Classification

- Numerous species
- *Bacillus anthracis*
- Anthracoides
  - *Bacillus cereus*
  - *Bacillus subtilis*
  - *Bacillus pumilus*
  - *Bacillus stearothermophilus*

General Characteristics

- Gram-positive rods arranged singly or in chains
- Aerobic or facultative anaerobes
- Form **endospores** which are heat resistant
- Most of the species are motile
  - *B. anthracis* is non-motile
- Majority of the species form complete haemolysis on BA
  - *B. anthracis* is non-haemolytic
- Catalase positive

General Characteristics

- Gram-positive rods
  - Straight or slightly curved
  - Single cells or in chains
  - Bamboo rod appearance
- Capsulated in tissues
- Forms spores:
  - When shed in the environment or grown on artificial media
  - Spores are oval, centrally placed and same diameter as the width of the cell

General Characteristics

- Vegetative cells: destroyed by heat at 60°C for 30 minutes
- Spores:
  - Relatively resistant to disinfectant and heat
  - Some can withstand dry heat at 140°C for 1-3 hours and boiling at 100°C
  - Can survive in the environment or in the hosts for long

Virulence factors

1. **Capsule**

A prominent **polypeptide** capsule

Consists of **poly-D -glutamic acid**

Observed in clinical specimens but not produced *in vitro* unless special growth conditions are used.

3 genes (*capA*, *capB*, and *capC*) responsible for synthesis of the capsule

Genes carried on plasmid *pXO2*

Virulence Factors

2. Exotoxin

Protective antigen (PA), **edema factor** (EF), and **lethal factor** (LF)

Nontoxic individually but form important toxins when combined:

- **PA + EF=edema toxin**, and **PA + LF =lethal toxin**

PA binds to one of two receptors on host cell surfaces

Host proteases cleave PA, releasing a small fragment and retaining a 63-kDa fragment (PA63) on the cell surface.

PA63 fragments self-associate forming a ringshaped complex of seven fragments (pore precursor or "prepore")

Exotoxin cont...

- The prepore can then bind up to 3 molecules of LF and/or EF through **competitive binding**
- Heptameric complex forms a transmembrane pore and releases LF and EF into the cell interior.

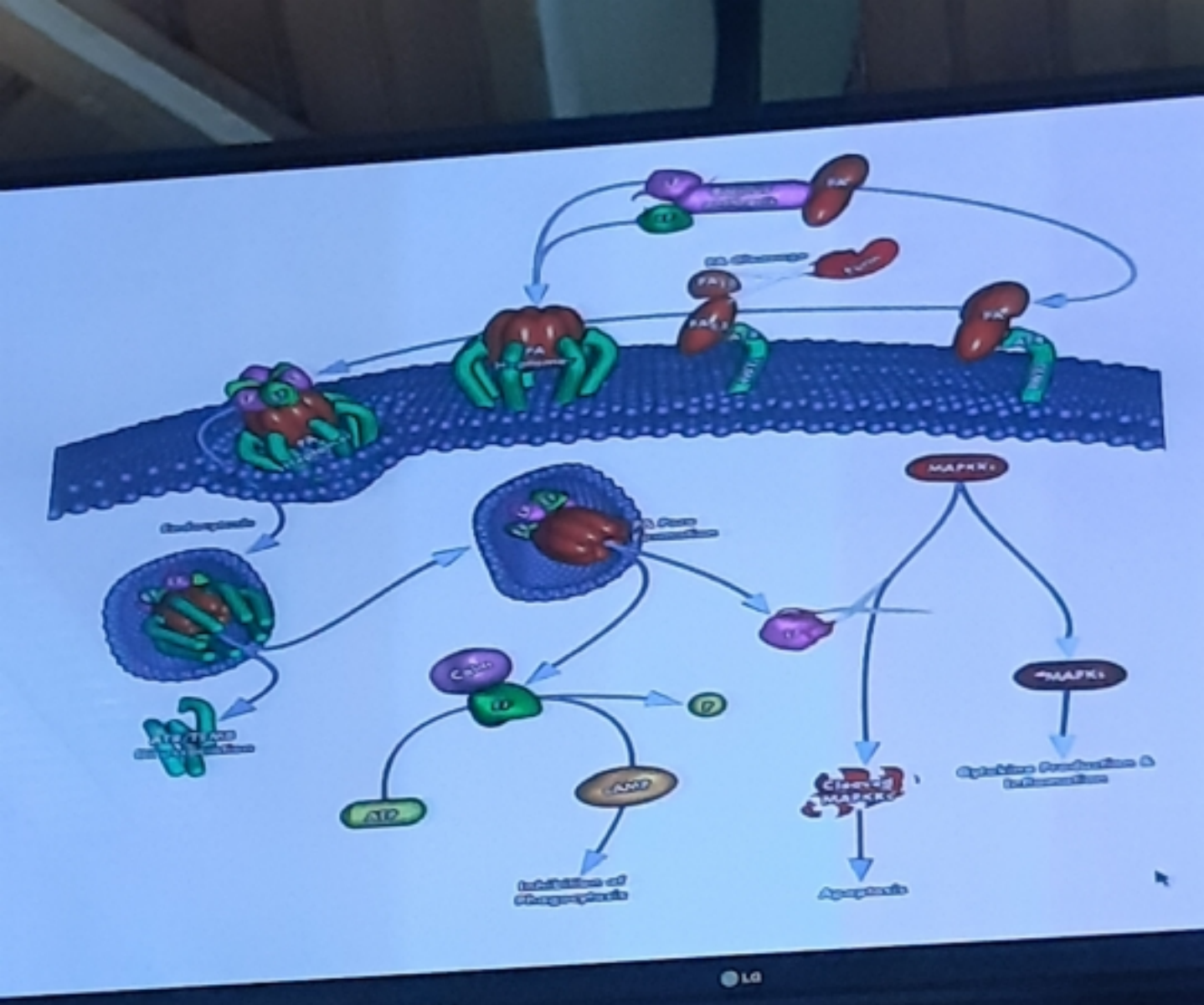
LF

- a **zinc-dependent protease** capable of cleaving mitogen activated protein (MAP) kinase, leading to cell death

EF

- a **calmodulin-dependent adenylate cyclase** that increases the intracellular cAMP levels and results in edema

*Genes for the protein components found on a large plasmid, *pXO1*


MAPK1
AMP
ATP
Apoptosis

Clinical Implications

- Causes **anthrax**
- Associated with outbreaks
- Primarily a disease of herbivores
- Human infections are accidental

Transmission

- Exists in the environment as a spore and can remain viable in the soil for decades
- Spores ingested by grazing herbivores
- Germinate within the animal to produce the virulent vegetative forms.
- Replicate and eventually kill the host
- **Products** (e.g meat or hides) from infected animals serve as a **reservoir for human disease**

Clinical Manifestations

1. **Cutaneous anthrax**
   - Small pimple or pustule appears at the site of inoculation 2-3 days after exposure
   - Ring of vesicles develop→coalesce to form an erythematous ring.
   - A small dark area appears in the centre of the ring and eventually ulcerates and dries(eschar)

Clinical Manifestations

2. **Inhalation anthrax/ Woolsorter's disease**
   - Spores included
   - Severe signs and symptoms
   - Starts as a non-specific illness: mild fever, fatigue and malaise 2-5 days after exposure
   - Sudden severe case with respiratory distress

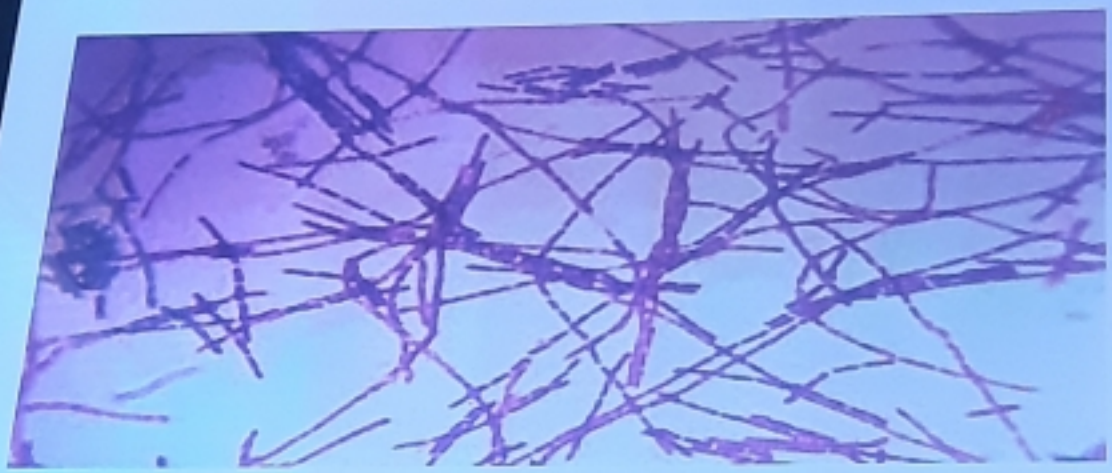
Clinical Manifestations

3. **GI anthrax**
   - Oropharyngeal/intestinal
   - Spores are inoculated into a lesion on the intestinal mucosa following ingestion of spores
   - Abdominal pain, nausea, vomiting, anorexia
4. **Injectional anthrax**
   - 1st recognised in 2001 in Norway
5. **Septicaemic anthrax**
   - Can arise as a complication of the other forms

Laboratory Investigations

- Specimen
  - fluid aspirated from cutaneous lesions,
  - sputum,
  - Cerebrospinal fluid
  - blood for culture.
  - Highly infectious
- Gram stain
- In smears from specimen, *B.anthracis* is capsulated
  - Polychrome methylene blue(**McFadyean**) stain; Giemsa, India ink

Gram stain



ositive McFadyean's Methylene Blue test:
ark/dark blue rods in a background of purple/pink
ained capsular material

Laboratory Investigations

ulture:

Grows aerobically and anaerobically (facultative naerobe)

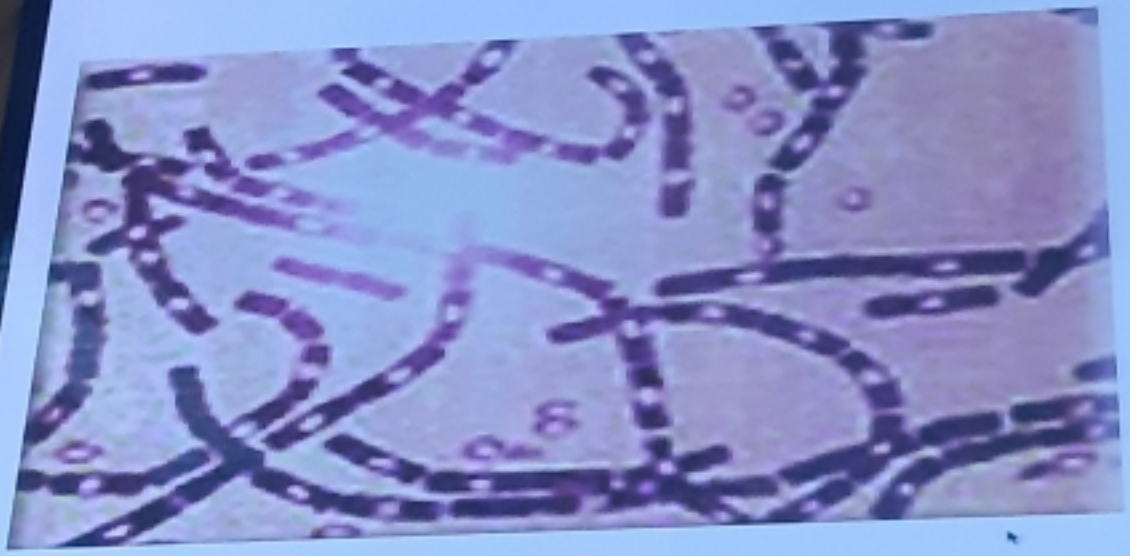
Temperature range for growth = 12–45 ºC; optimum 35–37 ºC; spore formation 25–30 ºC

A:

arge 2–5 mm in diameter, grey-white, irregular olonies with wavy edges

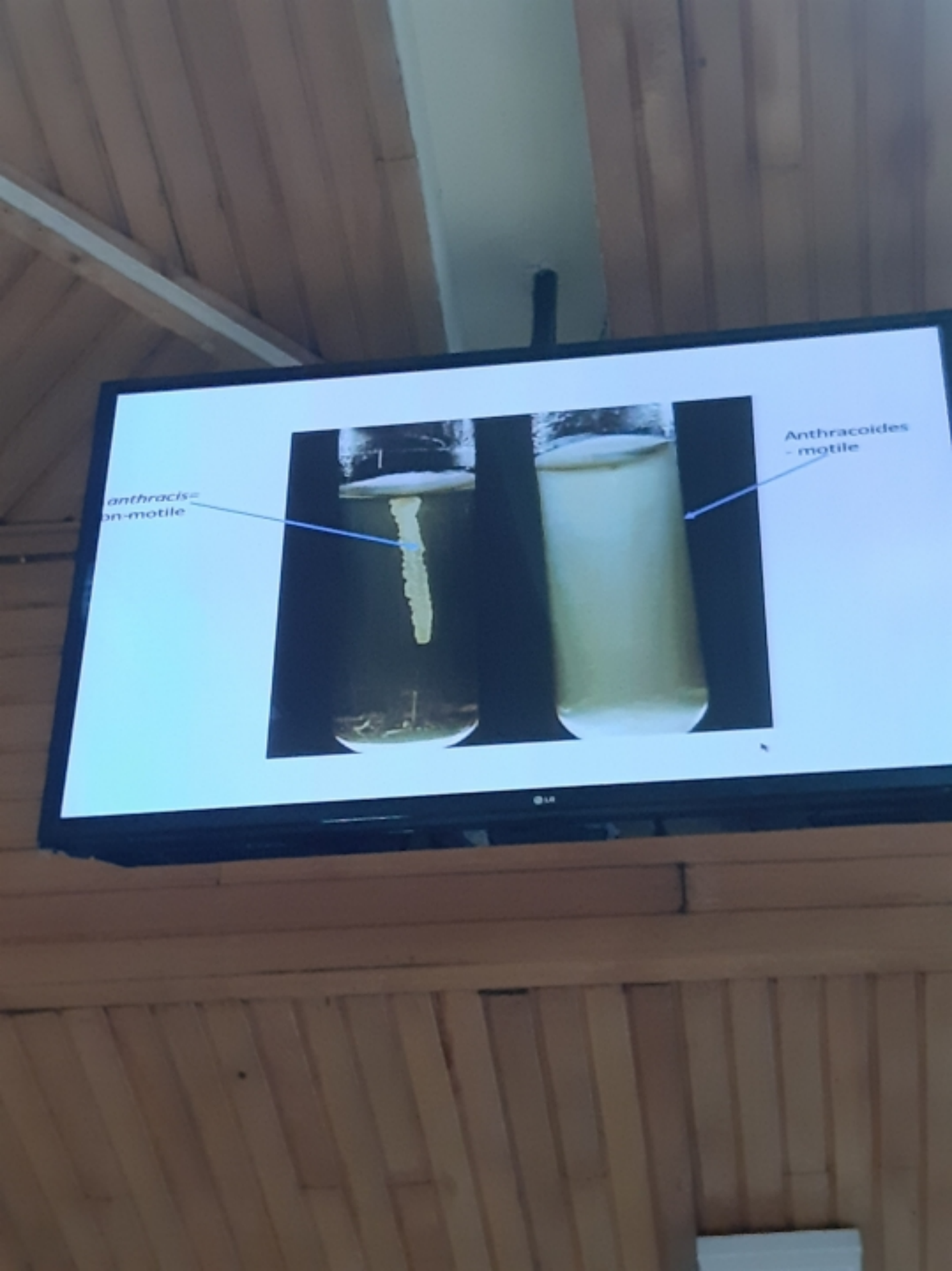
olonies nonhaemolytic or only slightly haemolytic

EMBA (Polymyxin, egg yolk, mannitol, bromothymol ue agar)– optional



Laboratory Investigations

- **Identification**
  - Catalase positive
  - Lecithinase production- inoculate on egg yolk; zone of **egg yolk precipitation**
  - Motility


anthracis=
on-motile
Anthracoides
- motile

reatment/Prevention/Control

Treatment

- Penicillin
- Chloramphenicol
- Tetracycline
- Erythromycin
- Streptomycin
- Fluoroquinolones
- First-generation cephalosporins

- Anthrax is resistant to many later-generation cephalosporins

Prevention and Control?

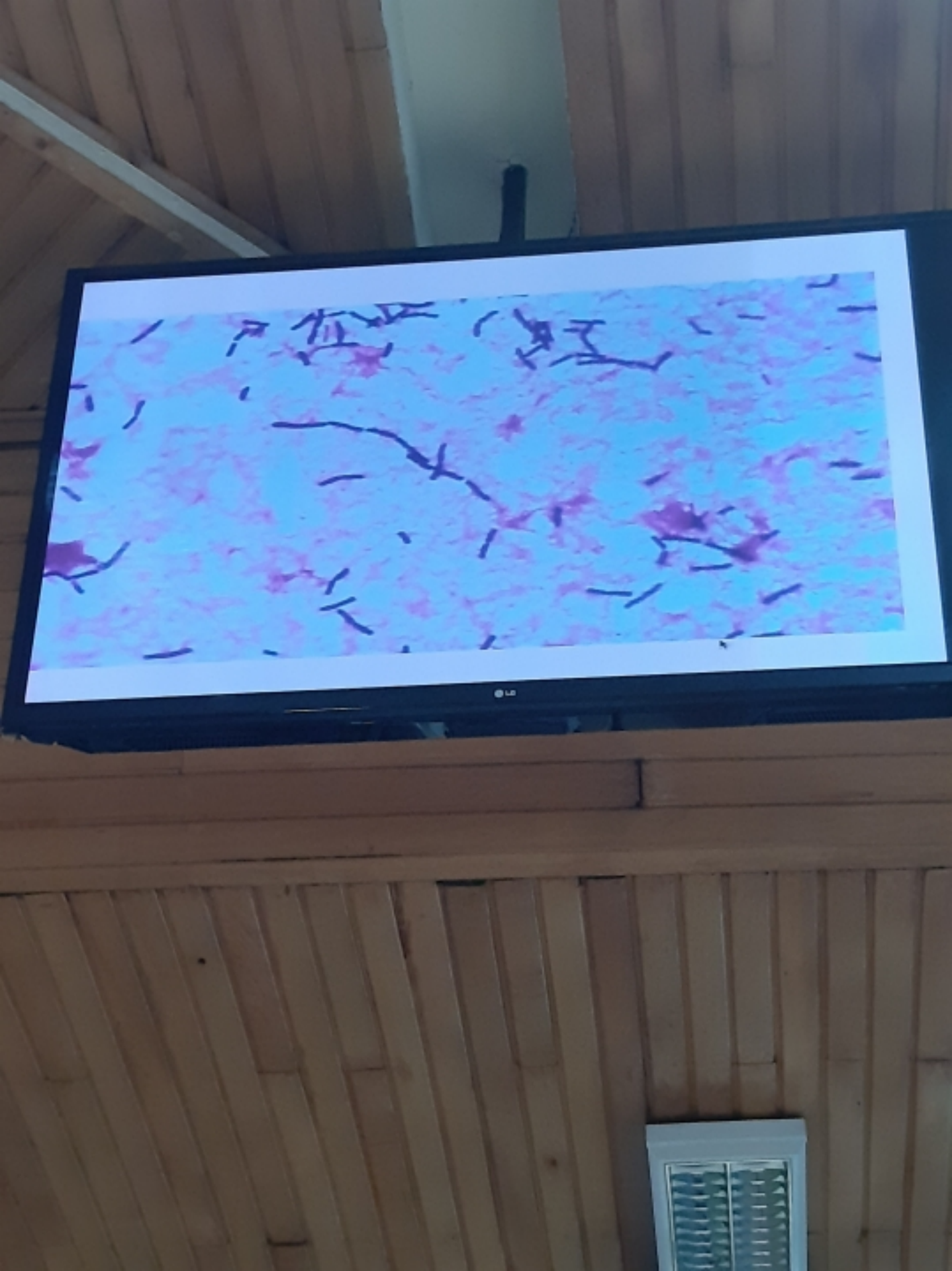
Anthracoides

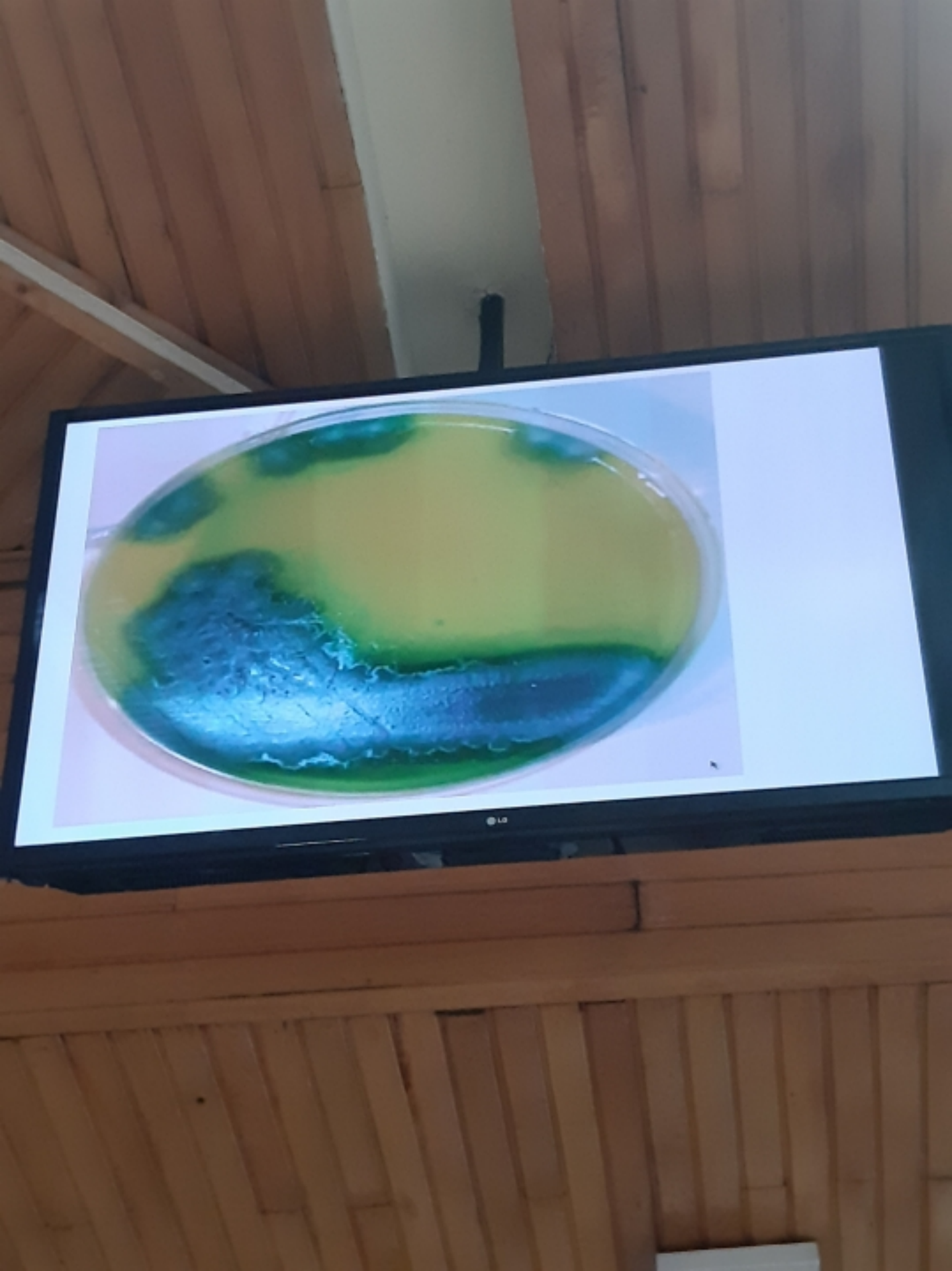
- Saprophytes-frequent contamination of cultures
- Primarily opportunistic pathogens
- Motile
- Not capsulated
- Not encountered as regular pathogens

Bacillus cereus

- Associated with

1. Food poisoning
   - Toxin-induced- rice and meat stews
   - Mild- diarrhoea and vomiting- a few hours
2. Severe eye infections
3. Anthrax-like progressive pneumonia,
4. Fulminant sepsis
5. CNS infections





Bacillus stearothermophilus

- Produces spores which are heat resistant
- Destroyed by heat at 121˚C in an autoclave for at least 12 minutes
- Useful for testing the efficacy of autoclaves


Lactobacilli

General characteristics

- Mainly saprophytes and commensals in humans and other animals
- Morphology
  - Gram positive bacilli
  - Non-motile
  - Non-spore forming
- Growth
  - Slow
  - Microaerophilic
  - PH 6.0

General characteristics

- Identification
  - Fermentation of carbohydrates with acid production
  - Tolerance to acidic conditions
- Species
  - *Lactobacillus acidophilus* complex
  - Oral cavity, GUT

General characteristics

- Play an important role in the health of the female GT
- Produce lactic acid from glycogen
- Lower vaginal PH and suppress overgrowth of microorganisms
- Low virulence(bacteraemia in immunosuppression)