ENTERIC BACTERIAL PATHOGENS

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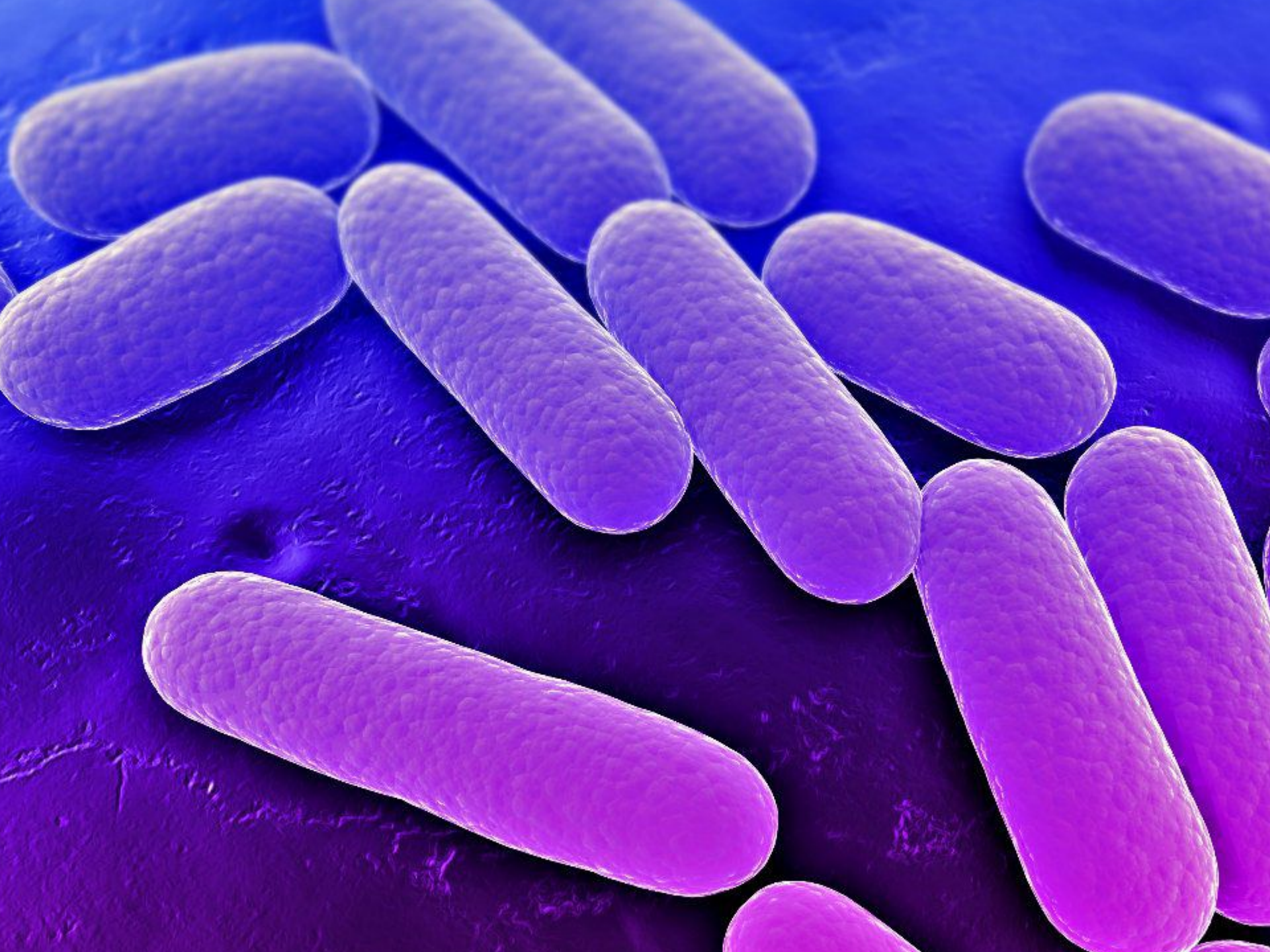


FAMILY ENTEROBACTERIACEAE

- are a large heterogeneous group of

Gram«-» rods whose natural habitat is the intestinal tract of humans and animals.

- **Genera (20)**

1. Escherichia

2. Salmonellae

3. Shigellae

4. Klebsiellae

5. Yersinia …..

FAMILY ENTEROBACTERIACEAE

Most of the members of Enterobacteriaceae are facultative anaerobes, ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors.

This family is characterized biochemically by the ability to ferment glucose with the production of acid or acid and gas, and to reduce nitrates to nitrites.

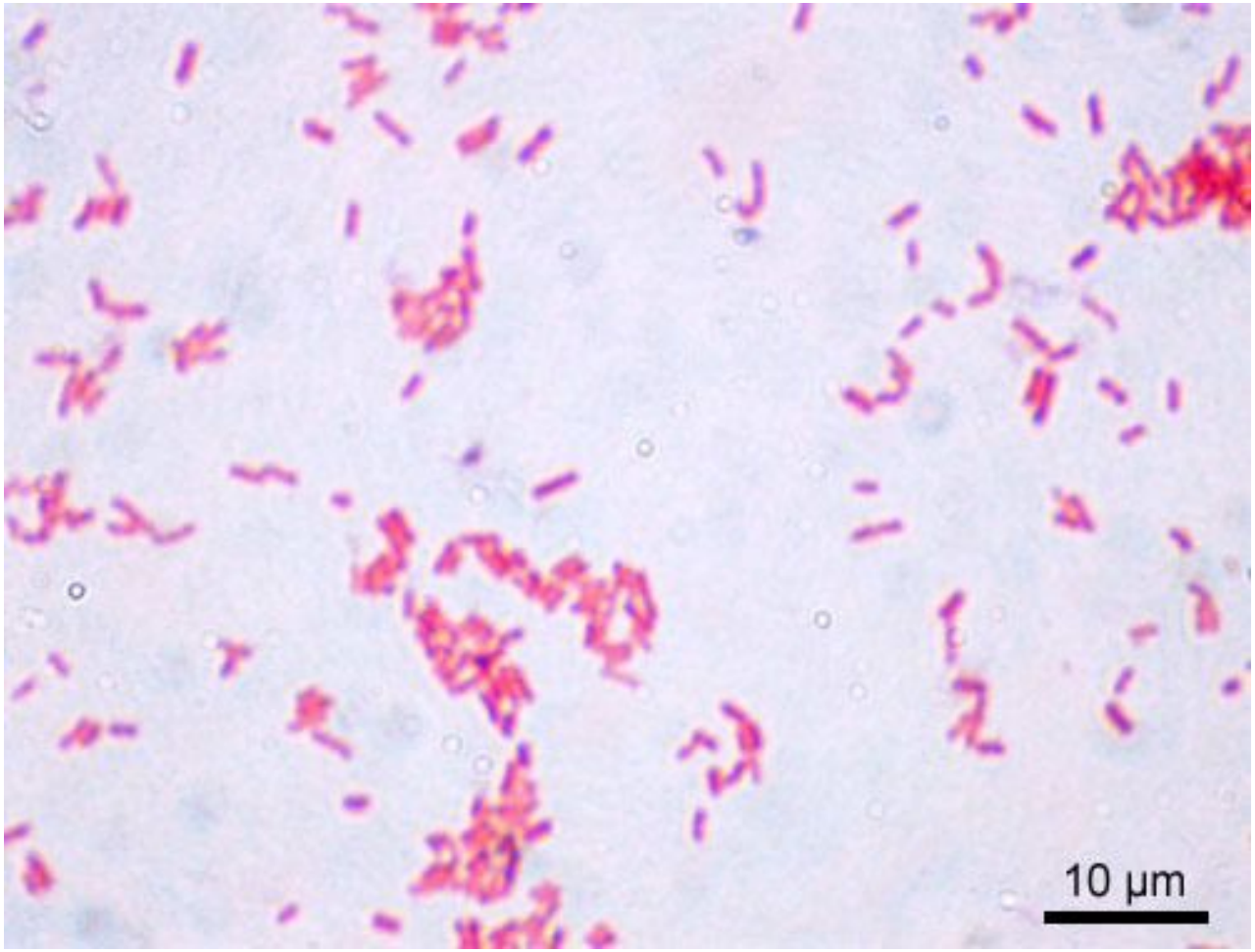
All members of this family are oxidase-negative.

FAMILY ENTEROBACTERIACEAE

- **Gram «-» rods**
- **Spores «-»**
- **Capsula «+» or «-»**
- **They are motile (*E.coli)* or non-motile (*Shigellae*)**
- **Facultative anaerobes**
- **T= 30-37ºC pH= 7,2-7,5**
- **Endo medium, Ploskirev medium, Levin medium, MacConkey agar.**

  Oxidase «-», Glucose «+», Lactose «-» ex.E.coli,
- **Mannitol «+», Indol «-» ex.E.coli, H2S «-» or «+».**

FAMILY ENTEROBACTERIACEAE

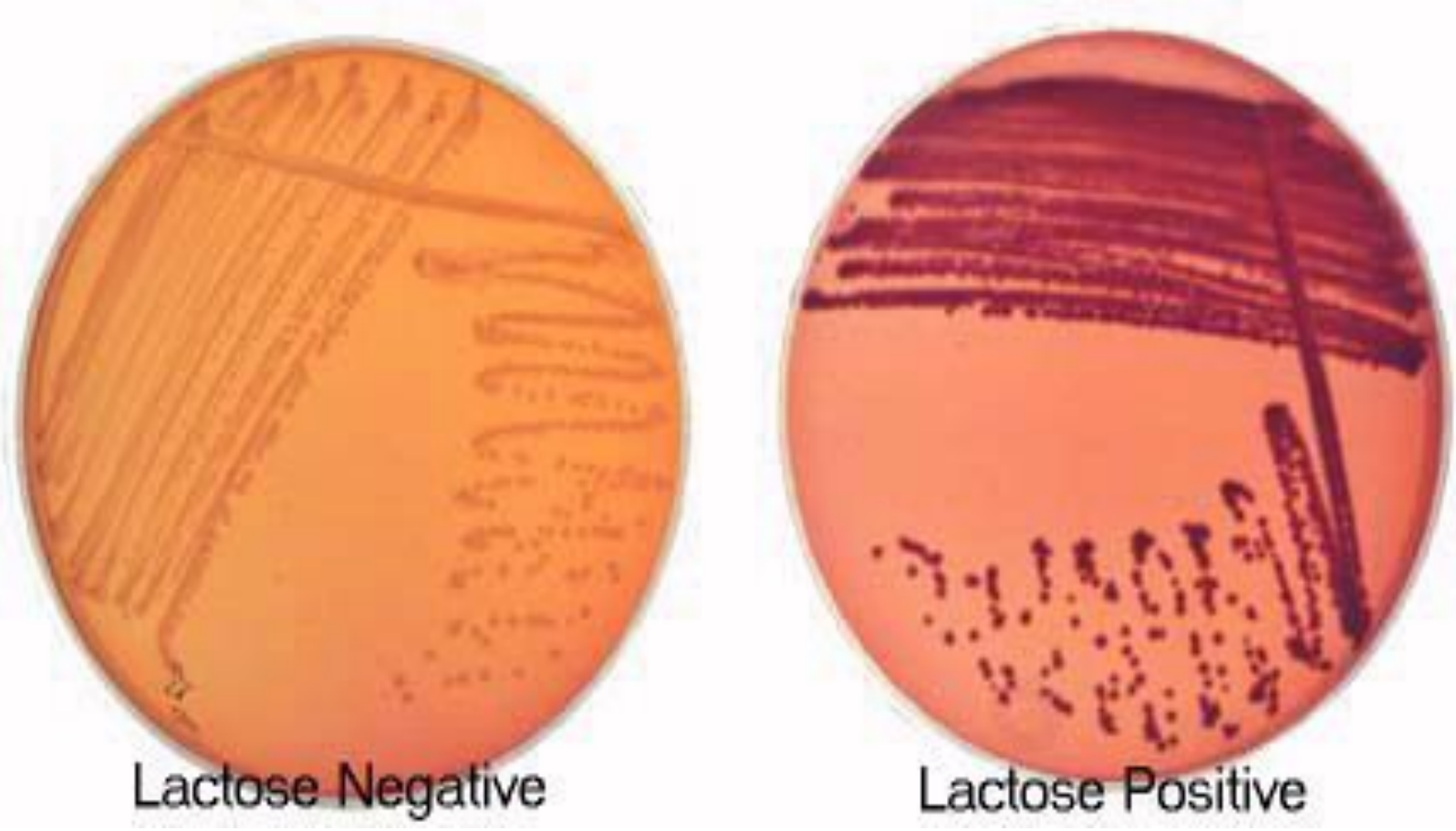


FAMILY ENTEROBACTERIACEAE

The differential culture media that contain carbohydrates and special dyes (indicators) are used to distinguish lactose-fermenting bacteria that form colored colonies from non-lactose-fermenting microorganisms producing colorless colonies on such differential media.

These culture media can allow rapid identification of enteric bacteria.

- Endo Medium

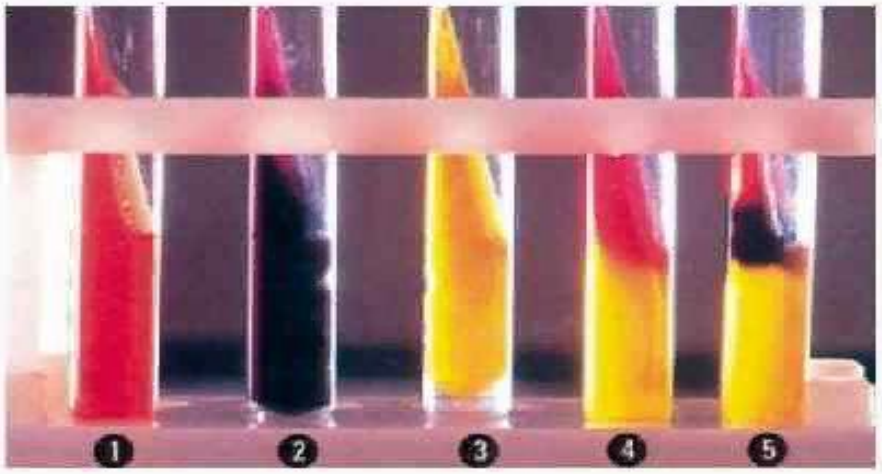


Kligler Iron Agar

Kligler Iron Agar

- Kligler iron agar is used for the differentiation of the Enterobacteriaceae members on the basis of their ability to ferment glucose and lactose and to liberate sulfides.
- Gas formed by carbohydrates fermenters is detected as bubbles or by splitting or displacement of the agar.
- Hydrogen sulfide production is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt.

Kligler Iron Agar



- Дифференциация представителей семейства *Enterobacteriaceae* на среде Клиглера: 1 - среда до посева; 2 - *Salmonella;* 3 - *Escherichia;* 4 - *Shigella;* 5 - *Salmonella* Typhi

Kligler Iron Agar

The lactose-positive and glucose-positive bacteria show both the slant and the butt yellow in color (E. coli).

The lactose-negative and glucose-positive bacteria show the yellow butt and the red slant (Salmonella, Shigella).

If the bacterium is glucose -negative and lactose-negative, both the butt and the slant remain red.

Hydrogen sulfide production is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt.

FAMILY ENTEROBACTERIACEAE

Enterobacteriaceae have a complex antigenic structure.

They are classified by more than 150 different heat-stable somatic O (lipopolysaccharide) antigens, more than 100 heat-labile K (capsular) antigens, and more than 50 H (flagellar) antigens.

In Salmonella Typhi the capsular antigen is called Vi- antigen.

The antigenic classification of Enterobacteriaceae often indicates the presence of each specific antigen.

FAMILY ENTEROBACTERIACEAE

- **Virulence factors:**
- ***Fimbriae***
- ***Enterotoxins***
- ***Hemolysins***
- ***Endotoxins***

FAMILY ENTEROBACTERIACEAE

- Epidemiology:
- They are pathogenic for human and animals.
- They are transmitted by the fecal-oral route.
- They may be responsible for hospital infections.
- The main clinical symptoms are diarrhea, vomiting, temperature.

FAMILY ENTEROBACTERIACEAE

- Microbiological diagnosis.
- Specimens : feces, vomit, food,urine, blood.

  Methods: bacteriological, serological, biological.

Salmonella

- **Family** – Enterobacteriaceae
- **Genus** – Salmonellae
- **Species** - S. enterica
- **Subspecies** – S. typhi, S. paratyphi A, S. paratyphi B, S. enteritidis, S. typhimurium
- The main taxonomic groups of salmonella are:
- **Family** → **Genus** → **Species** → **Subspecies** → **Serova**r

S. typhi was discovered in 1880 by K. Eberth and isolated in pure culture in 1884 by G.Gaffky.

Salmonella

Salmonella

Morphology:

- Gram «-» rods
- Spores «-» Capsula «-» They are motile
- **Cultural properties:**
- Facultative anaerobes Chemoorganotrophs
- Topt= 35-37º C pH= 6,8-7,2
- Endo medium, Ploskirev medium – pale pink colonies. Levin medium – blue colonies
- MacConkey agar- colourless colonies
- Bismuth-Sulfite agar – black colonies.
- In MPB they produce a uniform turbidity.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Bismuth Sulfite agar

Salmonella

Biochemical activity:

Glucose «+», Maltose «+», Mannitol «+» (acid)

S.paratyphi ferments carbohydrates with acid and gas formation.

Lactose «-» Sucrose «-»

- **Indol «-», H2S «+»**
- **Gelatin – does not liquefy**
- **Oxidase «-»**

Salmonella

- **<u>Antigenic structure</u>**:
- **O-somatic (serogroups), is destroyed by formalin.**
- **H – flagellar (serovars) , is destroyed by phenol.**
- **Vi – antigen is located on the surface of the bacterial cell , is destroyed by phenol and temperature.**

- *Kauffmann and White classified Salmonellae according their antigenic structure.*

Salmonella. Antigenic structure

Based on the presence of O-antigens, the Salmonella have been assigned to serogroups. The O group is designated by capital roman letter (A, B, C, D).

Each Salmonella serogroup can be identified by the slide agglutination test.

Salmonella. Antigenic structure

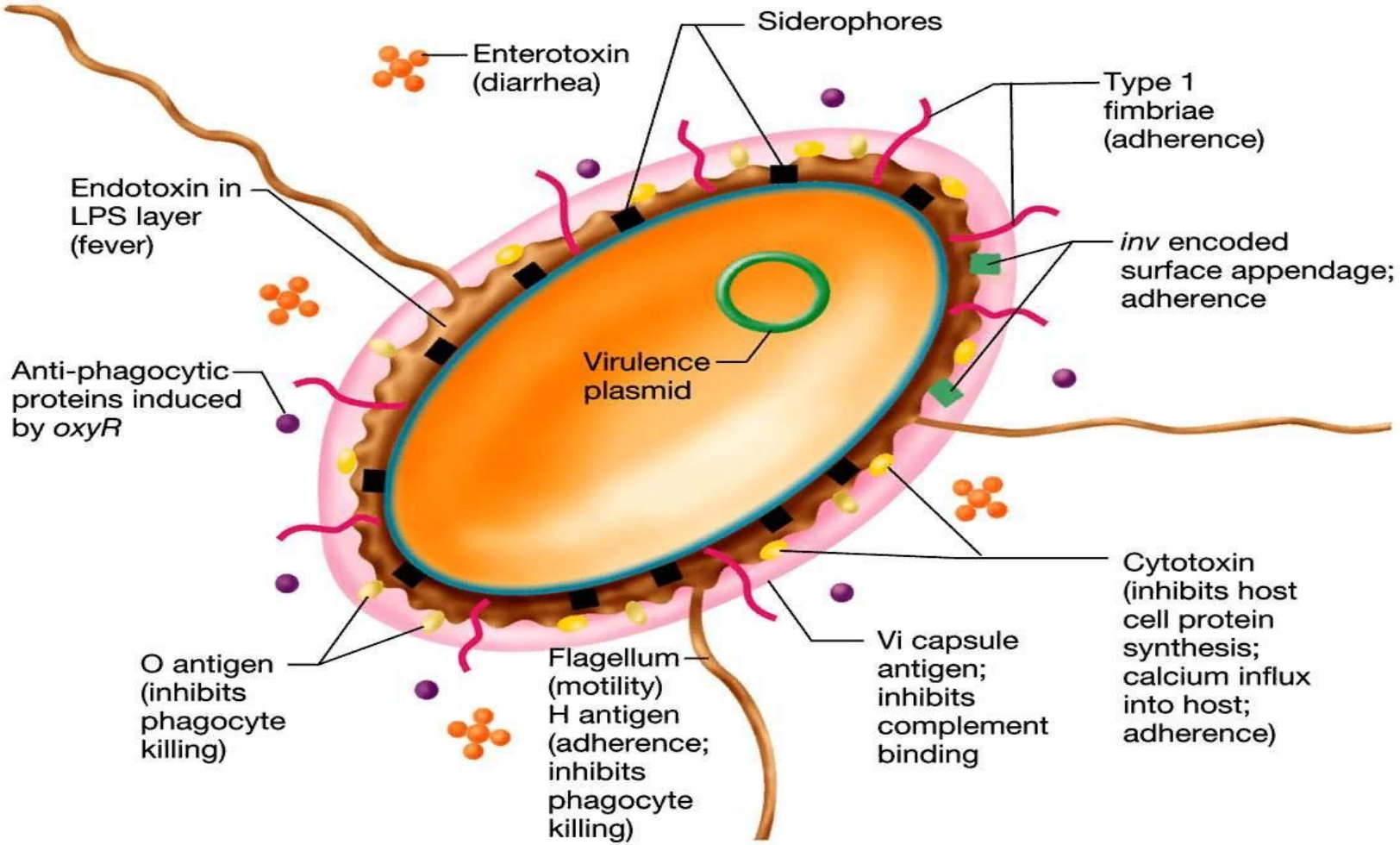
The H-antigens are designated by small roman letters and are kept in brackets (phase 1) and by arabic numerals for phase 2.

The use of specific H- antisera helps to identify the Salmonella serovars.

Salmonella

- **<u>Resistance:</u>**
- Survive in ice for several months.
- Survive in butter, meat, cheese, bread for 1-3 months.
- Survive in water and soil for several weeks.
- Susceptible to heat, t= 60-100º C.
- Susceptible to disinfectant solutions of phenol, chloramine.

Salmonella Virulence Factors



Salmonella Virulence Factors

The type III protein secretion system (T3SS) encoded by Salmonella pathogenicity island 1 (SPI-1) delivers effector proteins required for intestinal invasion and the production of enteritis.

SPI-1 encodes transcription factors that regulate the expression of some virulence factors of *Salmonella*, while other transcription factors encoded outside SPI-1 participate in the expression of SPI-1-encoded genes.

SPI-1 genes are responsible for the invasion of host cells, regulation of the host immune response, the host inflammatory response, apoptosis, and biofilm formation.

Salmonella

Salmonella are often pathogenic for humans or animals when acquired by the oral tract.

S. typhi, S. paratyphi A, and S. paratyphi B are the causative agents of enteric fevers.

Other species of salmonellae are the bacteria causing salmonellosis, a food-borne infectious disease (enteritis).

The enteric fevers are transmitted by the fecal-oral route.

Pathogenesis

The ingested salmonellae reach the small intestine, from which they enter the lymphatics (Peyer's patches) and then the bloodstream. After an incubation period of 10-14 days, fever, malaise and headache occur due to bacteriemia and toxicity of Salmonella by-products.

The skin may become dotted with small hemorrhages called "roseoles".

Gastrointestinal symptoms appear late in the course of the disease. Blood cultures are positive only in the first week of the disease.

At the beginning of the second week microbes are carried by the blood to many organs, including the liver, kidneys and the intestine. Salmonellae multiply in intestinal lymphoid tissue, kidneys and are excreted in stools and urine.

The stools and urine cultures give positive results on the second and third week.

Pathogenesis of enteric fever

- 1st stage – the ingestion
- 2nd stage – the invasion
- 3rd stage – bacteremia
- 4th stage – bacterial dissemination
- 5th stage – hyperergia and excretion
- 6th stage – final stage
- Immunity acquired is relatively stable but relapses and reinfections sometimes occur.

Laboratory Diagnosis

Blood cultures are positive only in the first week of the disease.

At the beginning of the second week microbes are carried by the blood to many organs, including the liver, kidneys and the intestine.

Salmonellae multiply in intestinal lymphoid tissue, gallbladder, kidneys and are excreted in stools and urine. The stools and urine cultures yield positive results on the second and third week.

A positive culture of bile establishes the presence of Salmonella genus in the biliary tract of carriers.

Salmonella Infections. Treatment .

Antimicrobial treatment.

Replacement of fluids and electrolytes are essential.

Susceptibility testing is an important method to choosing a proper antibiotic, because multiple drug resistance is a big problem in enteric bacteria.


Shigella

SHIGELLA

The causative agent of dysentery was described in 1888 by A. Chantemesse and in 1891 by A. Grigoryev and F. Widal.

In 1898 this organism was studied in detail by K. Shiga in Japan.

In 1900 S. Flexner in the Philippines isolated dysentery organisms.

Later other bacteria causing dysentery were discovered. According to the current International Nomenclature, all dysentery bacilli are grouped together in one genus known as *Shigella.*

SHIGELLA

Shigellosis (or bacillary dysentery) is a clinical condition characterized by fever, bloody diarrhea, and fecal leukocytosis. Classical bacterial dysentery is associated with infections caused by any of the four Shigella species:

Sh. dysenteriae, Sh. flexneri, Sh. boydii,

Sh. sonnei.

Taxonomy

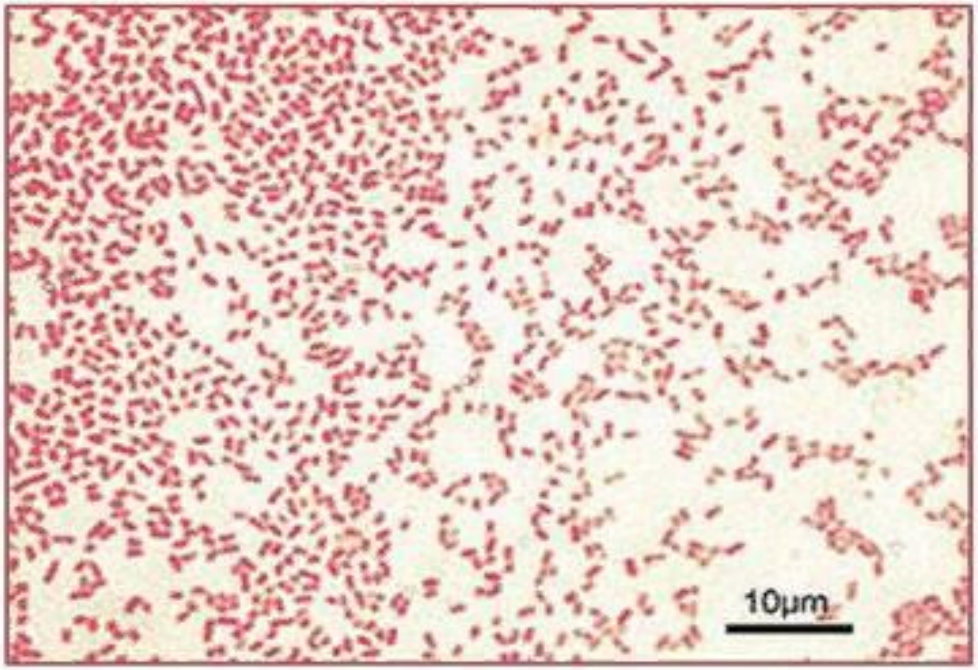
Family – Enterobacteriaceae

Genus – Shigella

Species - S. dysenteriae, S. flexneri, S. boydii,

S. sonnei.

Shigella



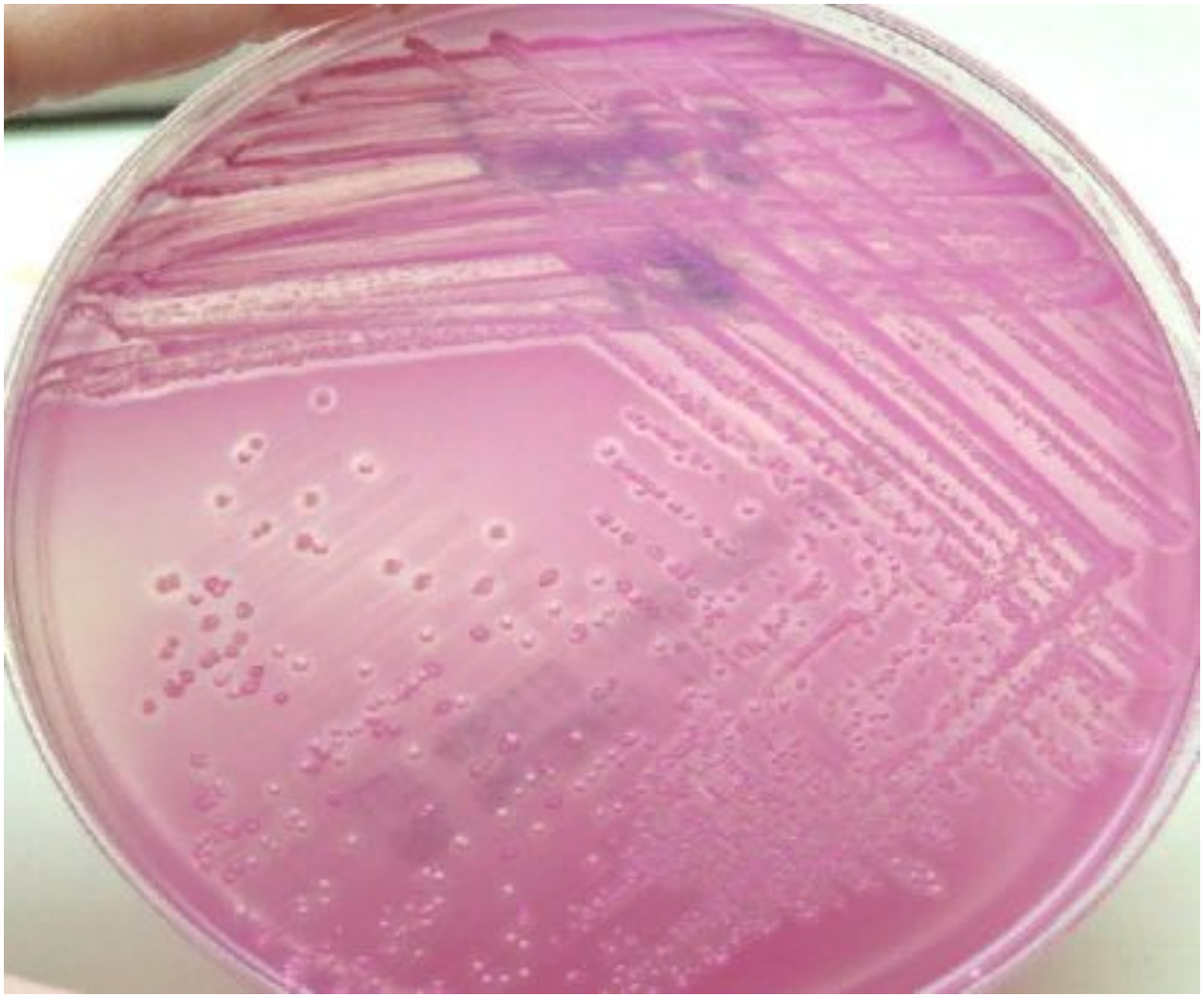
SHIGELLA

- **Shigella are slender gram-negative rods.**
- **Spores «-»**
- **Mirocapsula «+»**
- **They are non-motile**

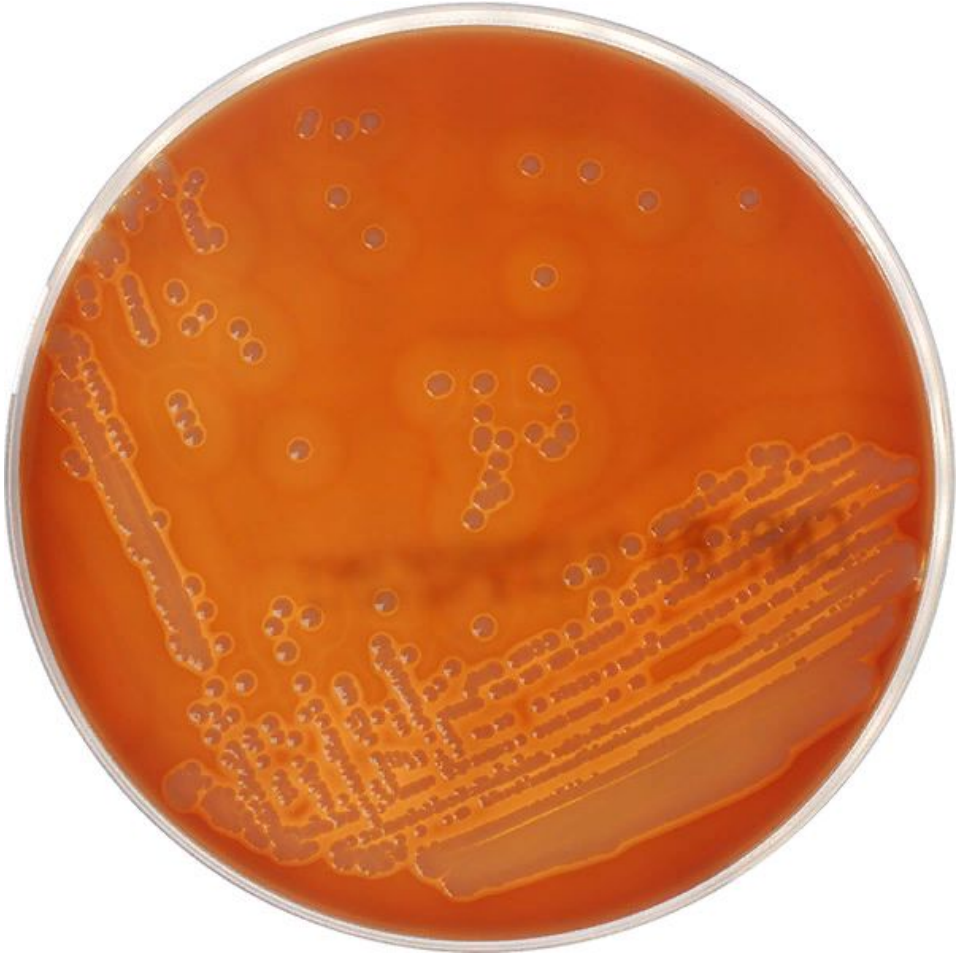
They are facultative anaerobes, but grow best aerobically.

Ploskirev agar

Endo agar



Levine Eosin Methylene Blue agar



SHIGELLA

All Shigella ferment glucose, form acid.

With the exception of Shigella sonnei, they do not ferment lactose.

Glucose «+» Lactose «-»

Mannitol «+» (S.dysenteriae «-») H2S «-»

Antigens: O – somatic antigen, K-surface antigen.

SHIGELLA

Shigella-induced infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare.

All Shigellae produce endotoxin.

Microabscesses in the wall of the large intestine lead to necrosis of the mucous membrane, superficial ulceration and bleeding.

Shigella dysenteriae also produces a heat-labile exotoxin that affects both the gut and the central nervous system.

Shigellosis

Shigellosis is the infection with fecal-oral route of transmission.

After a short incubation period (1-2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea.

A day or so later, as the infection involves the ileum and colon, the number of stools increase; they are less liquid but often contain mucus, pus and blood.

In children and the elderly, loss of water and electrolytes may lead to dehydration, acidosis, and even death.

Infection is followed by a type-specific immune response, but reinfection may occur.

Shigellosis. Diagnostic Laboratory Tests

SPECIMENS: Fresh stool, mucus flakes, and rectal swabs are taken for culture.

BACTERIOLOGICAL EXAMINATION:

The specimen is streaked on Ploskirev, (MacConkey or Levine EMB agar).

Colorless (lactose-negative) colonies are inoculated into the Kligler iron agar.

Organisms that not produce H2S and produce acid but not gas in the butt of the Kligler agar (glucose «+»), and that are nonmotile should be subjected to the slide agglutination test by specific Shigella antisera.

Shigellosis. Treatment and Immunoprophylaxis

AB - Ciprofloxacin, ampicillin, tetracycline, chloraphenicol

The eubiotics may be effective in limiting multiplication. There are no vaccines available for immunoprophylaxis of bacterial dysentery.

- isolation of patients and disinfection of excreta,
- detection of subclinical cases,
- sanitary control of water, food, and milk.