CORYNEBACTERIUM

Course Outcomes

- On completion of this course, the students will be able to: On completion of this course, the students will be able to: Perform sample collection from bacterial infections area and their diagnosis.

Course Objectives

- History & Introduction
- Classification
- Pathogenesis
- Laboratory diagnosis
- Treatment.

INTRODUCTION

- Corynebacteria / “Coryneform bacteria” – a group of non-spore forming, gram- positive bacilli, tend to be clubbed or irregularly shaped; (*coryne* = club)
- *Corynebacterium diphtheriae* the causative agent of Diphtheria is the major pathogen in this group.
- *Diphtheriods:* Normal commensals in throat, skin and conjunctiva.

HISTORY

- Hippocrates provided the first clinical description of diphtheria in the 4th century B.C.
- Bretonneu (1821), a French army surgeon, described the unique clinical characteristics of the disease, and used the term ‘dipht`erie’ to signify the tough leathery pseudomembrane that occurs in oropharynx and some times in nasopharynx;

  (*diphtheros* = leather)

HISTORY

- The bacterium that caused diphtheria was first described by Klebs in 1883, and was cultivated by Loeffler in 1884, who applied Koch's postulates and properly identified *Corynebacterium diphtheriae* as the agent of the disease.
- In 1884, Loeffler concluded that *C. diphtheriae* produced a soluble toxin, and thereby provided the first description of a bacterial exotoxin.
- Roux and Yersin (1888) discovered the diphtheria exotoxin and established its pathogenic effects.
- The antitoxin was described by von Behring(1890).

CORYNEBACTERIUM DIPHTHERIAE

MORPHOLOGY

- Slender Gram-positive rods, pleomorphic; easily decolousised;
- 0.6-0.8µ diameter and 3-6 µ length;
- Irregular swelling at one or both ends (‘club shaped’);
- Non-capsulate, Non-sporing and nonmotile
- Granules containing polymetaphosphate are seen in the cells;
- Take up bluish purple color against lightly stained cytoplasm, when stained with Loeffler’s Methylene Blue, and hence called ‘Metachromatic granules’;
- Also called, ‘volutin granules’ or ‘Babes Ernst granules’;
- They are often situated at poles- ‘polar bodies’

MORPHOLOGY

- Special stains for demonstrating the granules :
  - Albert's stain
  - Neisser's stain
  - Ponder's stain
- The bacilli are arranged in pairs, palisades or small groups; the bacilli lie at various angles to each other, resembling the letters, V or L;
- This is called, "Chinese letter pattern" or "cuneiform pattern";

CULTURAL CHARACTERISTICS

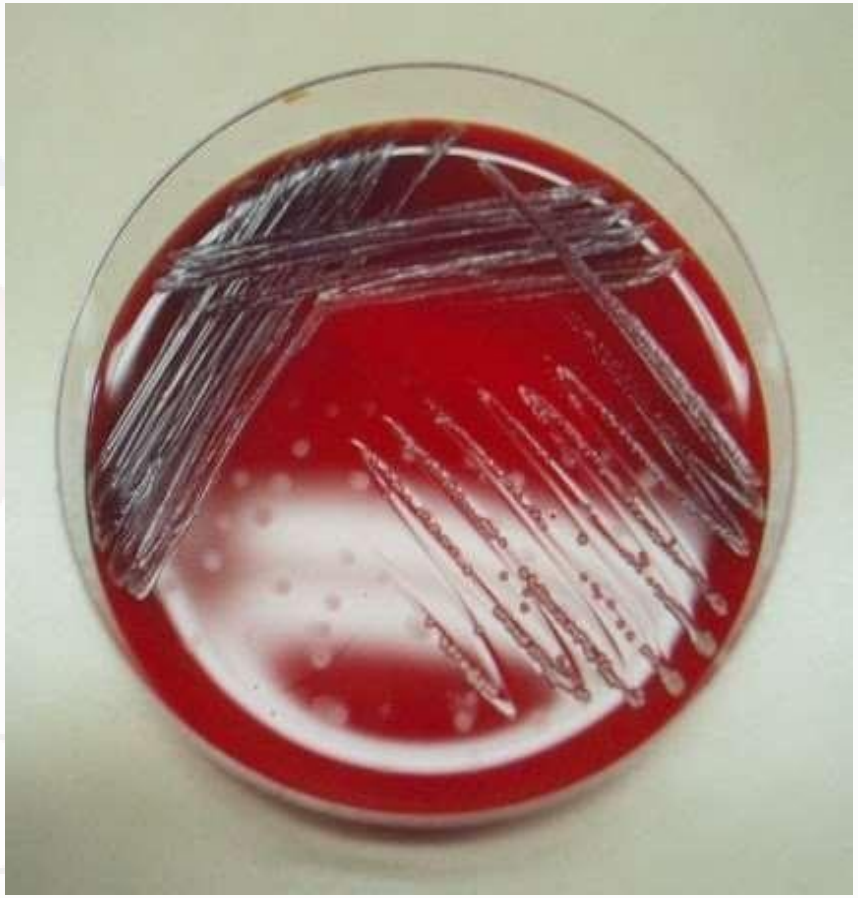
- Aerobe and facultative anaerobe;
- Optimum temperature is 37^0C
- Growth scanty on ordinary media;
- **Enrichment with**: blood, serum or egg is necessary for good growth;
- Potassium tellurite(0.04%) acts as a *'selective agent',* as it inhibits growth of most oral commensals and retards the growth of *Candida albicans* and *S.aureus;*

MEDIA FOR CULTIVATION

- Blood agar
- Loeffler’s serum slope
- Tellurite blood agar
- Hoyle’s tellurite lysed-blood agar
- Tinsdale’s medium (cystine added to tellurite containing agar)

COLONY CHARACTERISTICS

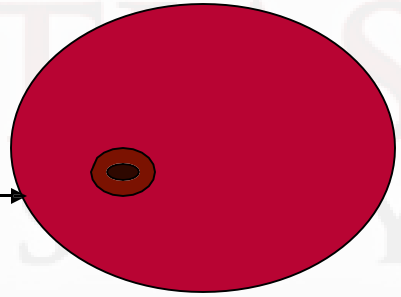
- Blood agar : small, granular and gray with irregular edges; Hemolysis may or may not present;
- Loeffler's serum slope:
  - Very rapid growth;
  - Colonies in 6-8 hrs
  - Initially circular white opaque colonies and acquire yellowish tint on incubation



COLONY CHARACTERISTICS

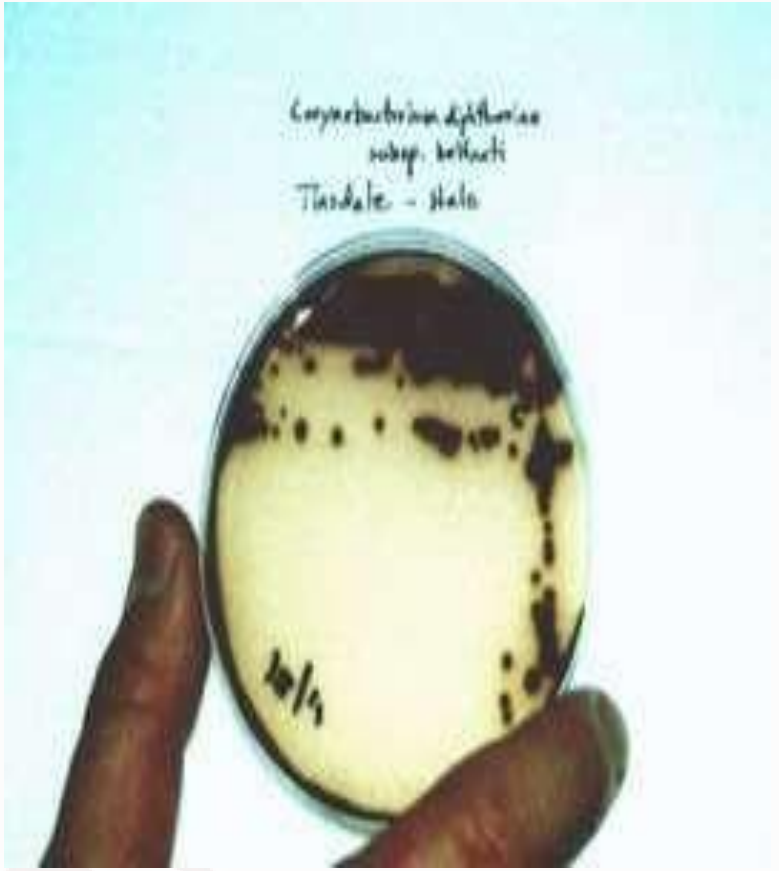
- Tellurite blood agar:
  - Growth slow; colonies seen after 48 hrs;
  - The colonies are brown to black with a brown-black halo because the tellurite is reduced to metallic tellurium;
  - Staphylococcus also produce such colonies

A diagrammatic representation →



COLONY CHARACTERISTICS

- Tinsdale's medium (also contain cystine in addition to tellurite):
  - **Grey black colonies with dark brown haloes** indicate C.diphtheriae and C.ulcerans (these contain cystinase)



BIOCHEMICAL REACTIONS

- Hiss serum sugars – for testing fermentation reactions;
- Ferment- *glucose, galactose, maltose and dextrose*; but not *lactose, sucrose, mannitol;*
- Proteolytic activity is absent;
- Do not hydrolyse urea;
- Do not form phosphatase;
- Produce cystinase (halo on Tinsdale's medium)

ANTIGENIC STRUCTURE

- Serotyping : Antigenically heterogenous
  - gravis: 13 types
  - intermedius : 4 types
  - mitis : 40 types

VIRULENCE FACTORS

- Virulent strains of diphtheria bacilli produce a very powerful exotoxin.
- The ‘virulence’ of diphtheria bacilli is due to their capacity to-
  - Establish infection and growing rapidly
  - Quickly elaborate an exotoxin
- Avirulent strains are common among convalescents , contacts and carriers, particularly those with extra-faucial infection

DIPHTHERIA TOXIN

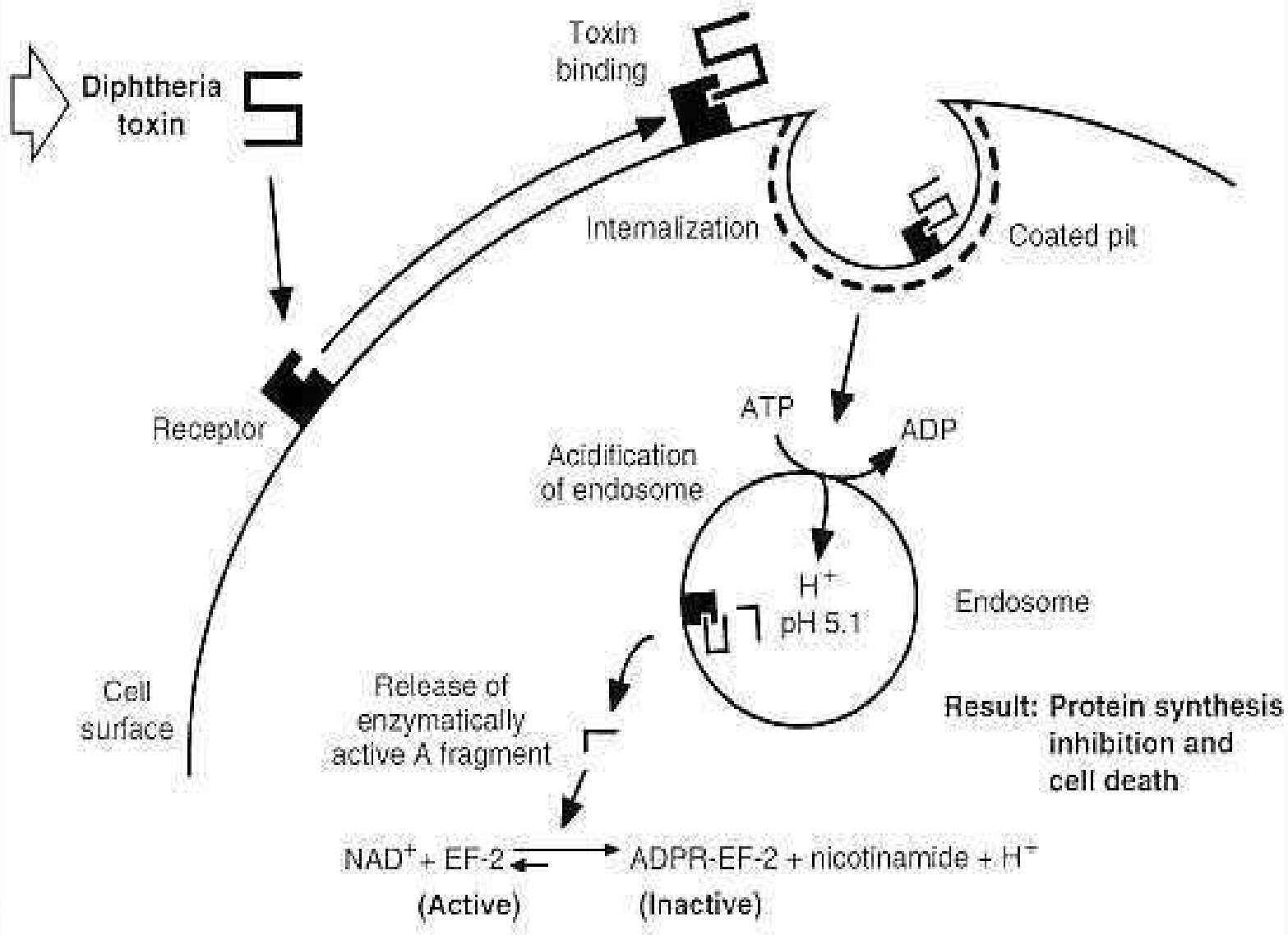
- The pathognomonic effects are due to the toxin;
- Almost all the gravis and intermedius strains and 80-85% of mitis strains are toxigenic
- Toxin is a protein;
- Two fragments, A and B;
- Extremely potent :
  - 0.1 µg lethal to guinea pig

Toxin – mechanism of action

- Fragment B : binds to a cell surface receptor and helps in transport of toxin into the cell;
- After entering the cell, A subunit is released ;
- A subunit catalyses the transfer of ‘adenosine diphosphate ribose (ADPR)’ from NAD+
- ADPRbinds with the elongation factor EF 2
- “ADPR-EF2” complex is inactive

 protein synthesis stops abruptly

 necrotising and neurotoxic effects of the toxin;


Diphtheria toxin
Toxin binding
Internalization
Coated pit
Receptor
ATP
ADP
Acidification of endosome
H+
pH 5.1
Endosome
Cell surface
Release of enzymatically active A fragment
Result: Protein synthesis inhibition and cell death
NAD+ + EF-2
(Active)
ADPR-EF-2 + nicotinamide + H+
(Inactive)

Reference

1. Dr. C P. Baveja, Text book of Microbiology for MLT, Second Edition, Arya Publication, 2017.
2. Dir. Prof. C P Baveja, Textbook of Microbiology, 4th edition, Arya Publication, 2013.

THANK YOU