

## **Family Enterobacteriaceae**

## General characteristics

The largest family among bacteria and constitute 50% of bacterial infections. More than 25 genera and 150 species.

**ALL are gram-negative bacilli** found as normal flora in the colon of **humans** and **animals** as well as wide spread in the environment like water, soil and vegetation.

All are aerobic or facultative anaerobic bacteria

All are oxidase negative but catalase positive

All ferment glucose with acid. This feature is used in the laboratory to distinguish them from the other large group of gram-negative bacilli that are NON-FERMENTERS (Pseudomonas and others).

All reduce nitrate to nitrite (Detected by uripath colorimetric strips indicated to UTI)

All release endotoxin (responsible for septicemia) and some produce exotoxins like enterotoxin

Some members are non pathogenic, other opportunistic pathogen when found outside of GIT, while others are true pathogens like Salmonella, Shigella, Yersinia and some types of E. coli

Resistant to antibiotics (Extended spectrum beta lactamase =ESBL)

There are 3 major antigens

1- O-Ag

2-K-Ag

3-H-Ag. These antigens are used for serotyping e.g. *Salmonella* has more than 2500 serotypes and *E. coli* more than 150 O types, 50 K and H types. e.g. *E. coli* O157:H7

One of the commonly used media in the laboratory to grow these organisms is the **MacConkey agar**. This plate contains lactose and it is useful in the diagnostic laboratory to distinguish those that ferment lactose from those that don't as a first step in identification. **Lactose fermenters** *E. coli*, *Klebsiella* and **lactose non-fermenters** are *Salmonella*, *Shigella* and *Proteus*.

# ***Escherichia coli***

## **General characters**

- 1- Is the **predominant** normal flora of colon among **aerobic and facultative anaerobic bacteria**. Also found in soil, water and foods. (E.coli from GIT called coliform and grow at 45C while E.coli from environment cannot)
- 2- **Majority** are **normal flora in the GIT** but become opportunistic pathogens when found outside of GIT like UTI, meningitis, while **minority** are **true pathogens** (cause diarrhea).
- 3- E.coli attached to the host cells via a common pili
- 4- Some strains are motile while others are non motile
- 5- Most strains are Lactose ferments and sorbitol fermenters while other strains are not.
- 6- Coliform bacteria mainly E. coli used as indicator of water contamination in routine microbiological examination of drinking water.
- 7- Antigenic structure (O =200 types (O157), H = 50 types (H7) , K= 50 types (K1)

**Don't forget that E. coli used in biotechnology for transformation and production of cloned gene.**

## **Virulence factors of E.coli**

### **Uropathogenic E.coli**

- 1- Fimbriae for adhesion
- 2- Exotoxins like hemolysin
- 3- Capsular antigen

### **Diarrhagenic E.coli**

- 1- Pilli
- 2- Exotoxins like ST and LT
- 3- Shiga toxin (EHEC)
- 4- LPS (septicemia and septic shock)

## Clinical infections

### Intestinal infections

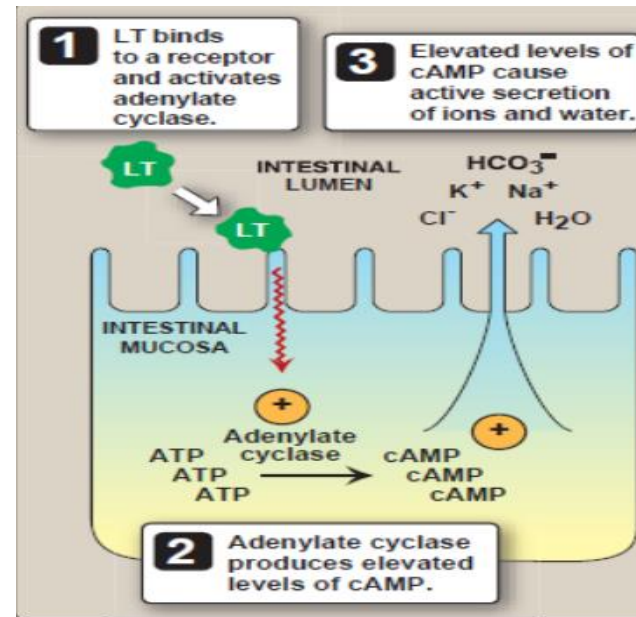
At least five types of intestinal infections that differ in pathogenic mechanisms have been identified

STRAIN	ABBREVIATION	SYNDROME	THERAPY <sup>1</sup>
Enteroto <b>toxicogenic</b> E. coli	E <b>T</b> EC	Watery diarrhea	Antibiotics may be useful. <sup>2</sup>
Enterop <b>athogenic</b> E. coli	E <b>P</b> EC	Watery diarrhea of long duration, mostly in infants, often in developing countries	Antibiotics may be useful. <sup>2</sup>
Enteroh <b>emorrhagic</b> E. coli	E <b>H</b> EC	Bloody diarrhea; Hemorrhagic colitis and hemolytic uremic syndrome (HUS)	Avoid antibiotics because of the possible risk of potentiating HUS.
Enterob <b>invasive</b> E. coli	E <b>I</b> EC	Bloody diarrhea	Rehydration and correction of electrolyte abnormalities.
Enterob <b>adherent</b> E. coli	E <b>A</b> EC	Persistent watery diarrhea in children and patients infected with HIV	Rehydration and correction of electrolyte abnormalities.

## 1- Enteropathogenic *E. coli* (EPEC):

Cause infantile diarrhea. The EPEC attach to mucosal cells in the small intestine, causing destruction of microvilli and development of characteristic lesions. Watery diarrhea results, which on rare occasions may become chronic. Shiga-like toxins are responsible for this destruction. It is self limited (don't require treatment only fluid replacement).

2- **Enterotoxigenic *E. coli* (ETEC):** ETEC are a common cause of watery diarrhea in children in underdeveloped countries and traveler's diarrhea in people visiting those countries. ETEC colonize the small intestine by pili and secrete **enterotoxin**, **heat stable (ST)** and **heat labile (LT)** which identical to cholera toxin. ETECs cause prolonged hypersecretion of chloride ions and water by the intestinal mucosal cells, while inhibiting the reabsorption of sodium. The gut becomes full of fluid, resulting in significant watery diarrhea that continues over a period of several days (No inflammation and self limited) (Figure 1).



**Figure 2.** The action of *Escherichia coli* LT (heat-labile toxin). [Note: ST (heat-stable toxin) activates guanylate cyclase, causing production of cGMP that also causes secretion.]

**3- Enterohemorrhagic E. coli (EHEC):** Also known as Shiga-toxin producing E. coli (STEC), or Verotoxin-producing E. coli (VTEC)

**EHEC** bind to cells in the **large intestine**, where they produce an **exotoxin** (verotoxin, or Shiga-like toxin), causing a severe form of copious, bloody diarrhea (hemorrhagic colitis) Like bacillary dysentery. Serotype O157:H7 is the most common strain of E. coli that produces verotoxin. This strain is also associated with outbreaks of a potentially life threatening, acute renal failure (hemolytic uremic syndrome, HUS) characterized by fever, acute renal failure, microangiopathic hemolytic anemia and thrombocytopenia in children younger than age five to ten years. The primary reservoir of EHEC is cattle.

**4- Enteroinvasive E. coli (EIEC)** cause a **dysentery-like syndrome** with fever and bloody stools.

**5- Enteroadherent E. coli (EAEC)** also cause **traveler's diarrhea** and **persistent diarrhea of young children**



## Extraintestinal infections

**1. Urinary tract infections (UTI):** *E. coli* is the most common cause of UTI (urethritis, cystitis, pyelonephritis)(70%), including cystitis and pyelonephritis. **Women are particularly at risk for infection (endogenous source)**

**2- Neonatal meningitis:** *E. coli* is a major cause of this disease occurring within the first month of life. The K1 (capsular) antigen is particularly associated with such infections. (other causes are: GBS, *L.mono*, *E.coli*, *Klebsiella pneumoniae*)

**3- Nosocomial (hospital-acquired) infections:** These include sepsis/ bacteremia, endotoxic shock, and pneumonia.

### Laboratory identification

#### Intestinal disease:

EIEC strains often do not ferment lactose, and may be detected on media such as Mac Conkey agar. These strain can be diagnosed by Sereny test by instilling bacteria into the guinea pig conjunctiva which cause keratoconjunctivitis (Figure 2).

EHEC, unlike most other strains of *E. coli*, ferment sorbitol slowly if at all, and may be detected on MacConkey sorbitol agar as non sorbitol fermentative and negative for beta glucuronidase which act upon MUG(4-methyleumbellyferyl  $\beta$ -D-glucoronide) other *E.coli* have this enzymes and convert MUG into fluorescent substance upon exposing to long wave length of UV light (Figure 3). Serological test using specific anti-O157 antisera.

Serological tests like ELISA for detection of LT, ST and Shiga toxin

PCR for detection of genes for LT, ST and Shiga toxin

Tissue culture to identify invasive or aggregative *E.coli*

## Sereny test

- Instillation of a suspension of freshly isolated EIEC or Shigella into the eyes of guinea pigs

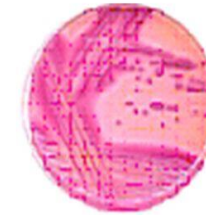


- Mucopurulent conjunctivitis

Fig.2. Sereny test for EIEC

## *E. coli* serotype differentiation

1. immunologic assay
2. growth on **MacConkey agar with sorbitol (S-Mac)**
  - most *E. coli* can ferment sorbitol (form pink colonies)
  - *E. coli* O157:H7 **does not ferment sorbitol** (colonies are clear/colorless)



*E. coli*

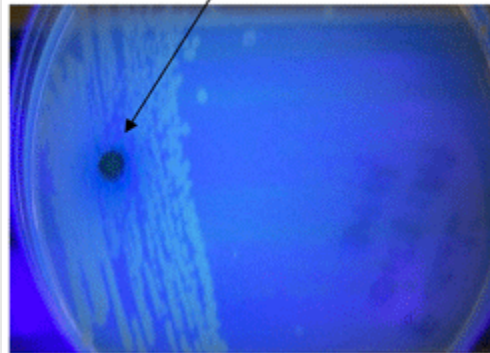


*E. coli* O157

Fig.3. Sorbitol non fermenter EHEC

Appearance of CC disc under UV (365nm) light. *E. coli* O157:H7 is glucuronidase (MUG) negative with no fluorescence, other *E. coli* are MUG positive and fluoresce.

*E. coli* O157:H7



*E. coli*

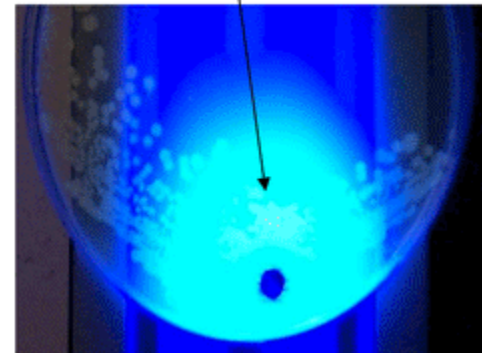


Fig.4. MUG negative EHEC

## **Extraintestinal diseases:**

Urine and CSF culture on Mac Conkey agar or Eosin methylene blue agar. Lactose fermenter colonies on Mac Conkey agar and metallic sheen colonies on EMB agar subjected to biochemical test like IMViC tests or using API 20 E. **Strain identification is performed by special reference laboratory using serological tests.**

## **Treatment and prevention**

Intestinal disease can be prevented by care in selection, preparation, and consumption of food and water. Maintenance of fluid and electrolyte balance is of primary importance in treatment. Antibiotics may shorten duration of symptoms; however, resistance is widespread. Extraintestinal diseases require antibiotic treatment. Antibiotic sensitivity testing of isolates is necessary to determine the appropriate choice of drugs.

## Selective media

### Mac Conkey agar

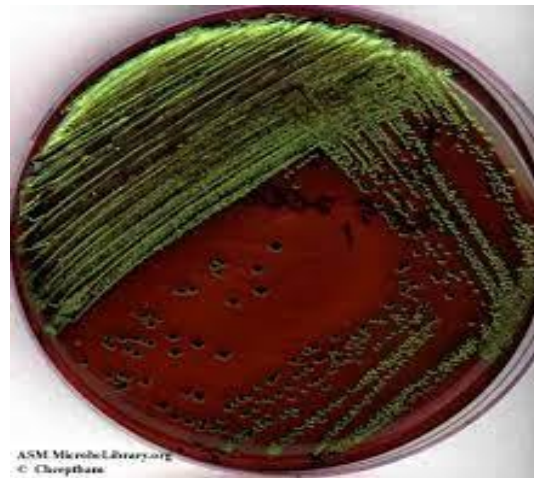
( pomegranate color)

**Pink colonies**



### Eosin methylene blue agar

**Metallic sheen colonies**



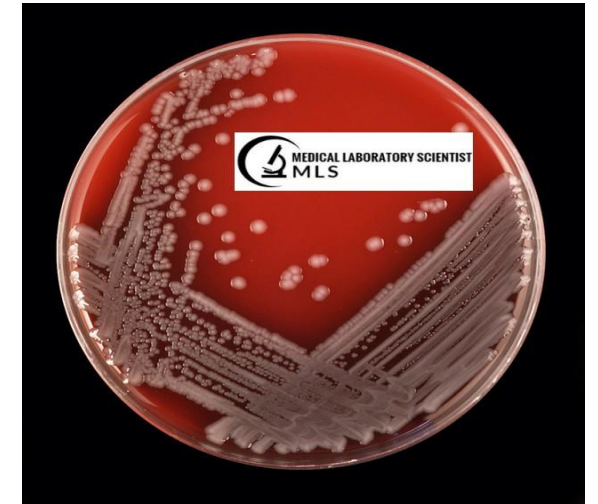
### CLED medium (Cysteine, lactose Electrolyte deficient media) (green color)

**Yellowish colonies**



### Blood agar

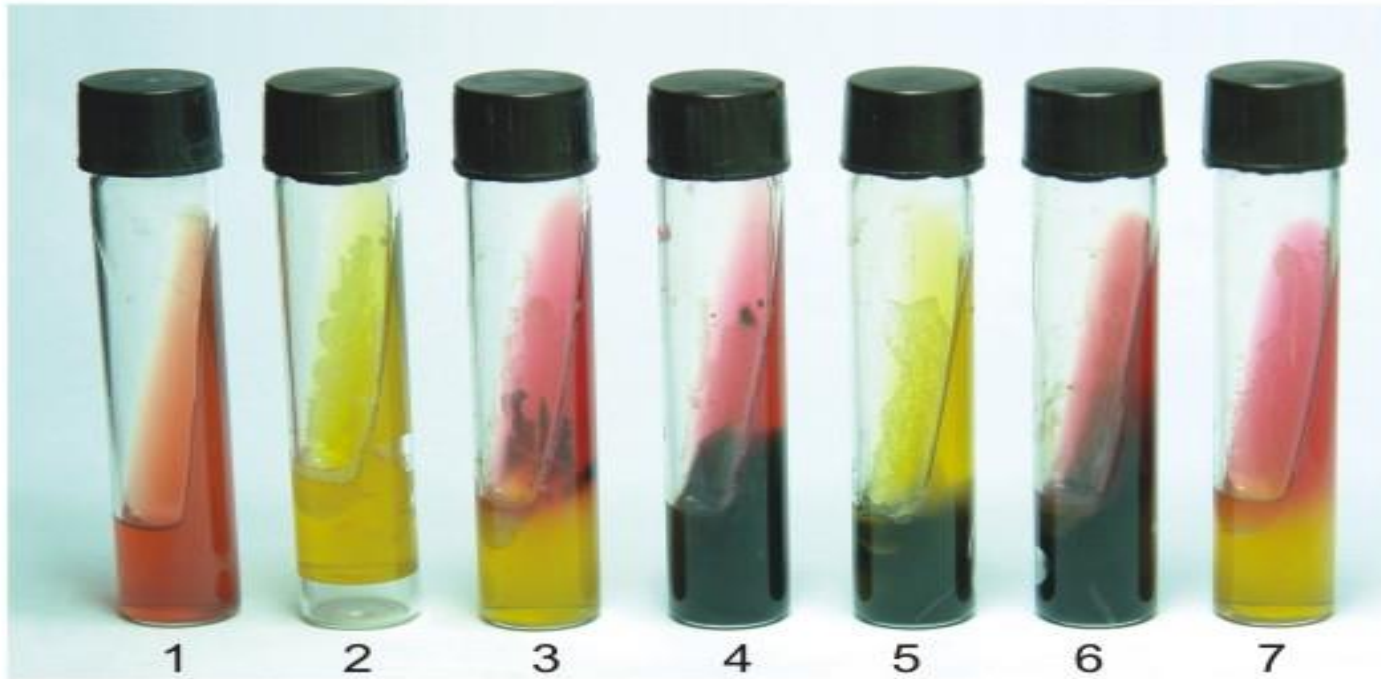
(E.Coli from UTI produce  
beta hemolysis)



### Sorbitol Mac Conkey agar

All E.coli strains ferment sorbitol (yellowish colonies)  
except EHEC, E.coli O157:H7)

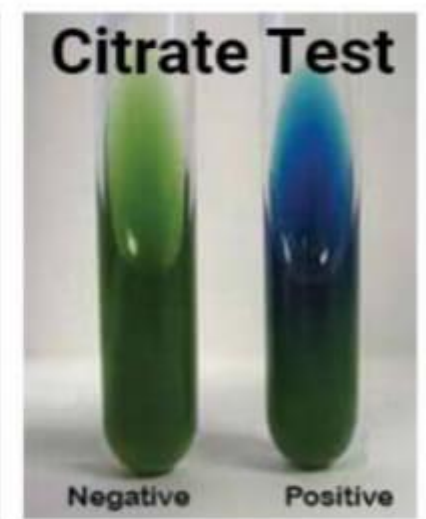
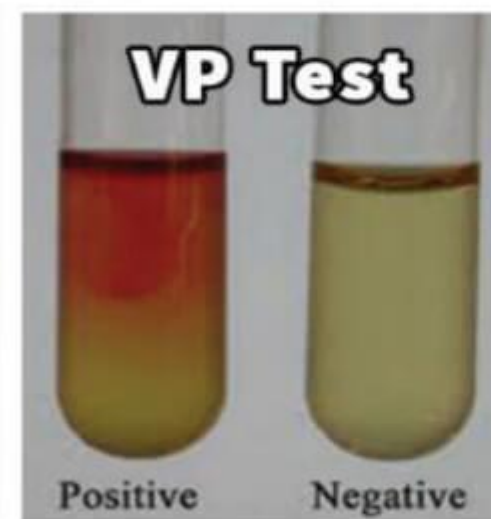
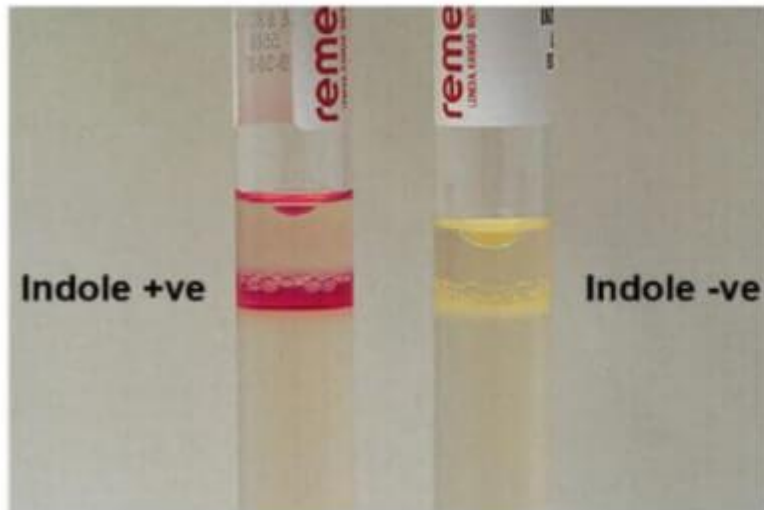




### **Triple Sugar Iron Agar (M021)**

1. Control
2. *Escherichia coli* ATCC 25922
3. *Salmonella Typhi* ATCC 6539
4. *Proteus vulgaris* ATCC 13315
5. *Citrobacter freundii* ATCC 8090
6. *Salmonella Typhimurium* ATCC 14028
7. *Shigella flexneri* ATCC 12022

# IMViC Tests



## Biochemical tests

**api® 20 E** CE 07221

REF : 25

Origine / Source / Herkunft /  
Origen / Origen / Προέλευση /  
Ursprung / Oprindelse / Pochodzenie :

1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4
ONPG	ADH	LDH	ODC	LCIT	H <sub>2</sub> S	URE	TDA	IND	LVP	LGEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO <sub>2</sub>	N <sub>2</sub>	MOB	McC	OF-O	OF-F									
+	+	+	+	-	-	-	+	-	-	-	+	+	-	+	+	+	+	-	+																
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Autres tests / Other tests / Andere Tests /  
Otras pruebas / Altri test / Outros testes /  
Άλλες εξετάσεις / Andra tester /  
Andre tests / Inne testy :

Ident. / Ταυτοποίηση :

*E. coli*

Imprimé en France / Printed in France

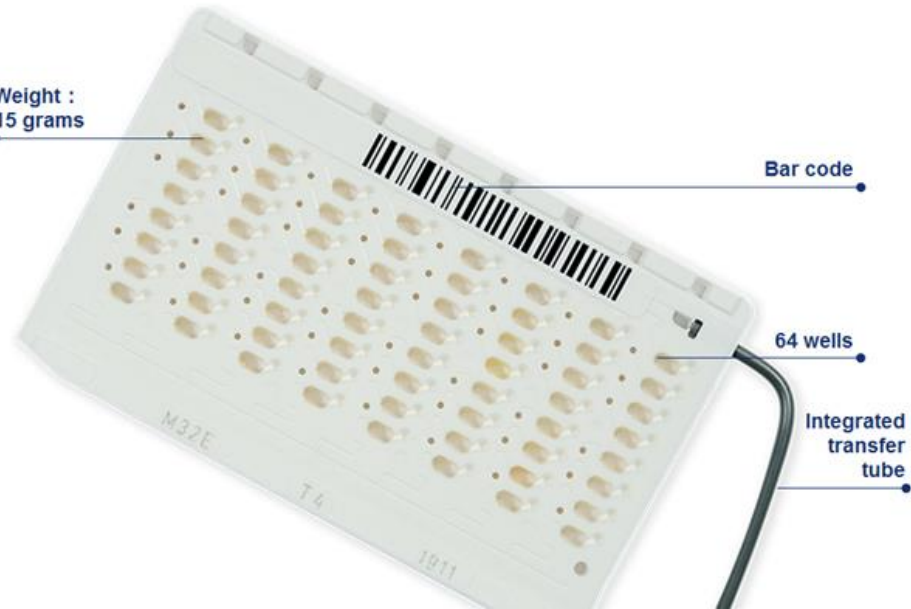




## Biochemical tests by full automated machine such as VITEK system



Weight :  
15 grams



Bar code

64 wells

Integrated  
transfer  
tube



## Genus: Klebsiella

### Characteristics:

Non-motile, lactose-fermenting, capsulated (produce large amount of capsule), gram-negative stout short rods.

### Main species of medical importance:

K. Pneumoniae

K.oxytoca

K. rhinoscleromatis

K. ozenae

*K. pneumoniae* is by far the most important species as a cause of infection in man.

### K.Pneumoniae + K.oxytoca

It is found as a commensal in the **intestinal tract, respiratory system** and also found in **moist environment in hospitals**. It is an important **nosocomial pathogen**. It causes:

- . Pneumonia as primary or secondary infections
- . Urinary tract infection
- . Septicaemia and meningitis (especially in neonates)
- . Wound infection and peritonitis

### K. rhinoscleromatis

It causes rhinoscleroma of nose and pharynx to extensive destruction of nasopharynx (hebra nose).

## **K.ozaenae**

It causes ozena manifesting with **foul smelling nasal discharge** leading to chronic atrophic rhinitis.

### **Laboratory diagnosis of klebsiella species:**

**Specimen:** Sputum, urine, pus, CSF, body fluid

**Smear:** Gram-negative rods

**Culture:** Large, mucoid, lactose-fermenting colonies on MacConkey agar. IMViC (-,-,+,+) non motile. TSI (A/A with gas production but without H<sub>2</sub>S production. Quellung reaction positive.

**Serology:** Capsular polysaccharide serotyping

**Treatment:** All klebsiellas are resistant to ampicillin and amoxicillin. Second and third generation cephalosporins, co-amoxiclav, aminoglycosides and quinolones are the mainstay of therapy. More recently klebsiellas that produce extended-spectrum beta lactamases (ESBLs) have appeared and this has resulted in resistance to a wide range of beta lactam antibiotics.

## **GENUS: ENTEROBACTER**

It is gram-negative **lactose fermenting motile rods**, and found as a commensal in the intestinal tract of humans and animals and moist environments.

Medical important species is ***Enterobacter aerogens***.

It produces **mucoid colony resembling klebsiella** on MacConkey agar.

Enterobacter aerogens is associated with **urinary tract infection, wound infection and septicemia** in immunocompromised and chronically debilitated patients.

## Chronic Atrophic Rhinosinusitis (ozena)

- Ozena: a disease of the nose in which there is **wasting** away of the **bony ridges** and **internal mucous** membranes.
- **Diffuse** atrophy of mucus membranes of nose & paranasal sinuses.
- Ciliated columnar epithelium of the nasal mucosa is **replaced** by stratified squamous epithelium.



## INTRODUCTION

- COINED BY:- VON HEBRA IN 1870

### DEFINITION

- Respiratory scleroma or rhinoscleroma is a progressive granulomatous disease commencing in the nose and later extending into the nasopharynx and oropharynx, the larynx and sometimes the trachea and bronchi.



## Genus: Serratia

The species of Serratia that most frequently causes human infection is *S. marcescens*. Serratia can cause extraintestinal infections such as those of the lower respiratory and urinary tracts, especially among hospitalized patients.

### Diagnosis

Specimen like sputum, urine and blood culture on nutrient agar, Mac conkey agar. A red pigment is produced by colonies of *S. marcescens* as shown in figure 5.

Biochemical tests

1- Non lactose fermenter

2- V-P: positive

3- TSI: A/A (No gas)

Citrate +ve

Motile

Indole negative

Dnase positive



Fig.5. Red pigments produced colonies of *S. marcescens*

## ***Proteus, Providencia and Morganella***

Two main species (many serotypes):

1. *Proteus mirabilis* (indole –ve )
2. *Proteus vulgaris* (indole +ve)
- 3- *P.rettgeri* ( urease negative)
- 4-*M.morganii*

### **Epidemiology**

The habitat is the human colon and the environment (soil and water). Transmission to the urinary tract is by ascending spread of faecal flora.

### **Laboratory Characteristics**

Gram-negative bacilli.

Non-lactose fermenters.

TSI: K/A with a large amount of H<sub>2</sub>S production (Figure 6)

Actively motile – exhibit “swarming”, a spreading type of growth (Figure 7) and have a strong fishy odour. They decompose urea rapidly by virtue of possessing the enzyme urease (Figure 8).

**Urease** – this enzyme is responsible for the alkalization of urine and subsequent damage to the renal epithelium in patients with urinary tract infections caused by *Proteus* spp. Urea is hydrolysed to NH<sub>3</sub> with a rise in pH – may precipitate salts, (Ca<sup>++</sup>, Mg<sup>++</sup>) causing calculi (renal stones).

**All of these genera are positive for phenylalanine deaminase test** (differentiated from other enteric bacteria)

### **Clinical infections**

1. Urinary tract infections – usually an ascending infection.
2. Wound infection.
- 3- Pneumonia and otitis media
- 4- Septicemia and meningitis



Fig.6. Swarming phenomenon



Fig.7. TSI



Fig.8. Urease test



## GENUS: CITROBACTER

It is gram-negative lactose fermenting motile rods, and opportunistic pathogen.

Medical important species is *Citrobacter freundii*.

*Citrobacter freundii* is associated with **urinary tract infection**, **wound infection** and **septicaemia** in immunocompromised and chronically debilitated patients.

## Genus: Salmonella

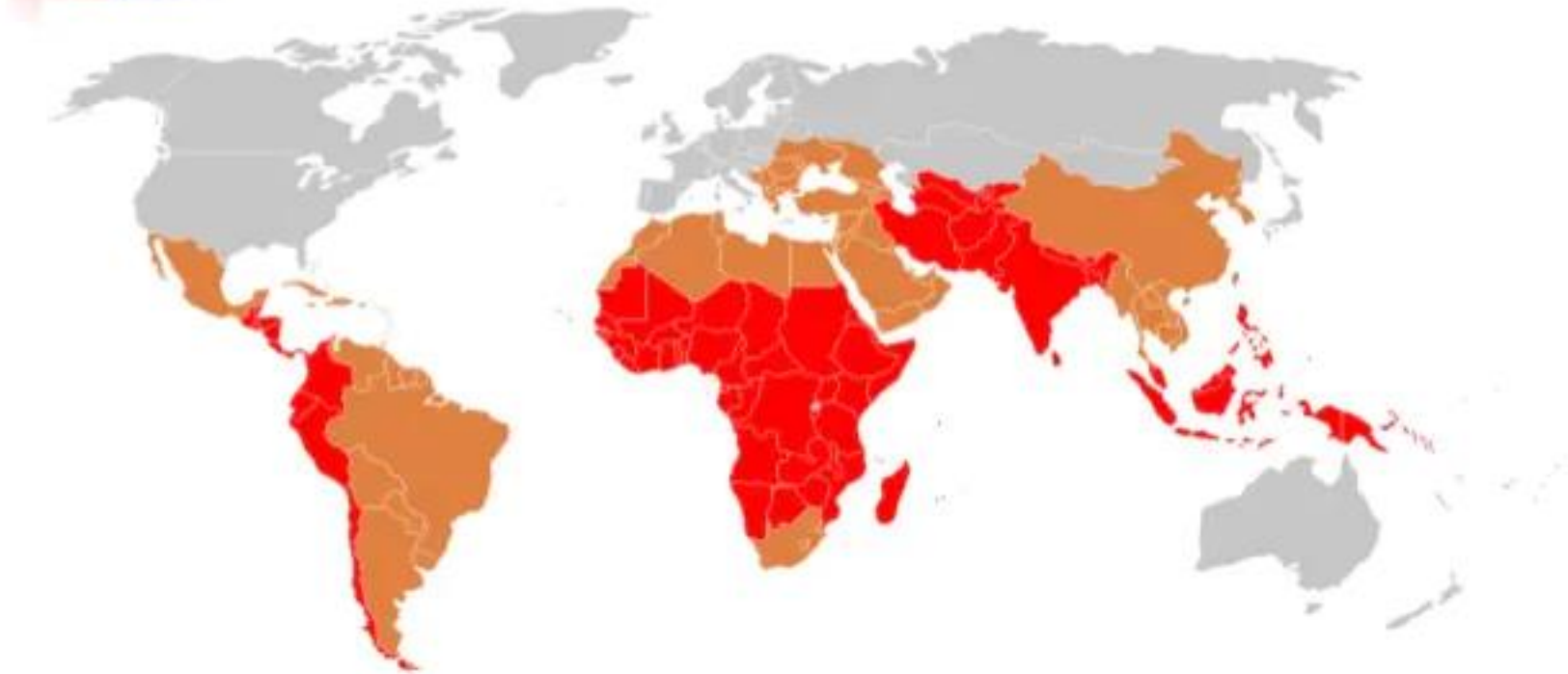
Currently, all strains are grouped in a single species: *S. enterica*, which has approximately 2500 different serotypes, or serovars including the clinically significant serotypes typhimurium and typhi. Most strains of *Salmonella* are Lac-ve and produce acid and gas during fermentation of glucose. They also produce H<sub>2</sub>S from sulfur-containing amino acids.

Those serotypes from animals and poultry can cause food poisoning (infection localized in GIT usually without bacteremia) and few serotypes namely *S. typhi* and *S. paratyphi* A, B and C cause typhoid and paratyphoid fevers respectively.

## Clinical findings

**1. Gastroenteritis:** This localized disease (also called salmonellosis) is caused primarily by serotypes enteritidis and typhimurium (non typhoidal salmonella). It is characterized by nausea, vomiting, and diarrhea (usually non bloody), which develop generally within 48 hours of ingesting contaminated food or water. Fever and abdominal cramping are common. In uncompromised patients, disease is generally self-limiting (48 to 72 hours), although convalescent carriage of organisms may persist for a month or more. More than 95% of cases of *Salmonella* infection are foodborne

## Typhoid fever is prevalent in many regions in the world



- High endemicity (>100 cases per lac per year)
- Medium endemicity (10 cases per lac per year)
- Low endemicity (<10 cases per lac per year)



**2-Enteric (typhoid) fever:** This is a severe, life-threatening systemic illness, characterized by fever and, frequently, abdominal symptoms. It is caused primarily by serotype typhi (typhoidal salmonella). Nonspecific symptoms may include chills, sweats, headache, anorexia, weakness, sore throat, cough, myalgia, and either diarrhea or constipation. About thirty percent of patients have a faint maculopapular rash on the trunk (rose spots). The incubation period varies from 5 to 21 days. A small percentage of patients become chronic carriers in the gall bladder. Complications can include intestinal hemorrhage and, rarely, focal infections and endocarditis.

### **Laboratory diagnosis:**

In patients with **diarrhea**, Salmonella can typically be isolated from stool by pre-enrichment technique using one of the enrichment media such as tetrathionate broth or thioglycolate broth then subculture on Salmonella selective media such as SS agar, Hekton Enteric agar XLD agar, Deoxycholate-Citrate agar. Biochemical test like TSI (K/A with little or H<sub>2</sub>S), urease –ve, Simmon citrate positive. Serological diagnosis.

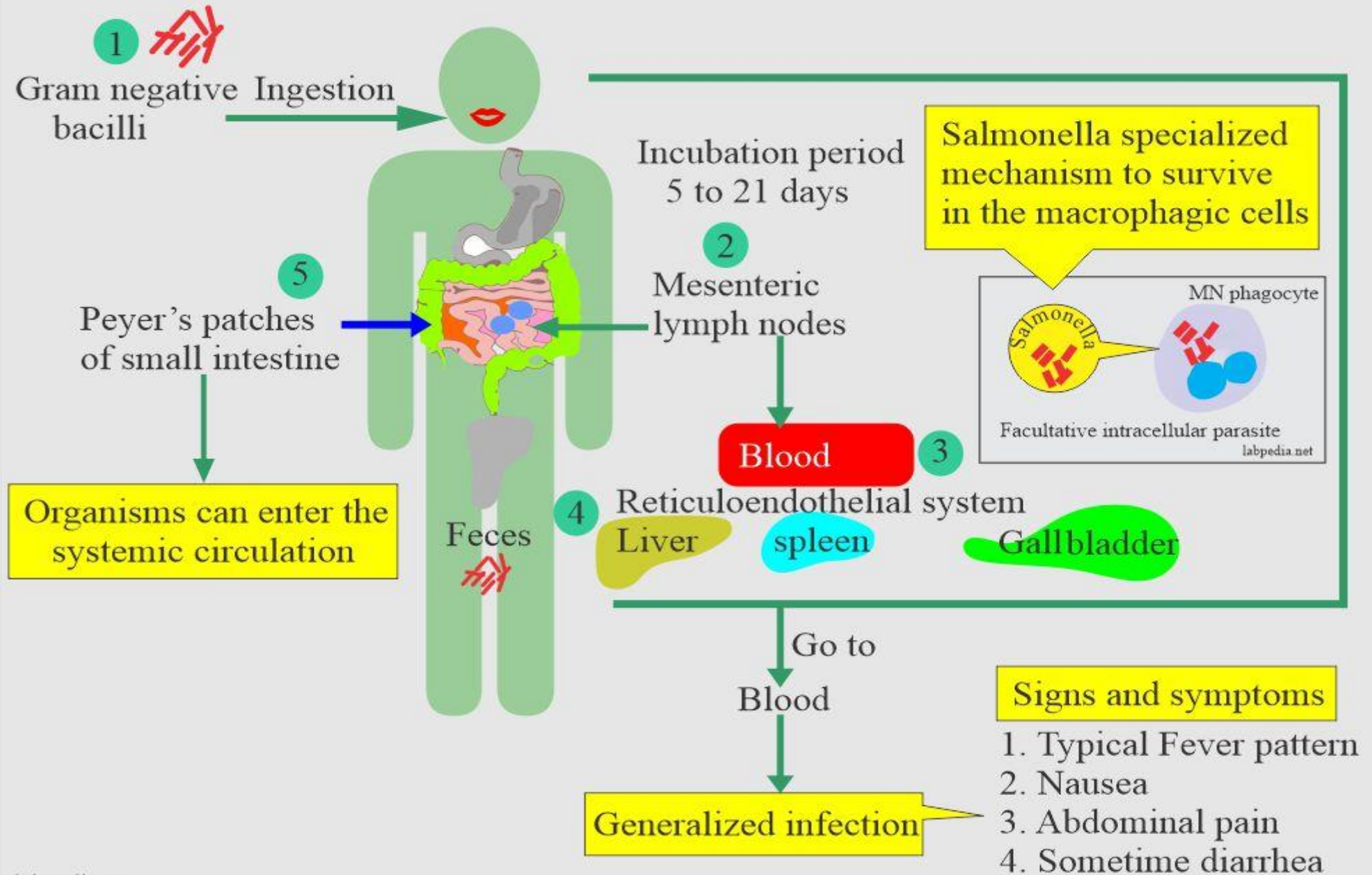
For enteric fever

#### **1. Isolation of the Causative Organism**

**a) BLOOD CULTURE** – During acute stage (Bacteremia).

#### **b) STOOL CULTURE**

Faeces are plated onto appropriate selective, differential and enrichment media. (Carriers)



## 1- Microbiological procedure **Blood Cultures**

- Bacteremia occurs early in the disease
- Blood Cultures are positive in

1<sup>st</sup> week in 90%  
2<sup>nd</sup> week in 75%  
3<sup>rd</sup> week in 60%  
4<sup>th</sup> week and later in 25%



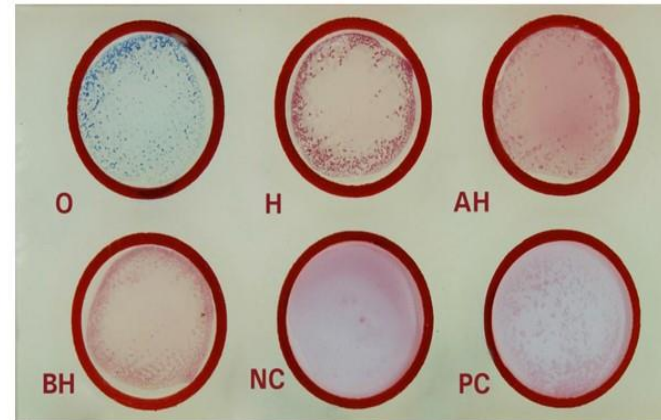
## Typhoid IgM /IgG Rapid Test



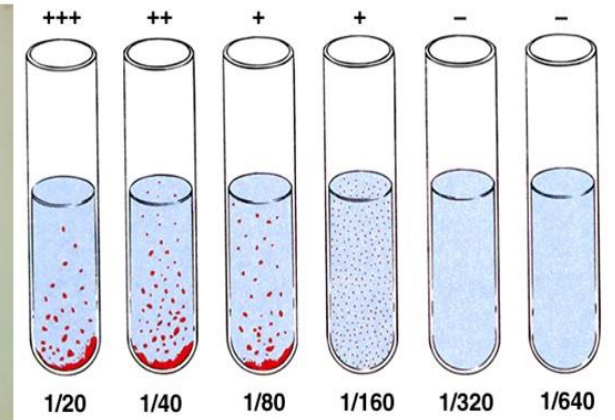




## Widal Test

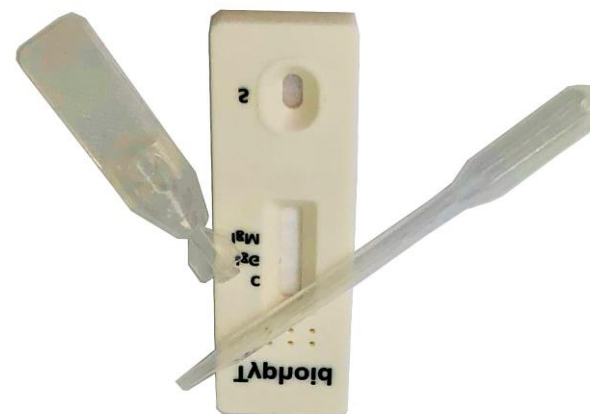


Rapid Slide Test



Quantitative Tube Test

[www.onlinebiologynotes.com](http://www.onlinebiologynotes.com)



Serological test like widal test to measure antibodies against both O and H antigens

**It should be stressed that the Widal reaction is not always reliable**

**Causes of false positive Widal test**

- Malaria infection
- Other acute febrile illness
- Poor quality reagent

**Causes of false negative Widal test**

- Specimen collected after antibiotic administration
- Specimen collected at early stage of diseases
- Technical errors

Treatment:

1. For cases

Chloramphenicol ,Fluoroquinolones, **3rd** generation cephalosporins

2. For carriers

Ampicillin followed by cholecystectomy

## Prevention and control

- Sanitary measures like hygienic food and drink handling, and avoid carriers from food handling until properly treated
- Vaccine using killed vaccine.

## GENUS: SHIGELLA

Species of medical importance are:

*S. dysenteriae* A cause severe form of bacillary dysentery

*S. flexneri* B

*S. boydii* C

*S. sonnei* D cause mild dysentery (Gram –ve , non motile and non capsulated)

**Disease** : shigellosis (bacillary dysentery= blood and pus in the stool). Usually its **localized infection in the large intestine without bacteremia.**

Shigellosis is a disease of wars, refugee camps, forced migration due to the lack of both sanitation, personal hygiene and overcrowding.

The disease is transmitted by feco-oral through drinking or eating of contaminated water and foods or by contaminated fomites like door handles, toilet handles, face clothes. **House fly** transmit the disease.

Remember that **Shigellosis is transmitted by 4Fs (Food, Fingers, Feces, Flies)**

Toxins: 1-endotoxin 2. Shiga toxin similar to verocytotoxin (VT-1) or Shiga like toxin (SLT-1 and SLT-2 ) produced by EHEC

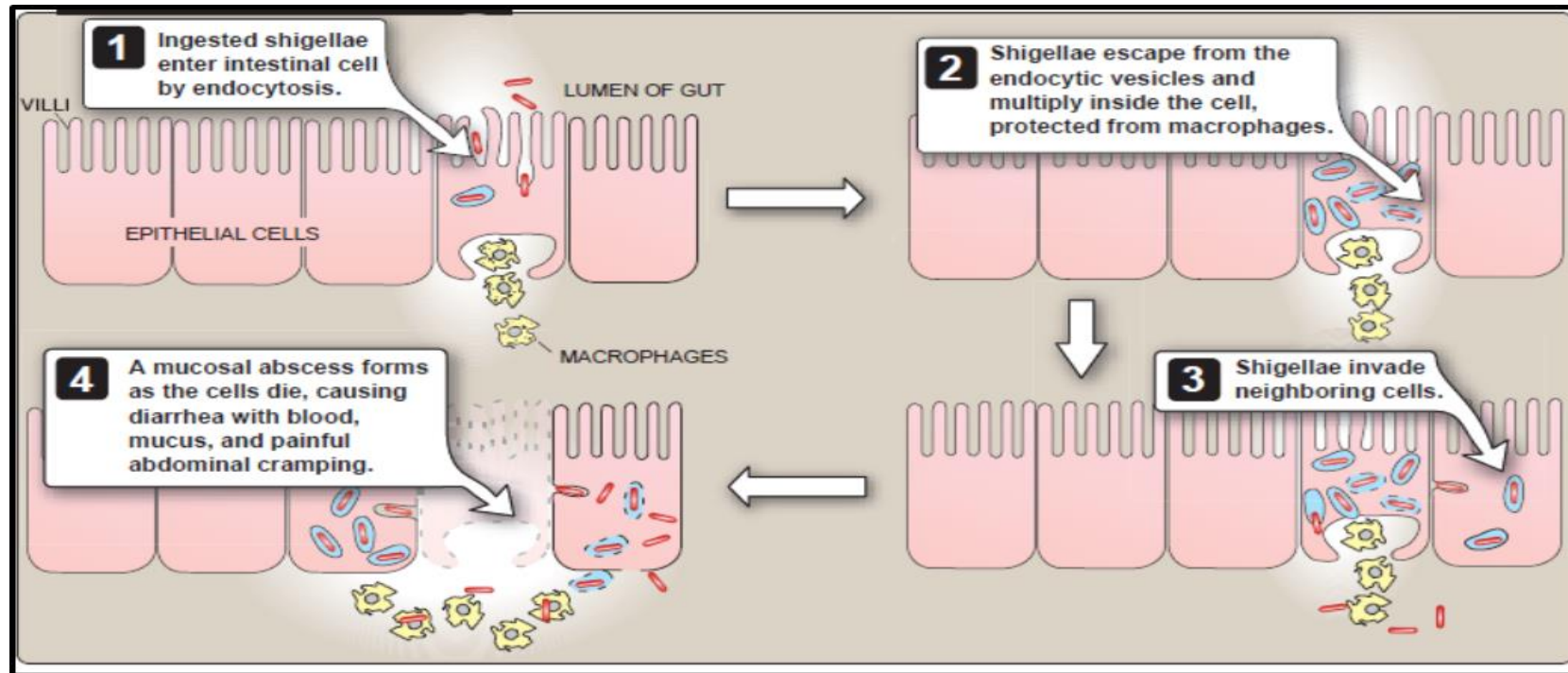
Figure 9. shows the pathogenesis of shigellosis, the site of infection is the terminal ileum and large intestine invading and destroying the villi producing inflammation due to Shiga toxin (**enterotoxin, cytotoxin and neurotoxin**) by Sh.dysentery type 1

## Clinical Presentation

**Infective dose : 100 bacilli (highly infectious )**

Incubation period usually between 2 to 3 days, but can be as short as 12 hours.

It may vary from a mild intestinal upset (“food poisoning”), lasting a short while (24 hours) and with no pyrexia, to a severe pyrexial illness with severe hemorrhagic diarrhea associated with severe abdominal cramps **tenesmus**). The stools **contain large gelatinous clots of blood** with abundant **mucus** and **pus**. The loss of water and electrolytes may lead to severe dehydration)



Shigella dysentery may **cause hemorrhagic colitis** and **hemolytic uremic syndrome**.  
Can cause **pseudomembranous colitis**  
Remember pathogen that produce pseudomembrane!!!!  
The patient is nervous due to **meningism** (irritation of meninges)

Fig.9. Mechanism of Shigella producing diarrhea



## Reiter's syndrome

# Reactive Arthritis

### Conjunctivitis



"Can't see"

### Urethritis



"Can't pee"

### Arthritis



"Can't climb a tree"

- Associated with **immune response** to **enteric** or **genitourinary** (*Shigella*, *Salmonella*, *Yersinia*) organisms (*Chlamydia*)
- **HLA-B27**
- **Conjunctivitis** - Discharge, erythema, burning, photophobia
- **Urethritis** - Dysuria, urgency, frequency, discharge
- **Arthritis** - Knees/Ankles/Feet





## Diagnosis

**Specimen:** Fresh stool or rectal swab (fragile bacteria)

**Culture:** Non-lactose fermenting colonies on MacConkey agar (except *S. sonnei*) and SS agar (no H<sub>2</sub>S production).

**Biochemical reaction:** TSI (K/A no gas and no H<sub>2</sub>S production. Motility test: **non motile, urease – ve, citrate –ve, KCN –ve, IMVIC all negative**). (Figure 10)

Serology using specific antisera to determine type of *Shigella*.

**Serology (antibodies) is not used to diagnose *Shigella* infections.**

## Treatment

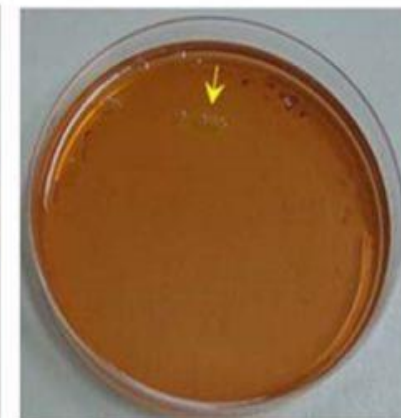
Ciprofloxacin, ampicillin, doxycycline, and trimethoprim– sulfamethoxazole are most commonly used.

## How to control the disease?

- (1) sanitary control of water, food, and milk; sewage disposal and fly control.
- (2) isolation of patients and disinfection of excreta.
- (3) detection of subclinical cases and **carriers**, particularly **food handlers**.
- (4) antibiotic treatment of infected individuals.



*Salmonella* on SS Agar



*Shigella* on SS Agar



SS Agar Plate  
(Salmonella-Shigella Agar)



*E. coli* on SS

## Fermentation of mannitol forms the basis of classification

All ferment mannitol except **Sh.dysentery**

Shigella sonnie late lactose fermenter

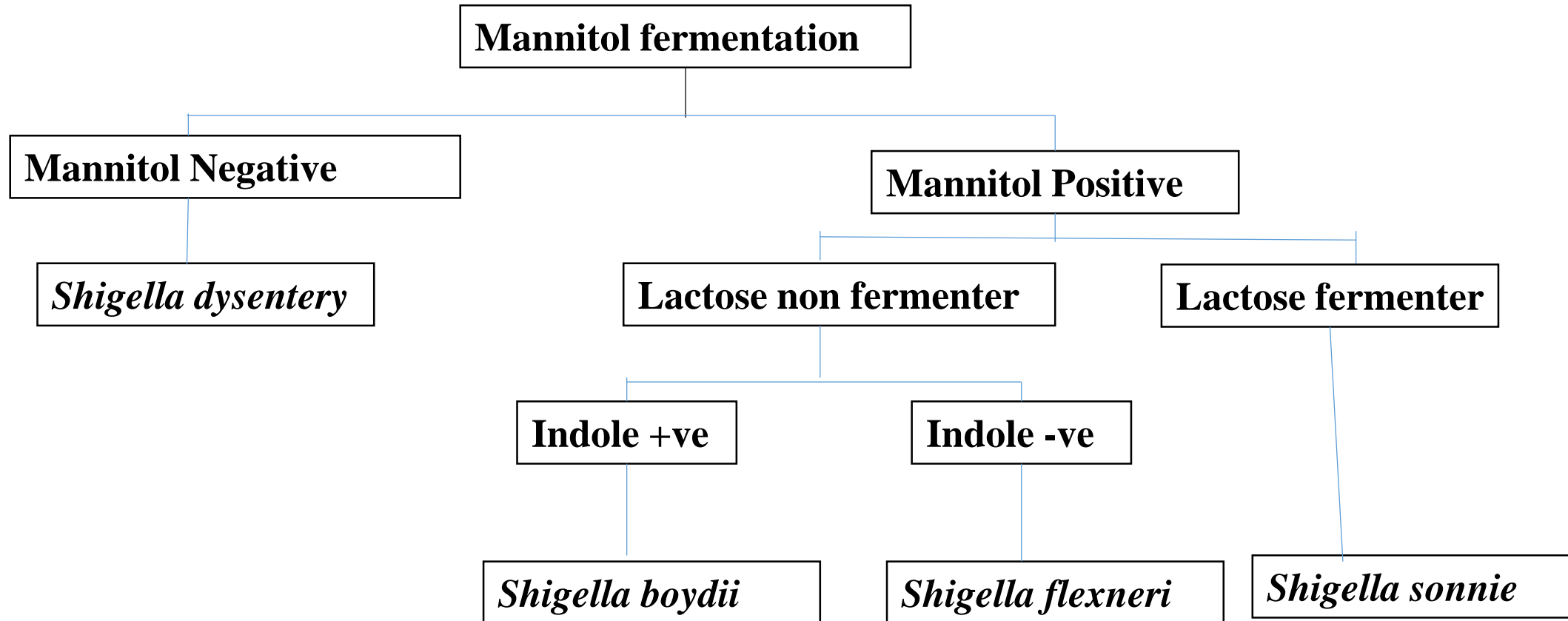


Fig.10.Biochemical reactions