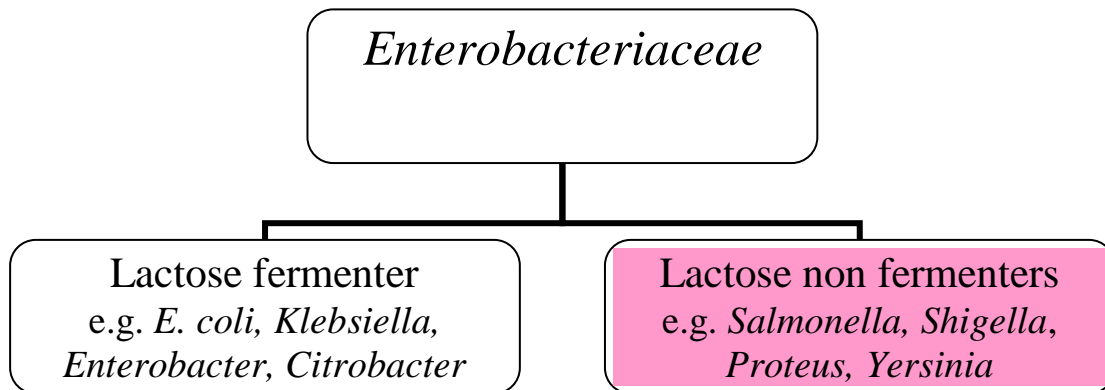


Practical 9

Enterobacteriaceae: I- Non Lactose Fermenters



I- *Salmonella* species:

- **General characters:**
- *Salmonella* species are:
 - Gram-negative rods
 - Oxidase negative
 - Ferment glucose
 - They do not ferment lactose and sucrose
 - Motile
 - Non capsulated
 - Non-spore forming
 - Facultative anaerobic
 - Characterized by O, H, and Vi antigens.
- **Two important members of *Salmonella* causing diseases:**
 - 1- *Salmonella* causing enteric fever which caused by either *Salmonella typhi* or *Salmonella paratyphi*
 - 2- *Salmonella* causing food poisoning which is caused by either *Salmonella enteritidis* or *Salmonella typhimurium*.
- **Enteric fever " Typhoid"**
 - Enteric fevers are severe systemic forms of salmonellosis.
 - The best studied enteric fever is typhoid fever, the form caused by *S typhi*, but any species of *Salmonella* may cause this type of disease.
 - The symptoms begin after an incubation period of 10 to 14 days.
 - Enteric fevers may be preceded by gastroenteritis, which usually resolves before the onset of systemic disease.
 - The symptoms of enteric fevers are nonspecific and include fever, anorexia, headache, myalgias, and constipation.
 - Enteric fevers are severe infections and may be fatal if antibiotics are not promptly administered.
 - Transmission via fecal-oral route = **person-to-person spread by chronic carrier** through fecally-contaminated food or water
 - 10-14 day incubation to signs of sepsis; sustained fever (delirium) for one to several weeks before abdominal pain and gastrointestinal symptoms

▪ **Laboratory diagnosis of typhoid:**

A- Direct diagnosis:

1- Specimen: Blood during 1st week, urine during 2nd week and stool during 3rd week

2-Isolation of microorganism:

- **From blood** using blood culture. Five to 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's 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 or tetrathionate broth which inhibits the growth of coliform and allow the growth of *Salmonella* and *Shigella*. Subculture is made on selective medium such as Salmonella-Shigella (SS) or deoxycholate citrate (DCA) agar which support the growth of *Salmonella* and *Shigella*. The suspected colonies are picked and identified by Gram stain and biochemical reactions.

3- Biochemical identification of Salmonella:

- *Salmonella* on MacConkey's agar they give yellow colonies, on SS agar they give pale yellow colonies with black edges due to H₂S production and on EMB they appear as colorless colonies. The biochemical reactions of *Salmonella* are outlined in the following Table.

Biochemical Test	Indole	MR	VP	CIT	Urease	TSI/H ₂ S	Motility
<i>Salmonella typhi</i>	-	+	-	-	-	A/Alk/+	Motile

II- *Shigella* species

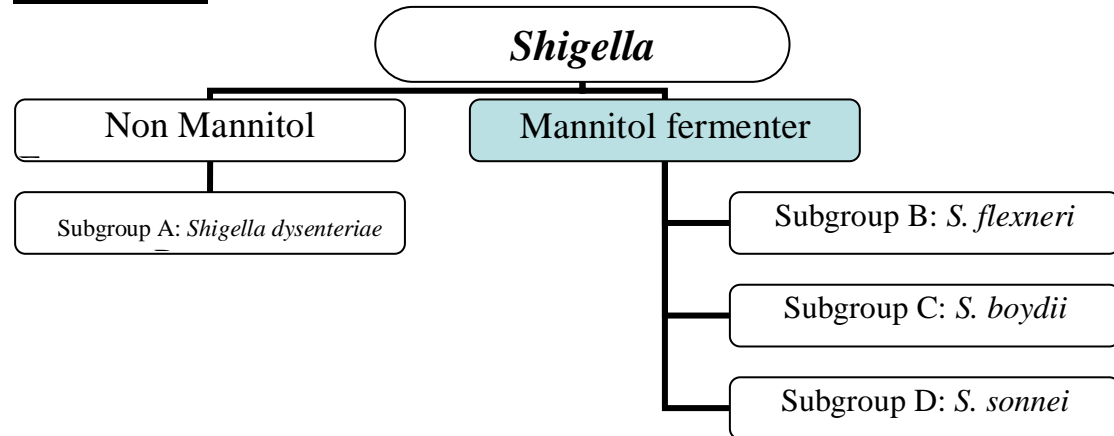
● The members of this genus are:

- Gram negative rods
- Non motile
- Non spore-forming
- Non capsulated
- Oxidase negative
- Ferment glucose
- Non lactose fermentating organism
- 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

Classification:



Clinical Syndromes (shigellosis):

- Ranges from asymptomatic infection to severe bacillary dysentery
- **Two-stage disease:** watery diarrhea changing to dysentery with frequent small stools with blood and mucus, tenesmus, cramps, fever
- **Early stage:**
 - Watery diarrhea attributed to the enterotoxin activity of Shiga toxin
 - Fever attributed to neurotoxin activity of toxin
 - Process involves:
 1. Ingestion
 2. Noninvasive colonization and cell multiplication
 3. Production of the enterotoxin by the pathogenic bacteria in the small intestine;
- **Second stage:**
 - Adherence to and tissue invasion of large intestine
 - Typical symptoms of dysentery
 - Cytotoxic activity of Shiga toxin increases severity
- **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 h then subculture on SS or DCA 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.
 - Biochemical reactions:

- **Shigella:** on MacConkey agar they give yellow colonies, on SS agar they give pale yellow colonies and on EMB they appear as colorless colonies. The biochemical reactions of *Shigella* are outlined in the following Table.

Biochemical Test	Indole	MR	VP	CIT	Urease	TSI/H ₂ S	Motility
<i>Shigella</i>	-	+	-	-	-	A/Alk/-	Non motile

Proteus species

General characters of *Proteus*:

- Gram negative rods
- Oxidase negative
- Ferment glucose
- Not ferment lactose
- Facultative anaerobes
- Non spore forming
- Actively motile:
 - a. Flagella (H antigen)
 - b. Swarms on lab media, producing spreading colonies
- Urease positive after 2-6 hrs (urea → NH₃+ CO₂)
- Grows well at alkaline pH

swarming of *Proteus mirabilis*



Major pathogens are *Proteus mirabilis* and *Proteus vulgaris*

- *P. mirabilis* causes urinary tract infections (UTIs): urease alkalizes 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.

Culture:

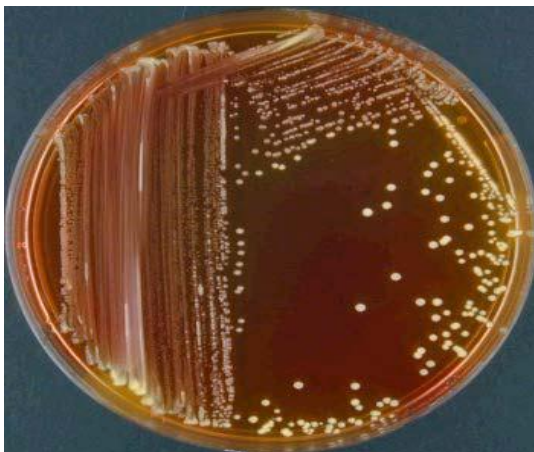
- On MacConkey agar → non lactose fermenters → pale yellow colonies
- On EMB agar → non lactose fermenters → colorless colonies
- On SS agar → non lactose fermenters → pale yellow colonies with black center
- On ordinary media, such as nutrient agar, blood agar, show swarming (successive waves on the 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:

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

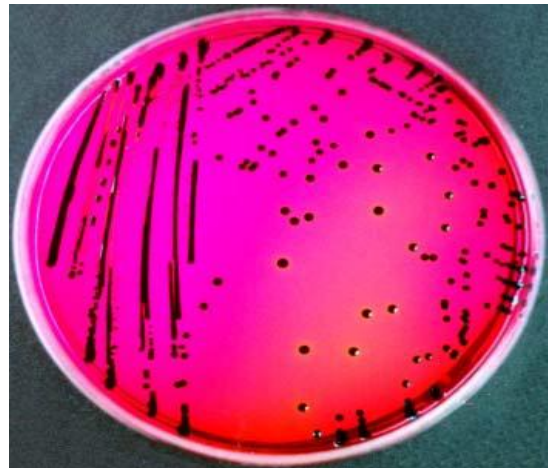
Biochemical Test	Indole	MR	VP	CIT	Urease	TSI/H ₂ S	Motility
<i>Proteus mirabilis</i>	-	+	-	-	+ (2-6 h)	A/Alk/-	Non motile

The differences between non lactose fermenter are summarized in the following table:

Items	<i>Salmonella</i>	<i>Shigella</i>	<i>Proteus mirabilis</i>
MacConkey	Pale yellow colonies	Pale yellow colonies	Pale yellow colonies
SS agar	Pale yellow colonies with black center	Pale yellow colonies	Pale yellow colonies with black center
EMB	Colorless colonies	Colorless colonies	Colorless colonies
Swarming	No swarming	No swarming	Swarm on non inhibitory medium
Indole	-	-	-
MR	+	+	+
VP	-	-	-
CIT	-	-	+
Urease	-	-	+ (2hrs)
TSI/H₂S	A/AIk/+	A/AIK/-	A/AIK/+
Motility	Motile	Non-motile	Motile



Shigella sp. on XLD



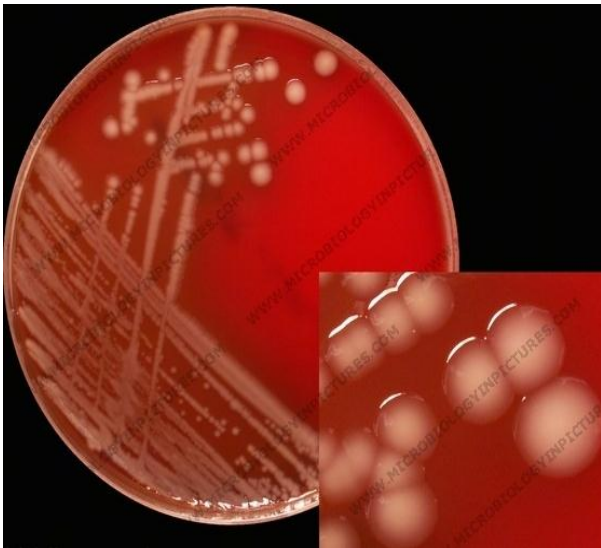
Salmonella on XLD. (Except for *S. typhi* which has white colonies)



Salmonella sp. growth on SS agar media (black colonies)



Klebsiella pneumoniae on MacConkey agar.



Klebsiella sp colonies on blood agar media. Some isolated strains are not haemolytic



E. coli on MacConkey media (lactose fermentor)

Swarming proteus



Escherichia coli

Salmonella
On SS agar media

Shigella