

## Enterobacteriaceae

General characters: Numerous interrelated bacterial flora of intestine are Gram-negative rods, motile only with peritrichate flagella or non-motile, non-sporing, non-acid fast, ferment glucose with or without formation of gas, reduce nitrates into nitrites, form catalase, oxidase negative and aerobic or anaerobic

### Classification

I. Based on action on lactose:

It is an old method. It has practical value in diagnostic bacteriology.

- a. Lactose fermenter e.g. *Escherichia coli*, *Klebsiella*
- b. Late lactose fermenter e.g. *Shigella sonnei*, *Paracolon*
- c. Non-lactose fermentation e.g. *Salmonella*, *Shigella*

II. Modern taxonomical concept :

Enterobacteriaceae may be classified into tribes, genera and species by their cultural and biochemical characters. The species are further classified as biotypes, serotypes, bacteriophage types and colicin types. At present there are five tribes as under :

Enterobacteriaceae

Tribe I Escherichieae Genus: *Escherichia*, *Edwardsiella*, *Citrobacter*, *Salmonella*, *Shigella*

Tribe II Klebsielleae : *Klebsiella*, *Enterobacter*, *Hafnia*, *Serratia*

Tribe III Proteae Genus: *Proteus*

Tribe IV Erwinieae Genus: *Erwinia*

Tribe V Yersinae : *Yersinia pestis*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*

### **ESCHERICHIA COLI**

It lives only in human or animal intestine. Detection of *E. coli* in drinking water is taken as evidence of recent pollution with human or animal excreta. *Escherichia coli* in contaminated water may be detected using PCR (rapid and sensitive), DNA probes, plating and biochemical tests.

**Morphology:** It is Gram-negative, non capsulated short, plump bacilli  $2 \text{ to } 4 \mu \times 0.4 \text{ to } 0.7 \mu$  in diameter and are motile. Spores are not formed.

**Cultural characters:** It is aerobic and facultative anaerobe growing on simple media Optimum temperature is  $37^{\circ}\text{C}$

1. Liquid broth: It shows uniform turbidity after 8 to 24 hours' incubation

2. **Nutrient agar:** Colonies appear after 12 to hours of incubation. They are circular 1 to 3 mm in diameter, smooth, colorless having entire edge with butyrous consistency Colonies are emulsified easily.

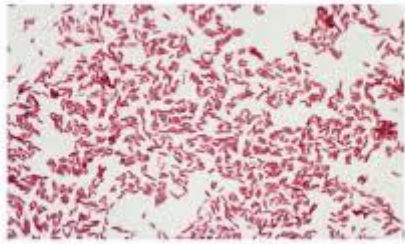
3. **MacConkey medium:** The colonies are pink due to lactose

4. **Blood agar:** Some strain may show zone of beta hemolysis

**Biochemical reactions:** It ferments lactose, glucose, sucrose, maltose and mannitol forming acid and gas. Indole and methyl red (MR) is positive. VP and citrate is negative. Urease is not hydrolyzed.  $\text{H}_2\text{S}$  is not produced.

**Resistance:** It can survive for months in soil and water. It is killed at  $60^{\circ}\text{C}$  in 20 minutes and chlorine (0.5 to 1 part per million). It is sensitive to streptomycin, tetracycline, chloramphenicol furadantin and nalidixic acid.

*E.coli* – Gram stained smear



## Laboratory Diagnosis

### 1. Hematological investigations :

- Total leukocyte count is usually within normal limits. In tissue invasion moderate-leukocytosis may be there.
- Differential leukocyte count: There may be increase in polymorphonuclear cells in tissue invasion.

**2. Bacteriological investigations:** Specimen: In urinary tract infection midstream urine is collected under aseptic conditions. Immediately the urine is examined and in case of delay it should be stored at 4°C.

In acute diarrhea a sample of feces or a rectal swab is collected. Pus may be collected on sterile cotton swab or sterile container.

**Smear Examination:** Centrifuged urine deposit is examined for pus cell, RBC and bacteria. Gram-stained smear from centrifuged deposit shows moderate to large number of pus cell and Gram-negative bacilli.

Here DNA probes for different enteropathogenic forms are quite reliable and useful. ELISA, precipitin test, radioimmunoassay are other useful tests to establish the identity of enteropathogenic strains.

**Culture:** Material is inoculated on blood agar plate and MacConkey plate. Lactose fermenting, Gram negative motile, indole positive, MR positive, VP negative and citrate negative is suggestive of *Escherichia coli*.

The count of organism should be more than lakh per ml (10<sup>5</sup> per ml) in urinary tract infection. It is called significant bacteriuria.

### **KLEBSIELLA**

It is found in the mucosa of upper respiratory tract, intestine and genitourinary tract. It is

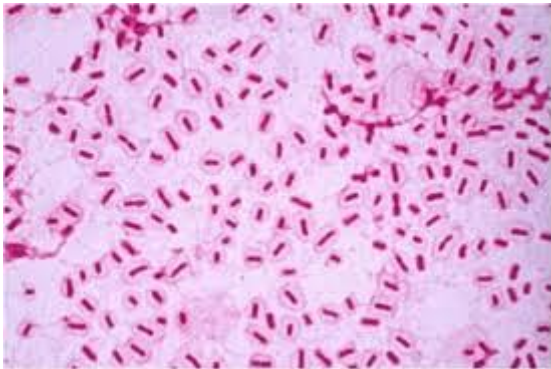
non-motile, capsulated, growing in ordinary media forming large mucoid colonies of varying degree of stickiness. It has been classified into 3 species on the basis of biochemical reactions.

1. ***K. pneumoniae***: It ferments sugar (glucose, lactose, mannitol) with production of acid and gas. Indole and MR are negative, VP and citrate are positive. It hydrolyses urea.

It may cause pneumonia, urinary tract infection and pyogenic infections. Serotype 1, 2 or 3 is usually responsible for pneumonia.

2. ***K. ozaenae***: It causes foul smelling nasal discharge (ozoena). Biochemical reactions are variable. It belongs to capsular type 3, 4, 5, 6.

3. ***K. rhinoscleromatis***: It causes rhinoscleroma. Organisms are seen intracellularly in lesion. It belongs to capsular type 3.



### Difference between Klebsiella and Escherichia coli

#### ***E. coli***

1. Non-capsulated
2. Motile
3. Indole and MR positive  
Citrate and VP negative
4. Urease negative
5. Colonies not mucoid and  
string test negative
6. Slender and long
7. Gas from glucose fills  
1/3rd of Durham's tube

#### ***Klebsiella***

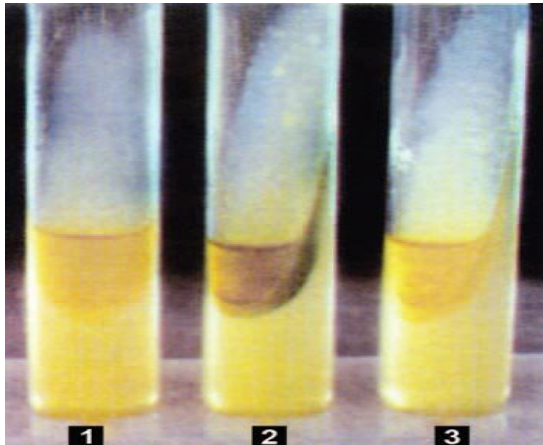
1. Capsulated
2. Non-motile
3. Indole and MR negative  
Citrate and VP positive
4. Urease positive
5. Colonies mucoid with  
string test positive
6. Short and thick
7. Gas from glucose fills  
more than 2/3rd of Durham's tube

## **PROTEUS**

**Morphology:** It is Gram-negative rods showing great variation in size,  $0.5 \times 1$  to  $3 \mu$ . It may be in long filaments or in granular form.

It is actively motile and show swarming motility, best seen at  $20^{\circ}\text{C}$ . It is non sporing and non-capsulated.

**Cultural character:** It is aerobic and facultative anaerobic. Culture emits characteristic putrefactive (fishy or seminal) odor. *Proteus vulgaris* and *Proteus mirabilis* show swarming type of growth at  $37^{\circ}\text{C}$  while swarming is absent in other species.



Phenylalanine agar:

1. Control; 2. *Proteus vulgaris*; 3. *Escherichia coli*



Swarming *Proteus* on blood agar media

**Broth:** It shows uniform and moderate turbidity after 18 to 24 hours of incubation.

There is powdery deposit and ammoniacal odor.

**Nutrient agar:** *Proteus vulgaris* and *Proteus mirabilis* swarm on solid media at  $37^{\circ}\text{C}$  after 12 to 18 hour incubation. Swarming may be due to progressive surface growth spreading from the edge of parent colony.

Swarming can be suppressed by:

- |                              |                               |
|------------------------------|-------------------------------|
| 1. Six percent agar in media | 2. Chloral hydrate            |
| 3. Sodium azide (1 : 500).   | 4. Alcohol (5 to 6%).         |
| 5. Sulfonamide               | 6. Boric acid (1 : 1000), etc |

Swarming does not occur on MacConkey agar medium.

**Biochemical reactions:** It forms acid and gas from glucose (except *Proteus rettgeri*). It characteristically deaminates phenylalanine to phenylpyruvic acid (PPA).

Hydrolysis of urea is another characteristic property of *Proteus*. It is MR positive and VP negative. It is nonlactose fermenter.  $\text{H}_2\text{S}$  is produced in *Proteus vulgaris* and *Proteus mirabilis*. Indole is not produced by *Proteus mirabilis*. *Proteus morganii* and *Proteus rettgeri* are  $\text{H}_2\text{S}$  negative. Citrate is positive in *Proteus rettgeri* and negative in *Proteus morganii*.

### **Laboratory Diagnosis**

**Hematological investigations:** Leukocytosis with increase in polymorphonuclear cells may occur when tissues are invaded.

**Bacteriological investigations:** On culture of material (urine, pus, sputum, etc.) we find swarming type of growth. It can be further identified by biochemical tests.

## SHIGELLA

It is found exclusively in the intestinal tract of man.

Morphology: It is non-motile, non-capsulated about  $0.5 \times 1$  to  $3 \mu$  in size.

**Cultural character:** It is aerobic and facultative anaerobic, grows readily in simple media with an optimum temperature of  $37^{\circ}\text{C}$  and pH of 7.4 .

**Broth:** There is uniform growth with mild turbidity after 12 to 24 hours' incubation. There is no pellicle formation.

Nutrient agar: After overnight growth colonies are small, 2 mm in diameter, circular, convex, smooth and translucent.

MacConkey agar: Colonies are colorless due to the absence of lactose fermentation except *Shigella sonnei* which ferments lactose late and forms pink colonies.

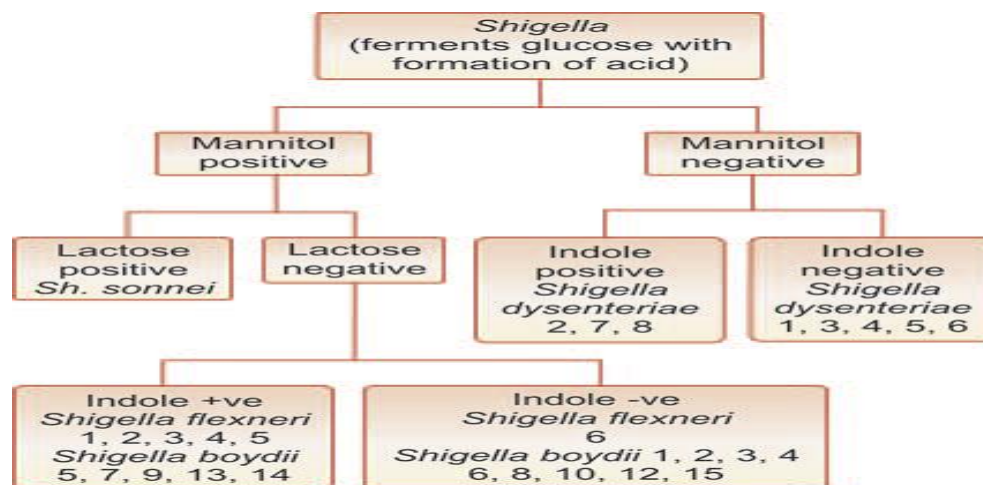
Desoxycholate citrate agar (DCA): It is a useful selective medium for *Shigella*

Resistance: It is killed at  $56^{\circ}\text{C}$  in 1 hour and 1 percent phenol in 30 minutes. Boiling pasteurization and chlorination kill the organism. In water and ice it survives and remain viable for 1 to 6 months.

### Biochemical Reactions :

It is MR positive and reduces nitrates to nitrites. It does not form  $\text{H}_2\text{S}$ , cannot utilize citrate and is inhibited in KCN. Catalase is positive except *Shigella dysenteriae* type I . Glucose is fermented with production of acid

and no gas (except Newcastle and Manchester biotypes of *Shigella flexneri* type 6). Fermentation of mannitol forms the basis of classification as shown above.



### Laboratory Diagnosis

**Collection of specimen:** Stools are collected under aseptic precaution and examined as under microscope.

Microscopic examination: Wet cover slip preparation shows large number of pus cells with degenerated nuclei, RBC and macrophages. Bacterial flora is considerably diminished.

A loopful of pus or blood tinged mucus from freshly passed fecal sample is cultured on MacConkey and DCA. Selenite broth is used as enrichment medium. After 12 to 18 hours of incubation colorless colonies (non-lactose fermenter) appear on.

**MacConkey medium.** These are tested for motility and biochemical reactions. Non motile organism which is urease, citrate KCN and  $\text{H}_2\text{S}$  negative, indole and MR positive suggestive of *Shigella*.

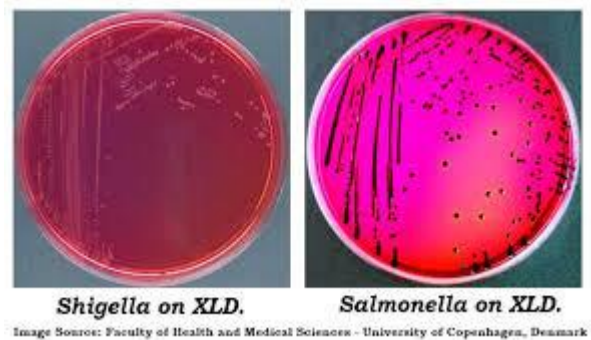
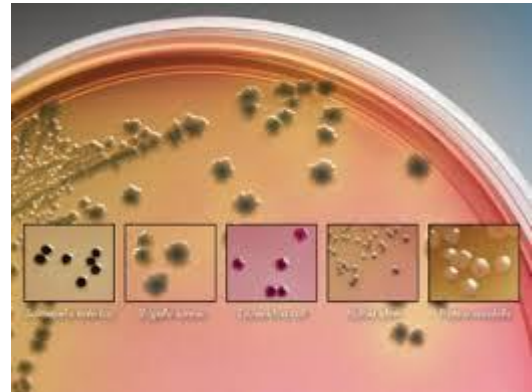
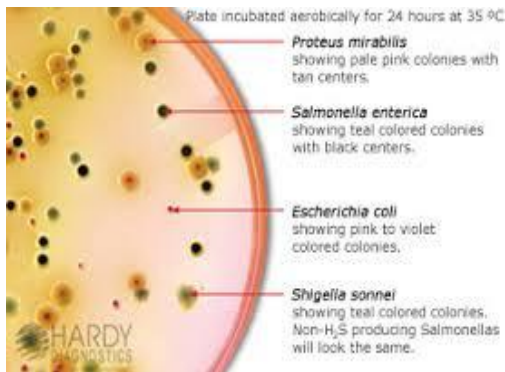
### Identification



is confirmed by slide agglutination with polyvalent and monovalent antisera.

An ECO RI generated 17 Kb fragment of *Shigella flexneri* serotype 5 virulence plasmid may identify specifically all *Shigella* species. This DNA hybridization technique may be useful in the identification of isolates of *Shigella* which fail to agglutinate with reference to *Shigella* antisera.

**Serology:** The fluorescent antibody technique has been employed for direct identification of shigellae in feces. It is of no value in diagnosis .



## **SALMONELLA**

Genus *Salmonella* is found in the intestine of man, animals and birds. Sometimes food (egg and meat) may be contaminated with this organism. It may cause enteric fever, gastroenteritis and septicemia.

**Morphology:** It is Gram-negative rods,  $2$  to  $4\mu \times 0.6\mu$  in size, motile (except *Salmonella gallinarum* and *Salmonella pullorum*). They are non-capsulated and non-sporing but may have fimbriae.

**Cultural character:** They are aerobic and facultative anaerobic, optimum temperature for their growth is  $37^{\circ}\text{C}$  and pH is 6 to 8.

**Broth:** It shows uniform turbidity after overnight culture. There is no pellicle formation.

**Blood agar:** Colonies are large 2 to 3 mm in diameter, circular low convex, translucent smooth and non-hemolytic .

**MacConkey's media:** It is non-lactose fermenter and colorless.

**Desoxycholate citrate media:** These colonies are non-lactose fermenter (colorless)

**Wilson and Blair bismuth sulfite medium:** Jet black colonies with metallic sheen due to  $H_2S$  formation may appear.

**Selenite F and tetrathionate:** These broth are commonly used as enrichment media.

**Biochemical reactions:** It ferments glucose mannitol and maltose forming acid and gas except *Salmonella typhi* which produces only acid and no gas. It does not produce indole but is MR positive, VP negative and citrate may be positive. Urea is not hydrolyzed and H<sub>2</sub>S is produced.

Laboratory Diagnosis

**1. Hematological investigations:**

a. Total leukocyte count in typhoid fever shows leucopenia. The count may be 3000 to 8000 per cu mm.

b. Differential leukocyte count: There may be lymphocytosis and monocytosis .

**2. Bacteriological investigations:** Organism may be isolated from blood, urine, feces, persistent discharge and in some cases from cerebrospinal fluids.

a. Blood culture: 5 to 10 ml blood of a patient is collected aseptically and is transferred into blood incubation of bottle at 37°C, 'culture bottles containing 100 ml of bile broth. After 24 to 48 hours they are sub cultured on blood agar and MacConkey, on which bacilli grow as non lactose fermenting, Gram-negative and motile organism .

b. Clot culture: Blood clot is cultured in 15 ml bile broth bottle (0.5% bile salts). It is more frequently positive.

c. Fecal culture: It is positive throughout .

Repeated cultures are required for successful isolation of organism. Fecal culture is useful more in cases who are on chloramphenicol.

Successful culture depends on use of enrichment and selective media. Fecal samples are plated directly on MacConkey, DCA and Wilson and Blair media. On MacConkey and DCA we find pale colonies. On Wilson and

Blair we find black colonies with metallic sheen. *S. paratyphi A* produce green colonies.

For enrichment, specimen are inoculated into one tube each of selenite F and tetrathionate broth. It needs 12 to 18 hours' incubation before subculture.

**Bile culture:** It is important for detection of carriers and in later stages of disease. Bile aspirated by duodenal tube is processed like fecal specimen.

**Urine culture:** It is less useful than blood and feces. Culture is positive in 2nd and 3rd week.

Clean voided urine is centrifuged and sediments are inoculated into enrichment and selective media.

Other material: Bone marrow culture is positive in most cases. Other specimens like rose spots, pus from lesion, CSF and sputum may be used for culture. At autopsy culture may be obtained from gallbladder, liver spleen and mesenteric lymph nodes.

**Serological test (Widal test):** *Salmonella* antibody appears at the end of first week. Widal test is used for this purpose. This is a test to measure H and O antibodies in the sera of patient. Two types of tubes are used :

a. Narrow tube with conical bottom (Dreyer's tube) for H agglutination.

b. Short round bottomed tube (Felix tube) for O agglutination.

Serial two fold dilution of patient serum and so on are mixed with equal volume of antigen (TO, TH, AO and

AH). At 37°C incubation is done for 4 hours and then rack is kept at 4°C overnight. H agglutination leads to formation of cotton wooly clump and O agglutination is seen as matted, granular irregular disc like pattern at the bottom of tube.

### **Interpretation of Widal Test**

1. Agglutination appears by the end of first week. The titer increases steadily till 4th week after which it declines.
2. Demonstration of rising titer of antibody by testing two or more samples are more Meaningful.
3. In single test 1/100 titer of O and 1/200 or more titer of H is significant.
4. In immunization, antibody against both S typhi and S. paratyphi will be there whereas in infection antibody will be seen only against infecting organism.
5. Immunized person or patients who have had prior infection may develop anamnestic response during unrelated fever. In anamnestic response there is temporary rise in H titer only, whereas in enteric it is sustained
6. Bacterial suspension should be free from fimbria otherwise false-positive result occurs.
7. Treated case may show poor agglutination response.

Tracing of typhoid carrier: Sewer swab technique is quite helpful in tracing of carriers .

This is done by following the gauze pads kept in sewers and positive for Salmonella typhi cultures, backwards from the main drain, ultimately lead to localization of the house of the carrier. However, typhoid carrier may be detected as follows

- a. Widal test may show raised antibody titers.
- b. Vi agglutination test is positive in a titer of 1/10 or more.
- c. Several stool cultures may help in the isolation of causative organism.
- d. Organism may be cultured from bile obtained after duodenal intubation.