Gram-negative facultative anaerobic bacilli

This group of gram-negative bacteria includes families Enterobacteriaceae, Vibrionaceae, Pasteurellaceae.

Family Enterobacteriaceae

One large family of gram-negative bacteria that have a considerable degree of relatedness is the Enterobacteriaceae. Enterobacteriaceae family can be divided into five tribes Escherichieae (subdivided into genera Escherichia, Edwardsiella, Citrobacter, Salmonella, Shigella), Klebsielleae, Proteae, Erwinieae, Yersinae. Enterobacteriaceae family includes 35 genera. The members of this group are common occupants of the large bowel of humans and animals and inhabit soil, water. Pathogenic, opportunistic and saprophytic species can be among enterobacteria. Enteric pathogens are the most frequent cause of diarrheal illnesses. Prominent pathogenic enterics include Salmonella, Shigella. The most important enteric opportunists are E.coli, Klebsiella, Proteus, Enterobacter, Serratia and Citrobacter.

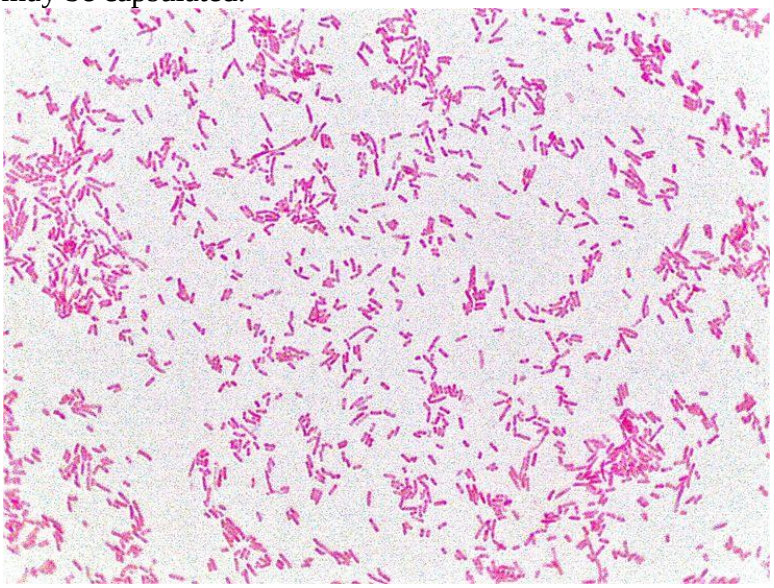
Morphology: they are short gram-negative rods with rounded ends and measure 1.5-5.0μm in length, 0.3-0.8μm in breadth. They do not form spores, they are facultative anaerobes. They differ in fermentative properties (they ferment a wide range of carbohydrates) and antigenic structure.

The genera in this family share several key properties: they ferment glucose, reduce nitrates to nitrites, oxidase-negative, catalase positive. According to the ability of enteric bacteria to ferment lactose, they are coliforms which include E.coli and other gramnegative normal enteric flora that ferment lactose rapidly (within 48 hours). Noncoliforms are generally non-lactose-fermenting or slow lactose fermenting that are either normal flora or regular pathogens. Antigenic structure is an important criterion, which is used for classification and identification of enteric bacteria. Enterobacteria have complex surface antigens: H (flagellar), K (capsular), O (somatic or cell wall antigen). Not all species carry H and K antigen, but all have O, the lipopolysaccharide involved in endotoxic shock.Several other tests are required to identify the level of genus (the production of indol, urease, methyl-red test, Voges-Proskauer reaction, citrate utilization test, carbohydrate fermentation).

Genus Escherichia

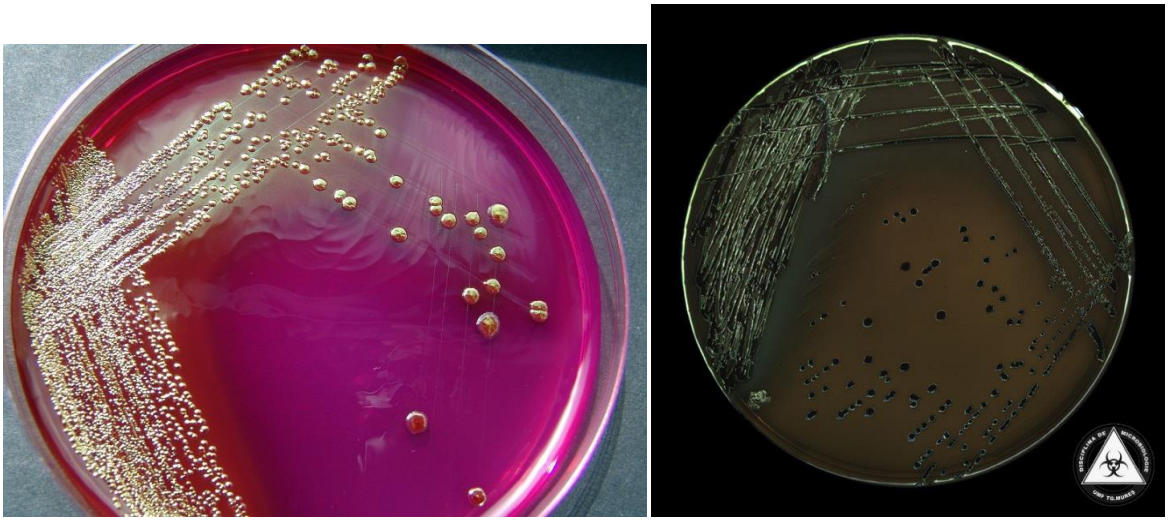
Escherichia coli normally inhabit the colon without causing the disease. When *E.coli* acquires virulence due to plasmids or genetic recombination, it cancause disease.

Morphology. *E.coli* are gram-negative bacilli, motile by peritrichous flagella, non- sporeforming, pathogenic strains may be capsulated.



***E.coli.* Gram staining**

Culture properties. *E.coli* are aerobes or facultative anaerobes. They grow in wide range of temperatures. Optimal growth of *E.coli* occurs at 37°C. *E.coli* grow well on ordinary media, e.g. MPA, MPB. On the solid media they form S- and R-forms of colonies. In the liquid media escherichia produce diffuse turbidity and bottom sediments. To identified *E.coli*, differential-diagnostic media - Mac-Conkey agar, Endo, Ploskirev, Levin' agar - are used. Colonies, developing on this media, will provide the separation of bacteria into lactose-ferments and non-lactose-ferments. Lactose fermenters develop red coloration with metalic shiny on the Mac-Conkey and Endo media, pink-purple coloration on the Ploskirev media, violet coloration on the Levin media.



***E.coli* culture properties**

Fermentative properties. *E.coli* ferment lactose, glucose, maltose and mannitol forming acid and gas. Indole and methyl red (MR) are positive. VP and citrate are negative. Urease is not hydrolysed. H_2S is not produced.

Antigenic structure: It has three antigens that are used to identify the organism in epidemiological investigations:

Somatic "O" antigen or cell wall antigen. This is thermostable, lipopolysaccharide antigen of the cell wall. By O antigen they are subdivided into 173 serological groups. The normal colon strains of E. coli belong to early O groups (1,2,3,4,5, etc.) and enteropathogenic strains belong to the latter O groups (26,55,111, 112,etc.).

"H" or flagellar antigen –H antigen is type specific. There are 75 H antigens. This antigen is thermolabile protein.

Capsular "K" antigen: there are 100 K antigens. (K antigen consists of three components A thermostable; B and L thermolabile). K antigen masks O antigen (for revealing O it is necessary to boil the culture and destroy the K antigen). K antigen is type specific too.

On the basis of O antigens, E. coli is subdivided into a number of O groups. Each O group is then divided into subgroups on the basis of K antigen. Each of these subgroups includes strains with different H antigens. Thus, on the basis of antigenic structure an antigenic formula is derived which fully reflects the antigenic properties of the strain. For example, E. coli O26:K60:H12. Specific

Resistance. *E.coli* are easily destroyed by heat and by common disinfectants. They are sensitive to drying and survive best in a high moisture environment. Respiratory care or anesthesia equipments are common sources of nosocomial infections.

1. Adhesive factors (fimbriae-which are chromosomally determined) and colonisation
2. Invasive factors which depend on surface proteins by which microbes penetrate into

intestinal epithelial cells, multiply and destroythem.

3. Exotoxins: **a)enterotoxins:** Enterotoxins: **heat labile protein toxin**, it activates adenylate cyclase in the enterocysts to form cyclic AMP. The accumulation of cAMP in the intestinal mucosa initiates the hypersecretion of electrolytes and fluids into the lumen, resulting in watery diarrhoea. **Heat stable** enterotoxin is a low molecular weight polypeptide and poorly immunogenic toxin. It activates guanylate cyclase causing the increased production of cyclic guanosine monophosphate (cGMP) and subsequent hypersecretion of electrolytes resulting in diarrhoea. **b)cytotoxin:** is phage encoded cytotoxin identical to Shiga toxin produced by Shigella dysentery, which can cause destroying of vessel's endothelium and intestinalwall.
4. Hemolysins: many strains of E. coli produce hemolysin. A larger proportion of E. coli strains recovered from extra-intestinal lesions of a man are haemolytic than are those isolated from humanfaeces.
5. Endotoxin - the somatic lipopolysaccharide surface O antigen, besides exerting endotoxin activity, also protects the bacillus from phagocytosis and the bactericidal effects of complement. Diseases caused by E. coli subdividedinto:

Endogenous: Causes diseases outside the intestinal tract. It causes urinary tract infection-cystitis, pyelonephritis. It can cause meningitis in association with the B streptococci; can cause sepsis (by the immunodeficiency). These infections are caused by conditionally pathogenic E. coli (the member of normal microflora of our organism) and named ***coli-bacteriosis.***

Exogenous: is an acute infection of the intestinal tract and is named ***esherichiosis*** (diarrhoea). E. coli causing diarrheal diseases is subdivided into 5 groups. They produce diarrhoea with different pathogenic mechanisms:

1. **Enteropathogenic E. coli (EPEC):** They cause coli-enteritis in infants and children (serogroups O26, O55, O111) usually occurring as institutional outbreaks but they can also cause sporadic diarrhoea in children and less often in adults. The pathogenesis of EPEC diarrhoea is not fully understood. EPEC do not ordinarily produce enterotoxins, nor they are invasive. They are seen to be adherent to the mucosa of the upper small intestine by superficial proteins, intimately attached to cup- like projections of the enterocyte membrane, causing disruption of the brush border microvilli (they produce inflammatory process and erosive surfaces). The pathogenesis is limited by endotoxin which causes inflammatoryreaction.

2. **Enterotoxigenic E. coli (ETEC)** causative agent of cholera-like diarrhoea infection. Thefirst step in pathogenesis is adherence of the bacteria to the cells of the jejunum and ileum by pili that protrude from the bacterial surface. ETEC is not invasive but produce enterotoxin, heat-labile toxin (functional blockater), which acts by stimulating adenylate cyclase, the resultant increase in intracellular cyclic AMP (cAMP) concentration stimulates cAMP-dependent protein kinase, causing an outpouring of fluid, potassium, and chloride from the enterocytes. Watery diarrhoea occurs (resembles a mild form of cholera). Its severity varies from mild watery diarrhoea to fatal diseases indistinguishable from cholera. Persons from developed countries visiting endemic areas often suffer from ETEC diarrhoea, a condition known as "travellers diarrhoea". Though plasmids with enterotoxin genes may be present in any strain of E. coli, in practice only a small number of serotypes become enterotoxigenic (e.g. O6, O8, O15, O25, O27, O167).

3. **Enteroinvasive E. coli (EIEC):** Causative agent of dysentery-like disease. These resemble shigella in many respects. Many of these strains are **non motile**, **do not ferment lactose or ferment it late** with acid, but without producing gas. Many of these show O antigen cross reaction with shigella. Clinically **EIEC** infection resembles shigellosis, ranging from mild diarrhoea to frank dysentery: bloody diarrhoea accompanied with inflammation**. EIEC** usually belong to serogroups O25, O114, O124, O144, O152, O154. They penetrate into enterocytes and produce heat-stable toxin (Shigella-like toxin) which inhibits protein synthesis by removing

adenine from rRNA of humanribosomes.

Enterohemorhagic E. coli (EHEC): EHEC strains have become well known as the cause of several out breaks of the diseaseas sociated withing estiononunder cooked hamburger or raw milk.

Cattle are suspected as the reservoir. These organisms produce verotoxin or shigella-like toxin, which is cytotoxin. This toxin is responsible for hemorrhagic colitis (an inflammation of the colon with bleeding). It penetrates into the blood and can defeat kidney. Many patients, especially children, infected by this organism produce stools combined with copious amounts of blood, but without fever. Sometimes it can have fatal result. The typical EHEC is serotypeO157:H7.

4. **Enteroaggregative E. coli (EAEC):** These strains are so named because they appear aggregated in a "stacked brick" formation on Hep-2cells or glass. They have been associated with persistent diarrhoea, especially in developing countries. They produce a low molecular weight heat stable enterotoxin called **EAST1**(enteroaggregative heat stable enterotoxin-1). In animal experiments they cause shortening of villi, hemorrhagic necrosis and mild oedema with mononuclear infiltration of the submucosa. Most of them are O-untypable, but many are H-typable.

Resistance: E. coli is more resistant to physical and chemical factors of the external environment than the other members of Enterobacteriaceae family. At 55° C the organism perishes in 1 hour, and at 60° C in 15 minutes. E. coli is sensitive to brilliant green and other disinfectants. E. coli is a common inhabitant of the large intestine of humans and mammals. The bacteria are excreted in great numbers with the faeces and are always present in the external environment (soil, water, foodstuffs, and other objects). Detection of E. coli in drink water is used as the indicator of faecal contamination (coli titer, coli index) **Coli-titer** is the minimal quantity of the water which contains one E. coli. It equals 300. **Coli-index** is the quantity of E. coli in one litre water. It equals 3.

As a member of normal flora E. coli (with bifidum bacteria, lactobacilli) participates in nutritional function by producing several vitamins B, K, D.

E. coli produce colicins (antibiotic like substances) which suppress exogenous and endogenous toxic products, and suppress the pathogenic flora (fungi, strepto-staphylococci, other intestinal pathogens). Antibiotic therapy inhibits the predominant normal flora and some diseases can occur (e.g., mycoses can occur and during antibiotic therapy we use anti fungal drugs). E. coli takes parts in stimulation of formation of immune system (due to presence of muramil peptide in their cellwall).

SEROLOGICAL CLASSIFICATION OF ESCHERICHIA

Category	Serogroup	Serotype
ETEC-enterotoxigenic E. coli	06.08.015.020 0.25.080.0115 0148.0159	06:H16.011:H27 0128:H7.0149:H10 0159:H20.
EIEC-enteroinvasive E. coli	029.0124.0144 0152.0167	028ac:H-.0124:H. 0124:H32. 0144:H-. 0159:H2
EPEC- enteropathogenic E. coli	055.086.0111. 0125.026	044:H34.055:H6. 0111ab:H12 0126:H7.0127:H9.
EHEC- enterohemorrhagic. E. coli	0157.0126.	0157:H7
EAEC- enteroaggregative E. coli		Very little is known

Immunity: During endogenous infection there is humoral immune response, but in this case

immunoglobulins haven't protective property. Immunodeficiency assists depressing phagocytosis. In the time of exogenous infection humoral immune response takes place too. IgG cross the placenta and from the blood it penetrates into the intestine. Here participate secretory IgA too.

The role and functions of normal microflora are numerous:

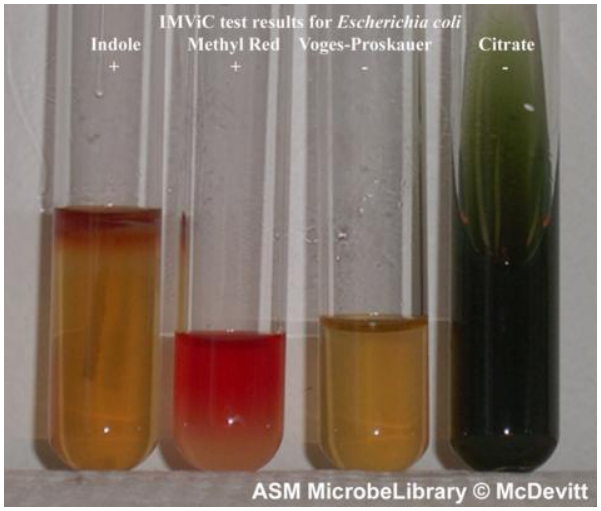
1. The normal microflora is non- specific defense factor of theorganism.
2. The normal microflora has antagonistic role against pathogenic and putrefactive (saprogenous) microflora due to production of lactic acid, acetic acid, antibiotics, bacteriocines; at powerful biological potential's expense can compete with foreign microflora.
3. The normal microflora participates in water - salt metabolism, regulation of gas composition of intestine, in metabolism of proteins, carbohydrates, fatty acids, cholesterin, nucleic acids, and also in production of biologically active compounds: antibiotics, vitamins (k, B group and others), toxins,etc.
4. The normal microflora participates in digestion and detoxication of exogenous substrates and metabolites, which is close to liver'sfunction.
5. The normal microflora participates in regulation of steroid hormones and bile salts as a result of excretion of metabolites from liver into intestine and following restitution init.
6. The normal microflora plays morphokynetic role in development of different organs and systems of the organism, participates in physiological inflammation of mucous membrane and in replacing theepithelium.
7. The normal microflora plays anti-mutagenous function due to the destruction of cancerogenic substances in the intestine. At the same time some bacteria can produce powerful mutagens. So, enzymes of intestinal bacteria transform artificial cyclomate into active cancerogene (cyclohexamine) for urinarybladder.
8. Exopolysaccharides (glycocalyx) of microorganisms, which is the part of biological membrane, prevent microbial cells from different physical-chemical influence. Intestinal mucous membrane is also under the protection of biologicalmembrane.
9. The normal microflora possesses a significant influence on formation and support of immune system. There are approximately 1,5kg of microorganisms in intestine, which antigens stimulate immune system. Natural non-specific stimulator of immunogeneses is muramyldipeptide, which is formed from bacterial peptidoglycane under the influence of intestinal lysozyme and other lytic enzymes. It results in abundant saturation of intestinal tissue with lymphocytes and macrophages, so, in norm the intestine is in chronic inflammation -`like conditions
10. The important function of normal microflora is participation in colonizativeresistance.

Laboratory diagnosis:

1. Bacteriological method-isolation of pure culture and identification(picture1)

2. Serological method: slide agglutination, tubeagglutination.

Microbiological diagnosis. Laboratory diagnosis is based on the bacteriological method. Collected specimens such as feces, vomiting, food, polluted water are cultivated on the differential-diagnostic media. Pure culture is identified according to the biochemical properties. E.coli split lactose, glucose, maltose, mannitol, produce indol, citrate utilization test is negative, methyl red test is positive, VP test is negative.



Agglutination test is used to determine serotype of isolated culture. IF and PCR can be used to detect enterotoxins in the pathological material.

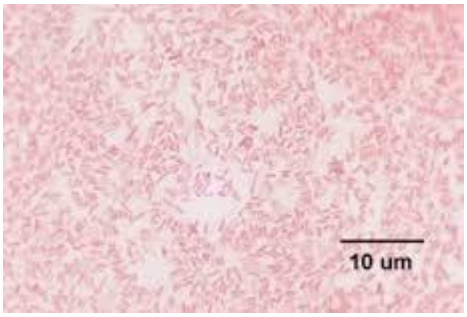
Treatment. UTI is usually treated with Bactrim (a combination of sulfamethoxazole and trimethoprim) or a fluoroquinolone. Pneumonia, meningitis, and sepsis are commonly treated with a third-generation cephalosporin and an aminoglycoside (_Antibiotics-penicillin, cephalosporins). Diarrheal syndrome is usually treated with fluids, electrolytes; coliproteicum bacteriophage also may be used. To recover intestinal microbiota in cases of dysbiosis eubiotics are used. Eubiotics: bifidobacterin; lactobacterin.

Prophylaxis. There is no specific prophylaxis. Nonspecific prevention is based on the compliance of sanitary regulations, sanitary control of the sources of water, food companies, food. Specific prophylaxis is not developed. Coliproteicum bacteriophage may be used for prophylaxis. . These infections are prevented by the practice of general hygienic measures in everyday life.

Genus Salmonella

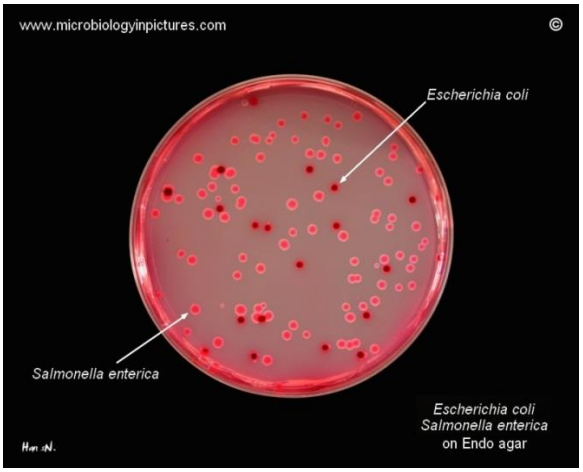
The genus Salmonella consists of bacilli that parasitise the intestines of a large number of vertebrate species and infect human beings, leading totyphoid and paratyphoid fevers, gastroenteritis, septicemia with or without focal suppuration, and the carrier state. The most important member of the genus is Salmonella typhi, the causative agent of typhoid fever.

Morphology. Salmonellae are gram-negative rods, predominantly motile with peritrichate flagella enterobacteria, around 0,7-1,5X2-5 μm in size. They don't form capsules and spores but may possess fimbriae.

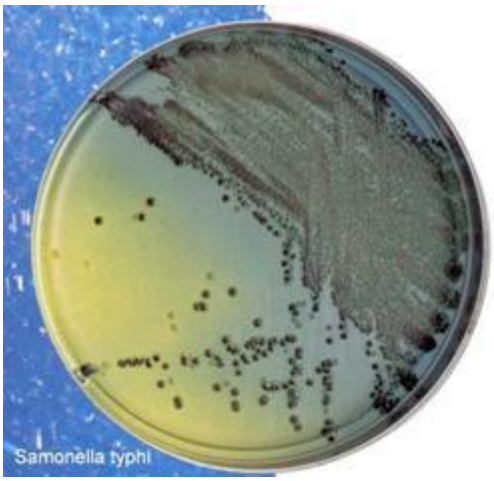


Salmonella. Gram staining

Culture properties. They are chemoorganotrophs and facultative anaerobes growing readily on ordinaryand bile containing media over a range of pH 6-8 and temperature 7°- 45°C (optimum is 37°C).Colonies are large, 2-3 mm in diameter, circular, convex and smooth. On the differential-diagnostic media (Mac-Conkey agar, Endo, Ploskirev, Levin'), colonies are colourless due to the absence of lactose fermentation. On Wilson and Blair bismuth sulphite medium, jet black colonies with a metallic sheen are formed due to the production of H_2S. *S. paratyphi* A and other species that do not form H_2S produce green colonies. Selenite broth and bile broth are commonly employed as enrichment media.



Salmonella on Endo agar



Salmonella on bismuth sulphite agar

Fermentative properties. Salmonellae ferment glucose, mannitol and maltose, forming acid and gas. They don't ferment lactose, sucrose and salicin, don't produce indol, don't hydrolyse urea. Salmonellae produce H_2S, except *S.paratyphi A*, *S.choleraesuis*. *S. typhi* and few other salmonella don't grow in Simmons' citrate medium as they need tryptophan as the growth factor.

Table Differential characteristic of Salmonella genus

Serotype	MR	VP	Citrate	Indol	H_2S	Lactose	Glucose	Maltose
S.typhi	+	-	-	-	+	-	A	AG
S.paratyphi A	+	-	-	-	-	-	AG	AG
S.paratyphi B	+	-	+/-	-	+	-	AG	AG
S. typhimurium	+	-	-	-	+	-	AG	AG

Antigenic structure: Salmonella possess three main types of antigens on the basis of which they are serologically typed. These are: **cell wall O, flagellar H, capsular Vi (virulence**) which are important for taxonomic and epidemiologic purpose. **F. Kauffman and P. White** classified the Salmonella into a number of groups according to antigenic structure (table 1). By the somatic **O antigen**, which is the outer polysaccharide of the cell wall Salmonella are subdivided into 65 serological groups (which are designated by capital letters A, B, C, D, ….) based on the presence of distinctive O antigen, in which O antigen factors were designated by Arabic numerals (1, 2, 3, etc.).

H antigen: This antigen present on the flagella is a heat labile protein. It is composed of two phases: phase I is specific, and the phase II is non-specific. Within each group this bacteriais

identified by the specific phase of H antigen (S. paratyphi A constitutes group A, paratyphi B belongs to group B (table).

Vi antigen -surface antigen envelops the O antigen. It is heat –labile acidic polysaccharide. It is destroyed by heating the bacteria at 100ºC for one hour. When fully expressed, it renders the bacterium inagglutinable by O antiserum but agglutinable by Vi antiserum.

Resistance. Salmonellae are relatively resistant to environmental factors. Salmonella species can survive several weeks in a dry environment and several months in water. They can withstand a pH in the range 4-9. Salmonella are not destroyed by freezing. They perish after being heated to 55 °C for one hour, or to 60 °C for half an hour.

Table1 KAUFMANN - WHITE SCHEME

Serogroup	O-antigen	H-antigen Phase I	H-antigen Phase II
A S. paratyphi A	1.2.12	a	/1.5/
B S. schottmuelleri (Paratyphi B)	1.4./5/.12	b	1.2
S. abony	1.4./5/.12	b	e,n,x
S. typhimurium	1.4./5/.12	i	1.2
S. derby	1.4./5/.12	f,g	/1.2/
S. wien	1.4.12.27	b	1,w
S. haifa	1.4./5/.12	z	1.2
S. heidelberg	1.4./5/.12	r	1.2
C S. hirschfeldii	6.7./Vi/	c	1.5
S. choleraesuis	6.7	/c/	1.5
S. montevideo	6.7	m,s,/p/	-
S. leopoldville	6.7	b	z
S. bonn	6.7	l,v	e,n,x
D S. typhi	9.12	d	-
S. enteritidis	1.9.12	g,m	/1.7/
S. dublin	1.9.12	g,p	-
S. rostock	1.9.12	g,p,u	-
S. moscow	9.12	b,g	-
S. gallinarum	1.9.12	s,q	-
E. S. london	3.10	l,v	1.6
S. anatum	3.10	e,h	1.6
S. amsterdam	3.10	g,m,s	-
S. Zanzibar	3.10	k	1.5

Virulent factors. Salmonellae posses many virulent factors, which provide for them mucous membrane invasion through M-cell (invasins), resistance to phagocytosis (Vi-antigen). Type III secretion systems and effectors molecules survive salmonellae in the macrophages. Endotoxin causes symptoms of intoxication. Enterotoxin causes activation of adenylate cyclaseand guanylate cyclasethat leads to the diarrhea.

Pathogenicity. Depending on the source of infection, ways of transmission, characteristics of the pathogenesis, salmonellae cause diseases such as typhoid and paratyphoid fevers, salmonellosis and nosocomial (hospital) salmonellosis.

SALMONELLA TYPHI
S. PARATYPHI A and S. PARATYPHY B

The **morphology** of the typhoid salmonella corresponds with the general characteristic of the Enterobacteriaceae family.

Cultivation: Salmonella are facultative anaerobes. The optimum temperature for growth is 37º

C. They grow on ordinary media at pH 6.8-7.2. On meat-peptone agar S. typhi forms semitransparent, large colonies (2-4 mm in diameter). Colonies of S. schottmulleri (S. paratyphi B) have a rougher appearance and mucus swelling round the colonies. This is a characteristic differential cultural property. On Ploskirev's and Endo's media S. typhi and S. paratyphi form colourless colonies due to the absence of lactose fermentation. On Wilson and Blair's brilliant-green bismuth sulphite medium black colonies with metallic sheen are formed due to production of H_2S. Salmonella paratyphi A and other species that do not form H_2S produce green colonies. Selenite broth and bile broth are employed as enrichment medias. In Rappaport media they grow and produce turbidity.

Fermentative properties: S. typhi does not liquefy gelatine; they ferment proteins and liberate hydrogen sulphide (H_2S); does not produce indole and reduces nitrates to nitrites. They ferment glucose, mannite, and maltose with acid formation. S. paratyphi ferments carbohydrates with acid and gas formation. Lactose, saccharose are not fermented. They are MR positive, VP negative, urea is not hydrolysed. S. paratyphi A and paratyphi B differentiated by the hydrogen sulphide production. S. paratyphi B formed H_2S, paratyphi A not (table 2).

BIOCHEMICAL CHARACTERS OF TYPHOID AND PARATYPHOID BACILLI AND E. COLI

Table 2

Microorganism	Glucose	Lactose	Maltose	Mannite	Saccharose	Indol	H_2S
E. coli	+AG	+AG	+AG	+AG	–	+	–
S. typhi	+A	–	+A	+A	–	–	+
S. paratyphi A	+AG	–	+AG	+AG	–	–	–
S. paratyphi B	+AG	–	+AG	+AG	–	–	+

(AG - acid and gas)
(A - acid)

By antigenic structure S. typhi in the D group, S. paratyphi A in the A group, S. paratyphi B in the B group and S. paratyphi B can cause infection in animal organism too (zooanthroponose).

Pathogenicity: The main virulent factors are endotoxin, Vi antigen.

Epidemiology and Pathogenesis: The source of infection is a patient, or far more frequently, a carrier. Man acquires infection by ingestion of contaminated water or food. Typhoid fever occurs in two epidemiological types. The first is endemic or residual typhoid that occurs throughout the year though seasonal variations may sometimes be apparent. The second is epidemic typhoid, which may occur in endemic or non-endemic areas. Typhoid epidemics are water, milk or foodborne. On reaching the gut, the bacilli attach themselves to microvilli of the ileal mucosa and penetrate to the lamina propria and submucosa. They are phagocytosed there by polymorphs and macrophages. The ability to resist intracellular killing and to multiply in these cells is a measure of their virulence.

For laboratory diagnosis of this infection it is necessary the pathogenesis of disease, which depends on different stages:

1. **Digestive stage –**penetration of microorganism intostomach.
2. **Mesenterial lymphadenitis stage:** On reaching the gut, the bacilli attach themselves to the epithelial cells of the intestinal villi and penetrate to the lamina propria and submucosa **(invasive stage).** After it they enter into the mesenteric **lymph nodes where they multiply.** This is **incubation period** which is usually 10-14 days. After it they enter the bloodstream and the next stage is developed (bacteremeia).
3. **Bacteremia period**(prodrome period) during which the bacilli are seeded in all the organs and tissues (liver, gall bladder, spleen, bone marrow, lymph nodes, lungs, kidney where further multiplication takes place). Prodromal period is usually 2-3 days during which non-specific symptoms such as fever, malaise and loss of appetite occur (The first week of thediseases).
4. **Parenchymatouse dissemination period (specific-illness period)** As bile is a good culture medium for the Salmonella, it multiplies abundantly in the gall bladder and **specific-illness period** occurs during which the overt characteristic signs and symptoms of diseases occur: headache, malaise, anorexia, a coated tongue and abdominal discomfort with either constipation or diarrhoea. Red rash (rose spots) appears on the skin during the second and or third week. Some develop psychoses, deafness or meningitis. Cholecystitis, arthritis, abscesses, periosteitis, nephritis, haemolytic anaemia, venous thromboses and peripheral neuritis are other complicationsfound
5. **Allergic-secreted period** when the bacteria are discharged into the intestine where involves the

Peyer's patches and lymphoid follicles of the ileum which had been previously sensitized by the Salmonellae in the initial stage. These become inflamed; undergo necrosis and slough off, leaving behind the characteristic typhoid ulcers and may be followed by perforation of the intestine and peritonitis and circulatory collapse. Ulceration of the bowel leads to the two major complications of the disease-intestinal perforation and hemorrhage.

6. **Convalescence (recovery period)** is slow**.** The typhoid-paratyphoid salmonellae together with products of their metabolism induce antibody production and promote phagocytosis. These processes reach their peak on the fifth-sixth week of the disease and eventually lead to recovery from the disease. In about 5-10% cases, relapse occurs during convalescence. The relapse rate is higher in patients treated early withchloramphenicol.

Clinical recovery does not coincide with the elimination of the pathogenic bacteria from the body. The majority of convalescents become **carriers** during the first weeks following recovery. Patients who continue to shed typhoid bacilli in faeces for three weeks to three months after clinical cure are called convalescent carriers. Those who shed the bacilli for more than three months but less than a year are called "temporary carriers"(acute carriers), and 3-5 per cent of the cases continue to excrete the organisms for many months and years after the attack and, for life (chronic carriers). Inflammatory processes in the gall bladder (cholecystitis) and liver are the main causes of a carrier state since these organs serve as favourable media for the bacteria, where the latter multiply and live for long periods. Besides, this typhoid-paratyphoid salmonella

may affect the kidneys and urinary bladder, giving rise to pyelitis and cystitis. In such lesions the organisms are excreted in theurine.

Immunity: Post infections immunity is tense, stable, lifelong, which depends on humoral and cellular immune response.

Prophylaxis: General measures amount to rendering harmless the sources of infection. This is achieved by timely diagnosis, hospitalization of patients, disinfection of sources, and identification and treatment of carriers. Of great importance is prevention of typhoid fever and paratyphoids are such measures as disinfection of water, safeguarding water supplies from pollution, systematic and thorough cleaning of inhabited areas, fly control, and protection of foodstuffs and water from flies. Regular examination of personnel in food-processes factories for identification of carriers is also extremely important.

In the presence of epidemiological indications specific prophylaxis of typhoid infections is accomplished by vaccination. The **TABte** vaccine was used which contains O and Vi-antigens of typhoid, paratyphoid A; B, and a concentrated purified and sorbed tetanus anatoxin. A new areactogenic vaccine (chemical vaccine) consisting of the **Vi-antigen** of Salmonella typhi has been produced. It is marked by high efficacy and used in immunization of adults and children under seven years of age. Specific bacteriophage can be used too.

Treatment: Patients with typhoid fever and paratyphoids are prescribed chloraminphenicol and the other antibiotics which act on gram-negative bacteria.

Laboratory diagnosis:

1. **Bacteriological:** Isolation of ***haemo-culture*** (bacteremic phase)**.** For this examination Rappaport media is used (MPB, 10% bile, glucose, indicator, float for indication of gase formation), in which differentiation of Salomella typhi and paratyphi is possible. If S. typhi is present fermentation of glucose with acid formation is occurs; for Paratyphi –acid and gas formation. A pure culture is isolated from faeces and urine copro-culture or urino-culture (recovery period). The test material is inoculated into bile broth; Ploskirev's, Endo's medias or bismuth sulphite agar(pict.2).
2. **Serological method –Widal tube agglutination reaction.** This is a test for the measurement of O and H agglutinins for typhoid and paratyphoid bacilli in the patient's sera (sufficient number of agglutinins accumulate in blood on the second week of the disease -specific illnessperiod).
3. **Passive hemagglutination test.**
4. **Skin-allergic test.**
5. **Diagnosis of carriers:** The detection of carriers is important for epidemiological and public health purpose. The demonstration of **Vi** agglutinins has been claimed to indicate the carrier state.

SALMONELLA-GASTROENTERITIS

(causative agents of food toxinfection)

The genus salmonella comprises many species and types of bacteria which cause food toxinfection (S. typhimurium; S. enteritidis; S. anatum; S.heidelberg, S. derby, S. haifa, S. infants). It may be caused by any salmonella except S. typhi. Salmonella gastroenteritis or food poisoning as distinct from typhoid fever and paratyphoids A and B is generally is anthropo-zoonotic disease, the source of infection being animal products.

Morphology: Morphologically Salmonella organisms possess the general characteristics of the family Enterobacteriaceae.

Fermentative properties: They do not liquefy gelatine and do not produce indole. The majority of species produce hydrogen sulphide and ferment glucose, maltose with acid and gas formation.

Resistance: Salmonella are relatively stable to high temperatures (60-75ºC), high salt concentrations, and to acids. They withstand 8-10 per cent solution of acetic acid for 18 hours, and survive for 75-80 days at room temperature.

A characteristic feature of foodstuffs contaminated by salmonella is that they show no changes which can be detected organoleptically.

Virulence factors are:

1. adhesion andcolonization.
2. enterotoxin which acts by adenylate cyclase mechanism It is necessary to note that salmonella's protein toxins have intracellular location and pass internal medium of macroorganism in the case of microbe's structuredestruction.
3. cytotoxin, which is similar to shigella exotoxin
4. different enzymes-protease, mucinase, decarboxylase,etc.
5. endotoxin

Pathogenesis: Human infection result from the ingestion of contaminated food. The most frequent sources of salmonella food poisoning are poultry, meat, milk, and milk products. Of great concern are eggs and egg products. Meat may be infected while the animal is alive or after its death.

Depend on virulence, infectious dose of bacteria and immune state of macroorganism, there are following clinical forms of infection:

1. food toxinfection
2. salmonellas' diarrhea
3.generalized (typhoid)

Intoxication develops in a few hours following infection. Masses of microbes ingested with the food are destroyed in the gastrointestinal tract and in the blood (bacteremia is infrequent). This results in the production of large amount of endotoxin which, together with the endotoxin entering the body with the ingested food, gives rise to intoxication. Salmonella produce exotoxin-enterotoxin too, which is similar to E. coli thermolabile toxin. The mechanism of enterotoxin is disturbances of water-salt metabolism, which cause diarrhoea.

Clinically, the disease develops after short incubation period (18-48 hours or less), with diarrhoea (with or without blood), which can vary from mild to severe, vomiting, abdominal pain and fever. It is self-limited, causes non-bloody diarrhoea, and does not require medical care except in the very young and very old. By the generalization of the processes the diseases are of long duration or become chronic.

- *It may vary in severity from the passage of one or two loose stools to an acute cholera- likedisease*
- *It usually subsides in 3-5 days, but in some cases more prolonged enteritis develops, with passage of mucus and pus in faeces resemblingdysentery.*
- *In a few, typhoidal or septicemic type of fever maydevelop.*

Immunity: Immunity acquired after salmonellosis is tense and type specific.

Treatment: Treatment of uncomplicated, non-invasive salmonellosis is symptomatic.

Antibiotics should not be used. But for the serious invasive cases antibiotic treatment is needed.

Prophylaxis: Veterinary-sanitary and other anti-epidemiological measures.

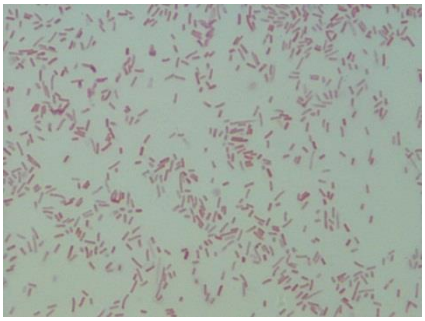
Control of salmonella food poisoning requires the prevention of food contamination. Food may become contaminated at various levels, from natural infection in the animal or bird, to contamination of the prepared food. Proper cooking of food destroyssalmonellae.

Laboratory diagnosis: Bacteriological method: Isolation of pure culture from the faeces of patients and food. In outbreaks of food poisoning, the causative article of food can often be identified by taking a proper history.

Genus Shigella

Shigella are typical members of the Enterobacteriaceae family. On the basis of biological and antigenic properties they are differentiated into four species: *S.dysenteriae*, *S.flexneri*, *S.boydii*, *S.sonnei*.

Morphology. Shigella are gram-negative, nonmotile, non-sporeforming, non-capsulated rods.



***S.sonnei* Gram staining**

Culture. Shigella are facultative anaerobes. They grow readily on ordinary media. Colonies are small, circular,shiny and smooth.On the solid media some species form S- and R-forms of colonies. In the liquid media they produce diffuse turbidity.On the differential-diagnostic media (Mac-Conkey agar, Endo, Ploskirev, Levin'), colonies are colourless due to the absence of lactose fermentation.Sometimes for shigella isolation from the feces it is necessary to use enrichment media - selenite broth.

Shigella on Endo agar

Fermentative properties. Shigella species generally don't ferment lactose, but *S. sonnei* can ferment lactose slowly after 24 hours. They typically are biochemically inert, do not produce gas from carbohydrates (with the exception of certain strains of S. flexneri). Shigella should also be urea hydrolysis negative. Indole reactions are variable, positive and negative, with the exception of S. sonnei, which is always indole negative.

Table Differential characteristic of Shigella

Serotype	MR	VP	Citrate	Indol	H_2S	Lactose	Glucose	Mannitol
S.dysenteriae	+	-	-	+/-	-	-	A	-
S.flexneri	+	-	-	+/-	-	-	A/AG	A
S.boydii	+	-	-	+/-	-	-	A	A
S.sonnei	+	-	-	-	-	slowly	A	A

Resistance. Shigella are resistant to environmental factors. Shigella are resistant to environmental factors. Shigella are well tolerated by drying, low temperature, but rapidly die by sunlight and by heating. Shigella are sensitive to disinfectants containing chlorine. They can be stored for several months on various objects. A favorable environment for the preservation of Shigella is food, especially dairy.

Antigenic structure. Shigella have O-somatic antigen. *S.sonnei* have K-antigen. On the basis of O-antigen, general characteristics and biochemical properties, the genus Shigella is divided into four species: *S.dysenteriae* (serogroup A, consisting of 12 serotypes); *S.flexneri* (serogroup B, consisting of 6 serotypes); *S.boydii* (serogroup C, consisting of 18 serotypes); and *S.sonnei* (serogroup D, consisting of a single serotype).

Virulent factors. Pili, outer membrane proteins, LPS mediate adhesion and colonization of shigella. K-antigen, group specific antigen and lipopolysaccharide protect shigella against phagocytosis. Lipid A of endotoxin inhibits lymphocyte activity. All species can cause invasion with subsequent intercellular distribution and reproduction in the epithelium of the mucous membrane of the large intestine. This ability is related to the operation of a large invasion plasmid having all species of Shigella. Pathogenic Shigella produce exotoxin which has cytotoxic, enterotoxic and neurotoxic properties.

Epidemiology. Shigellosis is endemic in developing countries were sanitation is poor.In developed countries, food or water-borne outbreaks occur sporadically.The source of infection is sick person or carrier. Diseases caused by *S.dysenteriae* are transmitted by contact-household way;infections caused by *S.flexneri* are transmitted by contaminated water; *S.sonnei* are transmitted alimentary. The healthy person is infected by ingestion or due to poor sanitation.

Pathogenesis. The portal of entry is GIT. Infection is initiated by ingestion of shigellae (usually via fecal-oral contamination). An early symptom, diarrhea (possibly elicited by enterotoxins and/or cytotoxin), may occur as the organisms pass through the small intestine. The signs of shigellosis are bacterial invasion of the intestinal epithelium and inflammatory colitis. Colitis in the rectosigmoid mucosa, with malabsorption, results in the characteristic sign of bacillary dysentery: unformed stools with blood and mucus.

Shigellosis has two basic clinical forms: 1) watery diarrhea associated with vomiting and mild to moderate dehydration; 2) dysentery characterized by a small volume of bloody, mucoid feces, and abdominal pain (cramps and tenesmus). Shigellosis is an acute infection with onset of symptoms usually occurring within 24-48 hours of ingestion of the etiologic agent. The average duration of symptoms in untreated adults is 7 days, and the organism may be cultivated from feces for 30 days or longer.

Immunity. Postinfectious immunity is type-specific, unstable and short-lasting.

Microbiological diagnosis. Although clinical signs may evoke the suspicion of shigellosis, final diagnosis is dependent upon the isolation and identification of Shigella from the feces. Blood-tinged plugs of mucus in fresh obtained feces specimens during the acute phase of disease, rectal swabs may be used for cultivation. Commonly MacConkey agar, Ploskirev agar, Endo agarare used. Following overnight incubation of primary isolation media at 37° C, colorless, non-lactose-fermenting colonies are streaked onto slants agar. Fermentative properties and slide agglutination tests with antisera for serogroup and serotype confirm the identification. Antibodies are found in patients only after the recovery.

Treatment. Oral rehydration should be given to prevent or correct dehydration.With proper hydration, shigellosis is generally a self-limiting disease, and the decision to prescribe antibiotics is predicated on the severity of the disease, the age of the patient, and the likelihood of further transmission of the infection. Effective antibiotic treatment reduces the average duration of illness from 5–7 days to approximately 3 days and also reduces the period of Shigella excretion after symptoms subside. Ampicillin, trimethoprim and sulfamethoxazole will eradicate sensitive organisms quickly from the intestine, but resistance to this agent is increasing. Ciprofloxacin is effective against multiple drug resistant strains. Bacteriophage for oral using can be recommended for the treatment. Intestinal bacteriophage liquid (Bacteriophagum intestinal fluids) includes mix of sterile filtrates of phagelysates of *S. flexneri* I, II, III, IV, VI serovariants, *S.sonnei* and other pathogens of intestinal diseases.

Antidiarrheal medications (loperamide) should not be used because of the risk of prolonging the illness.

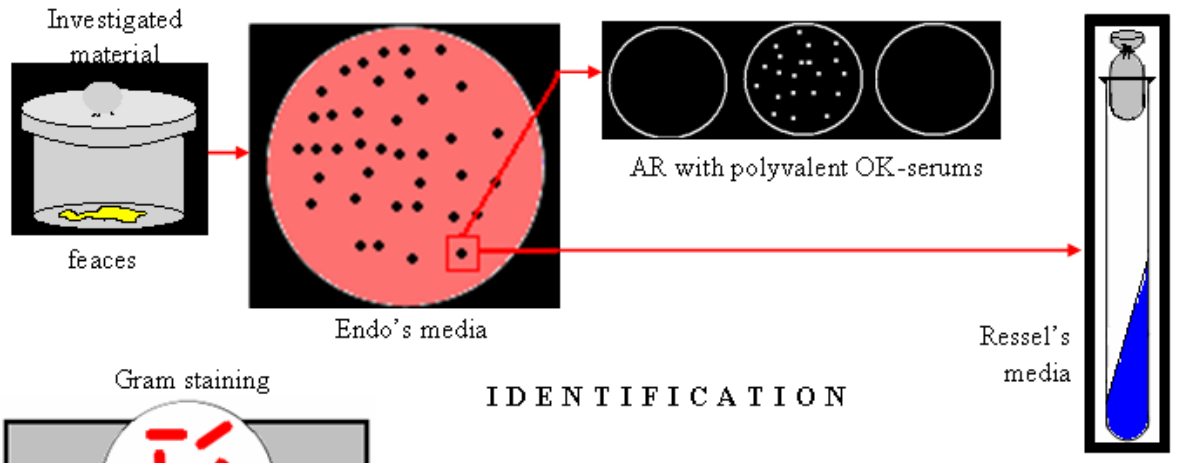
Prophylaxis. Nonspecific prevention is compliance with sanitary and hygiene rules of preparation, storage and sale of food products, water supply, personal hygiene. Specific prophylaxis is not developed.

Other coliforms of clinical importance mostly as opportunists are Klebsiella, Enterobacter, Serratia and Citrobacter. Proteus is non-coliform opportunistic bacteria.

ESCHERICHIA COLI

BACTERIOLOGICAL METHOD

BACTERIOLOGICAL METHOD



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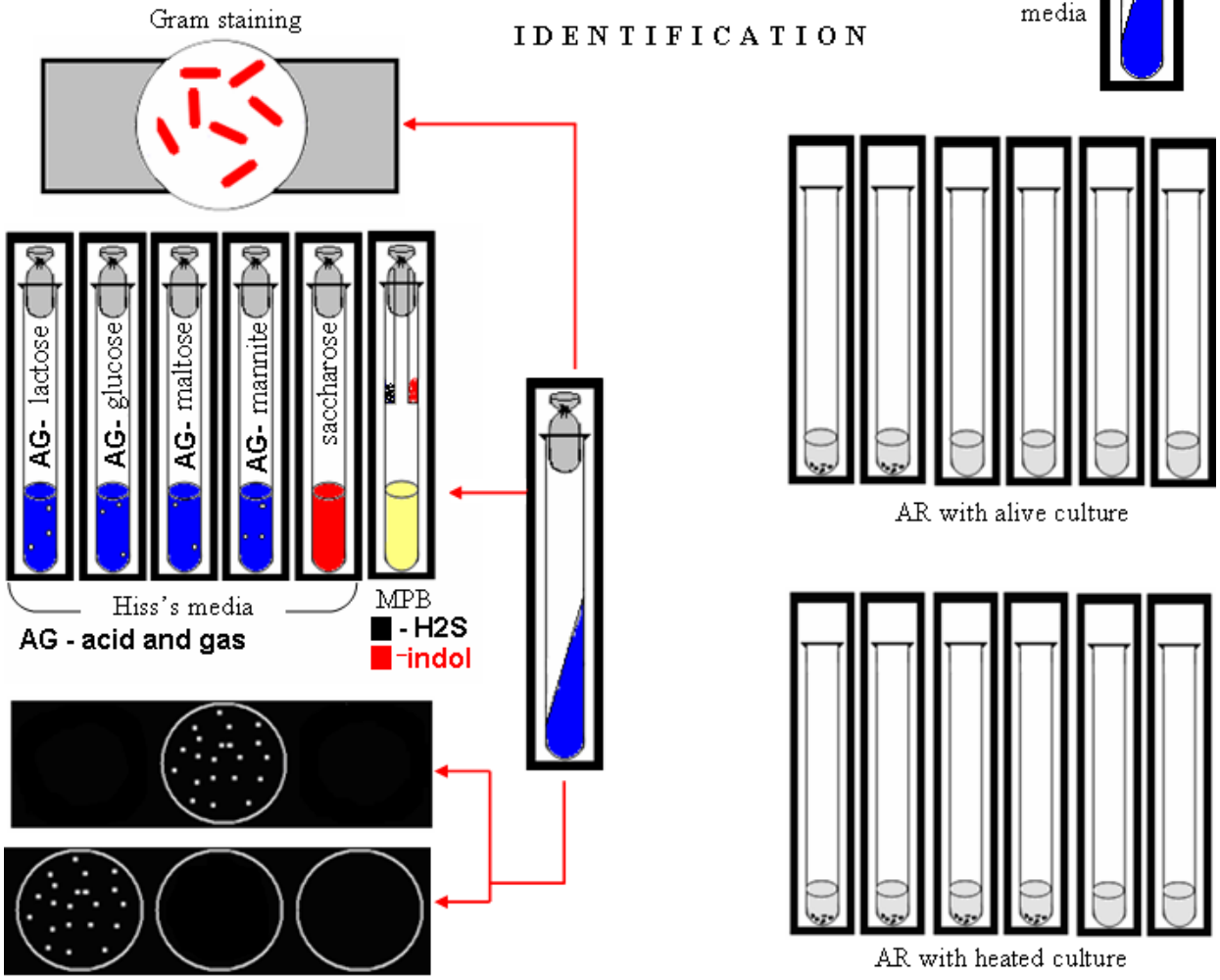
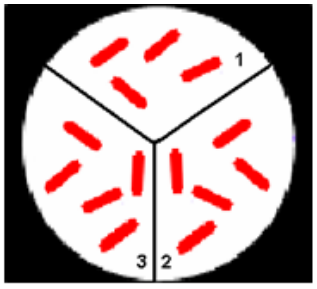


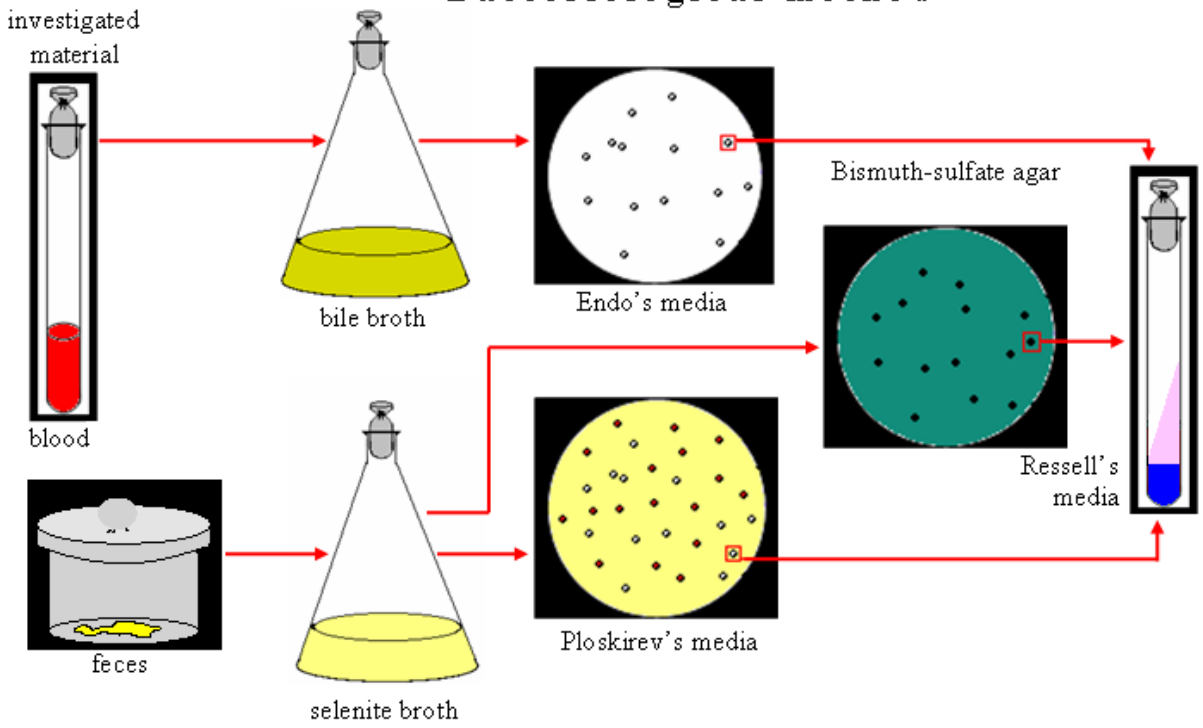


1.Salmonella typhi
2.Salmonella paratyphi
3.Salmonella schottmuelleri

Method

Bacteriological
Serological

Bacteriological method



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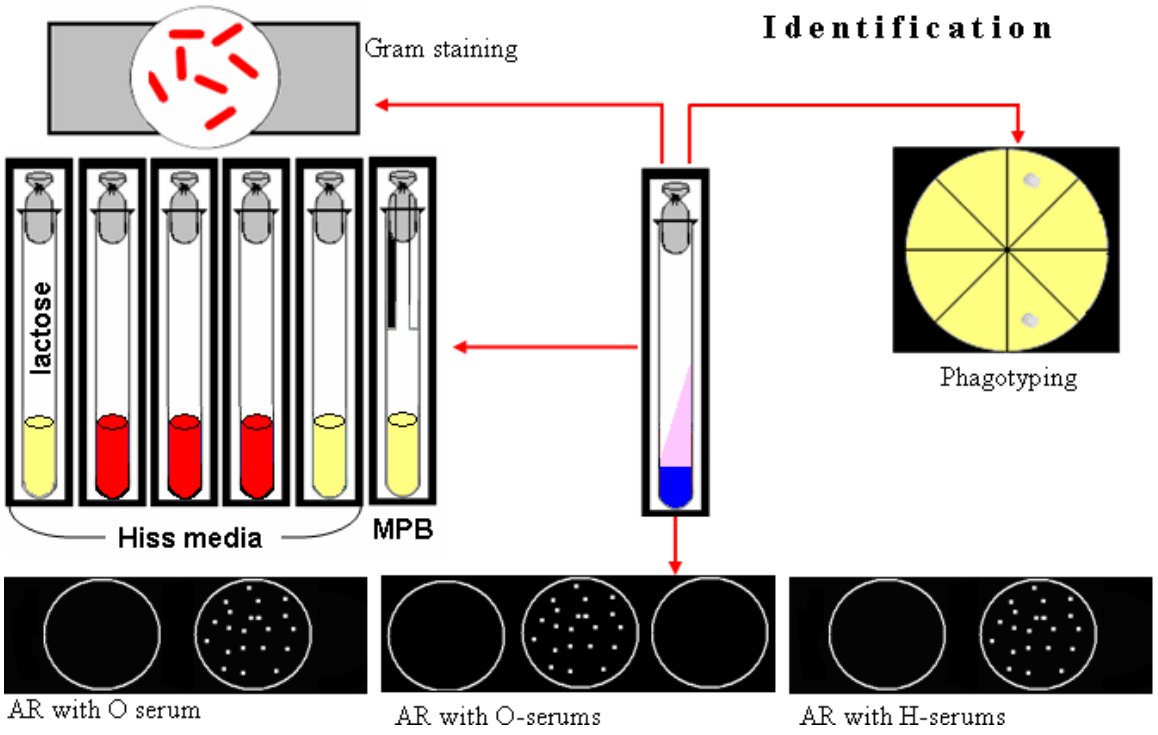
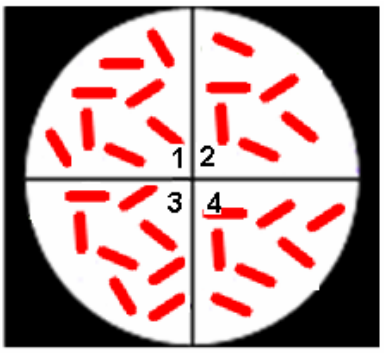


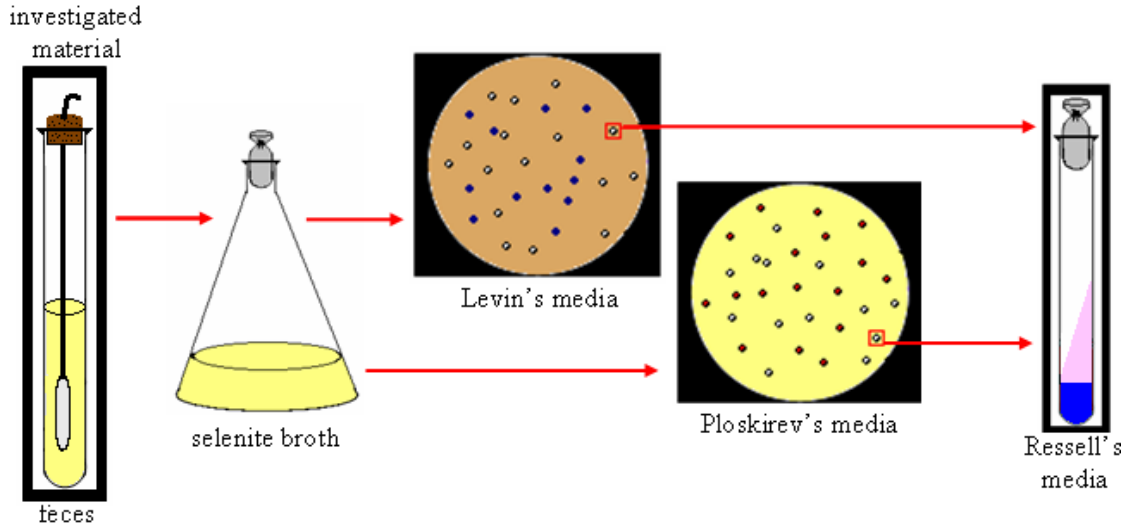


Shigella dysenteriae /1/
Shigella flexneri /2/
Shigella sonnei /3/
Shigella boydii /4/

METHODS

Bacteriological
Serological

Bacteriological method



Identification