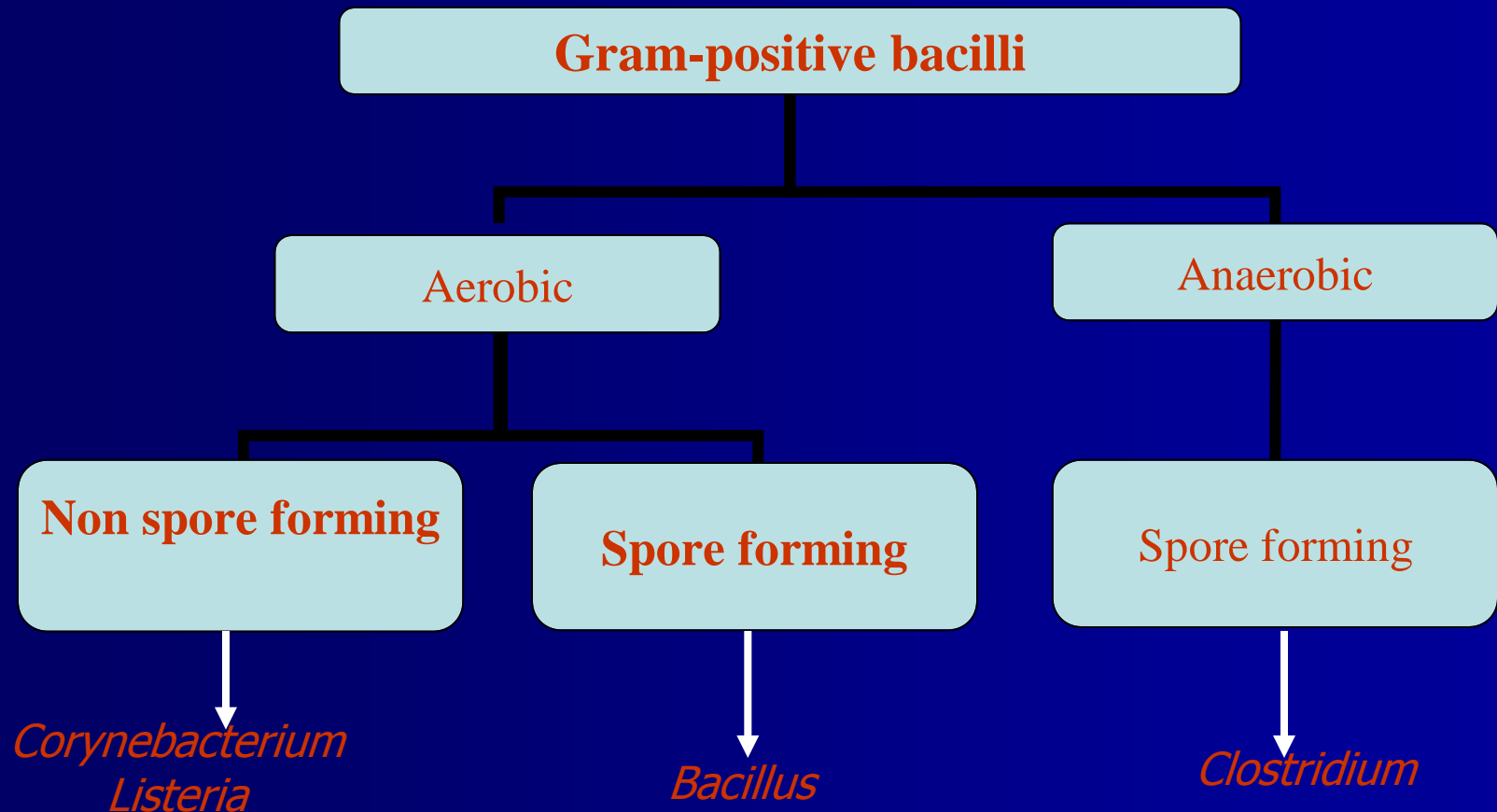
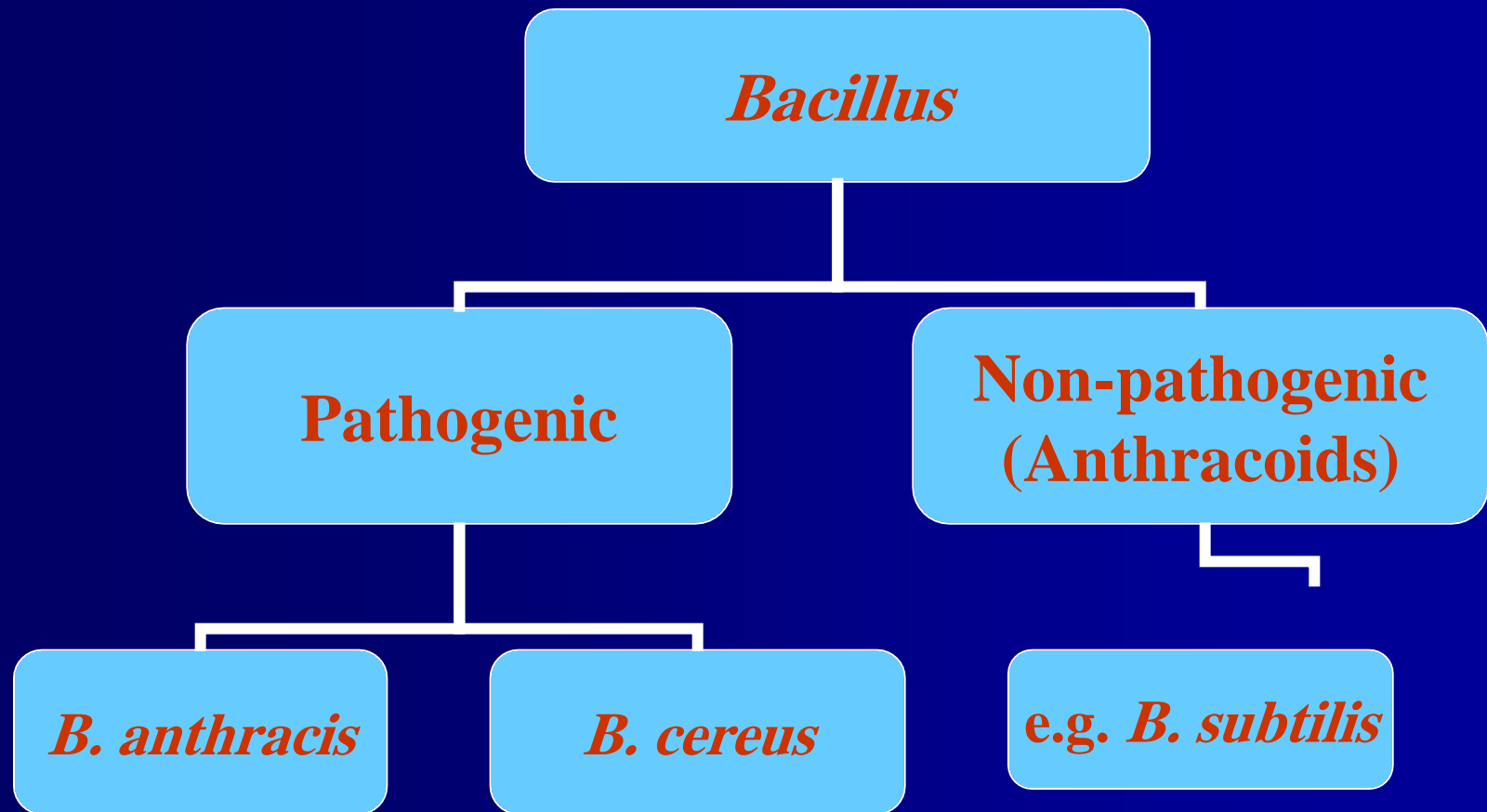


# Classification of Gram-Positive

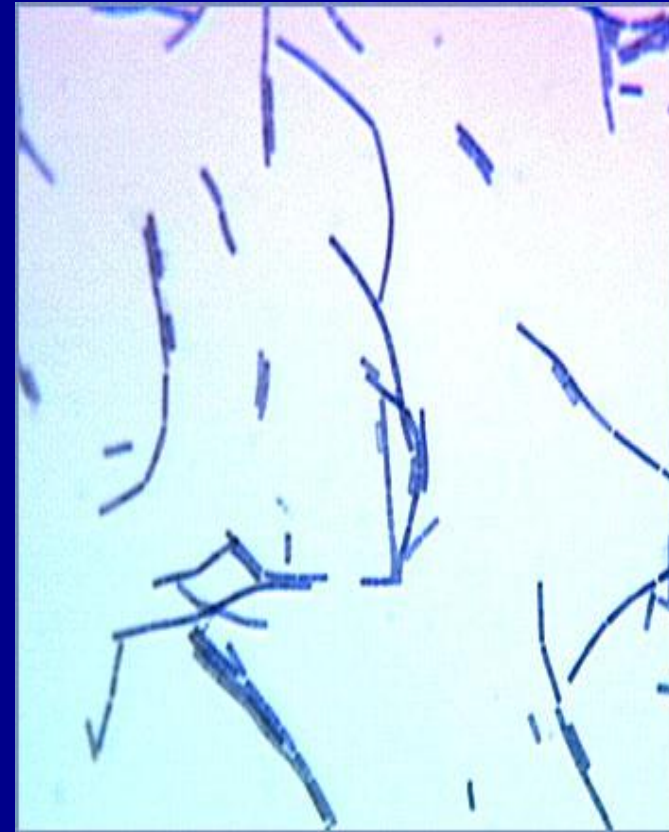


# Aerobic Spore Forming *Bacillus spp*



# General Characters of *Bacillus spp*

- Very large Gram positive bacilli
- Arranged in long chains
- Motile except *B. anthracis*
- Spore forming (outside the host)
- Capsulated (inside the host)
- Non Fastidious
- Facultative anaerobic
- Catalase positive
- It is found in soil habitats



# Disease Caused by *B. anthracis*

## Anthrax

- **Anthrax** is an acute infectious disease in man & animal caused by the spore-forming *B. anthracis*.
- Anthrax is **zoonotic disease**
- Anthrax is **occupational disease**
- Direct person-to-person spread of anthrax is extremely unlikely to occur.

# Types of Anthrax

- Cutaneous Anthrax (Malignant Pustule)
- Pneumonic Anthrax (Woolsorters disease)
- Intestinal Anthrax

# Virulence Factors

- **Poly-D-glutamyl Capsule**
  - Mediates the invasive stage of the infection
- **Anthrax toxin**
  - Mediates the toxigenic stage
    - The toxin consists of three distinct antigenic components, which is thermolabile protein.
    - Edema Factor (EF): necessary for edema production
    - Protective Antigen (PA): induces protective antitoxic antibodies in guinea pigs
    - Lethal Factor (LF): has a lethal effect of anthrax toxin

# *B. cereus*

- *B. cereus* is a normal inhabitant of soil
- Also isolated from food such as grains and spices
- *B. cereus* causes Two Types of food poisoning
  - **Emetic form or short incubation:**
    - It is caused by heat stable enterotoxin
    - Nausea, vomiting and abdominal cramps
    - Incubation period of 1-6 hrs
    - It resembles *S. aureus* food poisoning
  - **Diarrheal form or long incubation:**
    - It is caused by heat labile enterotoxin
    - Abdominal cramps and diarrhea
    - Incubation period of 8-16 hrs
    - It resembles food poisoning caused by *Cl. perfringens*

# Differential characteristics of *B. anthracis* & *B. cereus*

	<i>B. anthracis</i>	<i>B. cereus</i>
Hemolysis	No hemolysis	$\beta$ -hemolysis
Motility	Non-Motile	Motile



# Identification of *Bacillus Spp.*

## ■ Specimen

- Pastular exudates in malignant pustule
- Sputum in pneumonic anthrax
- Stool in intestinal anthrax (also in food poisoning by *B. cereus*)
  - Stool specimen is emulsified and heated to 80 C to kill non spore forming microorganism

## ■ Morphology

- Macroscopical (Cultural characteristics)
- Microscopical (Gram Stain, Spore Stain)

# Identification of *Bacillus Spp.*

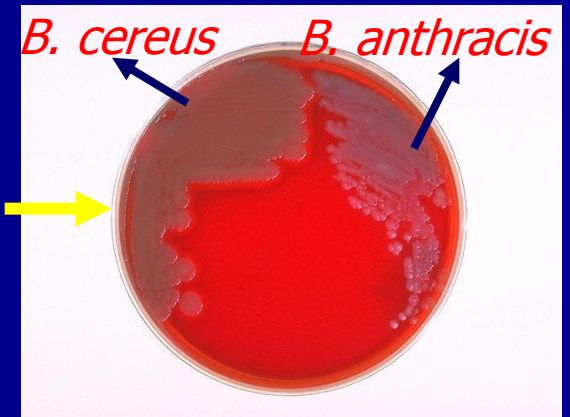
- **Cultural Characteristics**
  - **Grow on nutrient Agar**
    - On ordinary medium
      - Grow aerobically at 37C with characteristic mucoid or smooth colonies, which indicates the pathogenicity of organism (presence of capsule)
      - Rough colonies are relatively avirulent
    - Stab culture on gelatin medium results in inverted fire tree appearance.
  - **Growth on Blood Agar**
    - *Bacillus* species grow well on blood agar showing a double zone of hemolysis
    - *B. anthracis*, which grows well on blood agar without any hemolytic effect.

# Cultural Characteristics

Nutrient Agar



Blood Agar



# Identification of *Bacillus Spp.*

- **Morphology**
  - **Microscopical**
    - **Stain**
      - **Gram Stain**
        - Gram positive bacilli
        - Found in chains



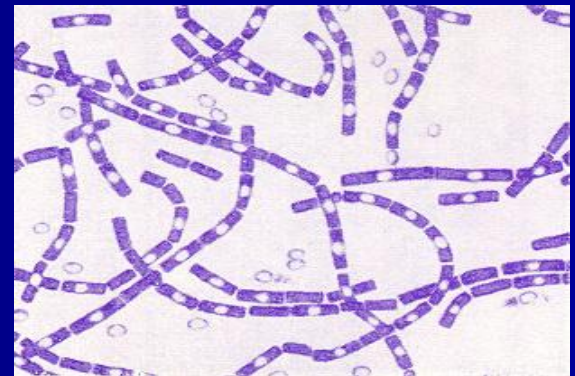
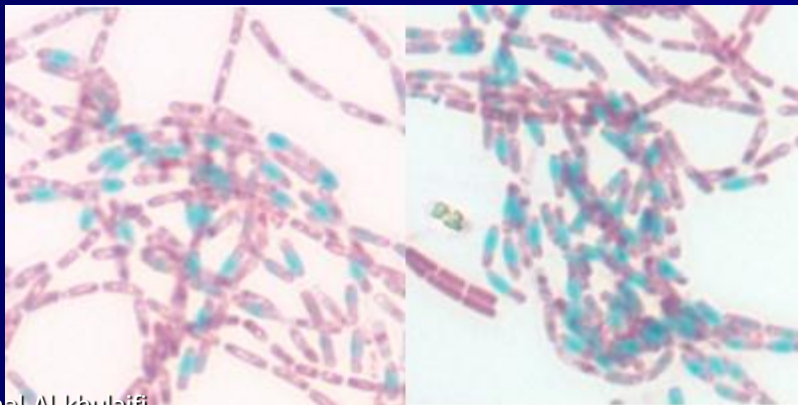
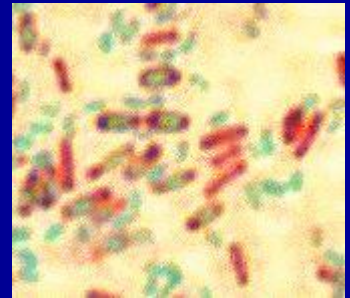
# Spore Stain Procedure

1. Make a heat fixed smear of *Bacillus*
2. Place the slide on the slide rack
3. Cover the smear with malachite green stain
4. Apply heat for 3-5 min without boiling and drying of the slide
5. Wash the slide gently in running water about
6. Counterstain with safranin for one minute
7. Gently rinse with water
8. Gently blot the slide dry, no rubbing, and let it air dry and examine with oil immersion optics.
9. Observe red vegetative cells and sporangia, and green endospores and free spores

# Identification of *Bacillus Spp.*

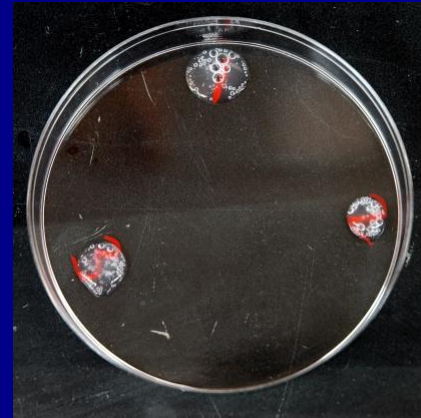
## ■ Spore Stain

- Bacillus spores are oval & central
- By spore staining technique (Malachite green & safranin) , the **spore** appears **green** while the **vegetative cells** appear **red**.



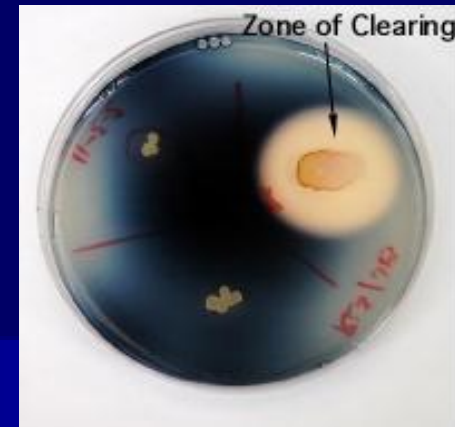
# Biochemical Tests:

## 1- Catalase Test



- All *Bacillus* species are catalase positive  
(Remember staphylococci are catalase positive)

# Starch Hydrolysis (Amylase Activity)



## ■ Principle

- Starch + Iodine  $\longrightarrow$  blue color
- Glucose + Iodine  $\longrightarrow$  No reaction

- Nutrient Agar containing 1% Starch + M.O  $\xrightarrow{\text{Amylase}}$  Glucose  
Appearance of colorless zone around the growth

Iodine  $\curvearrowright$

## ■ Procedure

- Inoculate nutrient agar plate containing 1% Starch with the M.O.
- Incubate the plate at 37 for overnight
- After incubation, flood the plate with Iodine solution

## ■ Result

- **Activity of amylase is indicated by a clear zone around the growth while the rest of the plate gives blue color after addition of iodine solution**



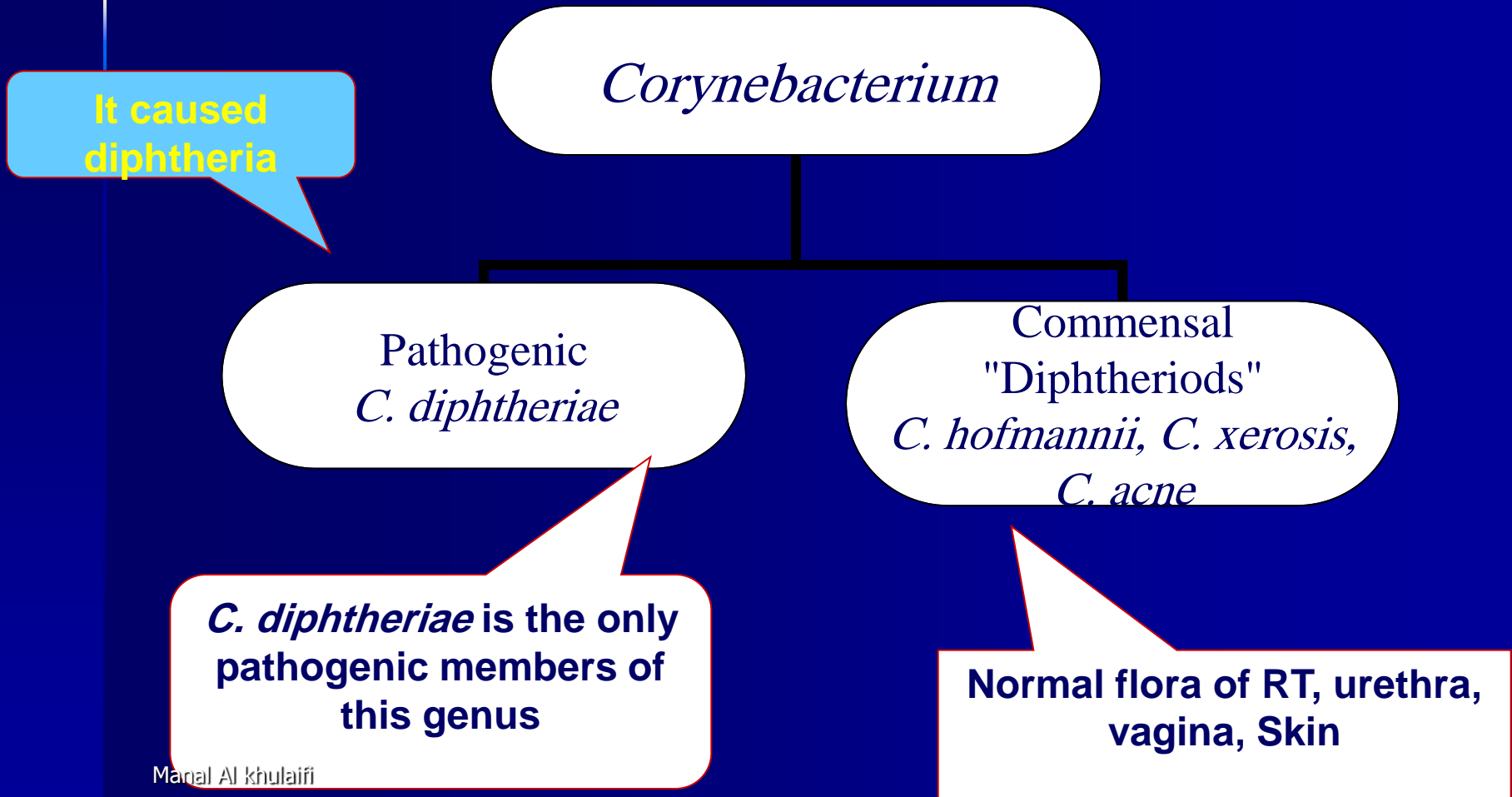
# Practical Work

- Gram Stain
- Spore Stain
- Catalase Test
- Starch hydrolysis

# Corynebacterium spp

- Gram positive bacilli, with characteristic morphology (club shaped and beaded)
- Non motile
- Non spore forming
- Non capsulated
- Facultative anaerobic
- *C. diphtheriae* is fastidious while diphtheroids are non-fastidious
- Catalase positive
- Oxidase negative

# Species of Corynebacteria



# Diagnosis of diphtheria

```
graph TD; A[Diagnosis of diphtheria] --> B[Clinical Diagnosis]; A --> C[Laboratory Diagnosis]; B --> D[Specific treatment must be never delayed for laboratory results]; C --> E[To confirm the clinical manifestation];
```

## Clinical Diagnosis

Specific treatment  
must be never delayed  
for laboratory results

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## Laboratory Diagnosis

To confirm the clinical  
manifestation

# Laboratory diagnosis of case

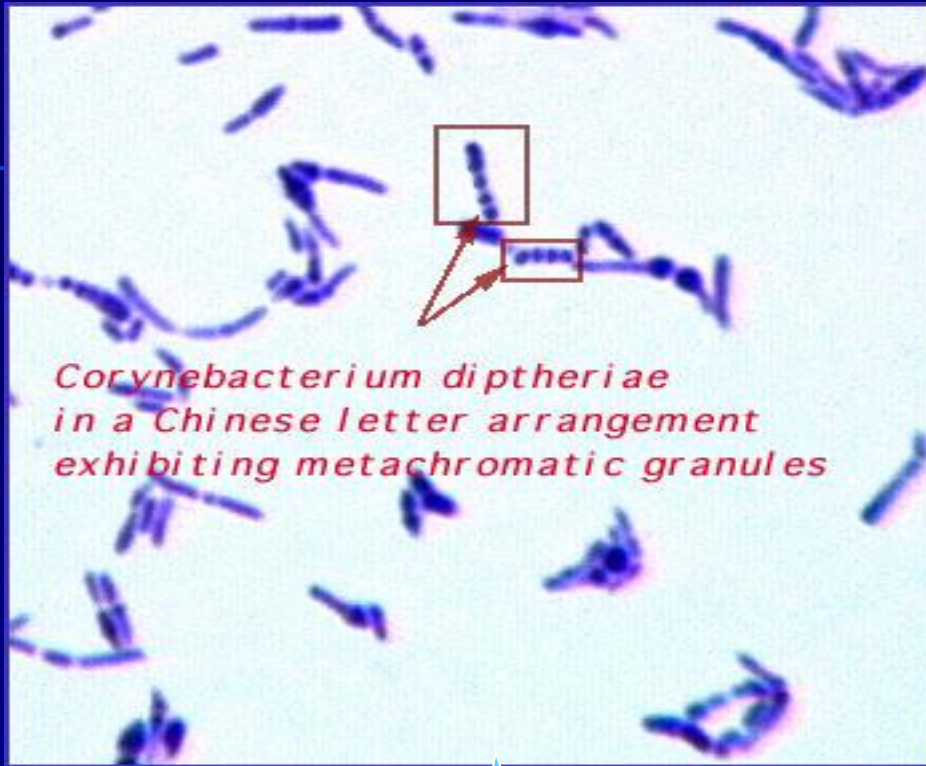
- **Specimen:** A throat swap
- **Culture:**
  - **On Loeffler's serum medium:**
  - Corynebacteria grow much more readily than other respiratory pathogens
    - Used to enhance the characteristic microscopical appearance of corynebacteria
    - The colonies of *C. diphtheriae* are small, granular, grey, smooth, and creamy with irregular edges



# Cultural characteristics

- **On blood tellurite agar (Mcloed's blood agar)**
  - It is selective medium for isolation of *C. diphtheriae* (Potassium tellurite)

- **Morphology**
- **Stain: gram stain:**
  - Gram +ve, nonspore forming nonmotile bacilli
  - Club-shaped (Coryne= club) arranged at acute angles or parallel to each other (Chinese letters appearance)
  - Beaded (metachromatic granules)
  - **Polychrome methylene blue stain:**
    - *C. diphtheriae* appears beaded due to the presence of intercellular “Metachromatic or volutin” granules
    - By stain, the granules appear red while the rest of organism appears blue.



Gram stain of  
*C. diphtheriae*

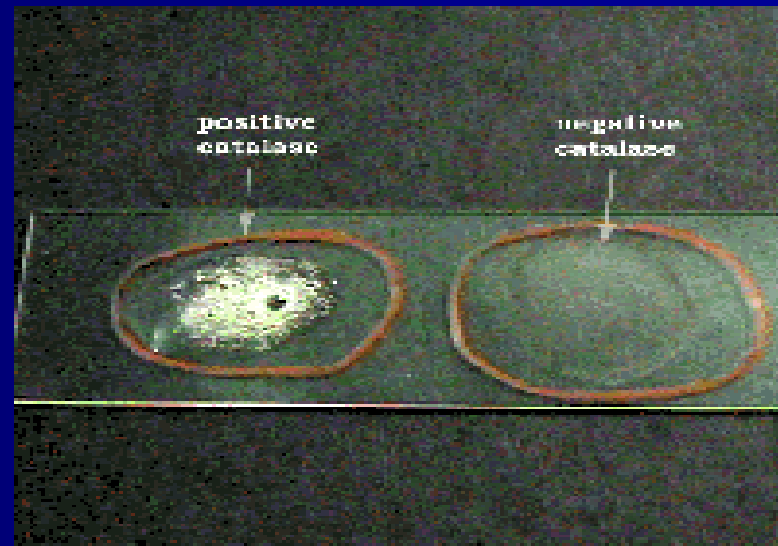
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*C. diphtheriae* on  
BTA



# Biochemical Reaction

- All *Corynebacterium* species are catalase positive (Also, *Staphylococcus* and *Bacillus* species are catalase positive)



# Diagnosis of Carrier

## I- Isolation of organism

## II- Detection of exotoxin Test for toxigenicity

Swap from throat & nose

Inoculation on Loeffler's  
Or BTA for 24 h/37 C

Diphtheria like M.O.

# Detection of exotoxin

I- *In vivo*

II- *In vitro*

Two guinea pigs are used

One is used as  
Test

The second is  
used as Control

Injected with  
diphtheria antitoxin

Both test and control injected with isolated MO

If both GP live

If control live & test  
die

Diphthroids Or non-toxigenic

*C. diphtheriae*  
i.e. non pathogenic

*C. diphtheriae*  
i.e. produce exotoxin

# In Vitro: Elek's Test

## ■ Principle:

- It is toxin/antitoxin reaction
- Toxin production by *C.diphtheriae* can be demonstrated by a precipitation between exotoxin and diphtheria antitoxin

## ■ Procedure:

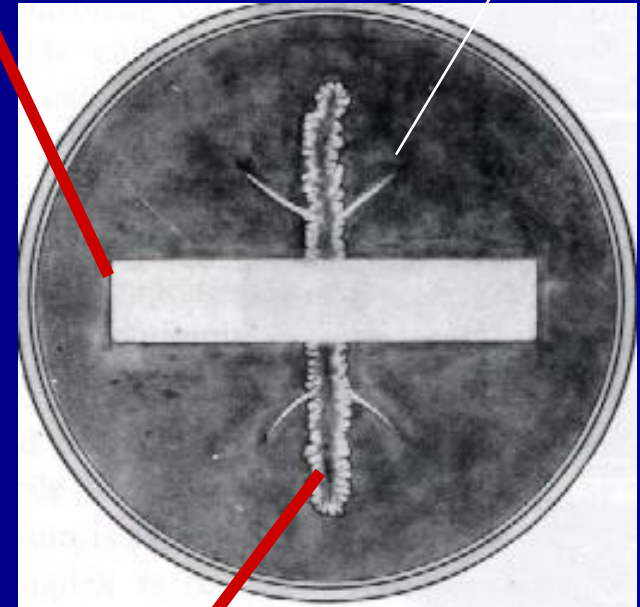
- A strip of filter paper impregnated with diphtheria antitoxin is placed on the surface of serum agar
- The organism is streaked at right angles to the filter paper
- Incubate the plate at 37C for 24 hrs

## Filter paper saturated with diphtheria antitoxin

### ■ Results:

- After 48 hrs incubation, the antitoxin diffusing from filter paper strip and the toxigenic strains produce exotoxin, which diffuses and resulted in lines four precipitation lines radiating from intersection of the strip and the growth of organism

Lines of precipitations



Inoculated M.O.

Positive Elek's Test