



# Culture Media of Enterobacteriaceae I

Clinical Bacteriology II  
CLS 413

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# General characteristics of the enterobacteriaceae

- ▶ Rod shaped
- ▶ Gram negative
- ▶ Catalase positive
- ▶ Oxidase negative
- ▶ Facultative anaerobes
- ▶ Most ferment glucose, some are LF
- ▶ Most reduce nitrate to nitrite

# Members of the Enterobacteriaceae Family

- ▶ E.coli:
  - ▶ lactose fermenter- releases acids as a product of fermentation. Color (Ph) indicators change the color of the medium in response to the acidic environment.
  - ▶ Usually produces dry LF colonies
  - ▶ Motile
- ▶ Klebsiella Pneumoniae:
  - ▶ Lactose fermenter
  - ▶ Mucoid LF colonies

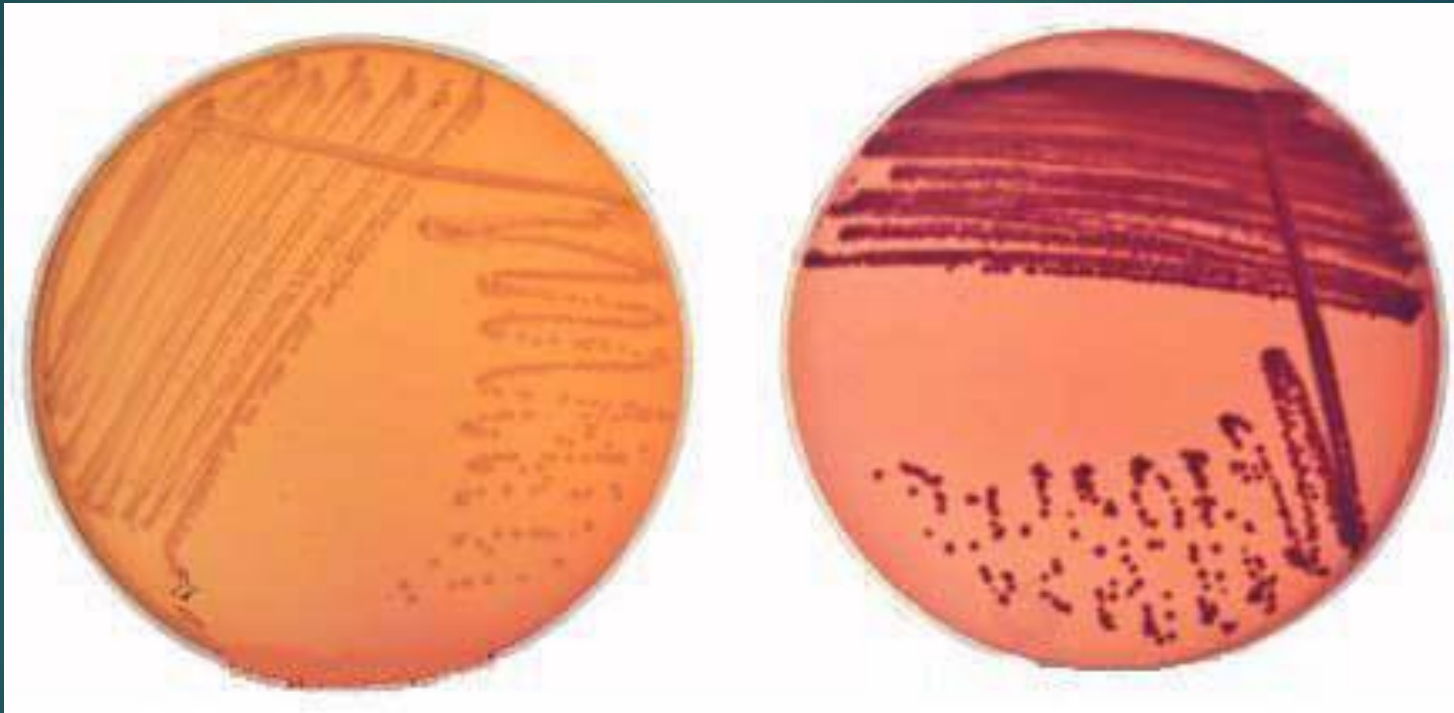
# Members of the Enterobacteriaceae Family

- ▶ Proteus:
  - ▶ Non lactose fermenter (NLF)
  - ▶ Tends to swarm on most culture media
  - ▶ CLED agar prevents swarming
  - ▶ Can produce H<sub>2</sub>S in sodium thiosulfate-containing media
  - ▶ Motile
- ▶ Salmonella:
  - ▶ NLF
  - ▶ Produces H<sub>2</sub>S in sodium thiosulfate-containing media
  - ▶ motile
- ▶ Shigella:
  - ▶ NLF
  - ▶ Non H<sub>2</sub>S producer

## MacConkey Agar:

- A selective and differential medium
- Selective for gram negative organisms
- Designed to isolate and differentiate enterics based on their fermenting characteristics
- Lactose is the only carbohydrate source in the medium
- Bile salts inhibit the growth of gram +ve organisms
- Neutral red is the pH indicator
- fermenters produce pink colonies. Non fermenters produce clear/colorless colonies

# Appearance of Lactose Fermenters and Non fermenters on Mac



## Cystein Lactose Electrolyte Deficient Agar (CLED):

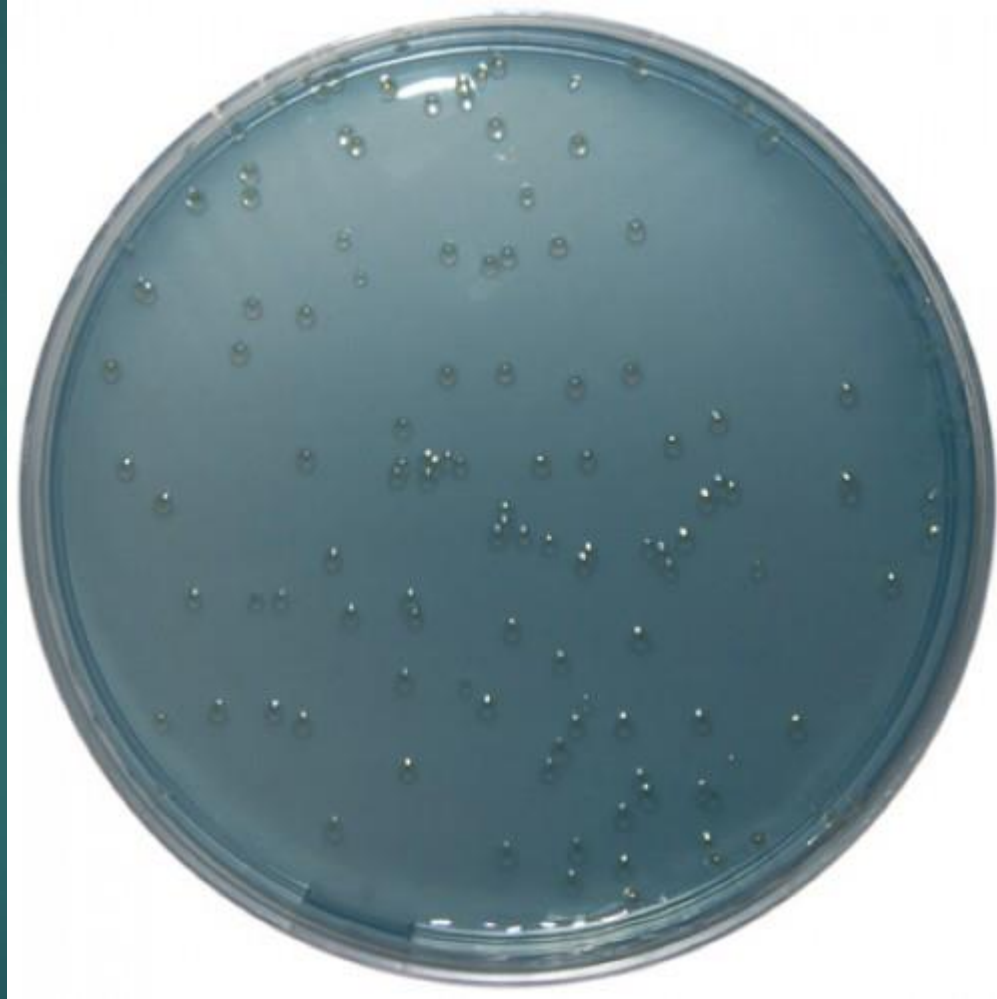
- ▶ A non selective differential medium
- ▶ Supports growth gram pathogens and gram positive contaminants
- ▶ Electrolyte deficient → inhibition of proteus swarming
- ▶ Bromothymol blue: pH indicator
- ▶ Most commonly used for urine culture
- ▶ Lactose fermenting bacteria produce yellow colonies
- ▶ Non fermenters produce blue colonies

# Swarming of proteus on nutrient agar

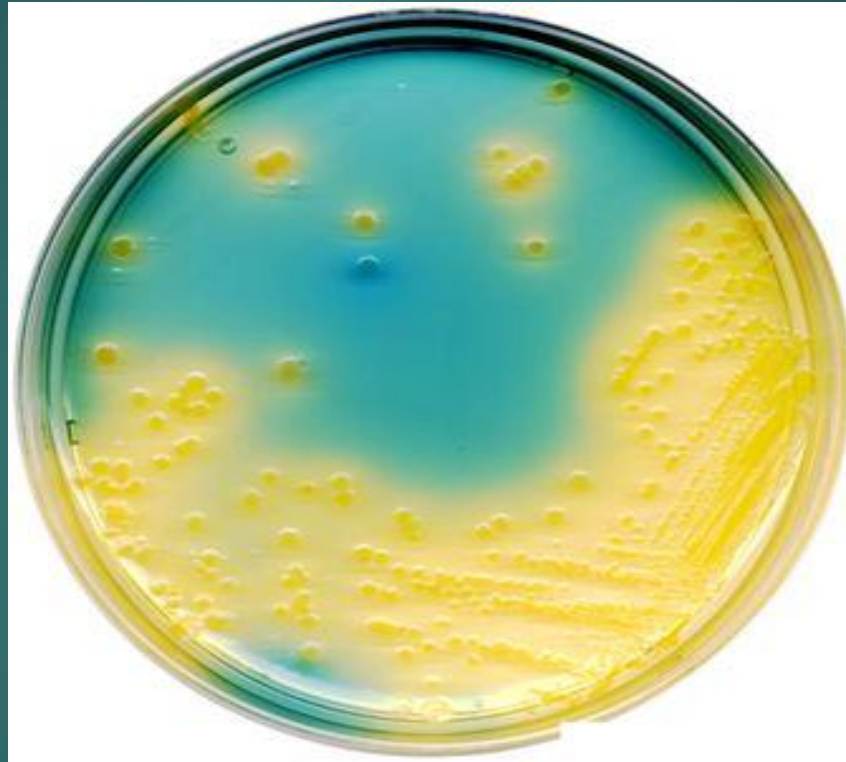




# Proteus on CLED Agar

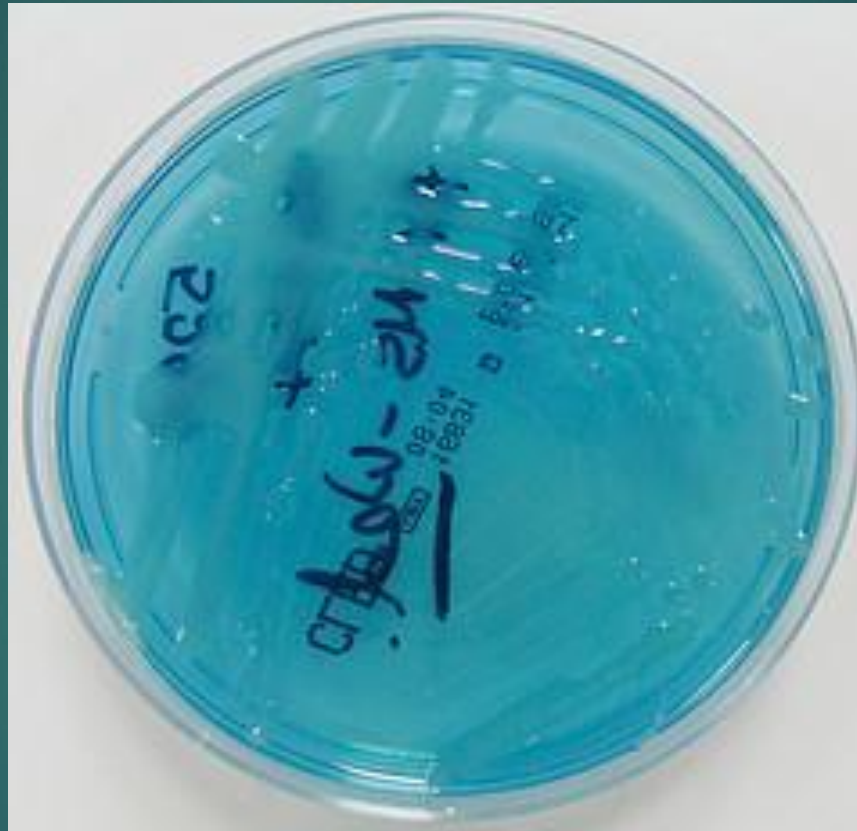


# Lactose Fermenters on CLED



E.Coli on CLED

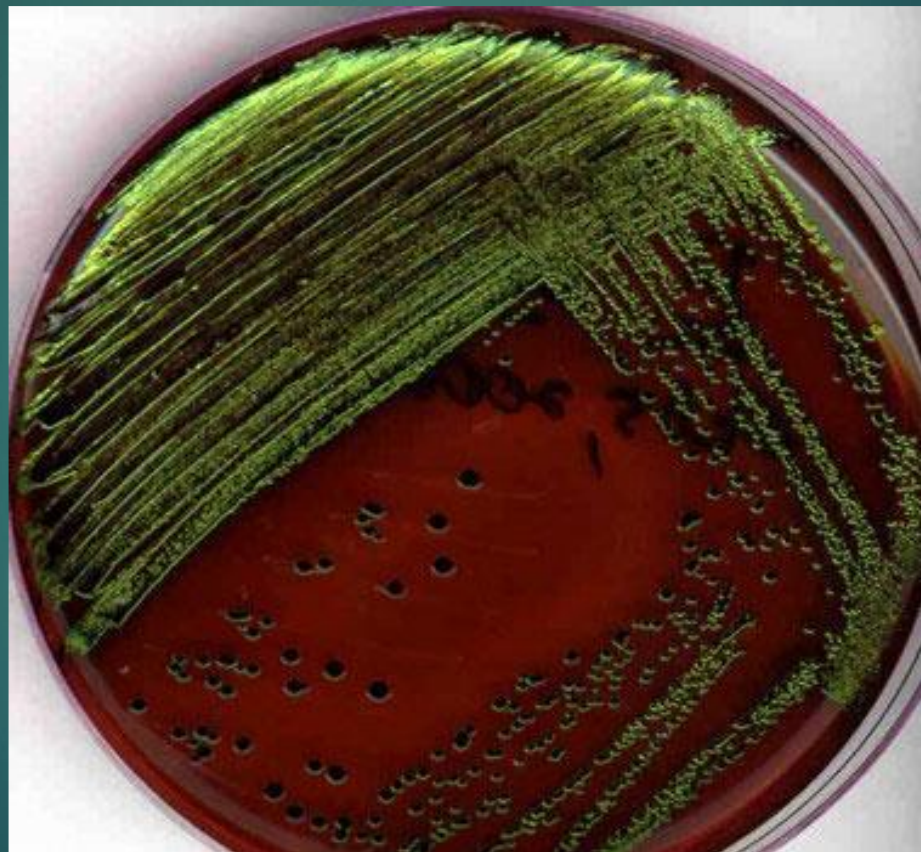
# Non Fermenters on CLED



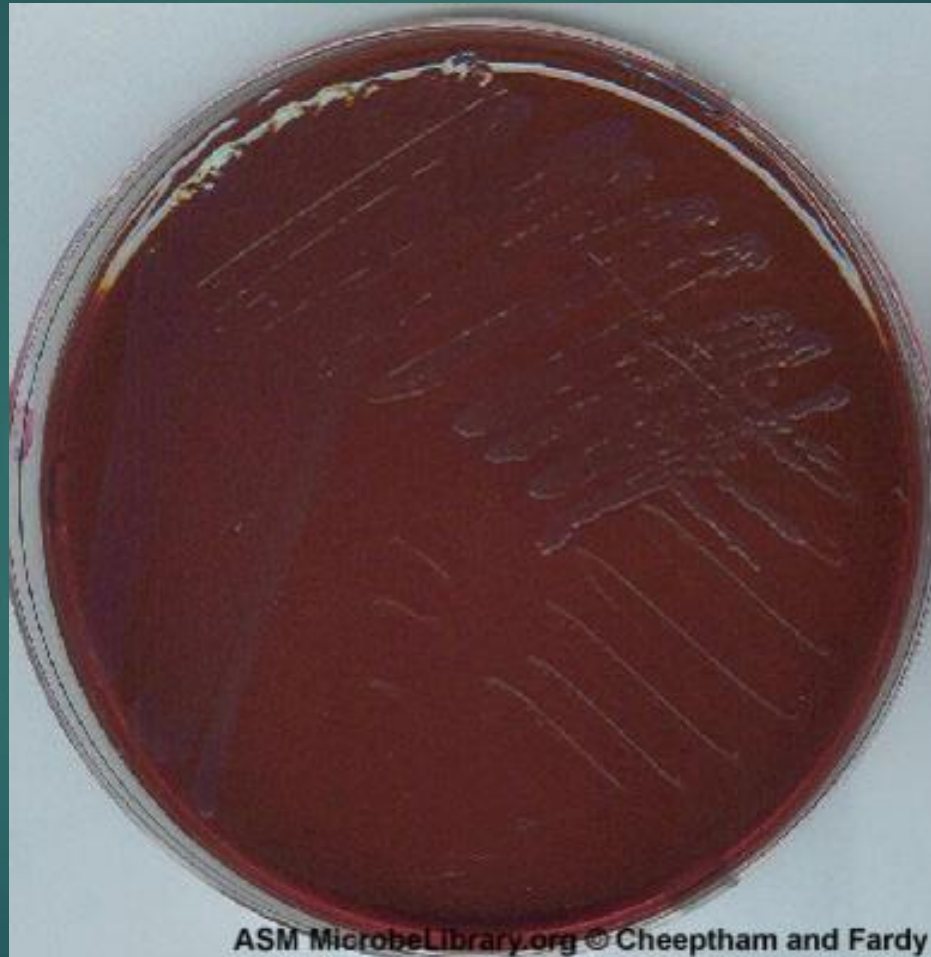
## Eosin Methylene Blue Agar (EMB):

- ▶ Selective and differential
- ▶ Selective for gram negative organisms
- ▶ Differentiates between LF and NLF
- ▶ Eosin and methylene blue inhibitory substances
- ▶ Lactose is the only carbohydrate source
- ▶ E.coli: LF with characteristic “green metallic sheen”
- ▶ Klebsiella produces colorless LF colonies (or colonies having the same color of the media)
- ▶ NLFs produce colorless colonies (colonies having the same color of the medium)

# E.Coli on EMB Showing Green Metallic Sheen



# Non Lactose fermenters on EMB



# Tasks to be done today



1. Prepare a smear from the provided organism and stain it by the gram stain method. View the slide under the microscope.
2. Culture the given organisms on your agar plates. Then incubate at 37 degrees Celsius. Return to the lab within 24 hrs to record your results. Growth patterns may change after that and your plate reading will be inaccurate.
3. View the demonstration plates and prepare a table of growth characteristics (which you will include in your report). This table must be completed today during this practical.
4. Prepare your lab report as described before
5. Make sure to include pictures of your results in your reports

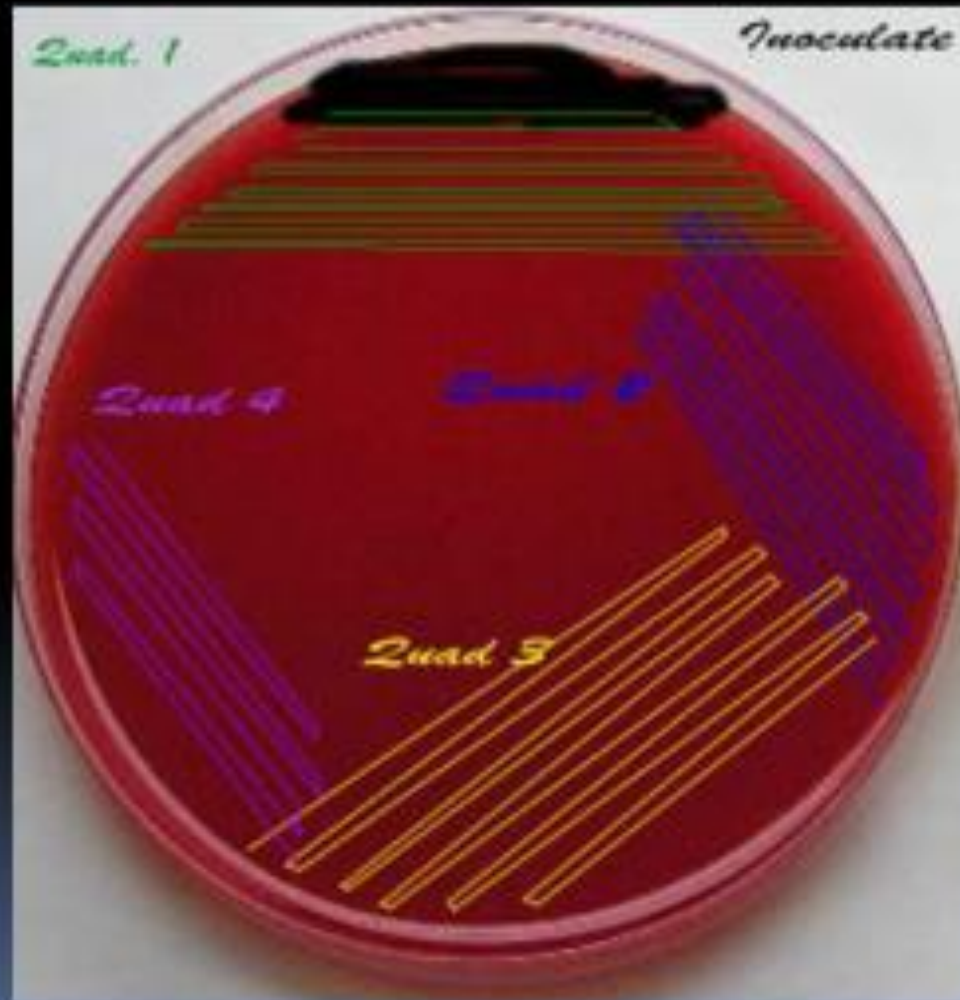
| Organism \ Media | E.coli | <u>Kleb.</u> | Salmonella | <u>Shigella</u> | Proteus |
|------------------|--------|--------------|------------|-----------------|---------|
| MAC              |        |              |            |                 |         |
| CLED             |        |              |            |                 |         |
| EMB              |        |              |            |                 |         |
| XLD              |        |              |            |                 |         |
| DCA              |        |              |            |                 |         |
| HE               |        |              |            |                 |         |

### Describe:

- Growth
- Color
- Lac fermentation
- H<sub>2</sub>S +/-
- Specific characteristics (mucoid colonies, dry, swarming..etc)



# Streaking a Plate



# Before you leave:

- ▶ Turn off your microscopes
- ▶ Turn off the incinerators
- ▶ Clean your bench with disinfectant spray and tissue
- ▶ Wash your hands