

Gram positive non spore forming non motile bacilli

Corynebacterium sp.

General character:

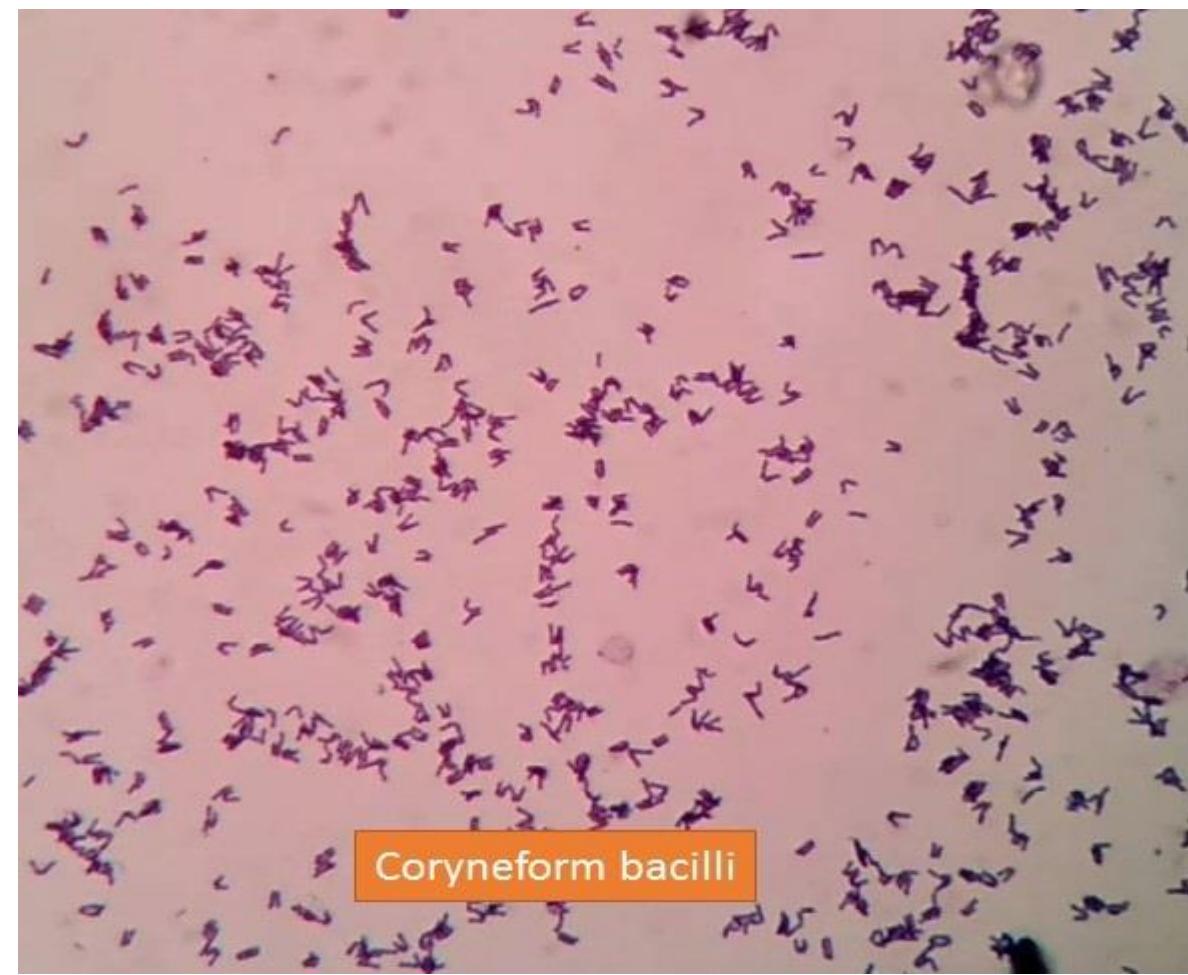
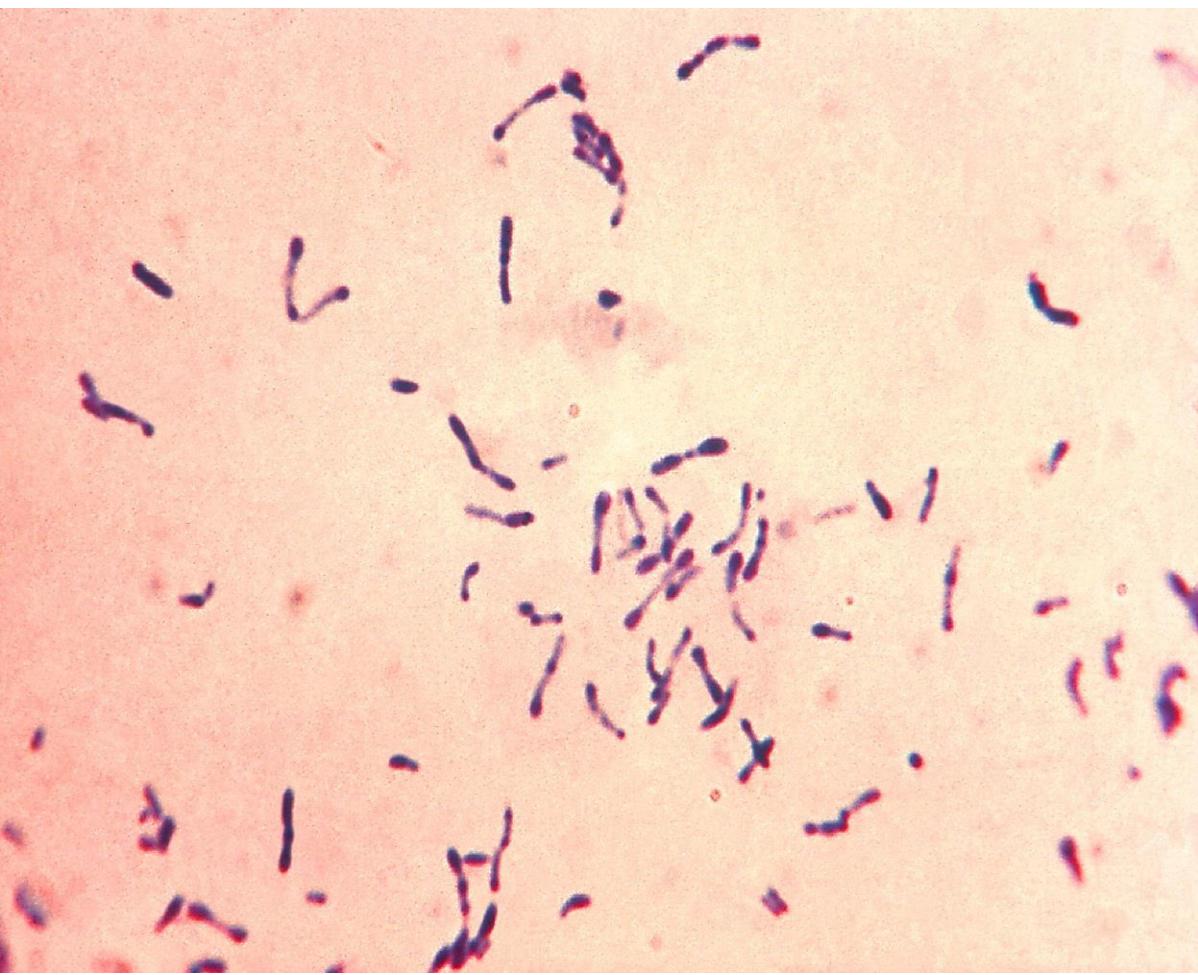
They are Gram-positive, non-acid fast and non-motile bacilli occurring in palisade or Chinese letter arrangement.

They frequently show club-shaped swelling (coryne=club shaped) at the ends and irregular staining.

They are found as normal flora of the skin and mucus membrane and named **diphtheroids**.

Because of their presence on skin and in the environment they are fairly frequently encountered **as contaminants** in laboratory cultures.

Corynebacterium diphtheriae is the pathogenic member and it shows **metachromatic granules** in Albert stained smear. It produces powerful **exotoxin** and causes a disease called **diphtheria**.



Coryneform bacilli

***Corynebacterium diphtheriae* a.k.a Kleb -Loeffler bacillus K.L.B**

Morphology

1- They are slender, pleomorphic, Gram positive, non-motile, non-spore forming club shaped bacilli with Chinese letter arrangement.

2- Contain metachromatic granules called volutine granules or babes –Ernst granules (polyphosphate) which appear as dark purple by **Alberts stain**, the feature can be used to differentiate from non-pathogenic species (diphtheroids).

3- Only lysogenic strains (infected with temperate phage carrying toxin) are pathogenic, therefore identification requires toxigenicity test (Eleks test).

Routes of infection

Droplet infection (main route)

Direct contact with skin lesions (less common)

Sources of infection

Diphtheria cases

Carrier cases

Pathogenesis

The bacteria colonize the mucus membrane of oropharynx or skin abrasion, then produce exotoxin (AB components). The toxin enter the cells and inhibit protein synthesis leading to necrosis and formation of pseudomembrane over the tonsils, pharynx and larynx. The pseudomembrane is firmly attached to the underlying tissues (Candida can also produce pseudomembrane but loosely attached). The toxin enters the blood circulation (No bacteremia but toxemia) and mainly affect the heart to cause myocarditis and peripheral neuropathy (demyelination).

Clinical findings

Clinically the patient presents with signs and symptoms of sore throat or pharyngitis. There is gray pseudomembrane (Figure 1) covering tonsils and pharynx that may lead to suffocation. In sever case there is swelling of the neck (bull neck) (Figure 2). In advance cases there is arrhythmia with difficulties in extremities, speech, swallow and vision.



Figure 1. Diphtheria pseudo-membrane in the throat.



Figure 2. Bull neck appearance due to the local cervical lymphadenopathy.

Diagnostic Laboratory Tests

Note: Specific treatment must never be delayed for laboratory reports if the clinical picture is strongly suggestive of diphtheria.

Swab from lesions should be taken before administration of antimicrobial drugs.

Smear examination reveals Gram positive bacilli with Chinese letter arrangement (Figure 3). Alberts stain shows metachromatic granules or volutine granules (Figure 4).

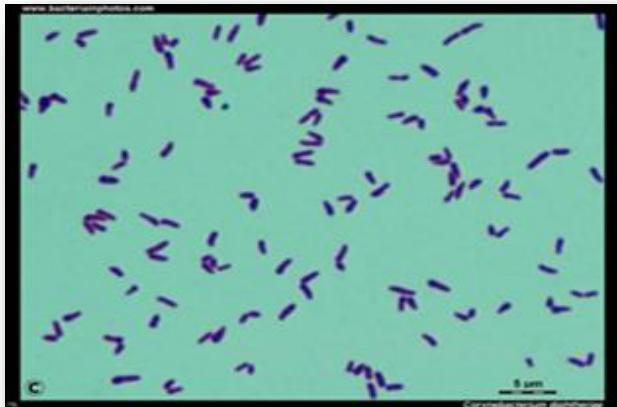


Figure 3. Gram stained smear revealing club shaped bacilli with Chinese letter arrangement.



Figure 4. Alberts stain revealing volutine granules.

Culture

The swab is inoculated on Lofflers serum slop agar (enhance metachromatin granules formation), blood agar (to exclude Streptococci) and on a selective medium such as a tellurite plate (eg, cystine-tellurite blood agar (CTBA) or modified Tinsdales medium) and incubated at 37 C in 5% CO₂. On cystine tellurite agar, the colonies are black with a brown halo (Figure 5).

Classification

3 morphological types of *C. diphtheriae* are found on tellurite containing media:

- △ **Mitis** ['maitɪs] – black colonies with a gray periphery
- △ **Gravis** – large, gray colonies
- △ **Intermedius** – small, dull gray to black.

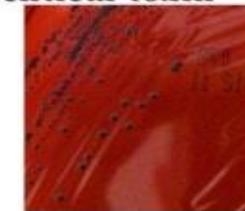
All produce an immunologically identical toxin



Mitis



Gravis



Intermedius

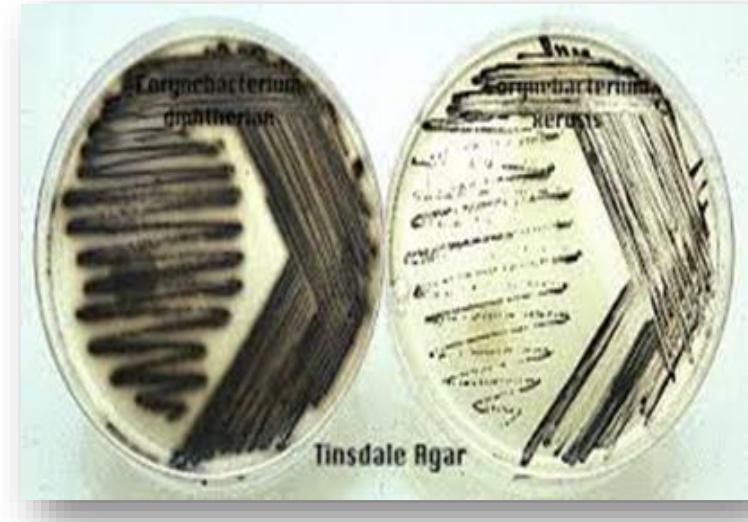


Figure 13. Black colonies of *C. diphtheriae* on Tinsdales agar.

The presumptive diagnosis and toxigenicity test should be performed because not all strains of *C. diphtheriae* are toxigenic only lysogenic strains toxigenic.

There are different method for this test

1- Animal inoculation (In vivo) using guinea pigs in which bacterial suspension is inoculated (subcutaneously) in two animals, one is protected by anti-diphtheria toxin as a control, while the second as a test. If bacteria is toxigenic will kill the test animal leaving the control animal live.

2- In Vitro test (Elek test): It is a precipitation test in which a filter paper strip is impregnated with anti-diphtheria toxin and placed on the surface of culture media then bacteria culture at right angle to filter paper. This plate is inoculated at 30°C for 24 to 48 hours. Toxin produced by bacterial growth will diffuse in agar and where it meets optimum concentration will produce line of precipitation. No precipitate will occur in nontoxigenic strain (Figure 6).

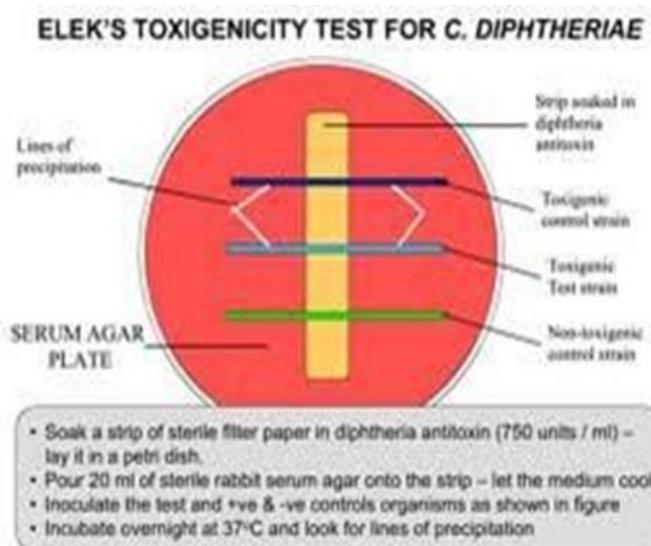


Fig.6. Elek test

PCR technique can also be used for detection of diphtheria toxin either from clinical specimens or from culture

Tissue culture test: This may be done by incorporation of *Corynebacterium diphtheriae* into an agar overlay of cell culture monolayers. Toxin produced may diffuse into cells and kills them.

Enzyme-linked immunosorbent assays can be used to detect diphtheria toxin from clinical *C diphtheriae* isolates.

An immunochromographic strip assay allows detection of diphtheria toxin in a matter of hours. This assay is highly sensitive.

Treatment

The antibiotics are of little value in treatment as they cannot deal with toxin already present in patient's body. Antitoxin should be given immediately if case is suspected as diphtheria since mortality rate increased with delay in starting antitoxic treatment. However, penicillin, preferably erythromycin or rifampicin or clindamycin, etc. may be used to prevent infection and to stop cases from becoming carriers. They may be used in dealing with established carriers.

Immunity

This infection can be prevented by vaccination using diphtheria toxoid. The vaccine is usually given as triple vaccine (DPT) with both pertussis and Tetanus at 6 months old. A booster dose is given at 18 months and another at school entry (6 years). This gives lasting protection.

Diphtheroids or Coryneform bacteria

They are non-pathogenic species found as normal flora of the mucus membrane of the skin, respiratory tract, genital tract and conjunctiva. They are commonly recovered from clinical specimens.

Morphologically distinguished from *C. diphtheriae* (Table 1).

TABLE 26.2: Differences between diphtheria and diphtheroids

<i>Diphtheria</i>	<i>Diphtheroids</i>
A. Morphology	
1. Gram-positive and thin	Gram-positive, short and thick
2. Metachromatic granules are more	They are less or absent
3. Pleomorphism present	Very little pleomorphism
4. Chinese letter arrangement	No such arrangement is seen here
B. Culture	
Growth on enriched media	May grow on ordinary media
C. Biochemical reactions	
Fermentation of glucose only	May ferment glucose and sucrose
D. Toxicity	
They are toxic	Usually non-toxic

Corynebacterium jeikeium is one of the coryneform bacteria most commonly isolated from acutely ill patients. It can cause disease in immunocompromised patients and is important because it produces infections, including bacteremia, that have a high mortality rate and because it is resistant to many commonly used antimicrobial drugs.

Corynebacterium urealyticum is a slowly growing species (48 hrs) that is resistant to antibiotics. As its name implies, it is urease positive. It has been associated with acute or chronic encrusted urinary tract infections manifested by alkaline urine pH and crystal formation. Colonies are characteristically pinpoint, whitish, opaque, smooth, convex, circular, and non-hemolytic

Arcanobacterium haemolyticum produces β-hemolysis on blood agar. It is occasionally associated with pharyngitis and can grow in media selective for streptococci. A *haemolyticum* is catalase-negative, similar to group A streptococci, and must be differentiated by Gram stain morphology (rods vs. cocci) and biochemical characteristics.

Propionibacterium acnes is found in acne pustule and is anaerobic. It produces lipases, which split off free fatty acids from skin lipids. These fatty acids can produce inflammation and contribute to acne. Tetracycline can inhibit lipolytic action.

A 5-year-old –boy presented with high fever, swelling of the neck and severe pharyngitis that covered by membrane that firmly attached to the underlying tissue.

1. Name the sample to be taken from this case
2. What is the suspected disease and the causative agent of this infection?
3. What is the main method used for presumptive diagnosis of this case?
4. What is the test used for final diagnosis of this case?
5. The fever of this case due to which of the following: septicemia or bacteremia or toxemia?
6. Is this disease preventable or not .If yes how?
7. What is the best treatment for this case?
8. Is antibiotics are effective for this case?
9. Why morphology (Gram stain and Alberts stain) is reliable for diagnosis of this case?
- 10.What is the nature of the vaccine used for preventing this infection?

Listeria monocytogenes

Gram positive short bacilli resembling diphtheroids as shown in figure 7.

Causes infections in both humans and animals (zoonotic).

Is an important food born pathogen.

Motile (tumbling end-over-end motility) at 22-28C but not at 37C.

Grow at 4C (cold enrichment).

Transmitted via contaminated foods and vegetables.

Can be differentiated from Diphtheroids by motility test and hemolysis (narrow zone).

Intracellular bacteria in which have certain protein (internalin)on the surface that induces phagocytosis and can escape from phagolysosomes (listeriolysin O) to be free inside cytoplasm. Then bacteria propelled by actin filaments to push out through cell membrane as elongated protrusions called filopods which engulfed by other host cells.

It causes two forms of perinatal infections, **early onset syndrome** called **granulomatosis infantisepticum**, which is a generalized infection of the baby in uterus that leads to **death** before or after delivery. **Late onset syndrome** occurs after **delivery till 3 weeks** and causes **meningitis**.

In causes **immunosuppressed adults meningitis**

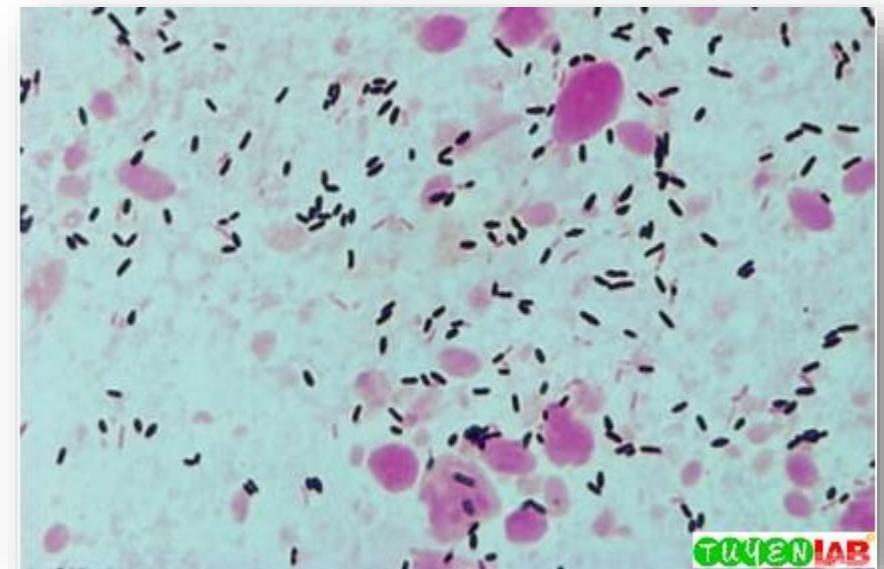


Fig.7. *Listeria monocytogenes*

In normal adults may cause food poisoning characterized by fever, chills, abdominal pain and diarrhea which is self-limited.

***Listeria monocytogenes* infections more common in**

Elderly people- bacteremia and meningitis

Pregnant women- abortion and still birth

Newborn-septicemia and meningitis

Immunocompromized people-meningitis and septicemia

Who has a higher risk of getting *Listeria* food poisoning?

Lessons from *Listeria* outbreaks: Food poisoning can happen to anyone. Each year, about 48 million people in the US (1 in 6) get sick from eating contaminated food. It can be especially dangerous for pregnant women and their newborns; older adults; and people with immune systems weakened by cancer, cancer treatments, or other serious conditions (like diabetes, kidney failure, liver disease, and HIV/AIDS). *Listeria* is a prime example of how germs that contaminate food can cause sickness and death in these groups.

Pregnant women, fetuses, and newborn infants



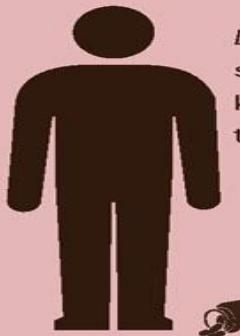
Listeria can pass from pregnant women to their fetuses and newborns. It can cause miscarriages, stillbirths, and newborn deaths.



Chancy cheese

LISTERIA OUTBREAK: Queso fresco (a type of soft cheese) sickened 142 people, killed 10 newborns and 18 adults, and caused 20 miscarriages.

People with weakened immune systems



Listeria can spread through the blood-stream to cause meningitis, and often kills. The weaker your immune system, the greater the risk.



Contaminated celery

LISTERIA OUTBREAK: Pre-cut celery in chicken salad served at hospitals sickened 10 people who had other serious health problems. Five of them died as a result.

Adults 65 or older



Listeria can spread through the blood-stream to cause meningitis, and often kills. The older you are, the greater the risk.



Tainted cantaloupes

LISTERIA OUTBREAK: Contaminated whole cantaloupes sickened 147 people in 28 states and caused one of the deadliest foodborne outbreaks in the US. There were 33 deaths, mostly in adults over 65, reported during the outbreak.

What foods are risky?

When it comes to *Listeria*, some foods are more risky than others. Meet some of the other foods where *Listeria* is known to hide.



Raw Sprouts



Raw Milk (unpasteurized)



Soft Cheeses



Deli Meats and Hot Dogs (cold, not heated)



Smoked Seafood

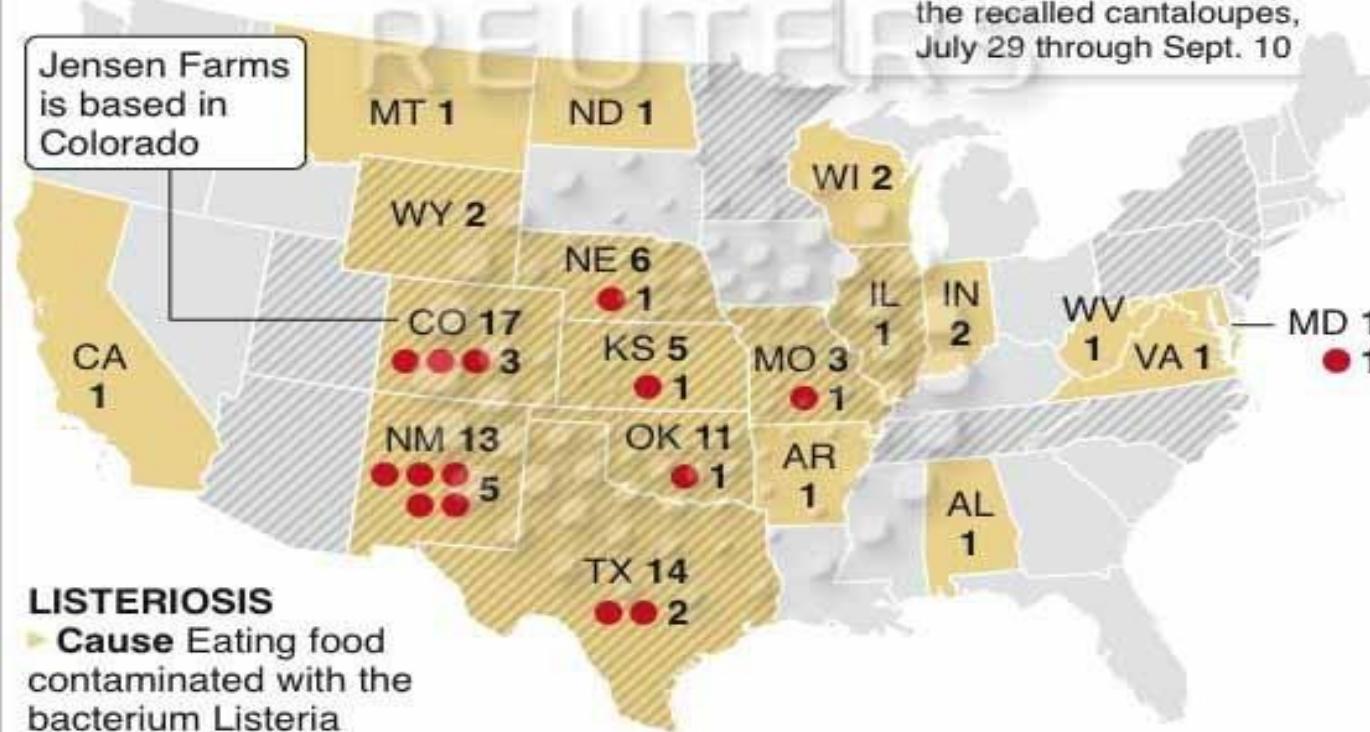
U.S. LISTERIA OUTBREAK

The Centers for Disease Control and Prevention said 19 states have reported infections from one of the four strains of listeria involved in the outbreak

AFFECTED STATES

As of 1500 GMT on Sept. 29, a total of 84 infected persons identified

Jensen Farms is based in Colorado



LISTERIOSIS

- ▶ **Cause** Eating food contaminated with the bacterium *Listeria monocytogenes*
- ▶ **Symptoms** Fever and muscle aches, often preceded by diarrhea or other gastrointestinal symptoms
- ▶ **Other symptoms** Headache, stiff neck, confusion, loss of balance, convulsions

Sources: Centers for Disease Control and Prevention, U.S. Food and Drug Administration

 REUTERS

Outbreak of Listeriosis in USA in 2011. 116 confirmed cases with 24 deaths due to the contaminated Cantaloupe



Laboratory diagnosis

If samples from animal products like meat, chicken or cheese follow pre-enrichment technique followed by enrichment using selective media

Fraser broth with supplement (ferric ammonium citrate , Nalidixic acid and Acriflavin, (30°C, 24 h). A loopful of the subsequent culture streaks on the surface of different agars (PALCAM listeria agar = Polymyxin, Acriflavin, Lithium-chloride, Ceftazidime, Esculin, Mannitol, with supplement, and OXFORD agar (Lab UK) with X122 supplement). Agar plates then incubate (37°C, 48 h) and observe for suspected colonies (greyish colonies surrounded by black halos, with sunken centers with possible greenish sheen on Oxford, or black colonies on PALCAM). Gram's stain, motility test at room temperature , catalase (+ve) and oxidase reactions (negative). Then confirm by PCR technique.

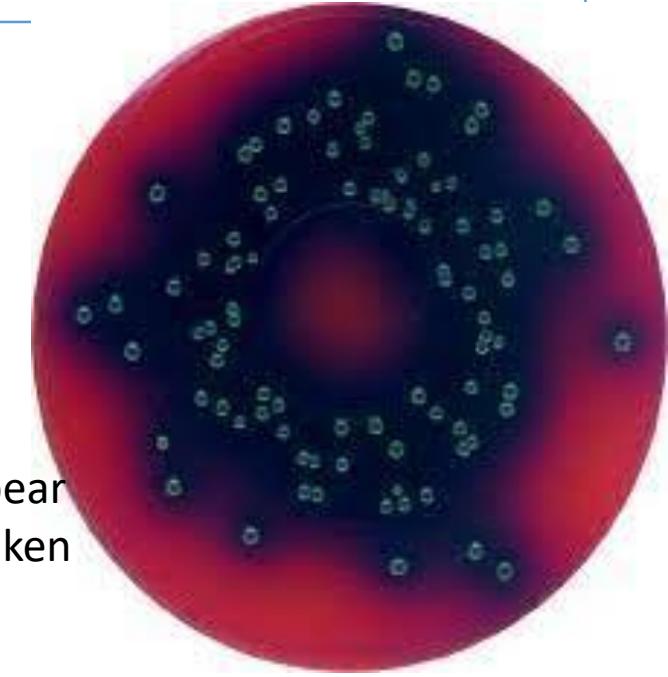


Figure : *L. monocytogenes* colonies on Palcam agar appear as grey-green with black-sunken centers and a black halo colonies

In case isolation from human cases (Vaginal swab, blood from babies, CSF or from placenta of abortion cases) culture on selective media like Oxford agar or on Blood agar then do gram stain, catalase test, oxidase test, motility at 22 C but not at 37C.

There is ELISA test for detection antilisteriolysin O IgG antibodies in the serum of aborted women

Treatment

Gentamicin plus ampicillin for severe infections

Bactrim (Trimethoprim +sulfamethoxazole) for food poisoning cases

How to prevent Listeriosis?

- Completely cook all meats and eggs.
- Carefully wash raw vegetables before eating.
- Keep raw meat away from raw vegetables and prepared foods. After cutting raw meat, wash the cutting board with detergent before using it for vegetables.
- Avoid drinking unpasteurized milk or foods made from such milk.
- Wash hands thoroughly after handling raw meat.

Case

A premature baby delivered by birth canal and developed high fever after one week. Procalcitonin test (A serological test used for detection of bacterial septicemia) was positive. Blood culture gave small hemolytic colonies. Gram stained smear from colonies revealed Gram positive club shaped bacilli arranged in Chinese letter like.

Questions

1. What is ur tentative diagnosis?
2. What make you to think for ur first diagnosis?
3. What is the source of this infection?
4. Which bacterium makes confusion with this pathogen?
5. What can cause in normal individuals?
6. Name two unique cultural characteristics of this pathogen?
7. Is this pathogen zoonotic?
8. Is this pathogen extracellular or intracellular?

Actinomycetes

Actinomycetes are Gram-positive, non-motile, non-sporing, noncapsulated filaments that break up in bacillary and coccoid form. Actinomycetes contain two medically important genera: *Actinomyces* and *Nocardia*

Features	<i>Actinomyces israelii</i>	<i>Nocardia asteroides</i> and <i>N. brasiliensis</i>
Gram stain	Gram +ve branching filamentous	Gram +ve branching filamentous
Weak acid fast stain	Non AF	Acid fast
Gases	Aerobic	Anaerobic
Habitat	Normal flora of mouth and genital tract	Soil
Source of infection	endogenous	exogenous
Disease	Actinomycosis Chronic indurated pyogenic infection with sinus drainage 1- Cervicofacial –poor dental and oral hygiene 2- Thoracic 3- abdominal. Cause IUD infection	Nocardiosis 1- Pneumonia with abscess 2- Brain abscess 3- Skin and cutaneous tissue abscess

Features

Actinomyces israelii

Nocardia asteroides and *N.brasiliensis*

Sulfur granules (macroscopic colonies)

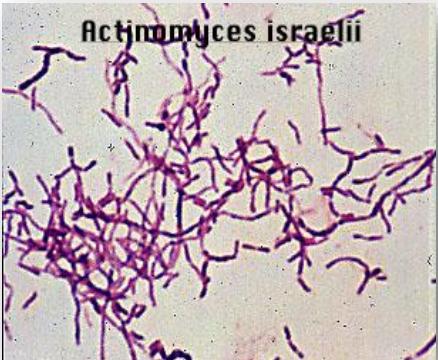
Present

Absent

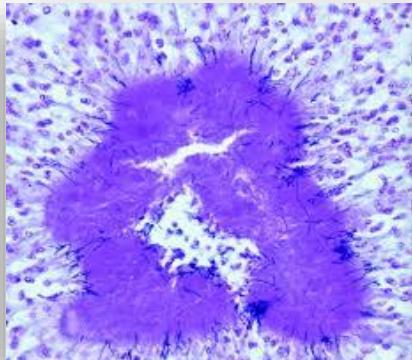
Treatment

Penicillin

Bactrim (Trimethoprim+ sulfamethaxazole)



A.israelii



Sun-rays appearance of sulfur granules



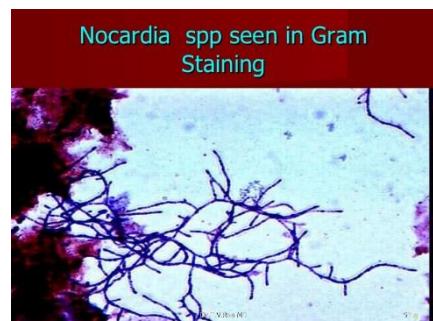
Sulfur granules discharging from abscess



Molar teeth appearance of *A.israelii* colonies



Acid fast branching filaments of *N.asteroieds*



Gram +ve branching filaments of *N.asteroides*

ACTINOMYCOTIC MYCETOMA

Mycetoma or Madura foot is localized, chronic granulomatous lesion of subcutaneous and deeper tissue involving mainly foot presenting as tumor with multiple discharging sinuses. Mycetoma is usually caused by fungi but may be caused by bacteria like actinomyces, *Nocardia streptomycetes*, *Staphylococcus aureus*, etc. In actinomycotic mycetoma color is from white to yellow whereas in fungus mycetoma it is generally black (Figure 8).



Fig.8. Madura foot