



CORYNEBACTERIA & MORAXELLA

Istar Dolapci, MD, PhD

Ankara University School of Medicine

Department of Medical Microbiology

Learning Objectives

- Identify the distinguishing characteristics of the species within the genera *Corynebacteria* and *Moraxella*
- Explain the media used for culture for this group of organisms, including the chemical principle and composition
- Correlate patient signs and symptoms with laboratory data, and identify the most likely etiologic agent

CONTENTS

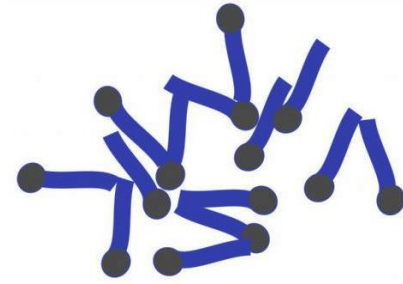
(Description Headings)

- ***Corynebacterium* spp.**
 - Physiology and Structure
 - Pathogenesis and Immunity
 - Epidemiology
 - Clinical Diseases
 - Laboratory diagnosis
 - Treatment, prevention & control
- ***Moraxella* spp.**

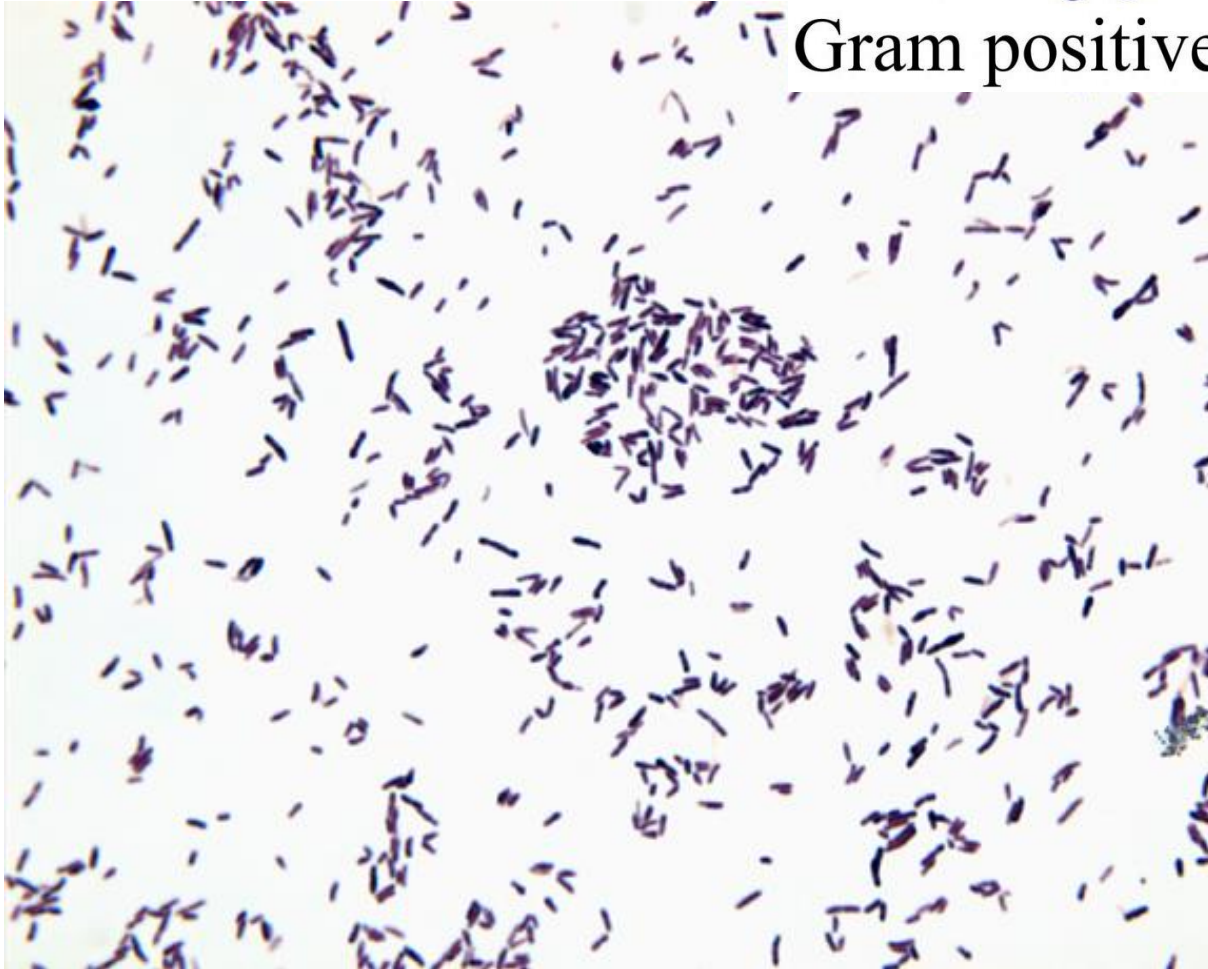
Corynebacterium spp.

- A large, heterogeneous collection of more than 100 species and subspecies...
- They have a cell wall with short-chain mycolic acid (in most species)
 - But they are not acid-fast!
 - They are stained with Gram stain

Corynebacterium spp.



Gram positive club shaped



Corynebacterium spp.

- Aerobic or facultative anaerobic
- Gram positive rods
- Nonmotile
- Catalase positive
- Ferment carbohydrates, producing lactic acid by product (most species but not all)
- Grow well on common laboratory media (many species)
 - Some species require supplementation of media with lipids for good growth

Corynebacterium spp.

- They normally colonize the skin, upper respiratory tract, gastrointestinal tract, and urogenital tract in humans
 - Can function as opportunistic pathogens
- Few are associated with human disease
 - e.g. *Corynebacterium diphtheriae*

Corynebacterium diphtheriae

Physiology and Structure

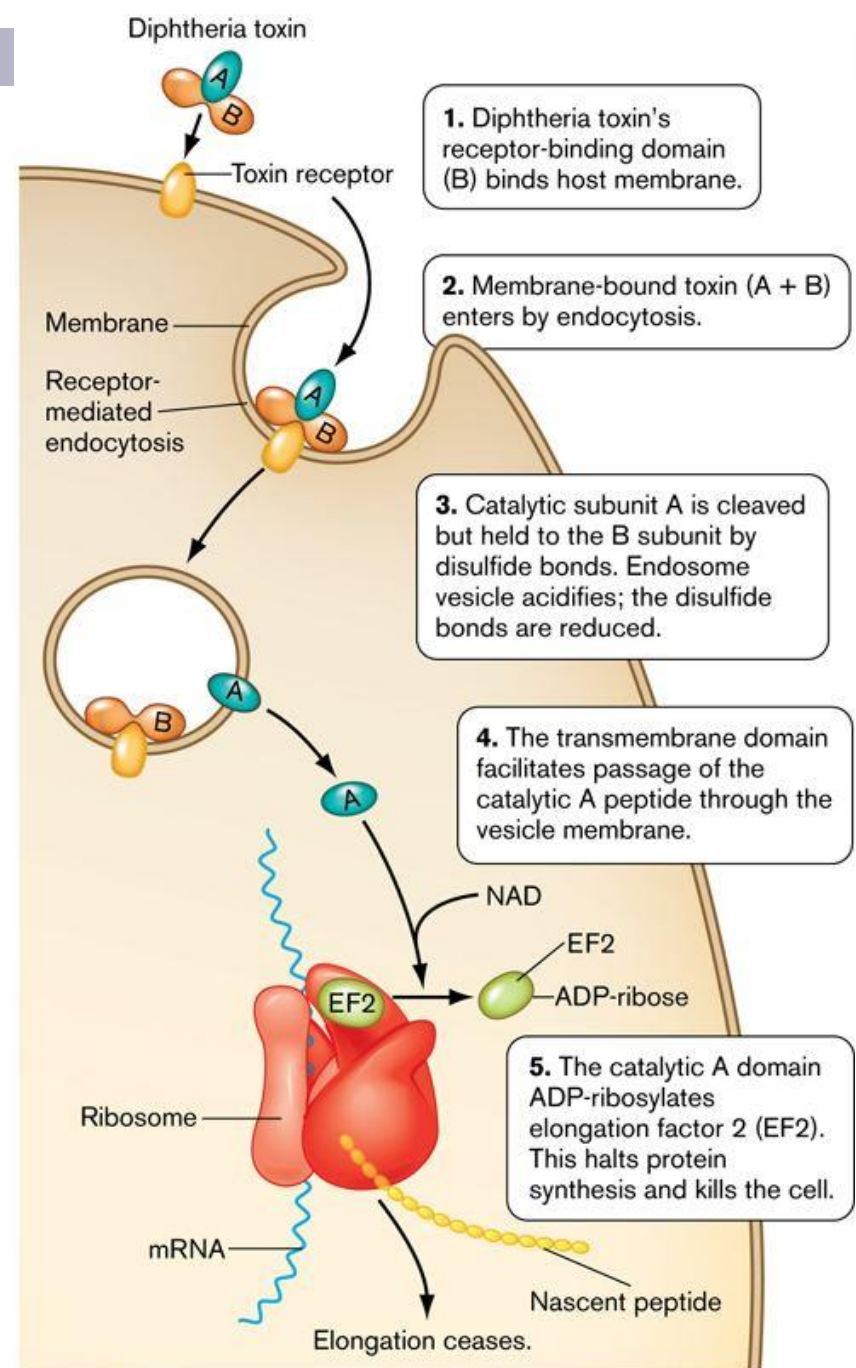
- *C. diphtheriae* is an irregularly staining, pleomorphic rod
 - 0.3-0.8 x 1.0-8.0 μm
- Grow on blood agar
 - Large, 1-3 mm colonies
- Four biotypes: *belfanti*, *gravis*, *intermedius*, and *mitis*
 - Most diseases are caused by biotype *mitis*

Corynebacterium diphtheriae

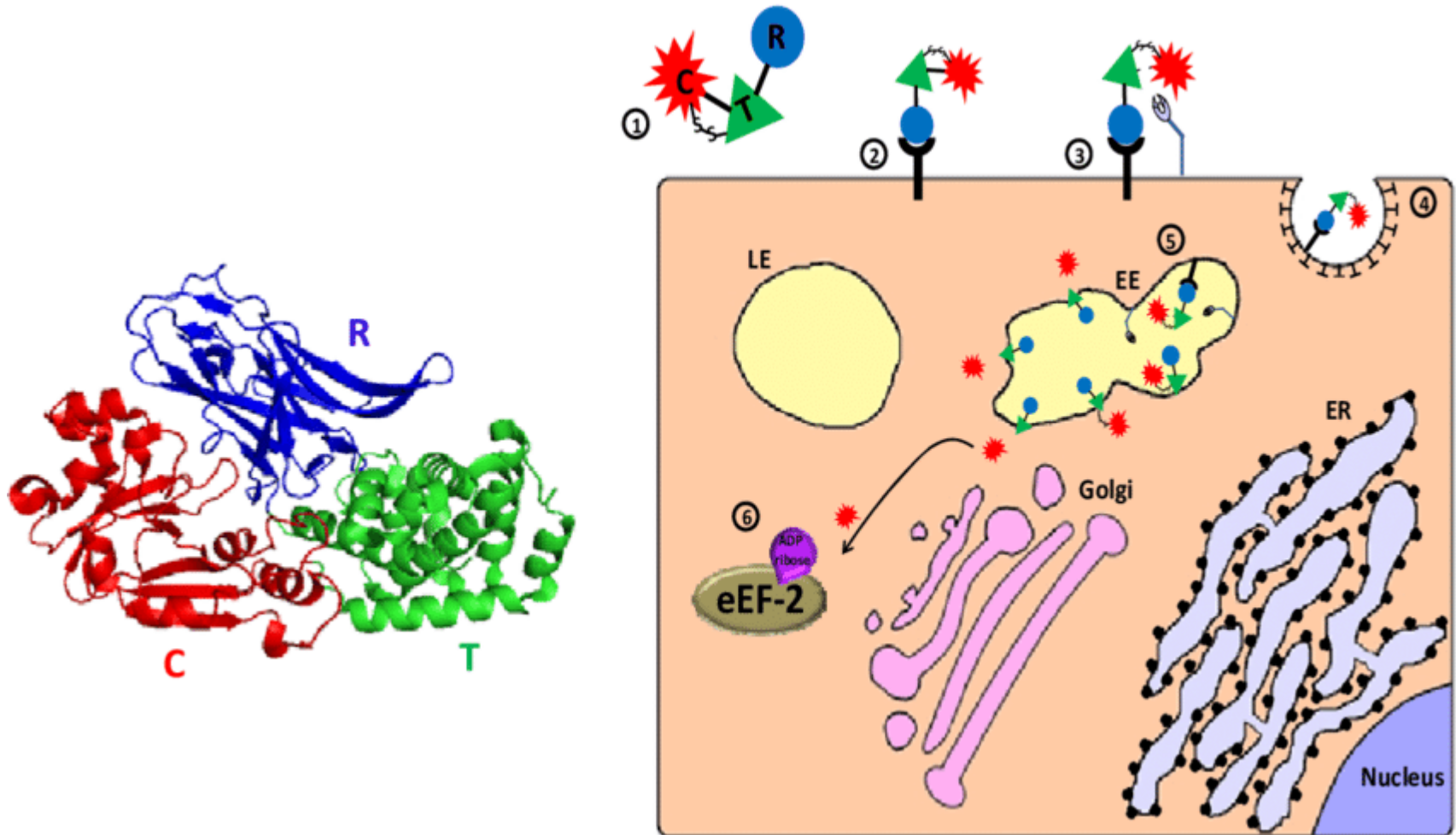
Pathogenesis and Immunity

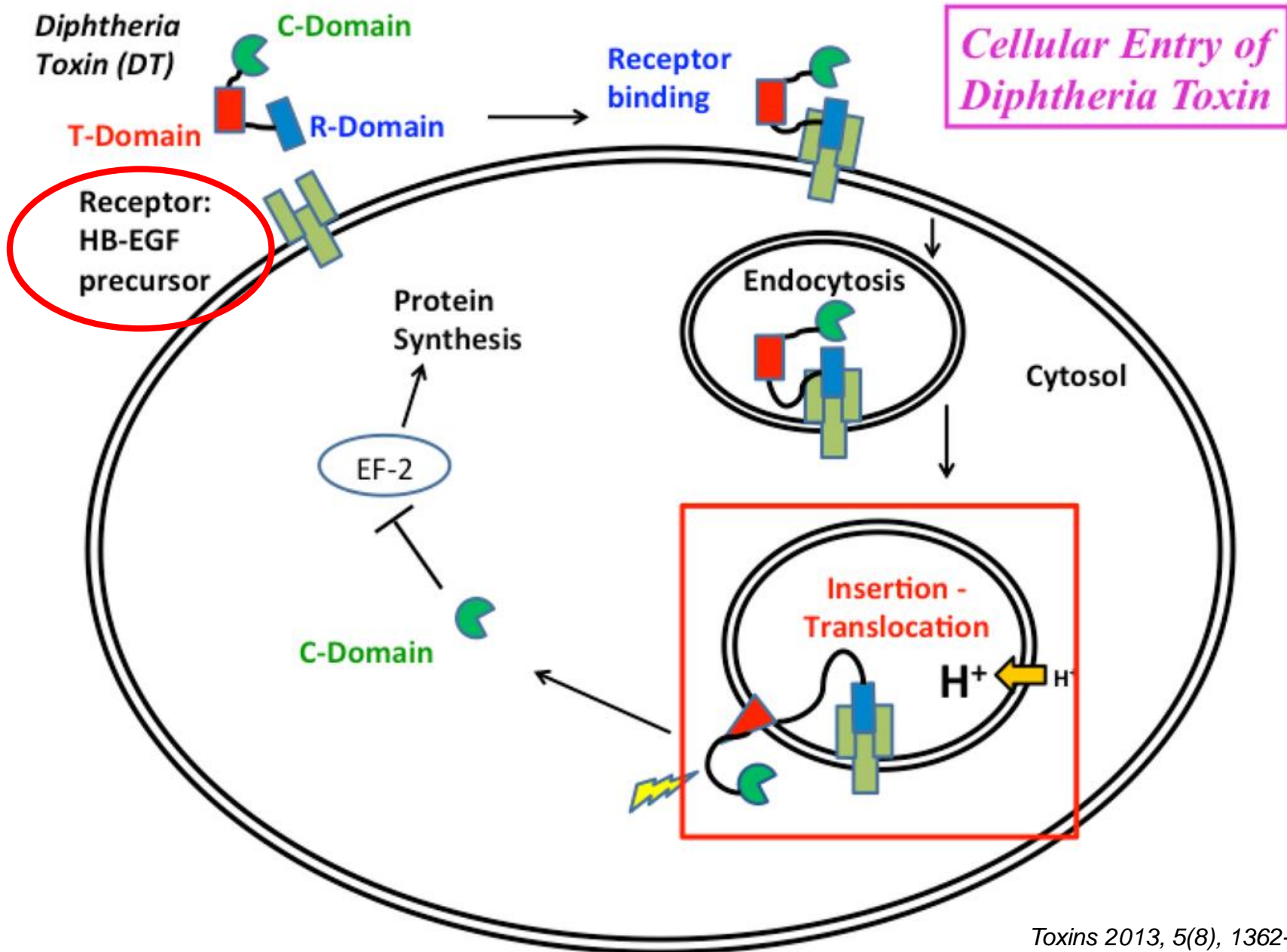
■ Major virulence factor: Diphtheria toxin

- This 58,300-Da protein is an example of the classic **A-B exotoxin**
- The exotoxin is coded by **tox gene**
- The gene is carried by a lysogenic bacteriophage, **β-phage**



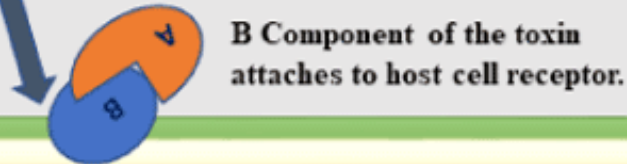
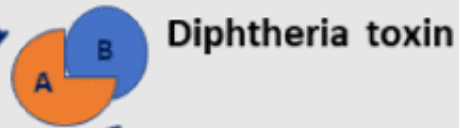
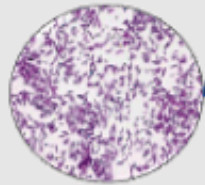
Diphtheria Toxin



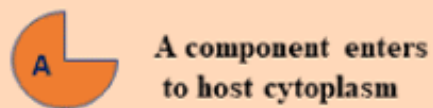


Diphtheria Toxin

Corynebacterium diphtheriae



Cell membrane



Inactivates elongation factor 2

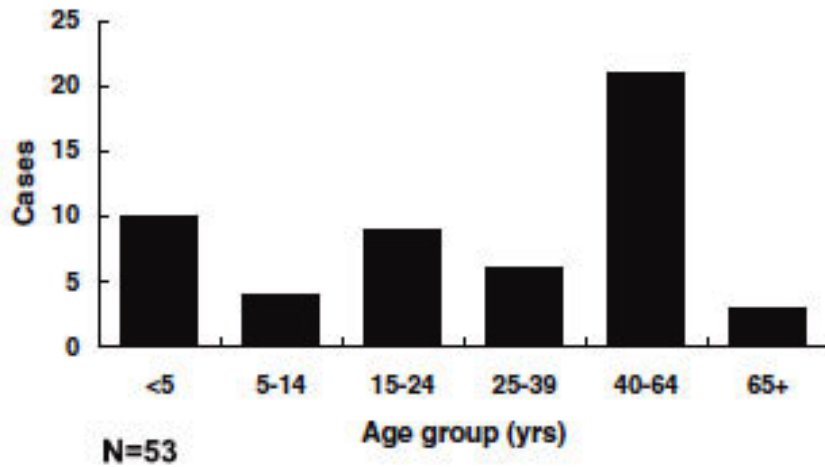
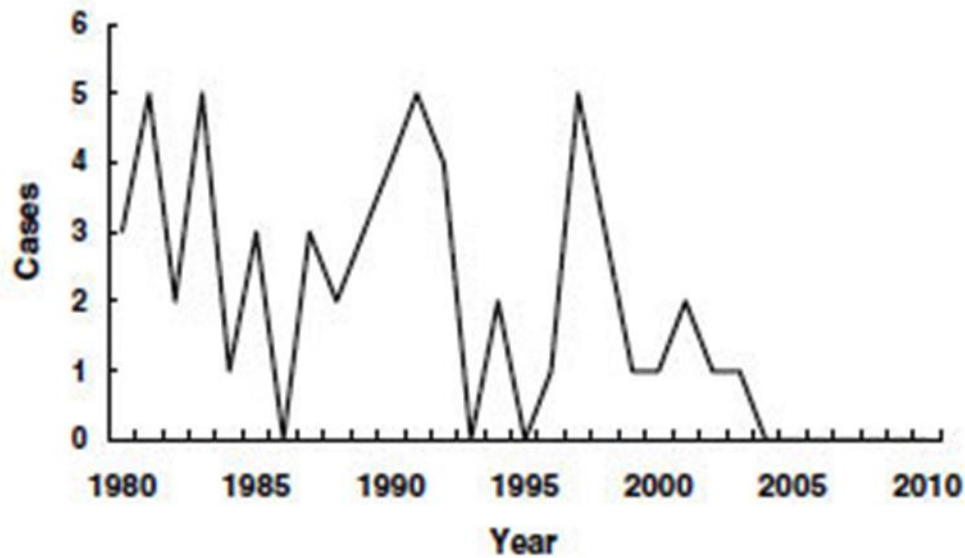
Prevents Protein
synthesis by
Ribosome

CELL DEATH

Corynebacterium diphtheriae

Epidemiology

- Worldwide distribution maintained in asymptomatic carriers and infected patients
- **Humans** are the **only known reservoir** for this organism
- Spread person to person by exposure to respiratory droplets or skin contact



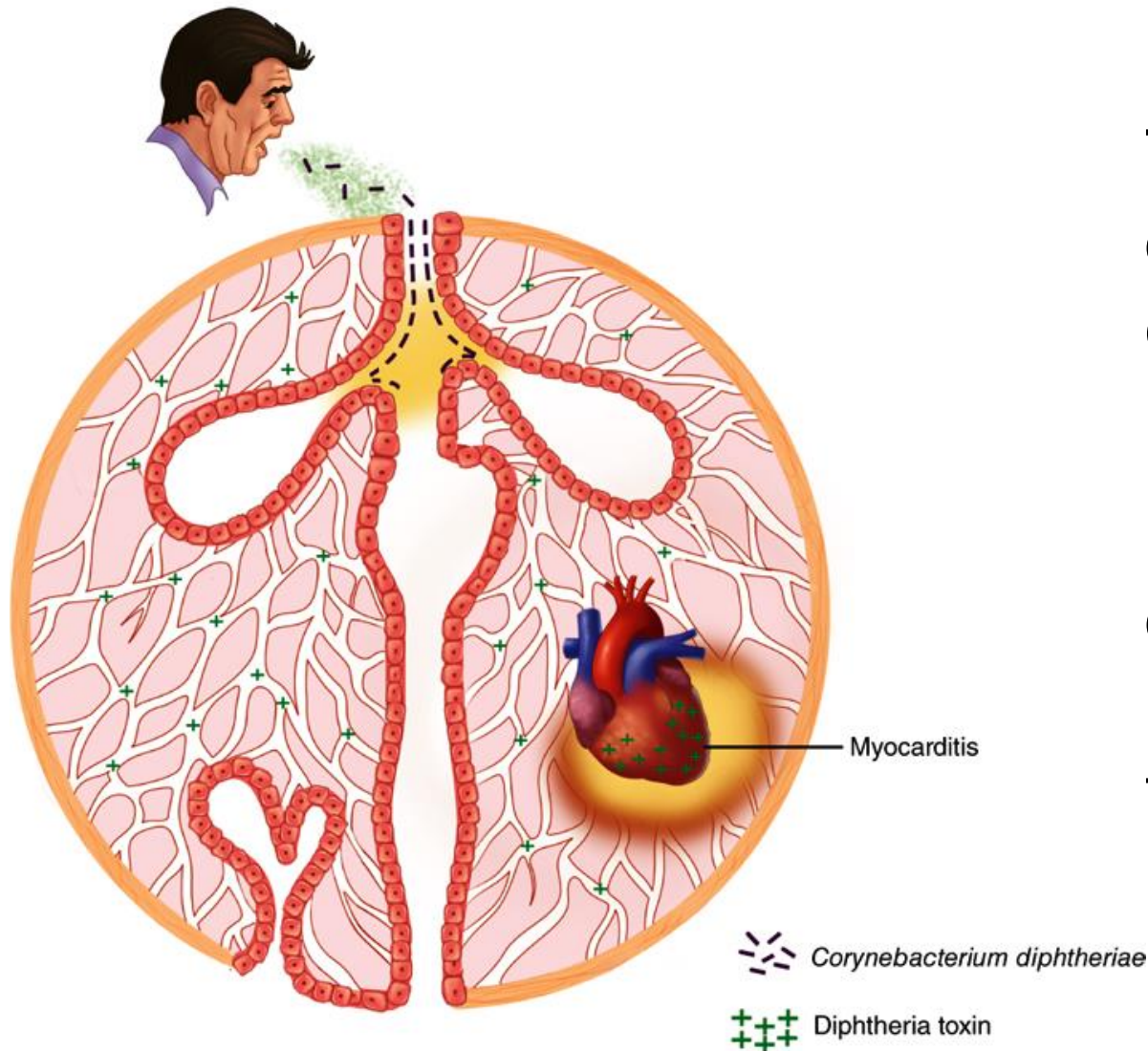
Corynebacterium diphtheriae

Clinical Diseases

The clinical presentation of diphtheria is determined by the;

1. Site of infection,
2. Immune status of the patient, and
3. Virulence of the organism
 - Toxigenic strains
 - Nontoxigenic strains

Diphtheria



The organism
does not need to
enter the blood to
produce disease
&
does not typically
invade deep
tissues



Corynebacterium diphtheriae

Clinical Diseases

1. Respiratory Diphtheria
2. Cutaneous Diphtheria

Respiratory Diphtheria

- Incubation period: 2- to 4-days
- Organisms multiply locally on epithelial cells in the pharynx
- Initially cause localized damage **as a result of exotoxin activity**

Respiratory Diphtheria

DIPHTHERIA



Experience these **SYMPTOMS?** Call your doctor.



sore throat



low fever



swollen glands
in the neck

The onset is sudden,
with;

- malaise,
- sore throat,
- **exudative pharyngitis**, and
- a low-grade fever

Respiratory Diphtheria

- A thick **pseudo membrane**
 - composed of bacteria, lymphocytes, plasma cells, fibrin, and dead cells
 - can cover the tonsils, uvula, and palate
 - firmly adheres to the underlying tissue
 - is difficult to dislodge without making the tissue bleed
 - **unique to diphtheria**



Respiratory Diphtheria

- Complications of diphtheria are attributable to effects of the toxin
 - The toxin, when absorbed, affects organs and tissues distant from the site of invasion
- The most frequent complications of diphtheria are **myocarditis** and **neuritis**
 - If myocarditis occurs early, it is often fatal
 - Neuritis most often affects motor nerves and usually resolves completely
 - Paralysis of the soft palate, eye muscles, limbs, and diaphragm etc.

Cutaneous Diphtheria

- is acquired through skin contact with other infected persons
- papule develops first and then evolves into a **chronic, nonhealing ulcer**
 - sometimes covered with a grayish membrane





Laboratory Diagnosis

Microscopy

Culture

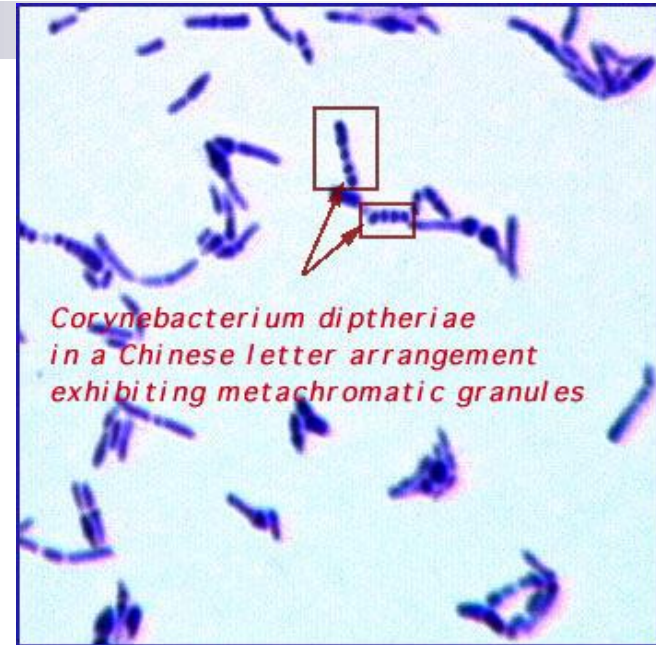
Identification

Toxigenicity testing

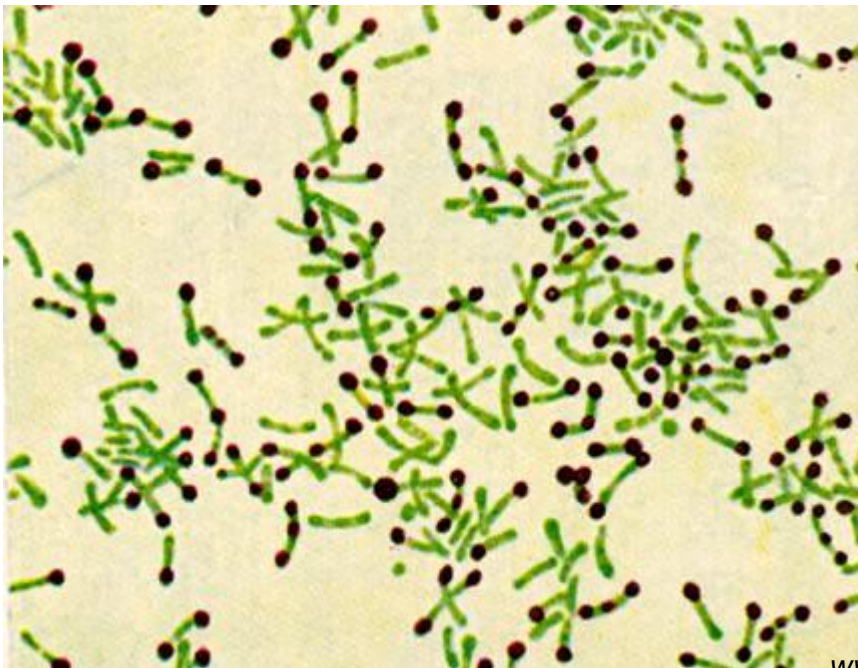
Laboratory Diagnosis

Microscopy

- Metachromatic granules
 - is not specific to *C. diphtheriae*
- Unreliable...



↓
Methylene blue stain



→ Albert's stain

Laboratory Diagnosis

Culture

- Specimen: from both the nasopharynx and throat
- Culture media:
 - A nonselective, enriched blood agar plate
 - A selective medium
 - Cysteine-tellurite blood agar [CTBA],
 - Tinsdale medium,
 - Colistin-nalidixic agar [CNA]

Laboratory Diagnosis

Culture

- Cysteine-tellurite blood agar [CTBA]
 - A long shelf life
 - Inhibits some strains of *C. diphtheria*
- Tinsdale medium
 - **The best medium** for recovering *C. diphtheriae* in clinical specimens
 - A short shelf life and requires addition of horse serum
- Colistin-nalidixic agar [CNA]
 - Commonly used for the selective recovery of gram-positive bacteria
 - A **practical alternative** medium



Laboratory Diagnosis



Corynebacterium diphtheriae on tellurite agar

Laboratory Diagnosis

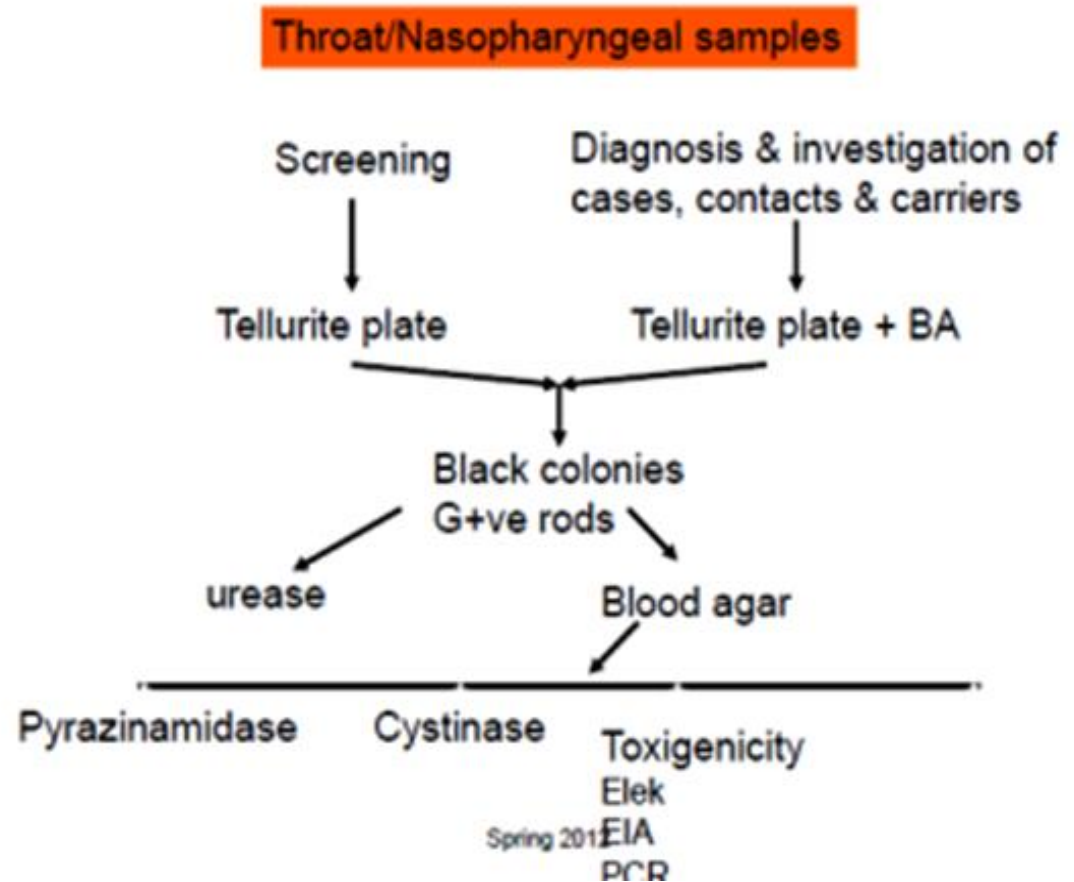


***Corynebacterium diphtheriae* in Tinsdale Agar medium**
Look for black colonies with brown halo

Laboratory Diagnosis

Identification

- Presence of cystinase
- Absence of pyrazinamidase

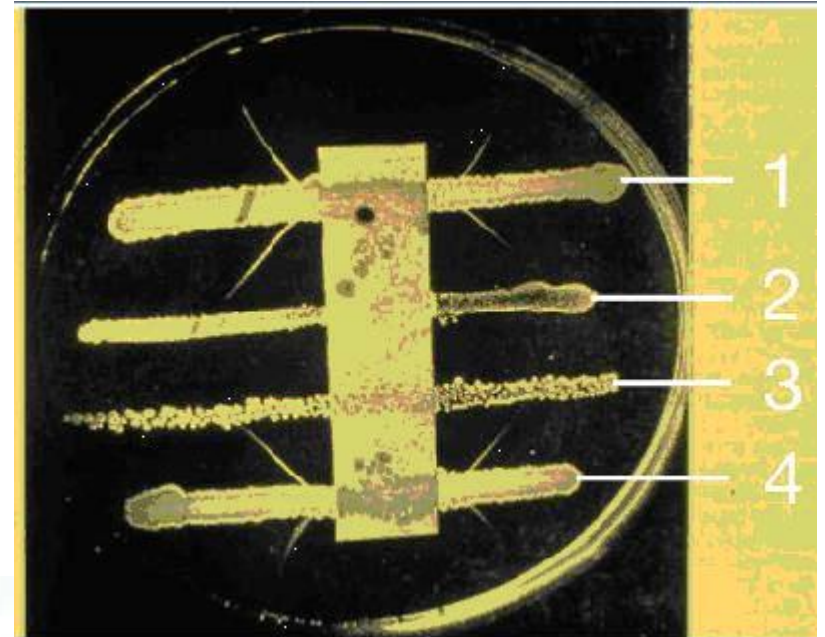


Spring 2011

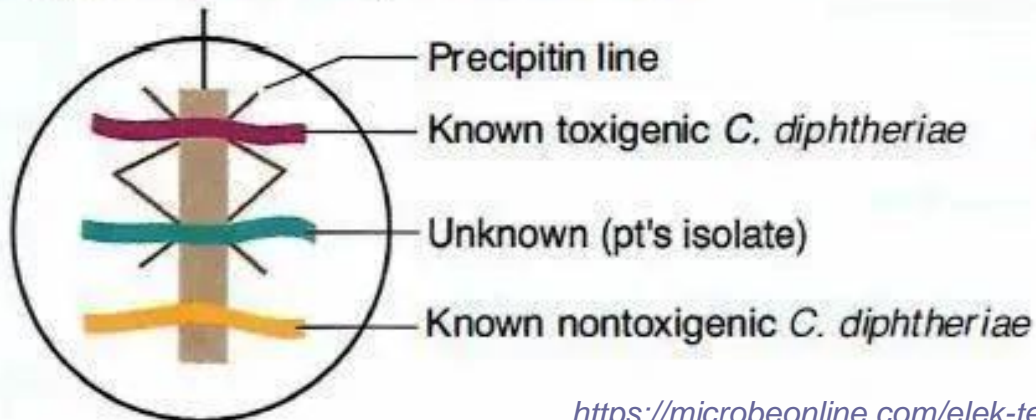
Laboratory Diagnosis

Toxigenicity testing

- Elek test
- PCR
- ELISA



Filter paper strip with *C. diphtheriae* antitoxin

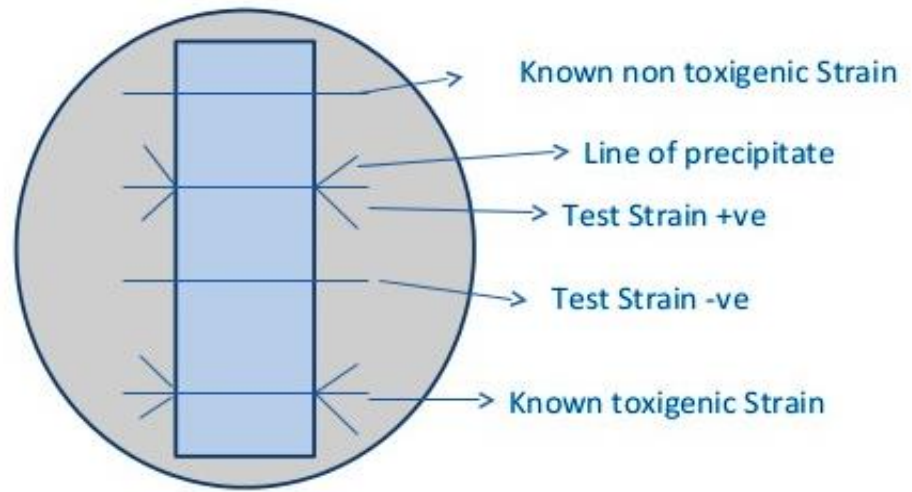


<https://microbeonline.com/elek-test-principle-procedure-results/>

<http://www.rahulgladwin.com/noteblog/bacteriology/what-is-an-eleks-test.php>

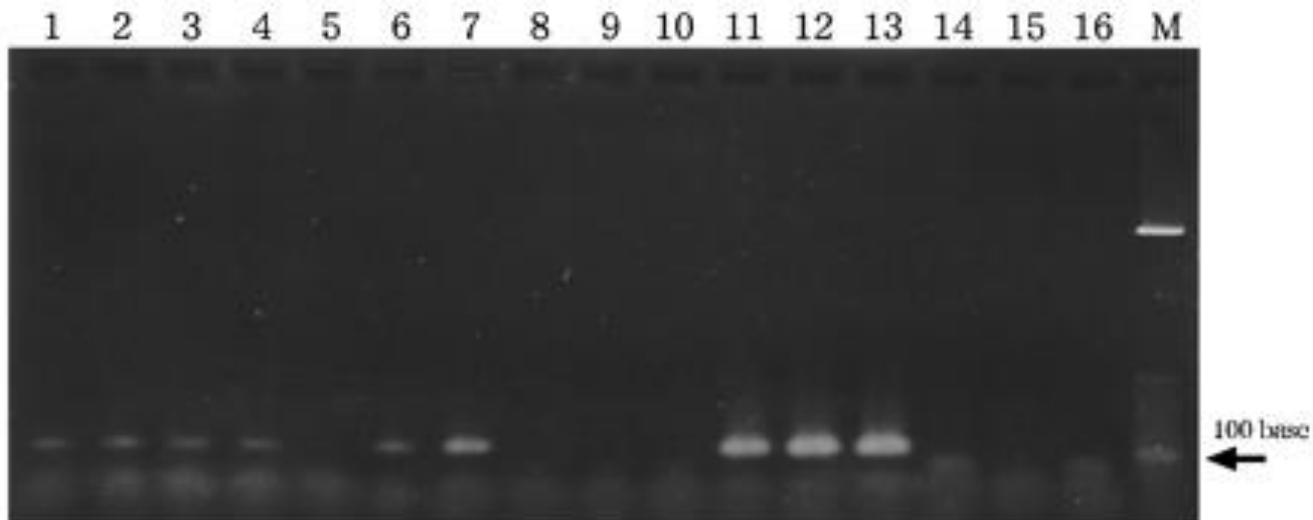
Elek Test

- A filter paper disk containing antitoxin (10 IU/disk) is placed on an agar plate
- The cultures to be tested for toxigenicity are spot inoculated 7–9 mm away from the disk
- After 48 hours of incubation, the antitoxin diffusing from the paper disk has precipitated the toxin diffusing from toxigenic cultures and has resulted in **precipitin bands** between the disk and the bacterial growth

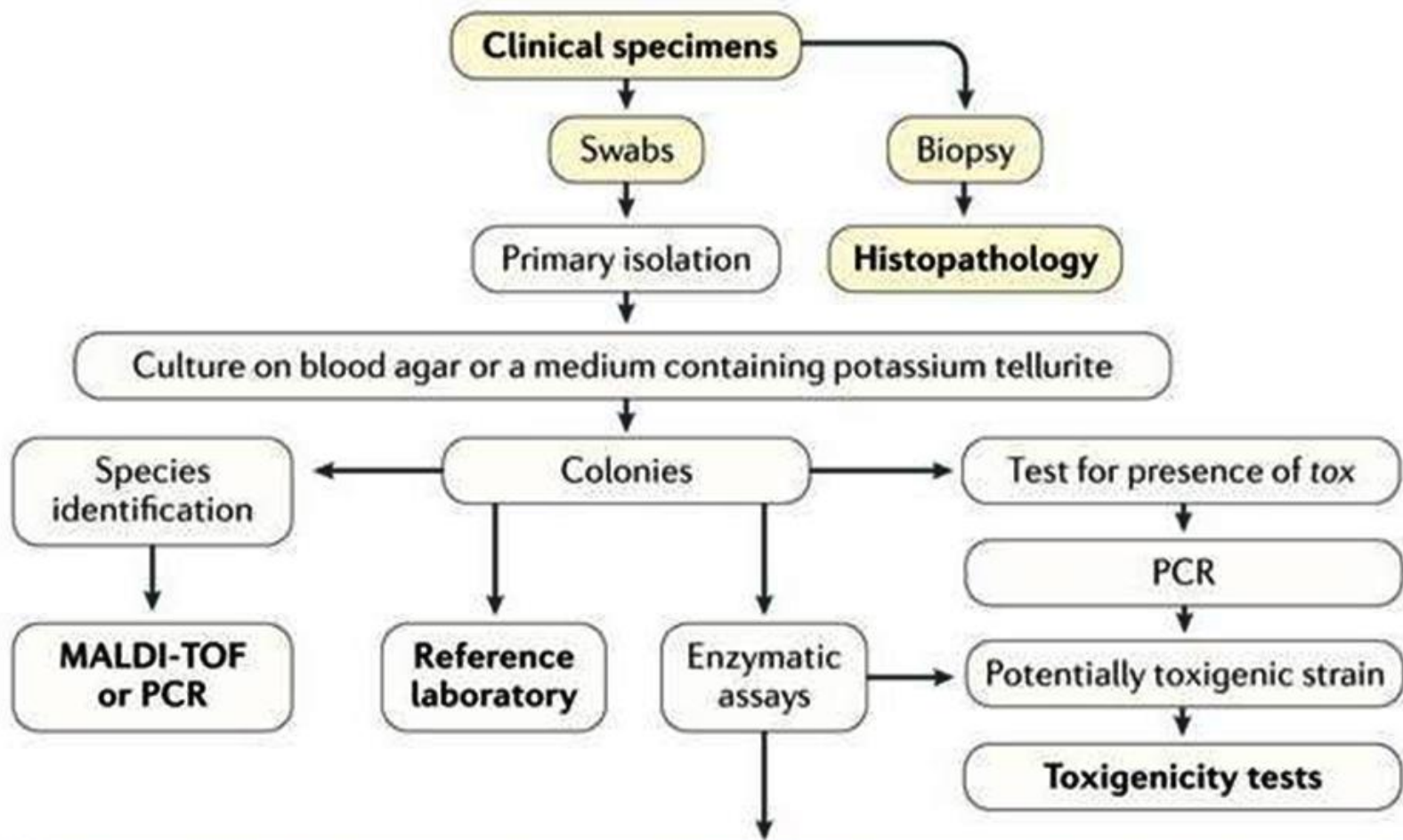


PCR

- An alternative method is the detection of the exotoxin gene by a polymerase chain reaction (PCR)–based nucleic acid amplification method
- This test can detect the *tox* gene in clinical isolates and directly in clinical specimens



Result of PCR analysis of the *C. diphtheriae* *tox* gene, using DNA templates;
JOURNAL OF CLINICAL MICROBIOLOGY, 2000, 38: 2400-02



Nitrate positive

- Biotypes of *C. diphtheriae* except belfanti

Nitrate negative

- *C. diphtheriae* except belfanti
- *C. ulcerans*
- *C. pseudotuberculosis*

Urease positive

- *C. ulcerans*
- *C. pseudotuberculosis*

Urease negative

- *C. diphtheriae*

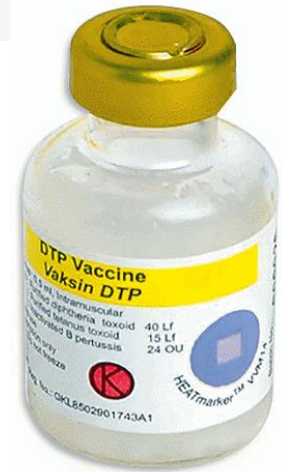
Pyrazinamidase negative

- *C. diphtheriae*
- *C. ulcerans*
- *C. pseudotuberculosis*

Treatment

- Diphtheria antitoxin
 - early administration is important
- Penicillin or erythromycin
 - can eliminate *C. diphtheriae* and terminate toxin production
- Immunization with toxoid
 - to enhance the production of protective antibodies

Prevention & Control



- DPT vaccine
 - At ages 2, 4, 6, 15 to 18 months, and 4 to 6 years
 - Booster vaccinations with diphtheria toxinoid, every 10 years

- People in close contact with patients who have documented diphtheria are at risk for acquiring the disease
 - Nasopharyngeal specimens for culture should be collected from all close contacts and antimicrobial prophylaxis with erythromycin or penicillin should be started immediately

Other *Corynebacteria* Species

Nonlipophilic *Corynebacteria* spp.

- *Corynebacterium ulcerans*
- *Corynebacterium pseudotuberculosis*
- *Corynebacterium minutissimum*
- *Corynebacterium amycolatum*

Lipophilic *Corynebacteria* spp.

- *Corynebacterium jeikeium*
- *Corynebacterium urealyticum*

Corynebacterium ulserans* & *Corynebacterium pseudotuberculosis

Organism	Clinical Features	Epidemiologic Features	Treatment
<i>C. ulserans</i>	Respiratory diphtheria	Normal microbiota: Humans and cattle Mode of transmission: Uncertain Zoonosis	No definitive guidelines. All are susceptible to vancomycin and teicoplanin
<i>C. pseudotuberculosis</i>	Lymphadenitis, ulcerative lymphangitis, abscess formation, respiratory diphtheria	Normal microbiota: Animals such as sheep, goats, and horses Zoonosis: Close animal contact, but infections in humans are rare	No definitive guidelines. All are susceptible to vancomycin and teicoplanin

Corynebacterium jeikeium* & *Corynebacterium urealyticum

Organism	Clinical Features	Epidemiologic Features	Treatment
<i>C. jeikeium</i>	Opportunistic infections; bacteremia	Immunocompromised patients at increased risk	Vancomycin
<i>C. urealyticum</i>	Urinary tract infections, including pyelonephritis; bacteremia	Risk factors include immunosuppression, underlying genitourinary disorders, antecedent urologic procedures, prior antibiotic therapy	Vancomycin

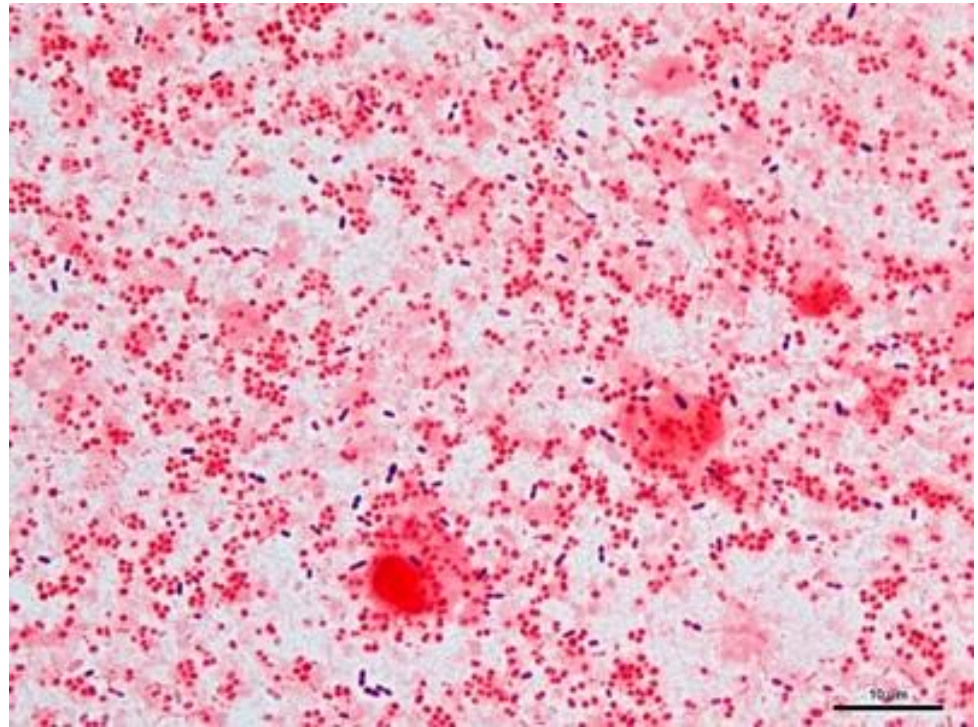
Pseudomonas and related bacteria

- Nonfermentative rods
 - Opportunistic pathogens of plants, animals, and humans

- Most clinically significant isolates are members of five genera:
 1. *Pseudomonas*,
 2. *Burkholderia*,
 3. *Stenotrophomonas*,
 4. *Acinetobacter*, and
 5. ***Moraxella***

- The most important species is *M. catarrhalis*
 - *strictly aerobic*,
 - *oxidase-positive*,
 - *gram negative diplococci*
 - considered to be a part of the normal oropharyngeal flora
 - can become pathogenic
- The cellular morphology of this species is more similar to that of *Neisseria* spp. than that of the other *Moraxella* spp.

Moraxella spp.



http://hit-microscopeweb.hc.msu.edu/Microbiology/Lab/S1-Resp_Image_11.html

***Moraxella* spp.**

When a breakdown of the patient's mucosal or epidermal defensive barriers occurs;

- Bronchitis
- Bronchopneumonia
 - in elderly patients with chronic pulmonary disease
- Sinusitis
- Otitis

Moraxella spp.

- rarely cause infection...
- low virulence...
- contaminants... ?



Moraxella spp./ Cultivation

- 5% Sheep blood
- Chocolate agar
- MacConkey agar
- Commercial blood culture systems
- Nutrient broths
 - Such as thioglycollate and brain-heart infusion
- 35°C in carbon dioxide or ambient air for a minimum of 48 hours
- Clinically important isolates should be sent to a reference laboratory for definitive identification

Moraxella spp.

Organism	Therapeutic Options	Potential Resistance to Therapeutic Options
<i>Moraxella</i> spp.	No definitive guidelines; generally susceptible to penicillins and cephalosporins	Beta-lactamase-mediated resistance to penicillins common

Moraxella spp.

Organism	Habitat (Reservoir)	Mode of Transmission	Spectrum of Disease and Infections
<i>Moraxella nonliquefaciens</i> , <i>Moraxella lacunata</i> , <i>Moraxella osloensis</i> , <i>Moraxella lincolnii</i> , <i>Moraxella canis</i> , <i>Moraxella atlantae</i>	Normal human microbiota that inhabit mucous membranes covering the nose, throat, other parts of the upper respiratory tract, conjunctiva, and, for some species (i.e., <i>M. osloensis</i>), the urogenital tract; may also colonize the skin	Infections are rare; when they occur, they are probably caused by the patient's endogenous strains; person-to-person transmission may be possible, but this has not been documented	Eye infections, bacteremia, endocarditis, septic arthritis, and, possibly, respiratory infections

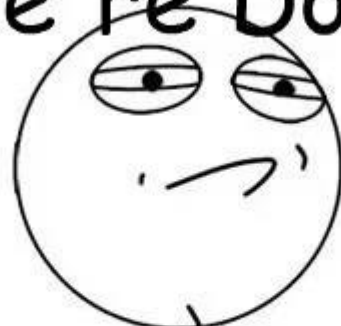
References

- Medical Microbiology; Murray, Rosenthal, Pfaller; 7th Ed; Elsevier Saunders; 2013
- Jawetz, Melnick & Adelberg's Medical Microbiology; Brooks G, Carroll KC, Butel J, Morse S (Eds); 27th Ed; McGraw Hill Lange; 2016
- Sherris Medical Microbiology; 6th Ed; Ryan KJ, Ray CG; McGraw Hill Education; 2014

THE END

THANKS FOR LISTENING 😊

We're Done.



Questions?