Enterobacteria

General Characteristics of Enterobacteria

The ***Enterobacteriaceae*** are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, *Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans.

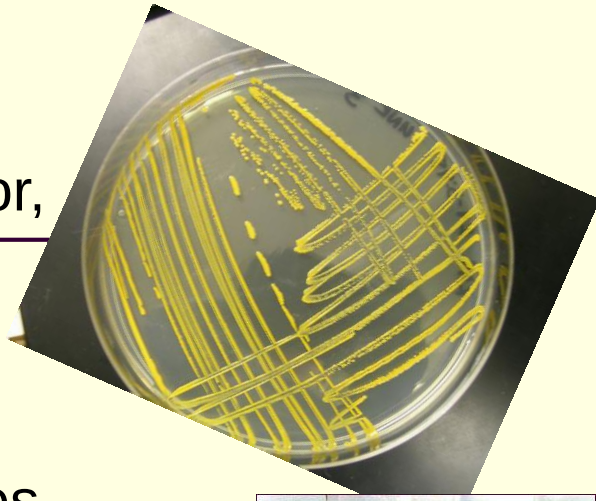
Morphology and staining: Gram-negative bacilli, 2-3x0.6 µm. Non-motile, or motile by peritrichous flagella. Non sporing.

Culture: grow well on ordinary media, e.g. blood agar, Mac-Conkey agar, CLED agar, Endo agar; aerobic and facultatively anaerobic; grow in wide range of temperatures.

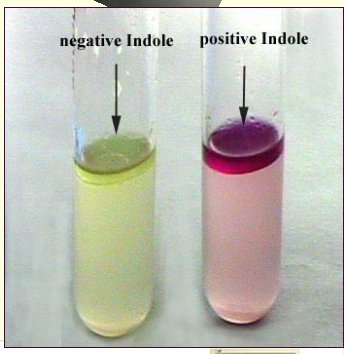
Toxins:

- Endotoxins are O antigens and are lipopolysaccharides consisting of sugars and lipid A. Present in the cell wall of Gram-negative bacilli, they are liberated when the bacterial cells lyse and are responsible for many pathological effects of enterobacterial infection.
- Exotoxins and proteins liberated extracellularly from the intact bacterium by some species of enterobacteria.

Identification:



- *Lactose fermentation:* e.g. Mac-Conkey agar or Endo agar contains lactose and a pH indicator, so lactose-fermenting colonies are pink.
On CLED medium, the colour of lactose fermentation is yellow.

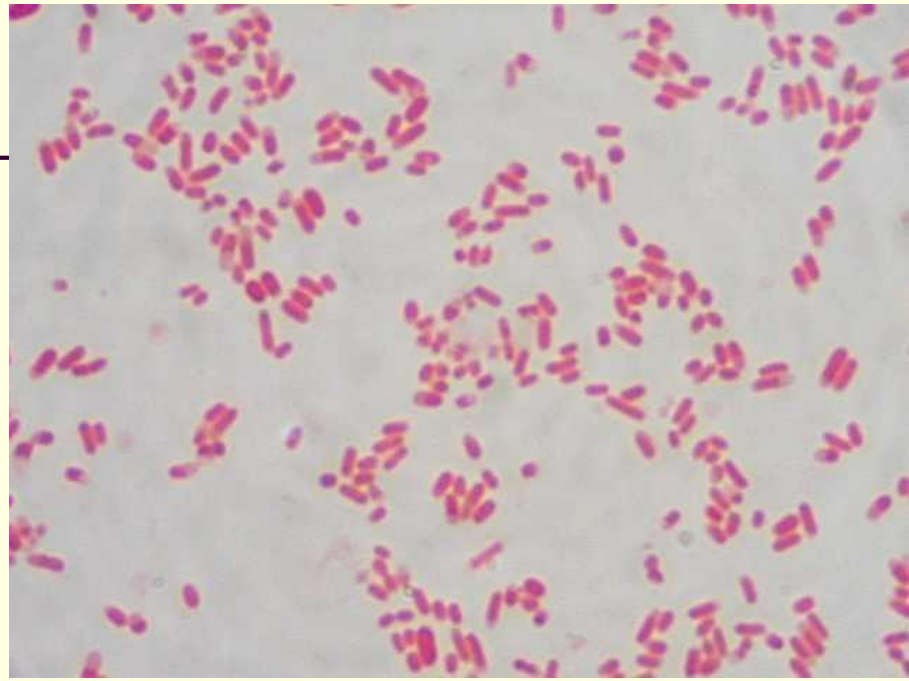


- *Biochemocal tests* are used to identify species of enterobacteria, usually by means of test kits based on 10 or 20 biochemical tests. Enterobacteria ferment glucose with or without formation of gas, reduce nitrates into nitrites, catalase positive, oxidase negative.

- *Serological tests* for somatic and flagellar antigens are used mainly for final identification of Salmonella and Shigella species.
- *Strain identification* within a species can also be done by bacteriophage typing or by analysis by restriction fragment length polymorphism of bacterial DNA.

Antibiotic sensitivity: the main antibiotics used against enterobacteria are: ampicillin/amoxycillin; aminoglycosides, trimethoprim, chloramphenicol, ciprofloxacin, cephalosporins. Nitrofurantion and nalidixic acid are used only for urinary tract infections.

Escherichia coli



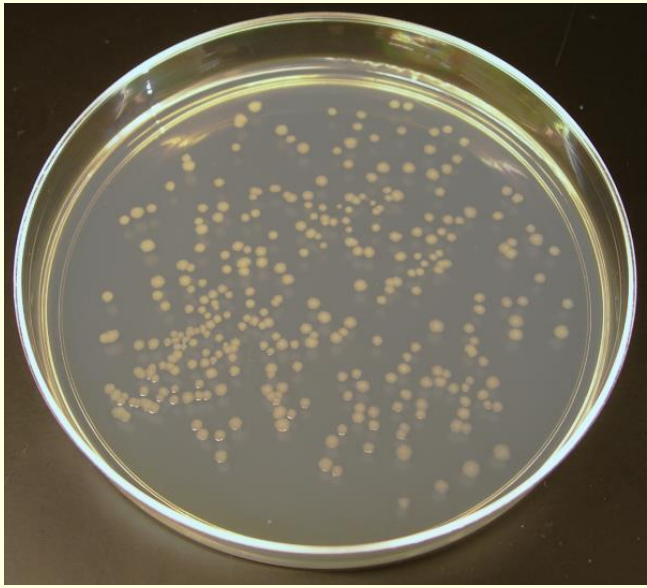
Family Enterobacteriaceae
Genera Escherichia
Species Escherichia coli

Morphology and staining: Escherichia coli is a Gram-negative rod, non-sporulating. The cells are about 2 μm long and 0.5 μm in diameter. Some strains of *E. coli* have capsule. The flagella of *E. coli* have a peritrichous arrangement.

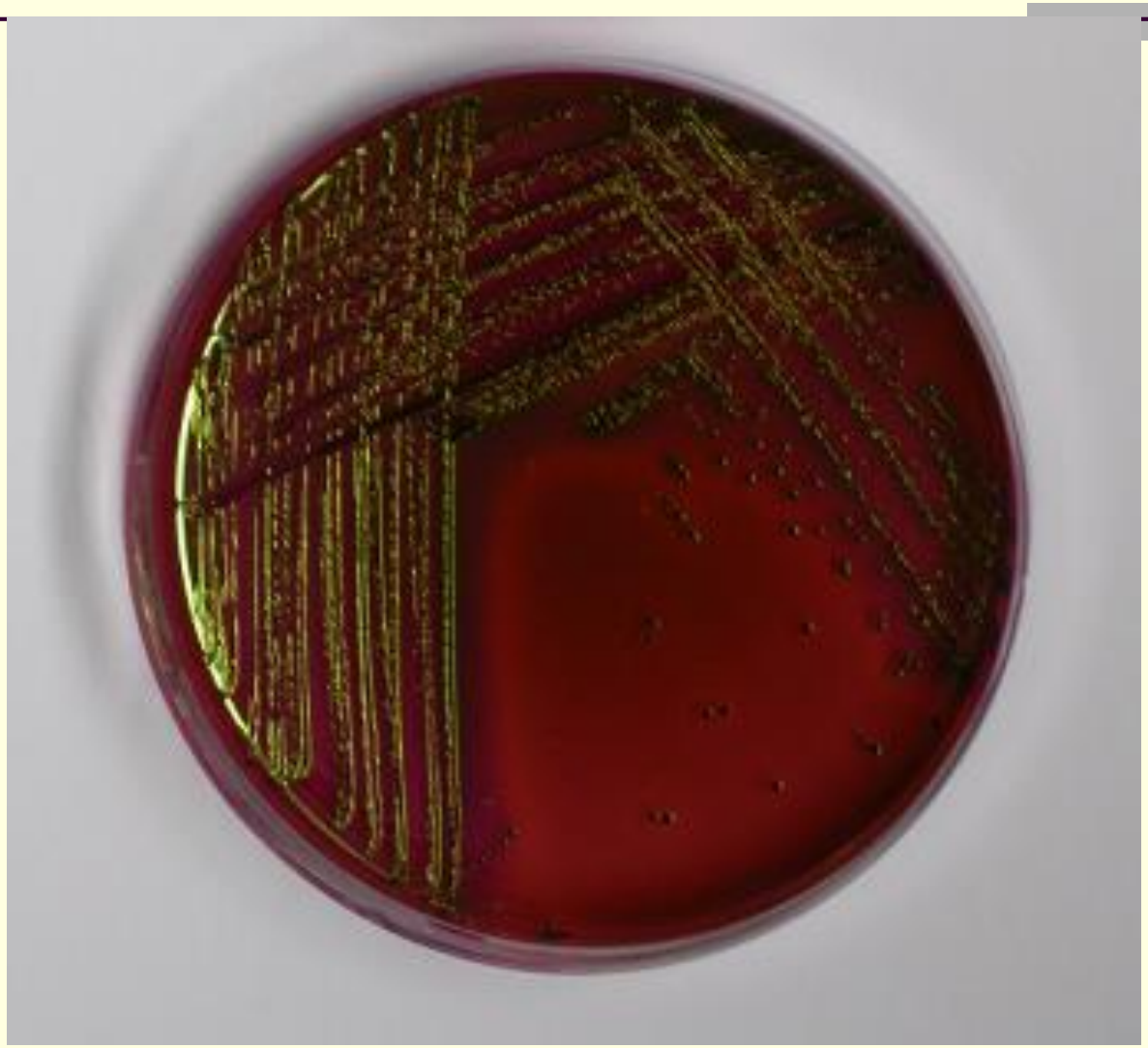
Cultural characteristic

E. coli is facultative anaerobic. It can live on a wide variety of substrates, grow well on ordinary media.Optimal growth of *E. coli* occurs at 37°C, but some laboratory strains can multiply at temperatures of up to 49°C.

After 24 hours'
incubation on MPA colony of *E. coli*
is 1 to 3 mm, circular,
convex, semi-transparent, smooth,
shiny, grayish-white.



E. coli give a metallic green color on EMB (Eosin methylene blue) agar.



Biochemical activity

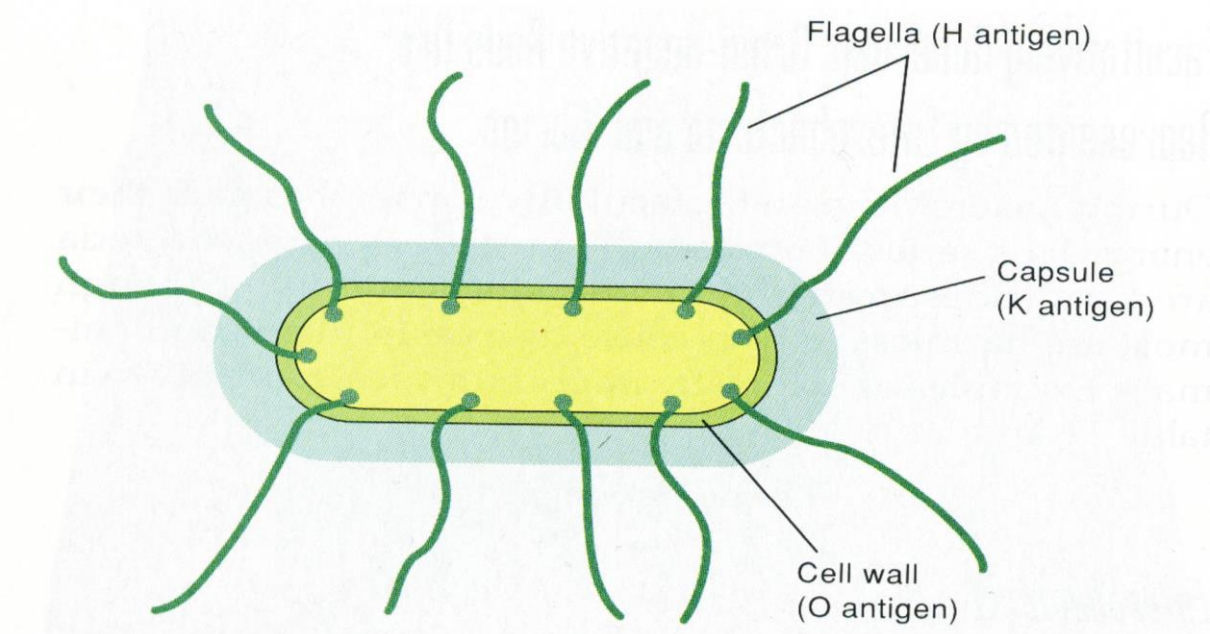
- Indole and methyl red (MR) is positive.
- Citrate is negative.
- Urease is not hydrolysed.
- H_2S is not produced.

- Carbohydrate utilization:

Escherichia coli produces acid and gas from fermentation of lactose, glucose, sucrose, maltose and mannitol.

Antigenic structure

Over 700 antigenic types (**serotypes**) of *E. coli* are recognized based on **O** (somatic antigen)**, H** (flagellar) **, and K** (capsular) **antigens**.



Schematic drawing of a typical enterobacterium. (The cytoplasmic membrane is not shown.)

Virulence Factors of Pathogenic E. coli

- *Enterotoxins*: these are of two types, both plasmid-coded: LT – heat-labile, and ST – heat-stable.
- *Vero cytotoxin* (VT) produces a cytopathic effect on Vero cells. There are two (VT1 and VT2), which are serologically distinct. Strains that produce VT (known as VTEC), notably E.coli 0157, cause diarrhoea with haemorragic symptoms.
- *Endotoxin (LPS)*
- Adhesins*:* fimbriae, intimin (non-fimbrial adhesin)
- Invasins: hemolysin, Shigella-like "invasins" for intracellular invasion and spread
- *Attaching-effacing mechanism:* some strains adhere to intestinal epithelium to cause erosion (effacement) of the microvilli, resulting in formation of characteristic structure known as *pedestals.*
- *Defense against immune responses***:** capsules, K antigens, LPS, antigenic variation.

Normal role

E. coli normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with food or water or with the individuals handling the child. In the bowel, it adheres to the mucus of the large intestine.It is the primary facultative organism of the human gastrointestinal tract. As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals.The commensal *E. coli* strains that inhabit the large intestine of all humans and warm-blooded animals comprise no more than 1% of the total bacterial biomass. The regular presence of *E. coli* in the human intestine and feces has led to tracking the bacterium in nature as an indicator of fecal pollution and water contamination.

Epidemiology of gastrointestinal infection

Transmission of pathogenic E. coli often occurs via fecal-oral transmission. Common routes of transmission include: unhygienic food preparation,farm contamination due to manure fertilization, irrigation of crops with contaminated greywater or raw sewage, feral pigs on cropland, or direct consumption of sewage-contaminated water. Food products associated with E. coli outbreaks include raw ground beef, raw seed sprouts or spinach, raw milk, unpasteurized juice, and foods contaminated by infected food workers via fecal-oral route.

Pathogenesis

Pathogenic strains of *E. coli* are responsible for three types of infections in humans: **urinary tract infections (UTI)**, **neonatal meningitis**, and **intestinal diseases (gastroenteritis)**.

E. coli is best known for its ability to cause intestinal diseases. Five classes (virotypes) of *E. coli* that cause diarrheal diseases are now recognized:

enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC).

Each class falls within a serological subgroup and manifests distinct features in pathogenesis.

Diarrheagenic *E. coli*: factors of virulence and characteristics of disease

- **ETEC**
  fimbrial adhesins
  non invasive
  produce LT and/or ST toxin
  watery diarrhea in infants and travelers; no inflammation, no fever

- **EIEC**
  nonfimbrial adhesins, possibly outer membrane protein
  invasive (penetrate and multiply within epithelial cells)
  does not produce shiga toxin
  dysentery-like diarrhea (mucous, blood), severe inflammation, fever

- **EPEC**

  non fimbrial adhesin (intimin)
  adherence of bacteria to intestinal cells
  moderately invasive
  does not produce LT or ST; some reports of shiga-like toxin
  usually infantile diarrhea; watery diarrhea with blood, some inflammation, no fever; symptoms probably result mainly from invasion rather than toxigenesis

- **EAEC**
  adhesins not characterized
  non invasive
  produce ST-like toxin (EAST) and a hemolysin
  persistent diarrhea in young children without inflammation or fever

- **EHEC**
  adhesins not characterized, probably fimbriae
  moderately invasive
  does not produce LT or ST but does produce shiga toxin
  pediatric diarrhea, copious bloody discharge (hemorrhagic colitis), intense inflammatory response, may be complicated by hemolytic uremia

Diagnosis

A definitive diagnosis of is made on the basis of **isolation** and **identification** of E.coli. **(*Bacteriological method*)**

Either MacConkey agar or EMB agar (or both) are **inoculated** with the the sample (faeces, urine, vomit, etc.)

On MacConkey agar, deep red colonies are produced as the organism is lactose positive, and this utilization will cause the medium's pH to drop leading to darkening of the medium.

Growth on Levine EMB agar would show black colonies with greenish-black metallic sheen.

Once isolated, E. coli can be **identified** by the gram-staining, carbohydrate utilization reactions, serologic methods such as agglutination reaction.

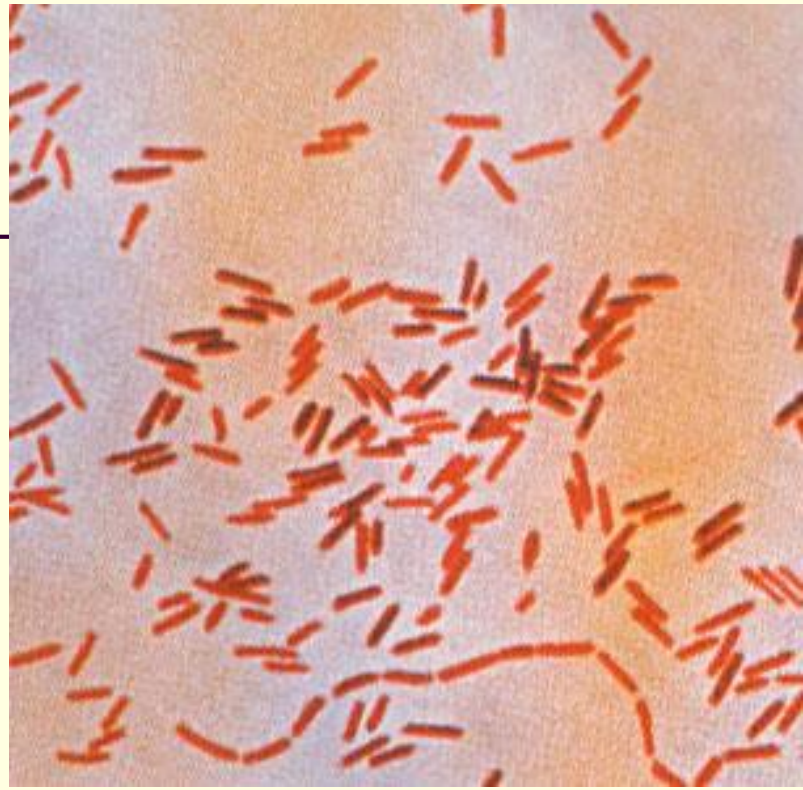
Serological method

To determine of specific antibodies of different classes in sera agglutination reaction are used.

Phagodiagnosis

Determination of phagovar of the isolated strain is important in identifying the source of infection.

Salmonella



Family Enterobacteriaceae
Genera Salmonella
Species Salmonella typhi,
Salmonella paratyphi A,
Salmonella paratyphi B

Morphology and staining: Salmonella spp. is a Gram-negative rod, non-sporulating, non-capsulating. The cells are about 1-3 μm long and 0.5 μm in diameter. The flagella of *Salmonella spp.* have a peritrichous arrangement.

Cultural characteristic

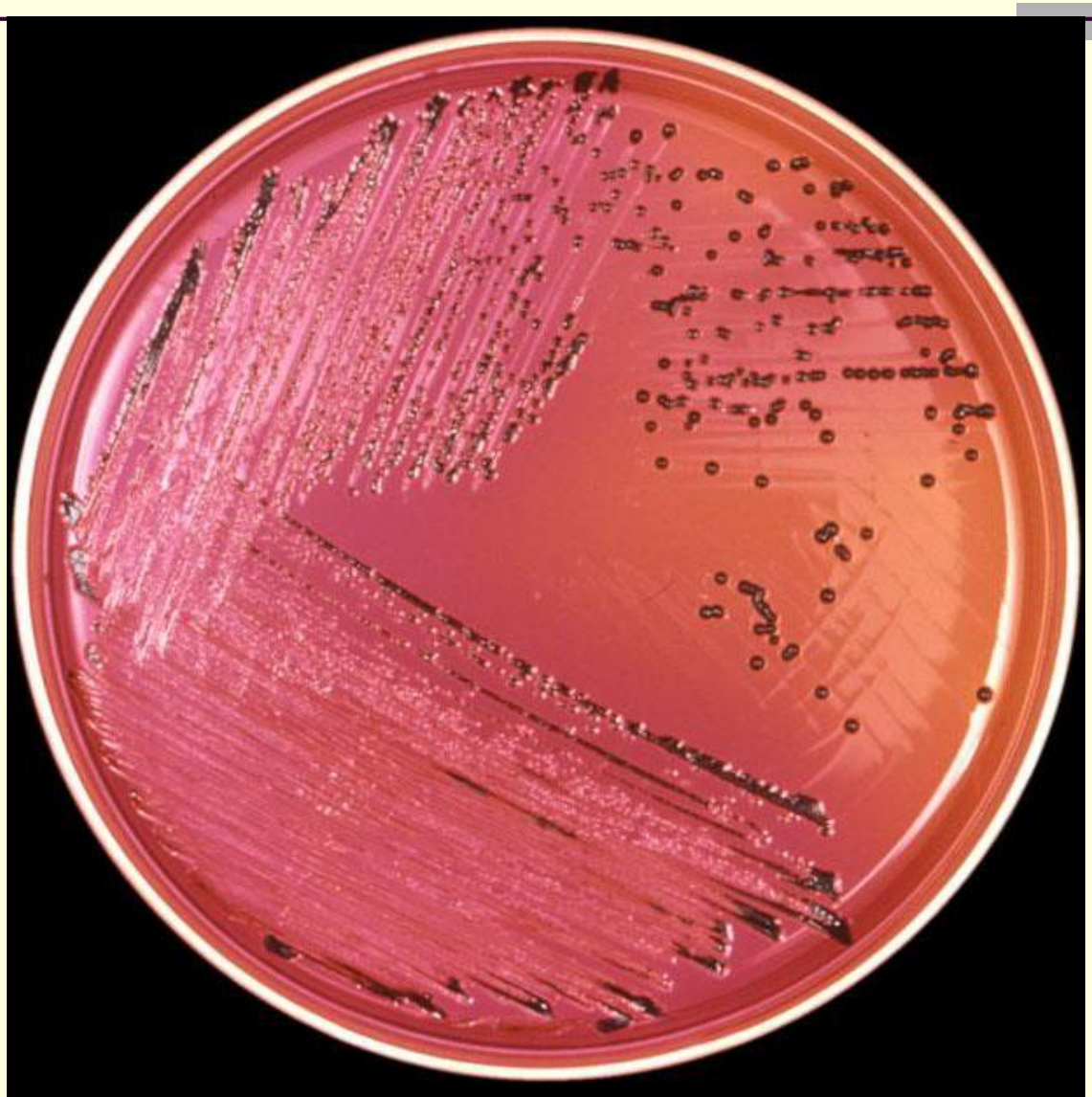
Salmonella typhi is facultative anaerobic. It can live on a wide variety of substrates, grow well on ordinary media.

Optimal growth of *E. coli* occurs at 37°C.

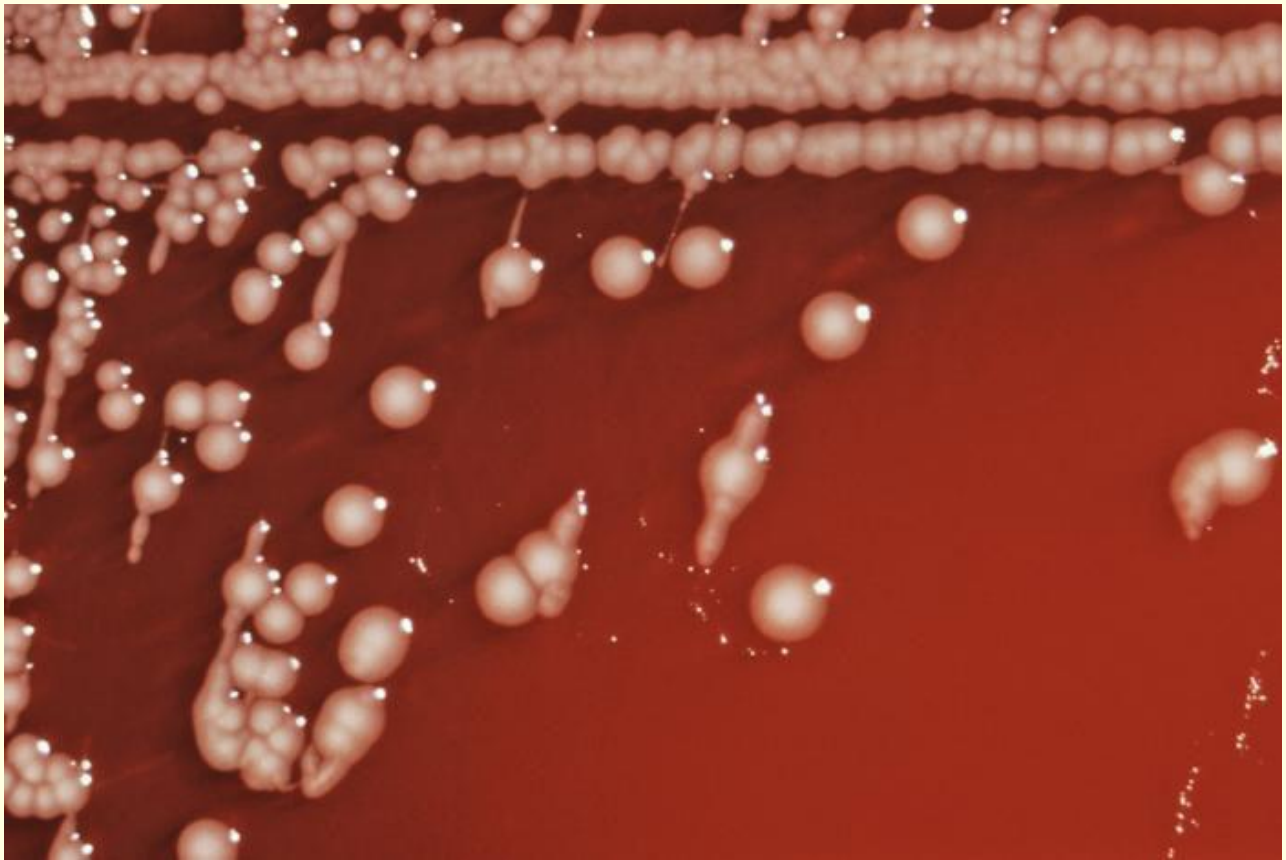
Some media are differential and nonselective, i.e., they contain lactose with a pH indicator, but do not contain any inhibitor for non salmonellae (e.g., bromocresol purple lactose agar). Other media are differential and slightly selective, i.e., in addition to lactose and a pH indicator, they contain an inhibitor for nonenterics (e.g., MacConkey agar and eosin-methylene blue agar).

The most commonly used media selective for *Salmonella* are SS agar, bismuth sulfite agar, Hektoen enteric (HE) medium, brilliant green agar and xylose-lisine-deoxycholate (XLD) agar. All these media contain both selective and differential ingredients.

Salmonella sp. after 24 hours growth on XLD agar. The presence of any black colored area indicates the deposition of hydrogen sulfide, (H_2S) under alkaline conditions.



Media used for *Salmonella* identification are those used for identification of all *Enterobacteriaceae.* Most strains grow on nutrient agar as smooth colonies, 2-4 mm in diameter. Most strains are prototrophs, not requiring any growth factors. However, auxotrophic strains do occur, especially in host-adapted serovars such as Typhi and Paratyphi A.



Biochemical activity

- Indole test negative
- Methyl red (MR) test is positive
- Citrate is positive
- Urease is not hydrolysed
- H_2S produced from thiosulfate
- Gelatin hydrolysis negative

- Carbohydrate utilization: Lactose negative

Salmonella typhi produces acid from fermentation of glucose, maltose and mannitol.

Salmonella paratyphi A, B produces acid and gas from fermentation of glucose, maltose and mannitol.

Antigenic structure

As with all ***Enterobacteriaceae,*** the genus *Salmonella* has three kinds of major antigens with diagnostic or identifying applications: somatic, surface, and flagellar.

Somatic (O) or Cell Wall Antigens are heat stable and alcohol resistant.

Surface (Envelope) Antigens - Vi antigen.

Flagellar (H) Antigens are heat-labile proteins.

Virulence Factors

- *Enterotoxin*: this enterotoxin causes water secretion in rat ileal loop.
- *Cytotoxin* that inhibits protein synthesis.

Both of these toxins are presumed to play a role in the diarrheal symptoms of salmonellosis.

- *Endotoxin (LPS).*
- Adhesins*:* fimbriae.
- Invasins: hemolysin, hyaluronidase, fibrinolysin, lecitinase
- *Defense against immune responses***:** Vi-antigens, LPS, antigenic variation.

Habitats

The principal habitat of the salmonellae is the intestinal tract of humans and animals.

Salmonella in the Natural Environment

Salmonellae are disseminated in the natural environment (water, soil, sometimes plants used as food) through human or animal excretion. Humans and animals (either wild or domesticated) can excrete *Salmonella* either when clinically diseased or after having had salmonellosis, if they remain carriers. *Salmonella* organisms do not seem to multiply significantly in the natural environment (out of digestive tracts), but they can survive several weeks in water and several years in soil if conditions of temperature, humidity, and pH are favorable.

Pathogenesis

Salmonella may produce 3 types of lesions: **Enteric fever; Septicemia; Food poisoning.**

Enteric fever: It is caused by S.typhi (70 to 85% in India), S.paratyphi A (15to 21%) and S.paratyphi B. Infection is through ingestion. The bacilli may enter the body through lymphoid of pharynx. In the gut organisms attach themselves with epithelial cells of intestinal villi and penetrate lamina propria and submucosa. They are phagocytosed by polymorph or macrophages. They enter mesenteric lymph nose to multiply there. Then they enter thoracic duct and subsequently bloodstream.

As a result there is bacteremia and organism are seeded in liver, gallbladder, spleen, bone marrow, lymphnode, lung and kidney, etc. In these organs further multiplication occurs. Then there is bacteremia and hence onset of clinical symptoms. The bacilli invade tissue, e.g. Payer's patches and lymphoid follicles of small intestine. The intestinal lesion ulcerates and hemorrhage or perforation may occur.

The organism liberates endotoxin which produces toxic symptoms like headache, anorexia, continuous fever and congestion of mucous membrane. Incubation period is 10 to 14 days. The typical features are step ladder pyrexia, palpable spleen and rose spots that fade on pressure and they appear in 2nd and 3rd week of infection.

S.paratyphi A and S.paratyphi B may cause paratyphoid fever resembling enteric fever.

- ***Septicemia:*** It is frequently caused by S. cholerae suis. It produces chills and spiked fever. Local lesions occur in various parts of body producing osteomyelitis, pneumonia, pulmonary abscess and meningitis, etc. The bowel is not invaded and fecal culture is negative.
- ***Food poisoning:*** It is by ingestion of contaminated food, e.g., meat and egg. Food poisoning is caused by S.typhimurium, S.enteritides, S.newport, etc. Incubation period is 12 to 48 hours. There is fever, vomiting, diarrhea (mucus and blood in stool). There may be ulceration of intestinal mucosa. There is no bacteremia.

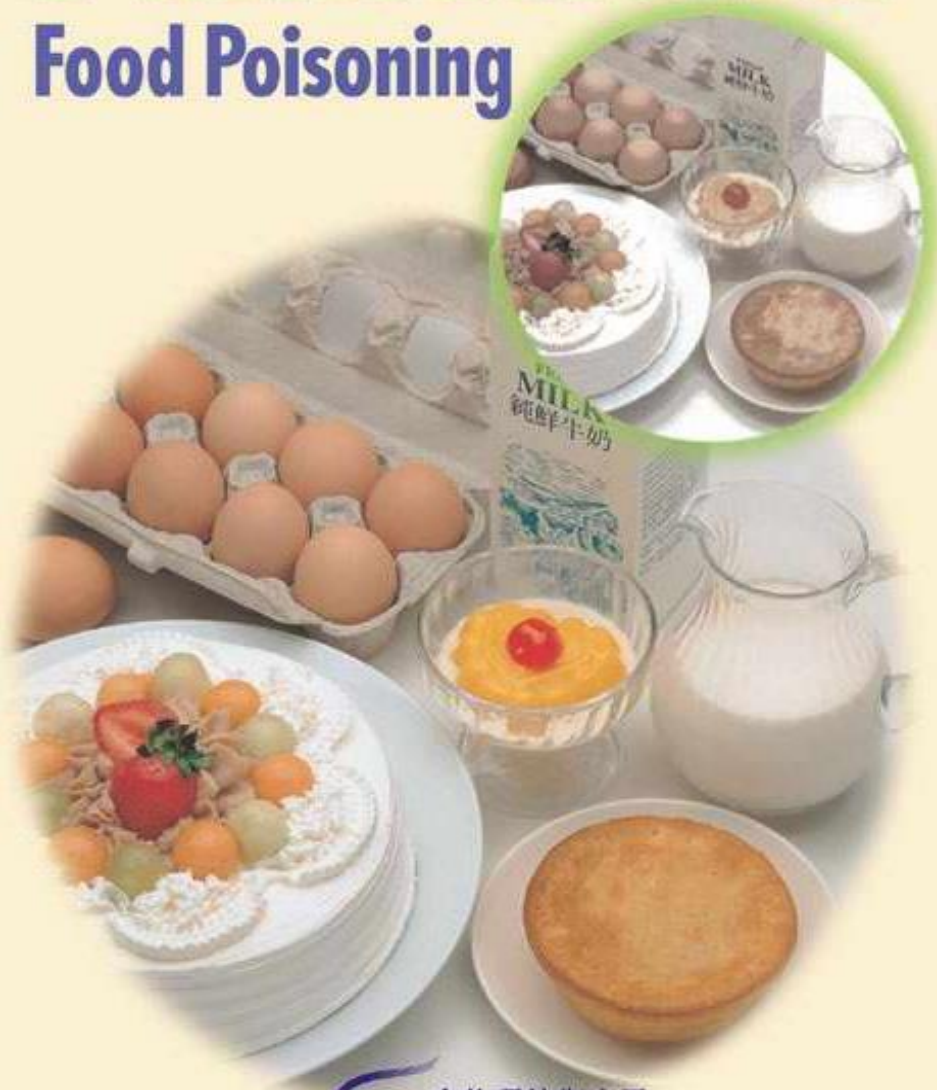
Epidemiology

Transmission of pathogenic Salmonella typhi, Salmonella paratyphi A, B often occurs via fecal-oral transmission. Common routes of transmission include: unhygienic food preparation,farm contamination due to manure fertilization, irrigation of crops with contaminated greywater or raw sewage, feral pigs on cropland, or direct consumption of sewage-contaminated water.

Food poisoning is by ingestion of contaminated food, e.g., meat and egg (next slide).

Salmonella

Food Poisoning



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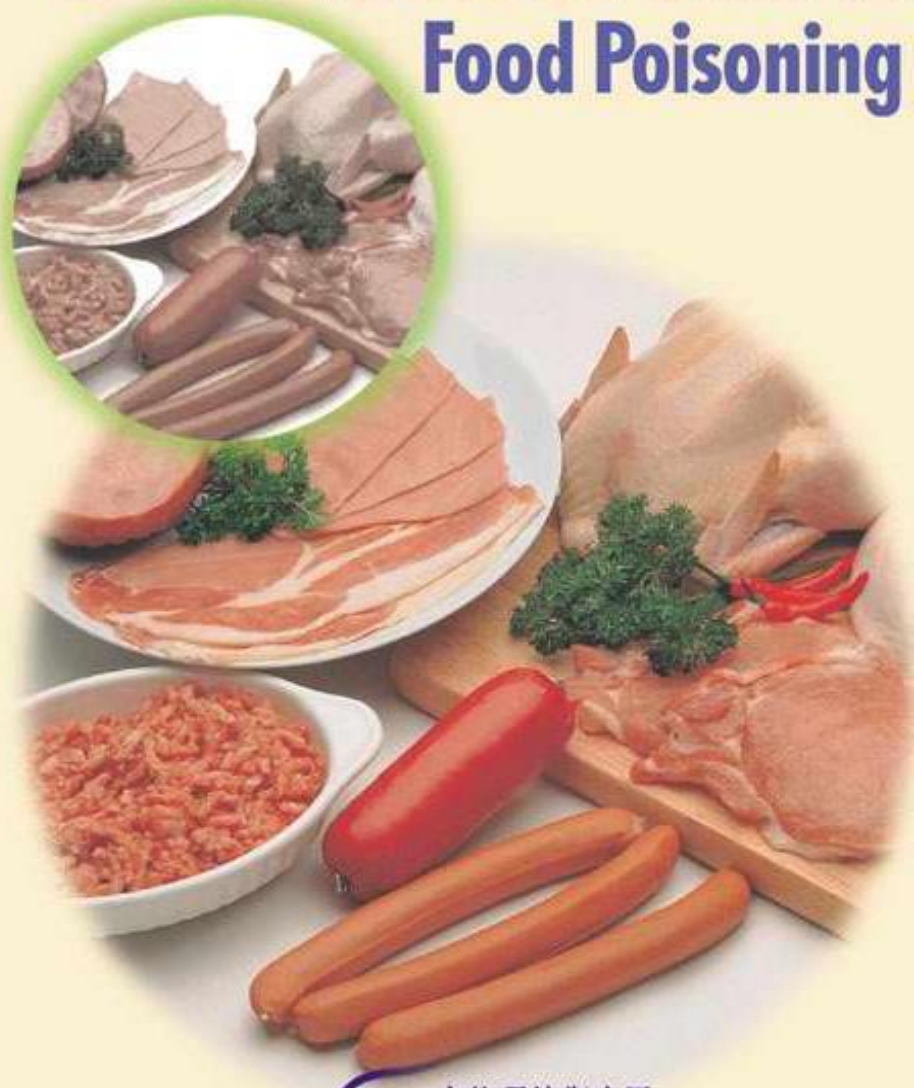
Salmonella

Food Poisoning



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Diagnosis

A definitive diagnosis of is made on the basis of **isolation** and **identification** of Salmonella spp. (***Bacteriological method***)

Organism may be isolated from blood, urine, feces, persistent discharge and in some cases from celebrospinal fluids. Either MacConkey or Endo agar are **inoculated** with the the sample. Culture on MacConkey or Endo agar (for pale, non-lactose-fermenting colonies) and on other indicator and selective media. Wilson and Blair bismuth sulphite medium: Jet black colonies with metallic sheen due to H2S formation may appear.

Once isolated, Salmonella spp. can be **identified** by the gram-staining, biochemical tests, serology (by identification of antigens).

Serological method

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

Specific prophylaxis

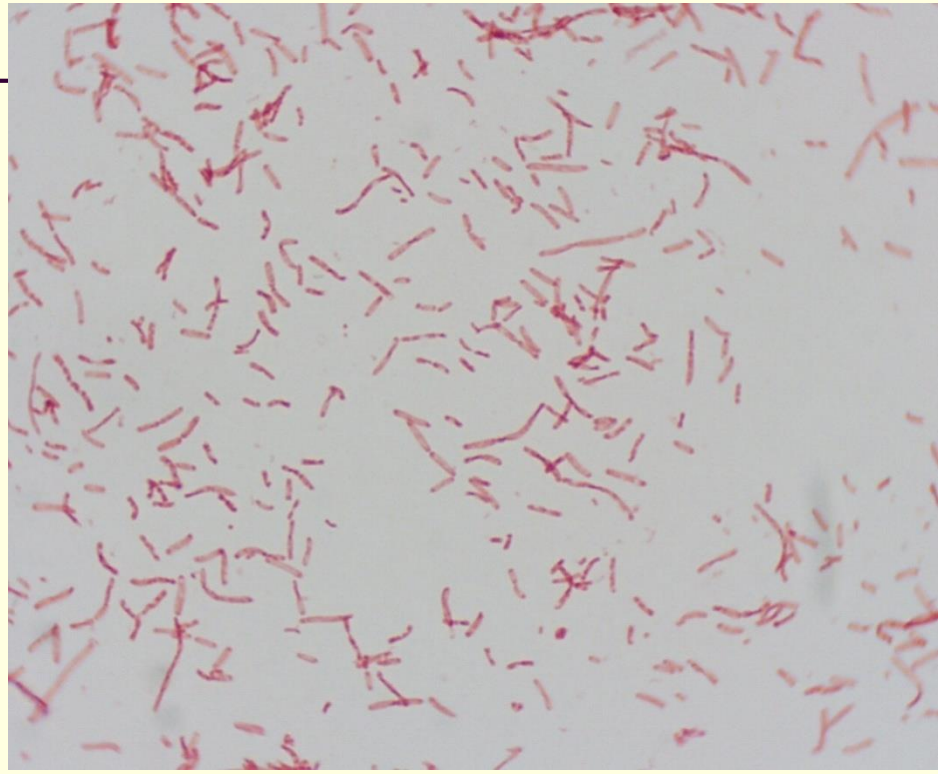
Specific prophylaxis consist of TAB vaccine containing S.typhi 1.000 million and S.paratyphi A and B 750 million each per ml of heat killed bacteria and preserved in 0.5% phenol. It is given in two doses of 0.5 ml subcutaneously at an interval of 4 to 6 weeks. However, encouraging results are noted with live vaccine (streptomycin dependent strain) and killed vaccine given as enteric coated tablets.

Three types of typhoid vaccines are currently available for use in the United States: (1) an oral live-attenuated vaccine; (2) a parenteral heat-phenol-inactivated vaccine; (3) a newly licensed capsular polysaccharide vaccine for parenteral use. A fourth vaccine, an acetone-inactivated parenteral vaccine, is currently available only to the armed forces.


Shigellosis and Cholera

Shigella

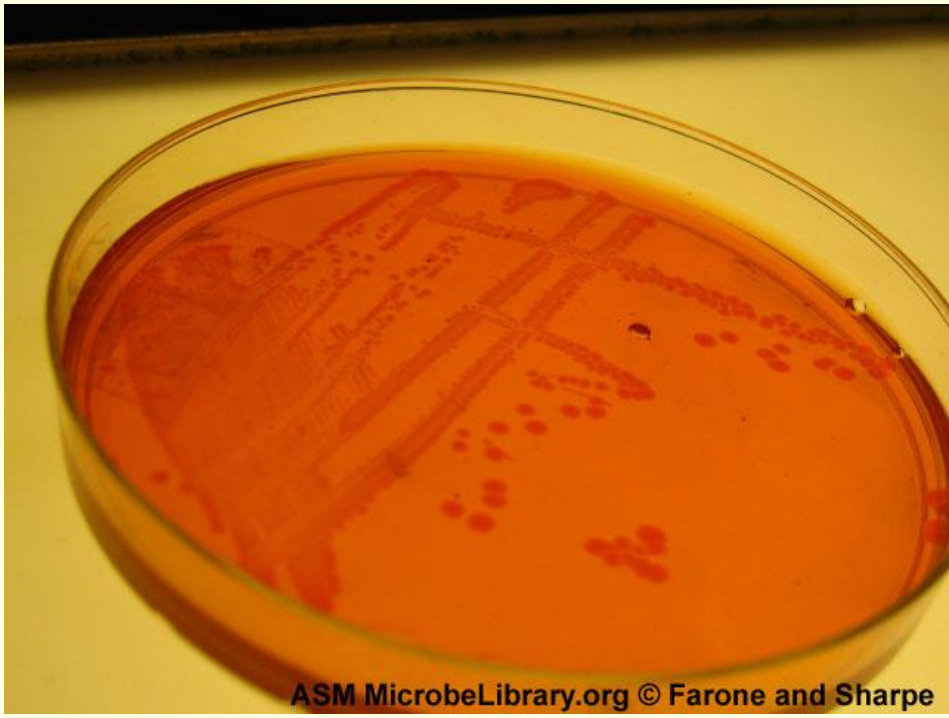
Family Enterobacteriaceae
Genera Shigella
Species Shigella dysenteriae
Shigella flexneri
Shigella boydii
Shigella sonnei



Morphology and staining: Shigella is a Gram-negative rod, non-sporulating. It is non-motile, non-capsulated, about 0.5x1μm to 3 μm in size.

Cultural characteristic

It is aerobic and facultative anaerobic, grows readily in simple media with an optimum temperature of 37^0C and pH of 7.4



Nutrient agar:
After overnight incubation, colonies are small, 2 mm in diameter, circular, convex, smooth and translucent.
Broth: There is uniform growth with mild turbidity after 12 to 24 hours' incubation.

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

Biochemical activity

Shigella (ferments glucose with formation of acid)				
Mannitol **positive**			Mannitol **negative**	
	Lactose **negative**			
Lactose **positive** ***S.sonnei***	Indole **positive** ***S.flexneri*** 1-5 ***S.boydii*** 5,7,9,13,14	Indole **negative** ***S.flexneri*** 6 ***S.boydii*** 1-4, 6,8,10-12,15	Indole **positive** ***S.dysenteriae*** 2,7,8	Indol **negative** ***S.dysenteriae*** 1,3-6

Antigenic structure

The genus *Shigella* has two kinds of major antigens with diagnostic or identifying applications: somatic and capsular.

Somatic (O) or Cell Wall Antigens are heat stable and alcohol resistant.

Capsular (K) antigens are heat stable.

On the basis of biochemical and serological properties shigella are classified into 4 groups

- *Shigella dysenteriae:* It consists of 10 serotypes.
- *Shigella flexneri:* It consists of 6 antigenic types and several subtypes.
- *Shigella boydii:* It consists of 18 serotypes.
- *Shigella sonnei:* On the basis of their capacity to form colicine, it is divided into 17 types.

Virulence Factors of Shigella

- *Endotoxin (LPS)* (contributes to the irritation of bowel wall)
- *Enterotoxin*: inhibits sugar and amino acid absorption in small intestine of human.
- *Vero cytotoxin* (VT) **,** also called the **Shiga toxin**, is produced by *Shigella dysenteriae.* The syndromes associated with shiga toxin include dysentery, hemorrhagic colitis, and hemolytic uremic syndrome.
- To aid its entry into the epithelial cell, the bacterial DNA encodes a number of plasmid and chromosomal proteins. These proteins are the invasion plasmid antigens (Ipa), surface presentation antigens (Spa), membrane excretion proteins (Mxi), and virulence proteins (Vir).

Epidemiology

Shigella is transmitted from an infected person to another usually by a fecal-oral route. Part of the reason for the efficiency of transmission is because a very small inoculum (10 to 200 organisms) is sufficient to cause infection.

Epidemics may be foodborne or waterborne. *Shigella* infections may be acquired from eating food that has become contaminated by infected food handlers. Vegetables can become contaminated if they are harvested from a field with contaminated sewage or wherein infected field workers defecate. *Shigella* can also be transmitted by flies. Flies can breed in infected feces and then contaminate food. *Shigella* infections can be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or even if someone with shigellosis swims or bathes or, worse, defecates, in it.

Pathogenesis

The bacteria are transmitted by the fecal-oral route, and through contaminated food and water. Once ingested, the bacteria survive the gastric environment of the stomach and move on to the large intestine. There, they attach to and penetrate the epithelial cells of the intestinal mucosa. After invasion, they multiply intracellularly and spread to neighboring epithelial cells, resulting in tissue destruction and characteristic pathology of shigellosis.

Clinical Disease

People infected with Shigella develop diarrhea, fever and stomach cramps starting a day or two after they are exposed to the bacterium. The diarrhea is often bloody. Shigellosis usually resolves in 5 to 7 days, but in some persons, especially young children and the elderly, the diarrhea can be so severe that the patient needs to be hospitalized. A severe infection with high fever may also be associated with seizures in children less than 2 years old. Some persons who are infected may have no symptoms at all, but may still transmit the bacteria to others.

Diagnosis

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

A definitive diagnosis of is made on the basis of **isolation** and **identification** of Shigella. (***Bacteriological method***)

A loopful of pus or blood tinged mucus from freshly passed fecal sample in cultured on MacConkey, Endo or DCA (Desoxycholate citrate agar). Selenite broth is used as enrichment medium. After 12 to 18 hours of incubation colorless colonies (non lactose fermenter) appear on MacConkey or Endo agar. These are tested for motility and biochemical reactions. Non motile organism which is urease, citrate, KCN and H_2S negative, indole and MR positive suggestive of Shigella. **Identification** is confirmed by slide agglutination with polyvalent and monovalent antisera.

Serological method

To determine of specific antibodies of different classes in sera indirect haemagglutination reaction are used.

The ***immunofluorescence test*** for Shigella recovery is employed in examining objects containing minor amounts of the causative agents and for rapid laboratory diagnosis of dysentery.

Treatment

Drugs like tetracycline or chloramphenicol are effective in
shigella infection. Treatment with antibiotic should be
continued for 5 to 7 days.
Also the antibiotics commonly used are ampicillin,
trimethoprim/sulfamethoxazole (also known as Bactrim or
Septra), nalidixic acid and the fluoroquinolone,
ciprofloxacin.
Appropriate treatment kills the bacteria present in the
gastrointestinal tract and shortens the course of the illness.

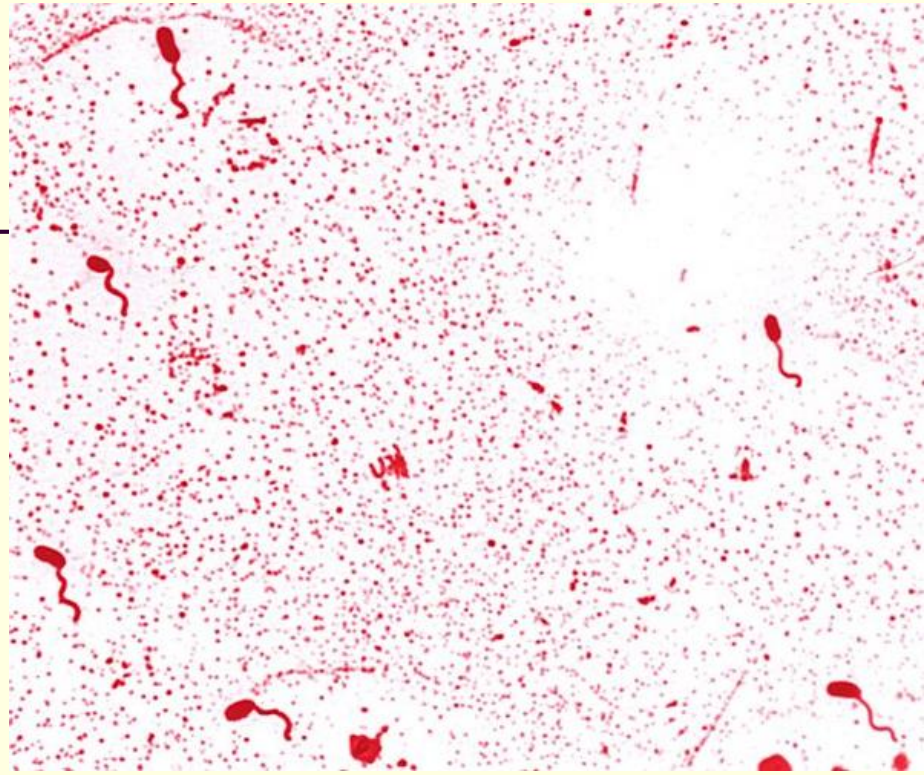
***Specific prophylaxis*:**

Oral live vaccine using streptomycin depended strains in polyvalent preparations have given highly significant protection against clinical disease.

Three approaches to *shigella* vaccine development that are under active investigation are:

1) parenteral O-specific polysaccharide conjugate vaccines;
2) nasal proteosomes delivering *Shigella* LPS; and
3) live, attenuated invasive *shigella* deletion mutants that are administered orally.

Vibrio cholerae



Family Vibrionaceae

Genera Vibrio

Species Vibrio cholerae

Patogenic for human biotype :

cholarae and el-tor

Morphology and staining: It is short Gram-negative straight or curved rods about 1,5 x 0.2 to 0.4 μm in size, motile by means of a single polar flagellum. Non-sporulating, non-capsulating.

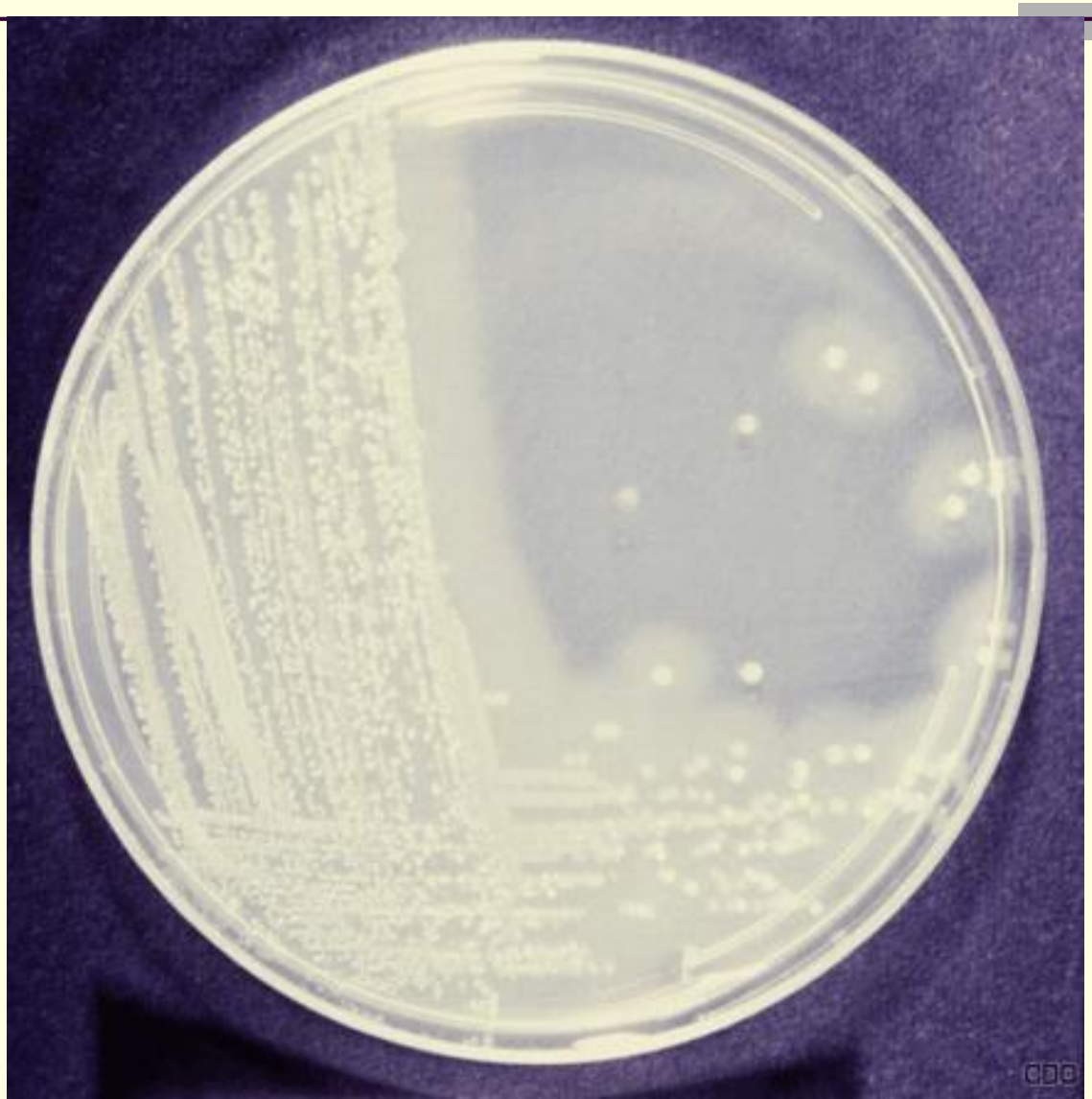
Cultural characteristic

- Cultural character: It is strongly aerobic. Growth occurs in alkaline pH (7.5 to 9.6) between 22 and 40^0C (optimum 37^0C). It grows well in simple media.
- Alkaline peptone water: Rapid growth about 6 hours with formation of thick surface pellicle. Turbidity and powdery deposits on prolonged incubation may be present.
- Nutrient agar: Colonies are moist, translucent, circular 1 to 2 mm in diameter with bluish tinge in transmitted light. The growth has distinctive odor.
- MacConkey (or Endo) agar: Colonies are colorless.
- Blood agar: Colonies show zone of green coloration around them which later become clear due to hemodigestion.

Special media:

- Alkaline bile salt agar pH 8.2 (BSA).
- Monsur’s gelatin-taurocholate tripticase-tellurite agar (GTTA).
- TCBS (thiosulphate citrate bile sucrose) medium: It contains thiosulphate, citrate bromothymol blue and sucrose. It is widely used.

Gelatin **agar** medium used in the identification of the bacteria *Vibrio cholerae*

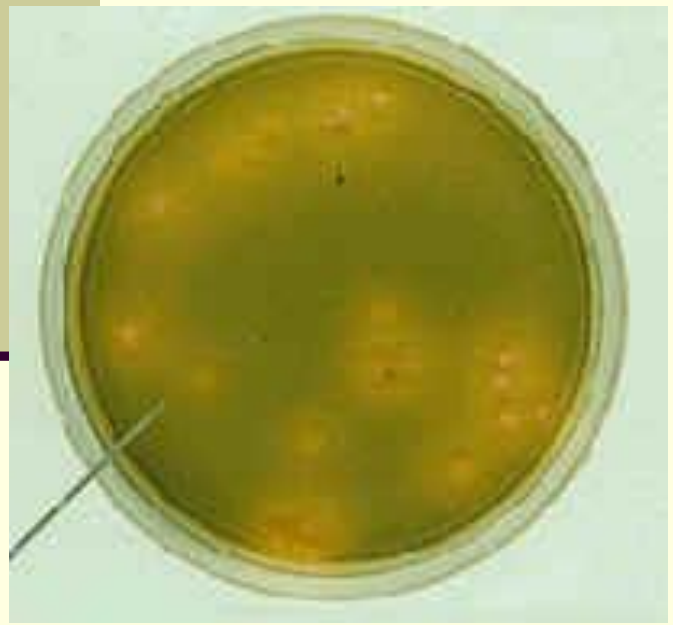


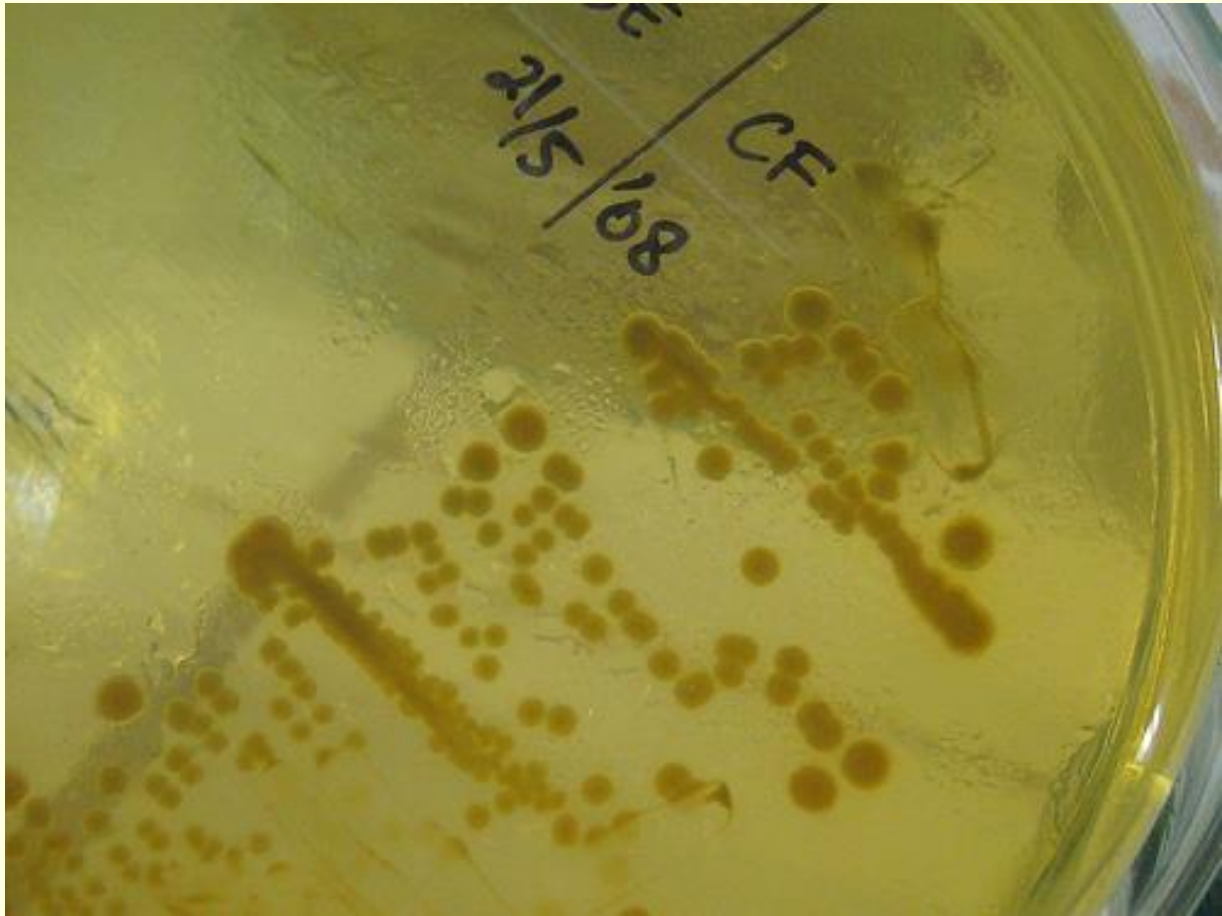
Thiosulfate Citrate Bile Salt Sucrose agar plate (TCBS)

Use : For the selective isolation of ***Vibrio cholerae*** from a variety clinical specimens and in epidemiological investigations.

Vibrio cholerae : Growth (yellow colonies)

Incubate : Aerobic 35° C, 24 hrs





Biochemical activity

- Oxidase is positive
- Indole test positive
- Urease test negative
- Gelatin hydrolysis positive
- Nitrate reduction positive
- Carbohydrate utilization

Vibrio cholerae destroy glucose, sucrose, maltose and mannitol with producing acid without gas

Resistance

Vibrio cholerae died by heat at 55^0C in 15 minutes.

It is destroyed by drying. The acidity of gastric juice at once kills them.

Antigenic structure

Antigenic variation has important role in the epidemiology and virulence of cholera. The ***flagellar antigens (H antigens)*** of ***Vibrio cholerae*** are shared with many water vibrios and therefore do not use in differing strains causing epidemic cholera.

O antigens, however, do differ strains of *V. cholerae* on the 139 known serotypes.

There are three different **O1 biotypes**, named **Ogawa, Inaba** and **Hikojima**.

Virulence Factors

- *Toxin production:* *Thermostable* Endotoxin *(LPS) and non* thermolabile enterotoxin called choleragen (exotoxin) and consists of subunits A and B. The cholerogen stimulates persistant and excessive secretion of isotonic fluid by the interstinal mucosa. Cholera toxin **activates the adenylate cyclase enzyme** in cells of the intestinal mucosa leading to increased levels of intracellular cAMP, and the secretion of H_20, Na^+, K^+, Cl^-, and HCO^{3-} into the lumen of the small intestine.
- *Motility and Chemotaxis*
- Adhesins: filamentous fimbriae, hemagglutinin, group of outer membrane proteins are products of the **acf** (**accessory colonization factor**) genes.
- Invasins: neuraminidase, mucinases, hyaluronidase, fibrinolysin, lecitinase, etc.

Epidemiology

Cholera occurs only in man. The disease is transmitted from mild and convalescent cases by contaminated water, milk, fruit, vegetable, etc. Flies may carry organisms from feces to food. Cholera is endemic in India, China, Japan and Indonesia.

Immunity after infection is short living. Gastric acidity seems an important defense against cholera. Ingestion of at least 10^{10} organisms is necessary to demonstrate some evidence of infection. However, neutralizing the acidity of the stomach lowered the number 10^{4} organisms.

Pathogenesis and Clinical signs

Its extreme manifestation, cholera is one of the most rapidly fatal illnesses known. A healthy person may become hypotensive within an hour of the onset of symptoms and may die within 2-3 hours if no treatment is provided. More commonly, the disease progresses from the first liquid stool to shock about 4-12 hours, with death following from 18 hours to several days.

The **clinical description** of cholera begins with sudden onset of massive diarrhea. The patient may lose liters of protein-free fluid and associated electrolytes, bicarbonates and ions within a day or two. This results from the activity of the cholera enterotoxin which activates the adenylate cyclase enzyme in the intestinal cells, converting them into pumps which extract water and electrolytes from blood and tissues and pump it into the lumen of the intestine. This loss of fluid leads to dehydration, anuria, acidosis and shock. The watery diarrhea is speckled with flakes of mucus and epithelial cells ("rice-water stool") and contains enormous numbers of vibrios. The loss of potassium ions may result in cardiac complications and circulatory failure. Untreated cholera frequently results in high (50-60%) mortality rates.

Diagnostic

A definitive diagnostic base on the **isolation** and **identification** of Vibrio cholerae **(*Bacteriological method*)**

Microorganism may be isolated from feces or vomitus.

Either TCBS or Monsur's agar are **inoculated** with the sample.

The culture of V. cholerae from feces or vomitus **indicates** cholera; however, definitive diagnosis requires agglutination and other clear reactions to group- and type-specific antisera.

Bacterioscopic method

A dark-field microscopic examination of fresh feces showing rapidly moving bacilli (like shooting stars) allows for a quick, tentative diagnosis.

Immunofluorescence also allows rapid diagnostic.

Diagnostic must separete from *Escherichia coli* infection, salmonellosis, and shigellosis.

Serological method

Serological test:

To determine of specific antibodies (agglutinins and vibriocidal AB) in patient's serum.

Treatment of cholera

Involves the rapid intravenous replacement of the lost fluid and ions. Following this replacement, administration of isotonic maintenance solution should continue until the diarrhea ceases. If glucose is added to the maintenance solution it may be administered orally. Most antibiotics and chemotherapeutic agents have no value in cholera therapy, although a few (e.g. tetracyclines) may shorten the duration of diarrhea and reduce fluid loss. It is susceptible to streptomycin, chloramphenicol and tetracycline.
Vibrio cholerae sensitive to macrolides, ciprofloxacin and aminoglycoside antibiotics too.

Specific prophylaxis

Vaccine is used for control of cholera infection.

Killed cholera vaccine containing killed suspension (12000 million V.cholerae per ml) containing equal number of Ogawa and Inaba serotypes is used nowadays which gives 40 to 60 % protection about one year.

The other vaccine preparations are procholeragenoid with killed Ogawa and Inaba, whole cholera vaccine with adjuvants like aluminium, liposomes or muramyle dipeptide, and live mutant vaccine strain (streptomycin resistant), B subunit toxoid (80 to 85% protection), etc. El Tor type vaccine make protection about 90 to 100% which lasts for 3 years.