** (receptor on epithelial cells), promoting phagocytosis
 - ▶ In phagolysosome, low pH activates the bacterium to produce **listeriolysin O** and two different **phospholipase C enzymes**
-



Listeria monocytogenes

Pathogenesis

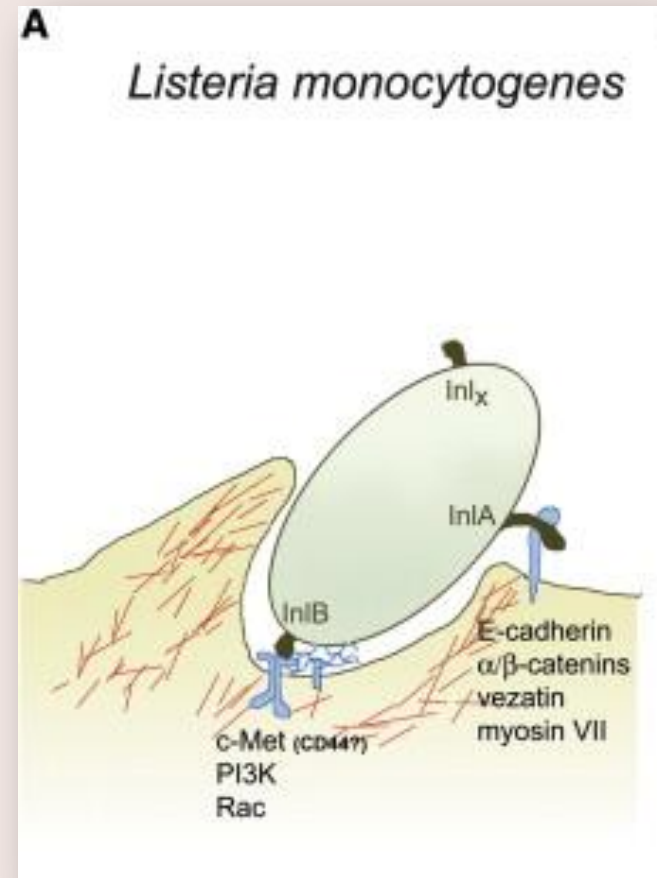
- ▶ Listeriolysin O and phospholipase C: lyse the membrane of phagolysosome and allow the listeriae to **escape into the cytoplasm** of the epithelial cell
 - ▶ The organisms proliferate, and then move to the cell membrane
 - ▶ **ActA** (another listerial surface protein) induces host cell actin polymerization, which propels listeriae to the cell membrane
-



Listeria monocytogenes

Pathogenesis

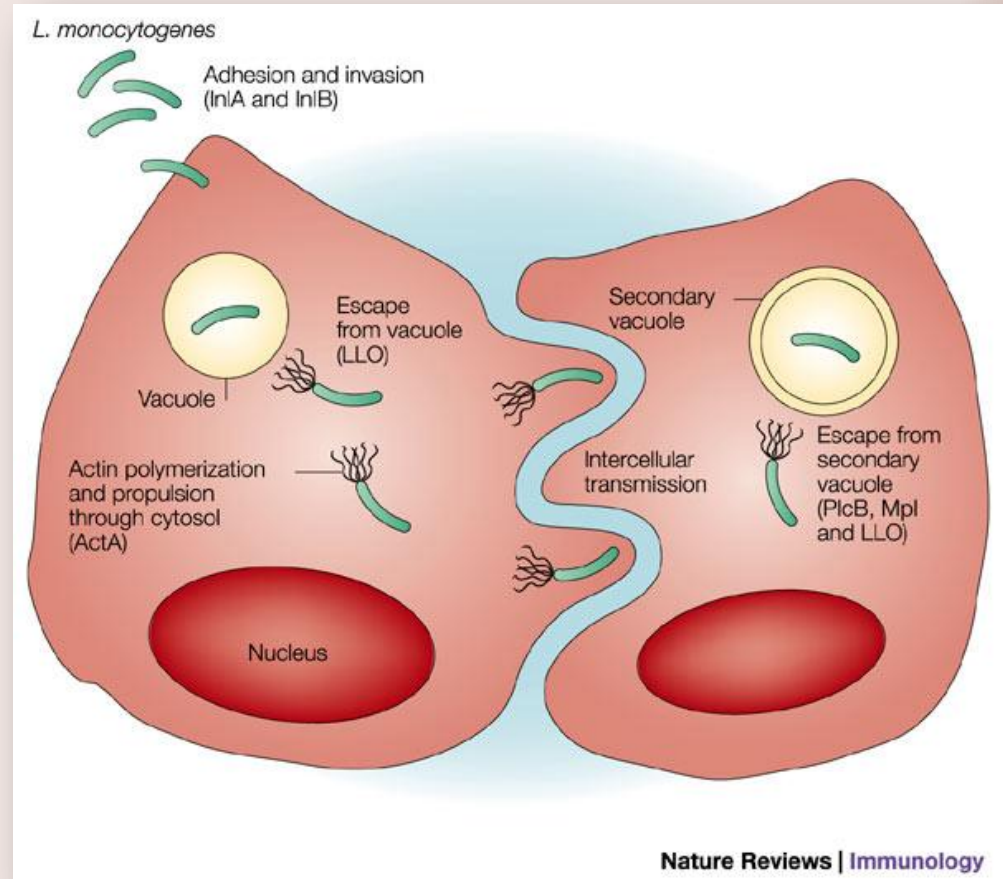
- ▶ Pushing against the host cell membrane, listeriae cause formation of elongated protrusions (**filopods**)
- ▶ These filopods are ingested by adjacent epithelial cells, macrophages, and hepatocytes
- ▶ The listeriae are released, and the cycle begins again



Listeria monocytogenes

Pathogenesis

- ▶ *L. monocytogenes* can move from cell to cell without being exposed to antibodies, complement or polymorphonuclear cells



Listeria monocytogenes

Epidemiology

- ▶ Source of infection:
 - ▶ Consumption of contaminated food
 - ▶ Undercooked processed meat, unpasteurized or contaminated milk or cheese, unwashed raw vegetables (cabbage)
 - ▶ Foods with small numbers of organisms can become heavily contaminated during prolonged refrigeration
 - ▶ From mother to child in utero or at birth



Listeria monocytogenes

Clinical diseases

- ▶ Neonatal disease
 - ▶ Early-onset disease
 - ▶ Late-onset disease
- ▶ Disease in adults
 - ▶ Disease in healthy adults
 - ▶ Disease in pregnant women or patients with cell-mediated immune defects



Listeria monocytogenes

Clinical diseases – Neonatal disease

▶ **Early-onset disease**

▶ **Granulomatosis infantiseptica**

- ▶ Acquired transplacentally in utero
- ▶ Disseminated abscesses and granulomas in multiple organs
- ▶ High mortality rate unless treated promptly

▶ **Late-onset disease**

- ▶ Acquired at or shortly after birth
 - ▶ **Meningitis** and **meningoencephalitis** with **septicemia**
-



Listeria monocytogenes

Clinical diseases – Neonatal disease

- ▶ Early-onset disease (Granulomatosis infantiseptica)



Listeria monocytogenes

Clinical diseases – Disease in adults

- ▶ **Disease in healthy adults**

- ▶ Asymptomatic or a mild influenza-like illness with or without gastroenteritis

- ▶ **Disease in pregnant women or patients with cell-mediated immune defects**

- ▶ Primary febrile bacteremia or disseminated disease with hypotension and meningitis
-



Listeria monocytogenes

Laboratory diagnosis

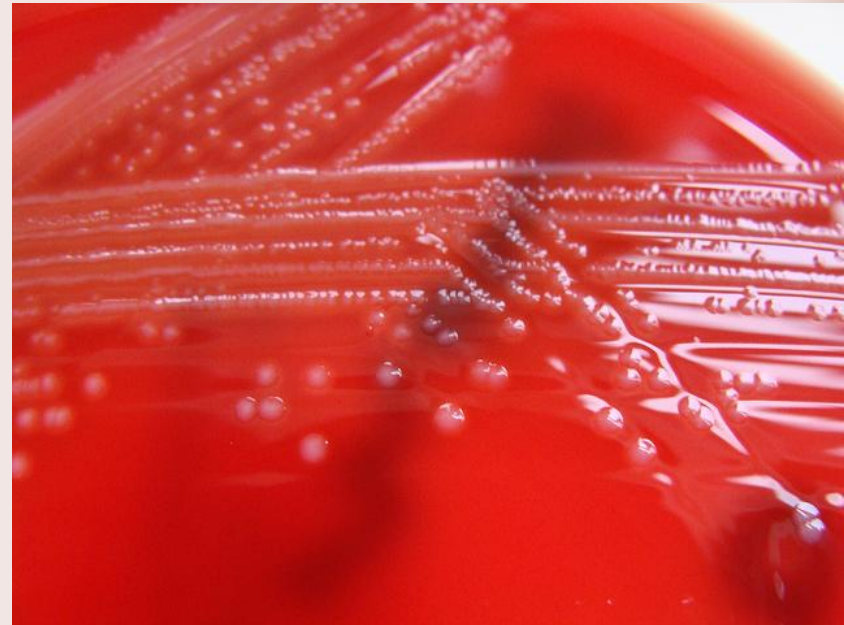
- ▶ Microscopy
 - ▶ Not sensitive; no organisms in the smears of CSF
- ▶ Culture
- ▶ Identification



Listeria monocytogenes

Laboratory diagnosis – Culture

- ▶ Grows on most conventional media
- ▶ Small, round colonies after incubation for 1-2 days
- ▶ Weak β -hemolysis on sheep blood agar



Listeria monocytogenes

Laboratory diagnosis – Culture

- ▶ Detection of listeriae in specimens contaminated with rapidly growing bacteria;
 - ▶ **Selective media**
 - ▶ **Cold enrichment** (storage of the specimen in the refrigerator for a prolonged period)
 - ▶ **CAMP test positive**
 - ▶ The **characteristic motility** of the organism in a liquid medium or semisolid agar
-



Listeria monocytogenes

Laboratory diagnosis – Culture

- ▶ *L. monocytogenes*: **CAMP test positive**



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Listeria monocytogenes

Laboratory diagnosis – Identification

- ▶ Biochemical, molecular and serologic tests
- ▶ 13 serotypes have been described
 - ▶ 1/2a, 1/2b and 4b: >95% human isolates
 - ▶ 4b: most of the foodborne outbreaks



Listeria monocytogenes

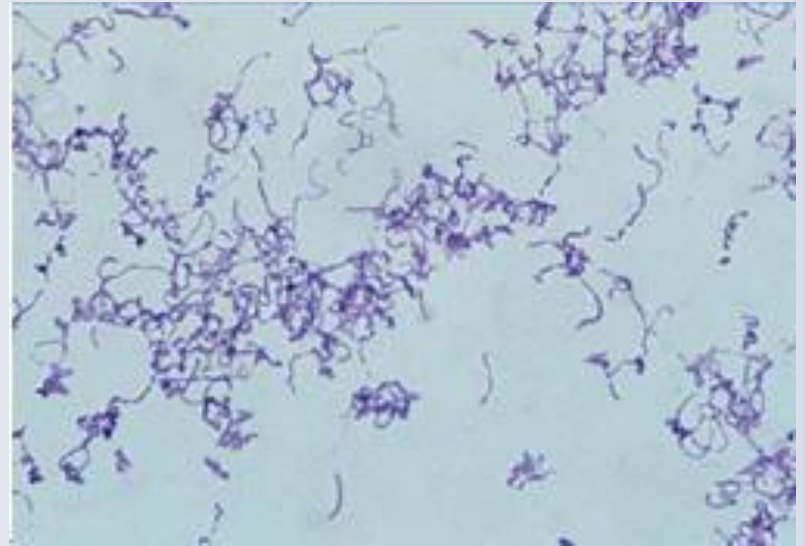
Treatment, prevention and control

- ▶ Gentamicin + penicillin or ampicillin
 - ▶ Trimethoprim-sulfamethoxazole
 - ▶ CNS infections in patients who are allergic to penicillin
 - ▶ Listeriae are ubiquitous and most infections are sporadic; prevention and control are difficult
 - ▶ Consumption of raw or partially cooked meats, unpasteurized or contaminated milk or cheese, and unwashed raw vegetables should be avoided
-



Erysipelothrix rhusiopathiae

- ▶ Slender, pleomorphic, Gram-positive rods that form long filaments
 - ▶ Distributed in land and sea animals worldwide
 - ▶ Colonization is particularly high in swine and turkeys
 - ▶ Causes erysipelas in swine
 - ▶ Disease in humans is less common
-



Erysipelothrix rhusiopathiae

- ▶ Disease in humans is zoonotic and primarily occupational
 - ▶ Butchers, farmers, fishermen, veterinarians, and etc...
- ▶ People are infected by direct inoculation from animals or animal products
- ▶ Three primary forms of human infection:
 - ▶ Localized skin infection (**erysipeloid**)
 - ▶ Generalized cutaneous disease
 - ▶ Septicemia: Uncommon; when present endocarditis (+)

Erysipelothrix rhusiopathiae

Clinical diseases – Erysipeloid

- ▶ The painful and pruritic skin lesion most commonly presents on the fingers or hands and appears violaceous with a raised edge
- ▶ No suppuration (different from streptococcal erysipelas)
- ▶ The resolution can be spontaneous but can be hastened with antibiotic therapy



Erysipelothrix rhusiopathiae

Laboratory diagnosis

- ▶ Gram stain of the specimen is typically negative
- ▶ Thin, Gram-positive rods associated with characteristic skin lesion and clinical history can be diagnostic
- ▶ Grows on most conventional media
- ▶ Incubated at 5%-10% CO₂ for 3 days or longer
- ▶ Blood cultures (-)



Erysipelothrix rhusiopathiae

Treatment, prevention and control

- ▶ Penicillin —→ both localized and systemic diseases
 - ▶ Patients allergic to penicillin;
 - ▶ Ciprofloxacin or clindamycin for localized cutaneous infections
 - ▶ Ceftriaxone or imipenem for disseminated infections
 - ▶ People at a higher occupational risk should use gloves and other appropriate coverings on exposed skin
 - ▶ Vaccination is used to control disease in swine
-

