
Chapter 13. Gram-positive Bacilli Infections

Table of Contents

CORYNEBACTERIUM SPECIES	1
<i>Corynebacterium diphtheriae</i>	1
BACILLUS SPECIES	4
<i>Bacillus anthracis</i>	4
<i>Bacillus cereus</i> Food Poisoning	6
OTHER GRAM-POSITIVE BACILLI INFECTIONS	6
<i>Listeria</i>	6
Actinomycetes	6

CHAPTER PREVIEW

- *Corynebacterium* species
- *Bacillus* species
- Other Gram-positive Bacilli Infections

Gram-positive bacilli of human importance include:

- *Corynebacterium* species
- *Bacillus* species
- *Clostridium* species: Discussed in Chapter 14, along with other anaerobic infections
- Mycobacteria (discussed in Chapter 15)
- Others: *Listeria* and Actinomycetes (discussed later in this chapter).

CORYNEBACTERIUM SPECIES

Corynebacteria are club-shaped gram-positive bacilli. *C. diphtheriae* is the most important species pathogenic to man; other species called *diphtheroids* are mainly skin commensals, and occasionally can be pathogenic to man.

Corynebacterium diphtheriae

Corynebacterium diphtheriae is the causative agent of *diphtheria*—a contagious disease, characterized by pseudomembrane formation over the tonsil. It commonly affects unvaccinated children.

Virulence Factors

The pathogenesis of diphtheria is mediated by diphtheria toxin; which acts by inhibiting protein synthesis.

- **Diphtheria toxin** is a bacteriophage coded toxin, comprises of two fragments—A and B

- *Fragment B* binds to the host cell receptors and helps in the entry of fragment A
- *Fragment A* is the active fragment, that gets internalized into the host cell and causes inhibition of protein synthesis by inhibiting elongation factor 2 (EF-2).
- **Transmission** occurs through inhalation of respiratory droplets (by coughing or sneezing)
- **Spread:** Organism does not invade, multiplies only at the local site, and secretes toxin. It is the toxin that enters the circulation and goes to various sites to produce various clinical manifestations.

Clinical Manifestations

Respiratory diphtheria is the most common form; characterized by—the presence of a tough leathery greyish white pseudomembrane, formed over the tonsils.

The *other manifestations* are cutaneous diphtheria and less commonly, toxic systemic complications such as myocarditis and neurologic manifestations.

Epidemiology

The incidence of diphtheria is greatly reduced after the introduction of widespread immunization. However, there are reports of a resurgence of cases in older children with incomplete booster doses. India still accounts for the maximum number of cases globally. The main source of infection is the carriers (nasal and throat), which is common in children.

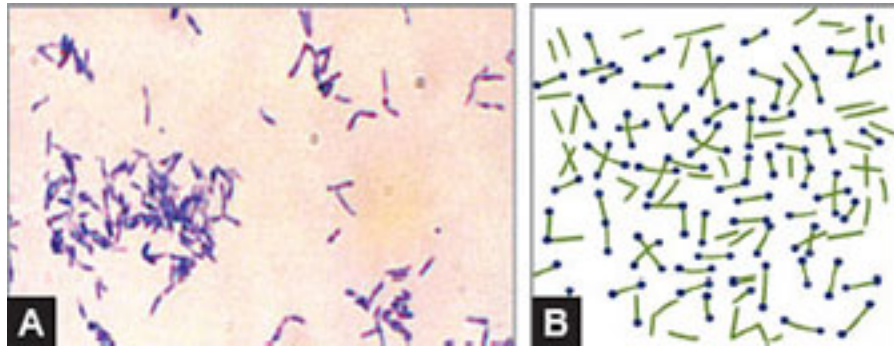
Laboratory Diagnosis

The role of laboratory diagnosis is to: (i) confirmation of clinical diagnosis, (ii) initiate the control measures, and (iii) for epidemiological purposes. However, treatment should promptly be started, not be delayed for obtaining laboratory results.

- **Specimens:** Throat swabs containing fibrinous exudates and a portion of pseudomembrane are the ideal specimens
- **Direct microscopy** of the specimen reveals:
 - *Gram stain:* Shows club-shaped gram-positive bacilli in Chinese letter or cuneiform arrangement, i.e. V- or L-shaped, due to dividing bacilli attached at an angle to each other at their ends (*Fig. 13.1A*)
 - *Albert's stain:* Shows characteristic green bacilli with bluish-black metachromatic granules at the poles (*Fig. 13.1B*).
- **Culture:** Important culture media are:
 - Enriched media such as blood agar and Loeffler's serum slope (*Fig. 13.2A*)
 - *Potassium tellurite agar:* It is a selective media. *C. diphtheriae* produces black colored colonies after 48h of incubation (*Fig. 13.2B*).
- **Identification** of the *C. diphtheriae* grown in culture is confirmed by various biochemical tests (e.g. serum sugar fermentation test) or by automated ID systems such as MALDI-TOF or VITEK
- **Toxin demonstration:** As *C. diphtheriae* can also be found as a colonizer in throat, demonstration of toxin (DT) production following isolation is important to establish the pathogenesis. Toxin demonstration can be done by:
 - Elek's gel precipitation test
 - Detection of DT by immunoassays

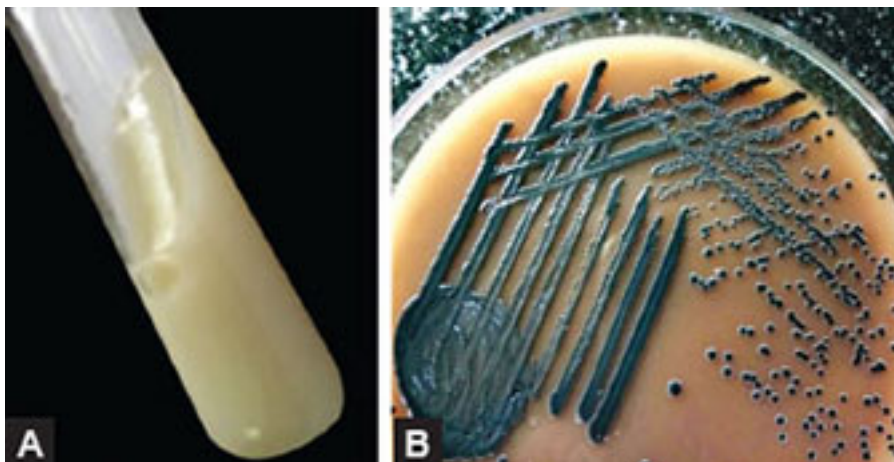
- Detection of gene coding DT by PCR.

Figs. 13.1A and B. *Corynebacterium diphtheriae*: A. Gram-stained smear shows V- or L-shaped bacilli with cuneiform arrangement; B. Albert's stain shows dark blue metachromatic granules at the ends of the green bacilli (schematic).



Source: A. Public Health Image Library, ID# /1943; Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

Figs. 13.2A and B. A. Loeffler's serum slope; B. Potassium tellurite agar shows black colonies.



Source: Department of Microbiology, A. JIPMER, Puducherry; B. PIMS, Puducherry (with permission).

TREATMENT

Diphtheria

Diphtheria is a medical emergency, and should be treated at the earliest. The treatment regimen comprises:

- **Anti-diphtheritic serum:** Passive immunization with anti-diphtheritic horse serum is the treatment of choice as it neutralizes the toxin.
- **Antibiotics** such as penicillin or erythromycin: It has a role if given early in treatment before toxin release. Antibiotics are also useful for the treatment of carriers.

Infection Control Measures

Patient should be placed in isolation room and all the steps of droplet precaution should be followed for the prevention of transmission of *C. diphtheriae* in hospitals (refer Chapter 38).

Vaccine

The diphtheria vaccine (toxoid) is given under the national immunization schedule as a combined vaccine along with pertussis and tetanus (DPT vaccine).

- **Children:** A total of seven doses are given
 - Three doses of pentavalent vaccine (DPT + hepatitis B+ *Haemophilus influenzae* b) at 6, 10, and 14 weeks of birth, followed by
 - Two booster doses of DPT at 16–24 months and 5 years, and
 - Another two booster doses of Td (tetanus toxoid, adult dose of diphtheria toxoid) at 10 years and 16 years.
- **A pregnant woman** also should receive two doses of Td at one month interval
- **Site:** DPT is given deep intramuscularly (IM) at the anterolateral aspect of the thigh
- **Thiomersal** (0.01%) is used as a preservative
- **Storage:** DPT should be kept at 2–8°C; if accidentally frozen then it has to be discarded
- **Protective titer:** Following vaccination, an antitoxin titer of ≥ 0.01 IU/mL is said to be protective.

BACILLUS SPECIES

Bacillus species are gram-positive spore-bearing bacilli.

- The important pathogens are *B. anthracis* (causes anthrax) and *B. cereus* (causes food poisoning)
- Other *Bacillus* species (called as anthracoid bacilli) are common laboratory contaminants.

Bacillus anthracis

Bacillus anthracis is the causative agent of anthrax, an important zoonotic disease transmitted by occupational exposure to infected animals such as cattle and sheep.

Pathogenesis

The pathogenesis of anthrax is mediated by two important virulence factors—

1. **Capsule:** It is polypeptide in nature, and acts by inhibiting phagocytosis
2. **Anthrax toxin:** It has three fragments; edema factor, protective factor and lethal factor.

Clinical Manifestations

B. anthracis is transmitted in three modes—contact, inhalation and ingestion. Accordingly anthrax in humans manifests in three forms.

1. **Cutaneous anthrax** (Hide porter's disease): It is the most common form (95%), characterized by black eschar surrounded by non-pitting called *malignant pustule* (Fig. 13.3)
2. **Pulmonary anthrax**: Wool sorter's disease (as commonly seen in workers of wool factory), characterized by hemorrhagic mediastinitis
3. **Intestinal anthrax** is very rare.

Laboratory Diagnosis

The useful specimens include—pus, sputum, blood, and CSF.

- **Gram stain:** *B. anthracis* appears as long chains of gram-positive bacilli with non-bulging spores, described as bamboo stick appearance
- **McFadyean's reaction:** Shows amorphous purple capsule surrounding blue bacilli (polychrome methylene blue stain)
- **Culture media:** Culture properties useful for identification are:
 - Medusa head appearance colonies on nutrient agar (seen under 10x microscope)
 - Dry wrinkled, nonhemolytic colonies on blood agar

Fig. 13.3. Malignant pustule.



Source: Public Health Image Library, ID# 1934/Centers for Disease Control and Prevention (CDC), Atlanta (with permission).

- Inverted fir tree appearance growth on gelatin stab agar.

TREATMENT

Anthrax

Ciprofloxacin or doxycycline are the drugs of choice, given for:

- 7-10 days for treatment of anthrax
- 60 days for post-exposure prophylaxis (along with anthrax vaccine)

Vaccine

Two important vaccines are available for anthrax.

- Live attenuated, non-capsulated spore vaccine
- Adsorbed (alum precipitated) toxoid vaccine containing the protective factor.

Bacillus cereus Food Poisoning

Bacillus cereus is a normal habitant of soil, also widely isolated from food items. It is an important agent of food poisoning in man; mediated by producing two types of toxins—

1. **Emetic toxin:** causes emetic type of food poisoning

- It is a preformed toxin (like *S. aureus* enterotoxin), that acts immediately after food intake, and therefore the incubation period is short (1–6 hours)
- Associated with the consumption of contaminated fried rice with emetic toxin.

2. **Diarrheal toxin:** causes a diarrheal type of food poisoning. Organism secretes this toxin only after entering the intestine, hence the incubation period is longer (8–16 hours).

OTHER GRAM-POSITIVE BACILLI INFECTIONS

Listeria

Listeria monocytogenes is a food-borne pathogen that can cause serious infections, particularly in neonates (neonatal meningitis and sepsis), pregnant women, and elderly people.

- Laboratory diagnosis involves CSF and blood culture
- Ampicillin is the drug of choice, given for 2–3 weeks in combination with gentamicin.

Actinomycetes

Actinomycetes are a diverse group of gram-positive bacilli arranged in chains or branching filaments. Important genera include:

- *Actinomyces*: They are anaerobe and non-acid fast; produce a clinical condition called actinomycosis, characterized by a painless, slow-growing mass with a cutaneous fistula in the cervicofacial region
- *Nocardia*: They are aerobe and acid-fast; cause pulmonary infection and a subcutaneous infection called actinomycetoma.

EXPECTED QUESTIONS

1. I. Write short notes on:

1. Laboratory diagnosis of diphtheria.
2. Diphtheria vaccine.
3. Clinical forms of anthrax.

2. II. Multiple Choice Questions (MCQs):

1. **Chinese letter pattern is observed in microscopy for _____?**
 - a. *C. diphtheriae*
 - b. *Bacillus anthracis*
 - c. *Clostridium* species
 - d. Mycobacteria
2. **Bamboo stick appearance is observed in microscopy for _____?**
 - a. Mycobacteria
 - b. *Bacillus anthracis*
 - c. *C. diphtheriae*
 - d. *Clostridium* species
3. **Selective medium used for *C. diphtheriae* is _____?**
 - a. Blood agar
 - b. Chocolate agar
 - c. LJ medium
 - d. Potassium tellurite agar

Answers

1. a	2. b	3. d
------	------	------