Family Enterobacteriaceae

Classification of Enterobacteriaceae according to Bergey's manual

Tripe	Genus
A- Escherichieae	1-Escherichia
	2-Edwardsiella
	3-Citrobacter
	4-Salmonella
	5-Shigella
B-Klebsielleae	6-Klebsiella
	7-Enterobacter
	8-Hafnia
	9-Serratia
C- Proteae	10- Proteus
D- Yersineae	11- Yersinia
E- Erwinieae	12- Erwinia



Coli form bacilli

- Coli form bacillus mean Escherichia, Citrobacter, Klebsiella and Enterobacter (lactose fermenter)
- > and to include the non lactose Fermenter Edwardsiella and Serratia.
 Escherichia
- The germs is named after Escherich, how was the first to describe this bacillus under the name bacterium colicommune (1885) the germs contain only when species name.

Escherichia coli

- > The bacteria are gram negative
- > Motile or non motile, non spore former
- row easily on ordinary culture media,
- Ferment lactose with gas production at 37C and 44C.
- Indole (+) MR(+) , VP(-), citrate (-) IMViC = + + - .
- > Intestinal parasite of man and animals
- capsules and fimbriae are found in many strains .





Nutrient agar

the colonies are large opaque grayish white circular, raised low convex with an

entire edge and smooth surface

Blood agar

Some strains produce beta haemolytic colonies

Macconkey's medium

The bacteria produce large bright pink colonies due to lactose fermentation.

Nutrient broth

There is profuse growth, producing general turbidity and deposit.





- > The bacteria ferment glucose, maltose, xylose, arabinose and glycerol with gas formation .
- Most strains (80%) ferment lactose ,
- indol (+), MR(+), VP(-), Citrate (-) : IMViC (+ + -)
- > H_2S (-) . Eijkman test is(±).

Antigentic structure :

- At least 160 types of O antigen , 90 K antigen and 50 H antigens have been recognized.
- ➤ the antigenic pattern of a strain is recorded as the number of the particular antigen it carries , as for example O 111 ,K 56 , H 4 .

The K antigen : is the envelope antigen located in the capsule or microcapsule covering the O antigen and renders the strain in agglutinable by the O antiserum Three kinds of K antigens have been

described as L , A and B .

H- antigen: the flagellar antigens are some times poorly developed, at least on primary isolation.

O- antigen: agglutination tests must be curried out on boiled or autoclaved cultures to overcome in agglutinability caused by K-antigens .numerous cross reaction occur between individual E. coli antigens and between O-antigens of Salmonella , Shigella and Citrobacter , organisms



In addition to the classical endotoxic activity associated with O-antigen , *E. coli* produce several soluble toxins . the important being two types of exotoxins , which are :

- **1-Enterotoxin**
- 2-Haemolysin
- **Enterotoxins**
- This toxins is produced by few strains of *E. coil*. the great majority of enteropathogenic strains of *E. coli* produce an enterotoxin.
- which mediates the movement of water and lons from the tissues in to the bowel lumen to give rise to diarrhea.
- > The process is similar to that occurring with cholera enterotoxin.



- Enteropathic strains produce two such toxins thermostable (ST) and thermolabile (LT).
 - There is a widely held view that the toxin having bound to sensitive cells acts in a manner analogous to the cholera toxin by activating cellular adenyl cyclase in epithelial cell of the small intestine lining to increase the level of cyclic adenosine-5-monophosphate (cAMP), which in turn brings about water and ion loss by increasing the permeability of the intestinal lining and thus produce diarrhea.
 - The ST does not stimulate adenyl cyclase activity or antibody production , it stimulates fluid secretion in the gut through the medication of cyclic-guanosinemonophosphate (cGMP)
 - > the test is usually performed in the ligated ileal loop.



Hemolysin:

Two kinds of haemolysins are produced a haemolysin, which is a solublehaemolysin found in cell free culture supernatants . the second , Beta haemolysin is a cell- bound haemolysin . these haemolysin do not appear to be associated with pathogens of infection with haemolytic strains .

Colicins :

The colicins are antibiotic like principles, broadly known as bacteriocins but they are often given more specific names to indicate the nature of the organism which produces them and those produced by *E. coli* are known as colicins .



- Colicins are proteins , but are some times associated with lipopolysaccharide of the
 O antigen complex .
- they act against other sensitive strains of E. coil as well as some closely related enterobacteria .
- Colicins bind to receptors on the outer membrane of sensitive cells, following this they contact the cytoplasmic membrane and disturb its function is some way.
- there appears to be no signification relationship between colicins and pathogenicity in E. coli .



Human pathogencity

- This bacteria is part of the normal intestinal flora (NIF) of man , and except for relatively few enteropathic strains are considered to be commensals at this site .
 It is potentially pathogenic at other sites in the body , where they may produce pyogenic infection . the following type of infections by E. coli may be produced :
 I- Urinary tract infection (UTI)
- 2-Diarrhea
- **3-Pyogenic infections**
- 4-Septicaemia



there are five groups of E. coli which can cause diarrhea

particularly in infants, but also in children and adults.

these groups have been labeled as:

1-Enteropathogenic E. coil (EPEC)

2-Enterotoxigenic E. coli (ETEC)

3-Enterovasive E. coil (EIEC)

4-Enteroadherant E. coil (EAEC)

5-Enterohaemorrhagic E. coil (EHEC)





The common clinical material referred to the laboratory may be :

1-Urine

- 2- stool
- 3- pyogenic material : a) pus b) swab c) C.S.F 4-septicaemia

Eijkman test

The test is based on the production of gas in Maccokey's broth at 44C and

Indole from peptone at 44C which is a characteristic of E. coli .





In 1962 Sakazaki and Murata described a group of enterobacteria that appeared to be quite distinct from all others .

The main habitat of these organisms appears to be the gastro - intestinal canal of snakes , but a number of isolation have been made from human faces , and a few cases of fabric diarrhea and systemic infection in man have also been reported . the organisms from a remarkably uniform group named Edwardsiella tarda The bacteria are motile with peritrichate flagella , non capsulated , ferment sugars and catalase (+) . ferments only glucose and maltose . H2S (+) , Indole (+) , MR(+) , VP(-) , citrate (-) : IMVIC = + + - - , gelatin liquefaction (-) .

Its pathogenic role is uncertain , but it has been isolated

from wounds , urine , blood and CSF in cases of meningitis

Genus Citrobacter

Gram negative , motile bacilli with peritrichate flagella ferment mannitol with gas production, may or may not ferment lactose Indole production variable (±) MR(+), VP(-) citrate (+) IMVIC = + + - + , H2S (+) found in soil and water and in intestinal canal of animals . some strains not only share somatic antigenic fractions with salmonellae, but must of them contained Vi antigen identical with or very closely related to that of salmonella typhi and salmonella paratyphi C. Some of the earlier strains to be investigated were classified as Salmonella (Salm. ballerup)



but they are now considered to be members of the Citrobacter group Citrobacter is a normal intestinal inhabitant . many strains formerly labeled paracolons belong to this group.

It may produce urinary tract infection , gall bladder , middle ear and rarely meningitis .

Genus klebsiella

- Friedlander, 1883 first described this bacillus as an aetiologic agent of pneumonia and so the formal name of Klebsiella pneumoniae was given to this organisms.
- > These organisms have also been called Aerobacter aerogenes, but it is generally accepted that the name Klebsiella aerogenes is preferable .
- The most commonly isolated members of the tribe klebsielleae is Klebsiella aerogenes (K. pneumoniae).originally it was thought to be the cause of the classic lobar pneumonia , the true agent of which is pneumococcus. The remaining two species of Klebsiella K. ozaenae and K. rhinoscleromatic are causative agent of chronic infection of the nasal mucosa and pharynx and are quite rare incidence.



Klebsiella aerogenes

- (Klebsiella pneumoniae, Aerobacter aerogenes, Friedlander bacillus)
 non motile,gram negative bacilli, capsulated, ferment common sugars
 with abundant gas production. Indole (-), MR(-) VP(+), citrate
 (+): IMVIC: - ++, do not liquefy gelatin
 - Found in the bowel and respiratory tract of man and animals and in soil and in water .
 - Mucoid strains produced the largest amount of polysaccharide and most of it was present as capsule or slime .



Cultural characters

Klebsiella grow well on ordinary culture media, on nutrient agar the colonies are grayish-white, mucoid. the bacteria are aerobic, optimum growth temperature is 37C.

- On Macconkey's medium the colonies are large, mucoid and pink.
- There is no haemolysis on blood agar, and it is urease positive, H2S negative .



Genus Enterobacter

Gram negative, motile bacilli, capsulated, ferment mannitol and gas from some sugars, but not starch. Indole(-), MR (-) VP(+) citrate (+) : IMVIC = - - + + . H2S (-), liquefy gelatin . Found in soil and water and in human intestinal tract. Resent studies indicate that Enterobacter infection occur less frequently than those caused by Klebsiella. The genus can be divided in to three species on the bases of the biochemical tests (E. cloacae, E. aerogenes and E. agglomerans)



These organisms can be differentiated from Klebsiella by the fact that all are motile and with the exception of E.agglomerans . most isolated of Enterobacter ferment lactose rapidly. The colonies Enterobacter strains may be some what mucoid, but the amount extracellular material formed is usually not great . the capsule of Enterobacter strains contain more slime antigen . over 90% of the strains are resistant to 100-1000 ug/ml cephalothin , and this have been proposed as a means of distinguishing Enterobacter from Klebsiella many strains possess B- lactamase activity.

Genus Hafnia

Gran; negative motile bacillus having peritrichals flag lla does not ferment lactose ,Indole(-),MR(-),VP(+),citrate(+) IMVIC= - -+ + . non capsulated and does not liquefy gelatin . Biochemical reactions are best demonstrated at 25C as some of them may be negative or irregular at 37C. Only one species is recognized Hafnia alvei. it is found in human and animals feces, sewage, soil and water.



Genus Serratia

- > Motile, small gram negative bacilli.
- Ferment mannital, sucrose, with the production of acid and some times a small bubble of gas. Indole (-), MR(-), VP(+) citrate(+): IMVIC= - - + + . liquefy gelatin rapidly.
- produce lecithinase , lipase and DNAase .
- some strains produce red and non diffusible pigment .
- Normally found in soil and water and some strains occur in animal body. The most familiar of the <u>Serratia</u> are red-pigmented variety of <u>Serratia</u> <u>marcescens</u> and Serratia <u>rubidaea</u>.

- > On agar after one or two days , the colonies are convex , pigmented , opaque center and transparent periphery with an irregular crenate edge
- The pigment prodigiosin is soluble in alcohol, ether, chloroform, acetone but is insoluble in water.
- All <u>Serratia</u> strains are actively proteolytic pigmented strains may simulate the appearance of blood in the sputum.
- The common infections include urinary tract and respiratory tract infections meningitis and wound infection.

Genus Proteus

- They were named proteus because of there pleomorphism, after the Greek God (proteus) who could assume an shape The bacteria are gram negative bacilli, usually motile by peritrichate flagella, many strains produce swarming growth on agar media, non lactose ferment, decompose urea, convert phenyalanin to phenylpyruvic acid.
- it is one of the most common bacteria in the soil and water containing decaying organic matter of the animal origin and usually occurs in large numbers in sewage.



Cultural characters:

- Growth occurs freely on ordinary nutrient media. One of the most characteristic properties of Pr. vulgaris and Pro. mirabilis is their ability to swarm on solid media like simple agar or blood agar.
- > The growth has fishy or seminal odor. On Macconkey's agar, the colonies are pale, non lactose fermenter, large, entire or finely radially striated edge.
- Biochemical reaction:

The genus Proteus can be divided in to species on the basis of biochemical reaction. All the strains ferment glucose and do not ferment lactose . MR(±), VP(-), Citrate(+), Indole(+) Catalase (+).



Phenyl pyruvic acid reaction (PPR)

- This test is specific test for the identification and differentiation of Proteus.
- The test is based on the ability of Proteus to transformed phenylalanine to phenylpyruvic acid.
- No other members of the family enterobacteriaceae possess such activity.

Antigenic structure :

- The presence of the flagellar and somatic antigens was demonstrated by Weil and Felix (1918).
- > non motile strains contain O-antigen.
- Certain Proteus strains are agglutinated by the serum of patients having typhus fever. These so called X strains contain an antigen common to the typhus rickettsiae and cause agglutinated of these bacilli.
- Weil and Felix reaction is of diagnostic value in typhus fever . in the Proteus OX-19, OXK, OX2 strains, the antigen is a part of the O-antigen and its specificity is determined by an alkali-stable carbohydrate hapten, which is also found in Rickettsia.



Pathogenicity :

- Proteus mirabilis accounts for the majority of Proteus infections in man, causing community as well as hospital-acquired urinary tract infections. Wound and burn infections are common occurrence.
- > Pneumonia and septicemia can also occur.
- ➢ There is evidence that Proteus urease is nephrotoxic favoring the intracellular infection of tubular epithelium and creating alkalinity in the kidney which leads to necrosis of renal tubular epithelium with the precipitation of magnesium and calcium salts and the formation of calculi.



Previously genus Proteus were divided in to :

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Pr. mirabilis H_2S(+) \longrightarrow Indole (-), maltose (-)
Pr. vulgaris swarming Indole(+), maltose (+)
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Pr. morganii H2S (-) → Citrate (-) , nicotinic acid requirement (+) *Pr. rettgeri* no swarming Citrate (+) nicotinic acid (-)

Now genus Proteus are classified as:

Pr. mirabilis

Pr. vulgaris

Pr. penneri

Pr. morgani changed to (Morganella morgani)

Pr. rettgeri changed to (Providencia rettgeri)



Genus Salmonella

- Gram negative bacilli, non spore forming, non capsulated, most strains are motile,
- Grow very easily on ordinary culture media, ferment glucose and mannitol with acid or acid and gas.
- > Do riot ferment lactose and sucrose , VP(-) , Indole is (-) exception is in rare strains , H_2S (+), Urea (-) , citrate (+).
- Intestinal pathogens of man and animals. More than 1800 types are included in this genus, Human pathogens produce typhoid and paratyphoid fevers and food poisoning, with the exception of <u>Sal</u>. <u>gallinarum</u> and <u>Sal</u>. <u>pullorum</u> all are motile by peritrichate flagella.

Cultural characters

On nutrient agar

The colonies after 24h. incubation are large, grayish white, moist, convex, smooth, circular, entire edge.

On Macconkey's medium

Non lactose fermenters and thus produce pale colonies.

On Deoxycholate citrate agar medium (DCA)

Colonies are pale due to absence of lactose fermentation.



Cultural characters

On Wilson and Blair medium

Brilliant green in the medium inhibits the growth of coliform and dysentery bacilli, but does not affect the growth of salmonella. Salmonella typhi gives rise to black colonies with a metallic sheen. reduction of sulphite to sulphide occurs in the presence of glucose. Sal .paratyphi-A and other species that do not form H2S produce green colonies

Selenite F and Tetrathionate broth

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These media inhibit the growth of coliform and other intestinal opportunists and thus may give pure growth of Salmonella and are thus enrichment media for Salmonella .



- Maltose, glucose and mannitol are fermented with the production of acid or acid and gas.
- > Salicin, lactose and sucrose are not fermented.
- Gelatin is not liquefied.
- > Indole (-) MR(+), VP(-), Citrate (+) except Sal. typhi and Sal. paratyphi-A

(-) (-+--) -H2S (+) except Sal. paratyphi-A (-)

➢ Gas is not formed by Sal. typhi and Sal. gallinarum—pullorum – Indole (-) except Sal. enteritidis and Sal. panama (+) (++-+)



- Antigenic structure of <u>Salmonella</u> has been studied in great detail by Kauffman and White .
- At least 12 antigens have been identified in <u>Sal</u>. <u>typhi</u> by haemagglutination test .
- ➢ The <u>Salmonella</u> are divided in to groups on the bases of somatic (O) antigens and the species within the groups are often differentiated on the bases of the flagellar (H) antigens.

Antigenic structure

Antigenisome of these species notable <u>Sal</u>. <u>typhi</u> also possess ,further somatic envelope antigen original described by Felix and Pitt (1934) as Vi antigen (Vi for virulence) which inhibits agglutination by the O- antibody.

Several strains may have fimbriae. fimbrial antigens are important in identification but cause confusion due to their non specific nature and wide – spread sharing among enterobacteria. > The O-antigen is less immunogenic than H-antigen the titre of O-antibody induced often infection or immunization is generally lower than that of the H- antigen, the H- antigen is strongly antigenic and induced antibody formation rapidly and in high titre following infection or immunized. > The Vi antigen tends to be lost on serial subculture. It is poorly immunogenic and only low titres of antibody are produced following infection.

Detection of Vi antibody is not helpful for diagnosing of cases typhoid fever, and hence Vi antigen is not employed in the widal agglutination reaction

Human pathogenicity:

Salmonellae cause the following clinical syndromes in man:

- > Enteric fever
- > Septicemia
- Gastro enteritis or food poisoning .

Salmonella infection in man is almost always acquired by

ingestion of the microorganisms, usually as contamination of food

, milk or water



Enteric fever :

- The term enteric fever includes typhoid fever caused by Salamonella typhi and paratyphoid fevers caused by Salmonella paratyphi A,B,C.
- > The frequency of the paratyphoid fevers as compared with typhoid fever varies indifferent localities, but most hospital records give a ratio of less than 1:10 in some regiens the proportion of paratyphoid my be as high 1:5 even more. The infection is acquired by ingestion and is thus through faecal oral route, it is possible that the bacilli may enter the body through the lymphoid tissues of the pharynx, but it is not known how frequent is this mode of entry is on reaching the gut, the bacilli attach themselves on the epithelial cell, of the small intestinal villi

The biochemical

- mechanism of penetration is not known, but the process appears to be similar of phagocytosis, as the bacteria approach the epithelium, the brush border begins to degenerate and the bacteria enter the cell.
- > They are then surrounded by inverted cytoplasmic membranes similar to phagocytic vacuoles.
- The Salmonella pass through the epithelial cells in to the lamina propria and submucosa. They are phagocytosed there by polymorphs and macrophages. The ability to resist intracellular killing and to multiply with, in these cells is a measure of their virulence.
- The organism enter the mesenteric lymph nodes, where they multiply and enter the blood stream.

- A transient bacteraemia follows during which the bacilli are seeded in the liver, gall bladder bone marrow, spleen, lymph nodes, lungs and kidneys where further multiplication takes place.
- The incubation period is usually 15 days, but may range from 5-20 days and appears to be related to the does of infection.
- During the first week of the infection, the symptoms consisting of headache, fever, malaise and general pains can be confused with a variety of other illnesses.



- As bile is a good culture media for the bacillus, it multiplies a abundantly in the gall bladder and is discharged continuously into the intestine.
- The clinical course of the disease is variable from a mild undifferentiated pyrexia to a rapidly fatal disease.
- A soft palpable spleen is a constant finding.
- > Hepatomegaly also is a common feature.
- Rose spots that fade on pressure appearance on the skin during the second or third week.



Laboratory diagnosis

- **1- Direct evidence** of isolation of the bacilli from the patients urine or bile
- **2- Indirect evidence** by the demonstration of signification titer of antibodies in the serum (widal test).
- Isolation of organism from blood (Blood culture) of the patient constitutes a positive laboratory diagnosis.
- > Bacteraemia occurs early the disease and blood cultures are positive .
- in approximately 90% of cases in the first week of fever, 75% of cases in the second week, 60% in the third week 25% there after till the surer of pyrexia.
- > This is method of the choice in all suspected case of typhoid fever.
- > Blood cultures rapidly become negative on treatment with chloramphenicol

- Bone marrow cultures may positive for Sal. typhi after the blood becomes negative about 4-5 ml of blood is collected by vein puncture under strict precaution and inoculated into a culture bottle containing 3050m1 of 0.5% bile broth. This is mix and incubated at 37C.
- The large volume of blood is collected to trap more organisms the lame volume of the culture medium neutralized inhibitor substances in the blood.
 After 24h. incubation, the bile broth is subcultured on Macconkey's agar or Shigella
- Salmonella(SS)agar.
- On these media the non lactose fermenting Salmonella produce colorless, opaque or translucent colonies: The colonies are picked up for motility and biochemical reaction, then diagnosis confirmed by using antisera.

Widal agglutination reaction: (widal test)

- The antibodies against the causative organisms appear in the blood of a patient from 7th to 10th day after the onset of the typhoid and rarely in salmonella gastro enteritis.
- Tests should be curried out for both H and O antigen of enteric organisms likely to be prevalent and encounted in the area, In India it is common to test against <u>Sal</u>. <u>typhi</u> and Sal, <u>paratyphi-A</u> whereas great Britain <u>Sal</u>. <u>typhi</u> and <u>Sal</u>. <u>paratyphi</u> <u>-B</u> are common organisms responsible for enteric infection.



In widal test the titer of the antibody, from the first appearance in the serum gradually rises, may reach its maximum about the end of the third weeks after Which it declines gradually, A rising titer on repeat testing is therefore of high significance in the sero-diagnosis of infection.

H antibodies persist longer than O-antibodies and it is more significant and reliable a total leucocytic count may be helpful, a leucopaenia with a relatively lymphocytosis in seen. Eosinophils are markedly decreased or even absent.

Shigella dysenteriae

- Shigellosis also presents a significant risk to travelers from developed countries when visiting in endemic areas, and sporadic food or water-borne outbreaks occur in developed countries.
- The pathogenic mechanism of shigellosis is complex, involving a possible enterotoxic and/or cytotoxic diarrheal prodrome, cytokine-mediated inflammation of the colon, and necrosis of the colonic epithelium. The underlying physiological insult that initiates this inflammatory cascade is the invasion of Shigella into the colonic epithelium and the lamina propria. The resulting colitis and ulceration of the mucosa result in bloody, mucoid stools, and/or febrile diarrhea.



Shigella dysenteriae

- Gram-negative, facultative anaerobes of the genus Shigella are the principal agents of bacillary dysentery.
- > This disease differs from profuse watery diarrhea, as is commonly seen in choleraic diarrhea or in enterotoxigenic Escherichia coli diarrhea, in that the dysenteric stool is scant and contains blood, mucus, and inflammatory cells. > In some individuals suffering from shigellosis, however, moderate volume diarrhea is a prodrome or the sole manifestation of the infection. Bacillary dysentery constitutes a significant proportion of acute intestinal disease in the children of developing countries, and this infection is a major contributor to
 - stunted growth of these children.



Shigella a Highly Infectious Bacteria

- Shigella is one of the most infectious of bacteria and ingestion of as few as 100-200 organisms will cause disease.
- Most individuals are infected with shigellae when they ingest food or water contaminated with human fecal material.
- Shigella can survive up to 30 days in milk, eggs, cheese or shrimps.



Morphology & Physiology

> Small Gram-negative, facultatively anaerobic, coliform

Bacillus, non-motile (no H antigen)

Possess capsule (K antigen) and 0 antigen

K antigen not useful in serologic typing, but can interfere with 0

antigen determination, 0 antigens: A, B, C, D correspond respectively

to the four species

- > Non-lactose fermenting
- Bile salts resistant: trait useful for selective media fermen

Reduce nitrates (N03 to N02 or N2)





HABITAT AND TRANSMISSION

•Shigella species are found only in the human intestinal tract.

•Carriers of pathogenic strains can excrete the organism up to two weeks after infection and occasionally for longer periods.

• Shigella are killed by drying. Shigella are transmitted by the fecal-oral rout.

• The highest incidence of Shigellosis occur in areas of poor sanitation and where water supplies are polluted.

PATHOGENICITY

Shigella dysentery's form a powerful exotoxin, it is associated with epidemics of bacillary dysentery.

In man, shigellosis begins with symptoms of _____ gastro-enteritis which is accompanied by abdominal pain and diarrhea.

As it progresses, diarrhea becomes more frequent and is usually accompanied colicky pain.

PATHOGENICITY

- Later diarrhea losses its fecal characteristic and is followed by mucus with pus and blood.
- The disease is usually accompanied by fever and marked prostration. It is also known that children are more frequently attacked than adult persons and the symptoms are more severe.



LABORATORY DIAGNOSIS

The only satisfactory method of laboratory diagnosis is to cultivate the bacilli from the patient.

In the early stages of acute shigellosis, isolation of the causative organism from the feces is usually accomplished without difficulties by using the same special media and methods employed for salmonella