

# **Enterobacteriaceae**

## **Lab-6**

# Enterobacteriaceae

- **Lactose Fermenters** e.g. *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*.
- **Non Lactose Fermenters** e.g. *Salmonella*, *Shigella*, *Proteus*.

# GENUS: *Salmonella*

## ❖ Characteristics:

- Gram-negative rods, Oxidase negative and Facultative anaerobic.
- *Salmonella* does not ferment lactose and produce H<sub>2</sub>S.
- Motile, Non-spore forming and Non capsulated.
- Characterized by O, H, and Vi antigens.

*Salmonella* is never considered part of the normal intestinal flora! It is always pathogenic and **can cause 4 disease states in humans:**

- 1. Typhoid fever (Enteric fever)** which caused by either *S. typhi* or *S. paratyphi*.
- 2. Gastroenteritis (diarrhea) or Food poisoning** which is caused by *S. enteritidis*
- 3. Carrier state**
- 4. Sepsis.**

# Laboratory diagnosis of typhoid:

## A- Direct diagnosis:

1- **Specimen:** Blood, urine and stool.

### 2- Isolation of microorganism:

From blood. 5-10 ml of blood is taken aseptically from patient during the 1st week of infection, add to 50-100 ml sterile nutrient broth and incubate at 37°C for 24 hrs.

Subculture is done on MacConkey agar which shows **pale yellow colonies** in positive case i.e. **non lactose fermenter**.

From stool by culture on enrichment medium such as **selenite F broth** which inhibits the growth of coliform and allow the growth of *Salmonella* and *Shigella*.

Subculture is made on selective medium such as **(SS) agar** which supports the growth of *Salmonella* and *Shigella*.

The suspected colonies are picked and identified by **Cultural characteristics** and **biochemical reactions**.

### 3- Cultural characteristics:

- On MacConkey agar they give **yellow colonies**.
- On SS agar they give **pale yellow colonies** with **black centers due to H<sub>2</sub>S production**.
- On EMB they appear as **colorless colonies**.

## 4- Biochemical identification of *Salmonella*:

The biochemical reactions of *Salmonella* are outlined in the following Table.

Biochemical Test	I	MR	VP	CIT	Urease	H <sub>2</sub> S	Glucose F.	Lactose F	Motility
<i>Salmonella typhi</i>	-	+	-	-	-	+	+	-	Motile

F: Fermentation

## **B- The indirect diagnosis or Serological diagnosis:**

- In the 2nd week of the disease, antibodies against Salmonella are present in the patient's serum and can be detected by: Widal test.



# GENUS: *Shigella*

- Gram negative rods, Oxidase negative and Facultative anaerobes.
- *Shigella* does not ferment lactose and does not produce H<sub>2</sub>S.
- Non motile, Non spore-forming and Non capsulated
- They are the causative agent of **bacillary dysentery**.

# Four species:

1. *Shigella dysenteriae*: causes most serious form of bacillary dysentery
2. *Shigella flexneri*: most common cause of shigellosis in underdeveloped countries
3. *Shigella sonnei*: most common cause of shigellosis in developed countries
4. *Shigella boydii*: causes mild intestinal upset.

# Laboratory diagnosis:

## Specimen: Stool or rectal swap

Stool culture is done from mucous bloody part of stool on enrichment media such as **Selenite broth** at 37°C for 24 hrs. then subculture on **SS agar**. The suspected pale yellow colonies is picked up and examined by:

**Gram stain:** Gram negative bacilli, non-motile, non-spore forming and non-capsulated.

## Cultural characteristics:

On MacConkey agar they give **yellow colonies**.

On SS agar they give **pale yellow colonies**.

On EMB they appear as **colorless colonies**.

# Biochemical reactions:

- The biochemical reactions of Shigella are outlined in the following Table.

Biochemical Test	I	MR	VP	CIT	Urease	H <sub>2</sub> S	Glucose F.	Lactose F	Motility
Shigella	-	+	-	-	-	-	+	-	Non motile

**Selenite Broth** is a selective enrichment medium used for cultivation of *Salmonella* and some *Shigella* sp. Specimens from feces, urine, water and foods may be inoculated even if quantity is very low. Sodium selenite inhibits gram positive cocci as enterococci and gram negative bacteria as coliforms.

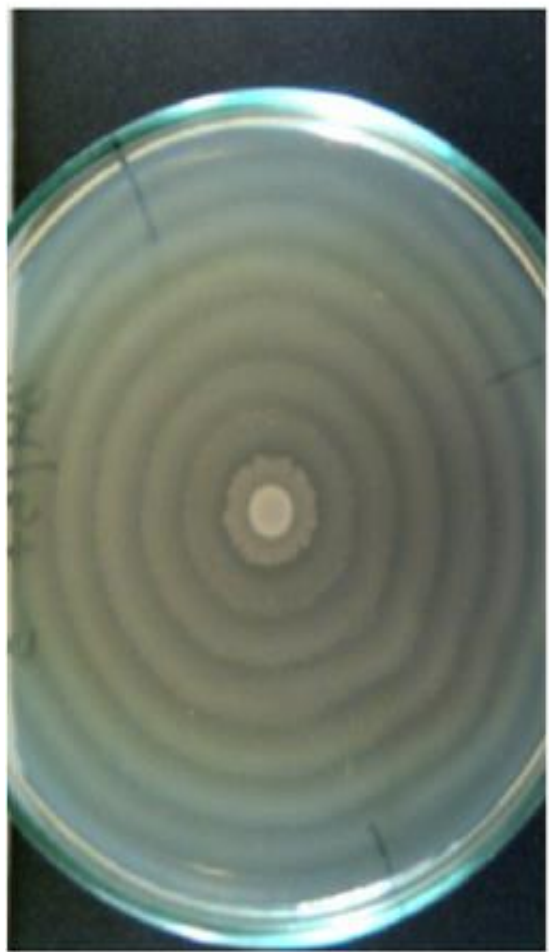
# GENUS: *Proteus*

- **Characteristics:**
- Gram negative rods, Oxidase negative and Facultative anaerobes.
- *Proteus* does not ferment lactose and produce H<sub>2</sub>S.
- Non capsulated and none spore forming.
- Actively motile and show swarming.
- Urease positive after 2-6 hrs (urea → NH<sub>3</sub>+ CO<sub>2</sub>)
- Grows well at alkaline pH

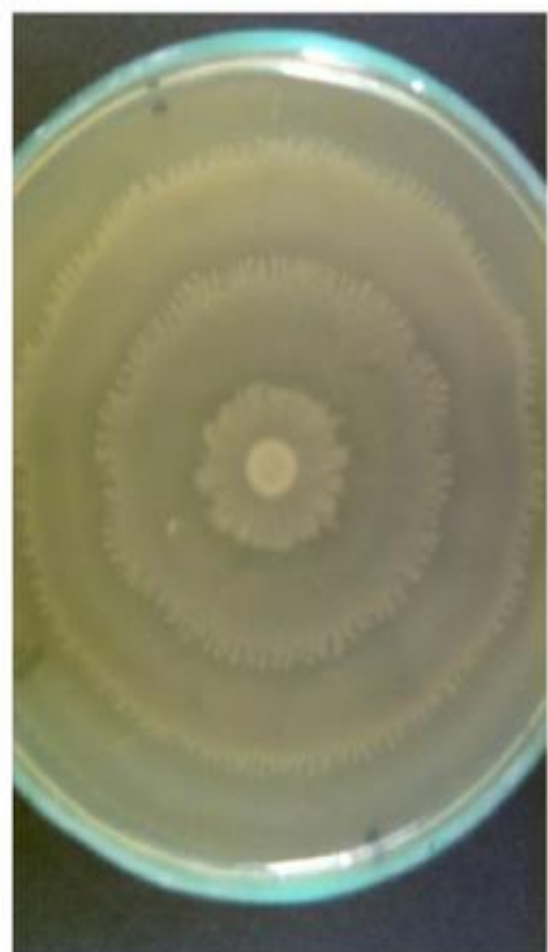
- Discontinuous swarming produces concentric circles around the point of inoculation.



A



B



C





- **Major pathogens** are *Proteus mirabilis* and *Proteus vulgaris*.
  - **P. mirabilis** causes **urinary tract infections (UTIs)**: urease alkalinizes urine → precipitation of calcium and magnesium salts → stone formation → renal epithelium damage
  - **P. vulgaris** causes **nosocomial infections** (pneumonia, bacteremia) and UTIs.

# Laboratory diagnosis:

**Specimen:** Urine or Stool.

**Cultural characteristics:**

- On **MacConkey agar** they give **pale yellow colonies**.
- On **EMB agar** they give **colorless colonies**.
- On **SS agar** they give **pale yellow colonies with black colour**.
- On **ordinary media**, such as **nutrient agar, blood agar**, show **swarming** (successive waves on the surface) due to high motility of *Proteus*. The suspected is picked up and examined by:

**Gram stain:** Gram negative bacilli, motile, non-spore forming and non-capsulated.

**Biochemical reactions:** The biochemical reactions of Proteus are outlined in the following Table.

Biochemical Test	I	MR	VP	CIT	Urease	H <sub>2</sub> S	Glucose F.	Lactose F	Motility
<b>Proteus mirabilis</b>	-	+	-	-	+ (2-6 h)	+	+	-	<b>motile</b>

# Identification of Gram's -ve rods

