Enterobacteriaceae

Characteristics

Bacteria belonging to the family *Enterobacteriaceae* are:

- Gram-negative rods up to 3 μm in length which ferment glucose and a wide range of other sugars.
- Are oxidase-negative.
- They are catalase-positive, non-spore forming facultative anaerobes which grow well on MacConkey agar because they are not inhibited by the bile salts in the medium.
- Reduce nitrates to nitrites
- Some species, notably *Escherichia coli,* ferment lactose.
- The motile enterobacteriaceae have peritrichous flagella.

The family contains more than 28 genera and over 80 species. Less than half of the genera are of veterinary importance. The term 'coliform', formerly only used to describe enterobacteria capable of fermenting lactose, is now sometimes used to describe other members of the family.

Enterobacteria can be arbitrarily grouped in three categories:

1- Major pathogens: - The major animal pathogens *E. coli, Salmonella* species and *Yersinia* species can cause both enteric and systemic disease.

2- Opportunistic pathogens: - occasionally cause clinical disease in locations other than the alimentary tract.

3- Non-pathogens:- without pathogenic significance for animals, such as *Hafnia* and *Erwinia,* can be isolated from faeces and the environment and may contaminate clinical specimens (Fig. 1).

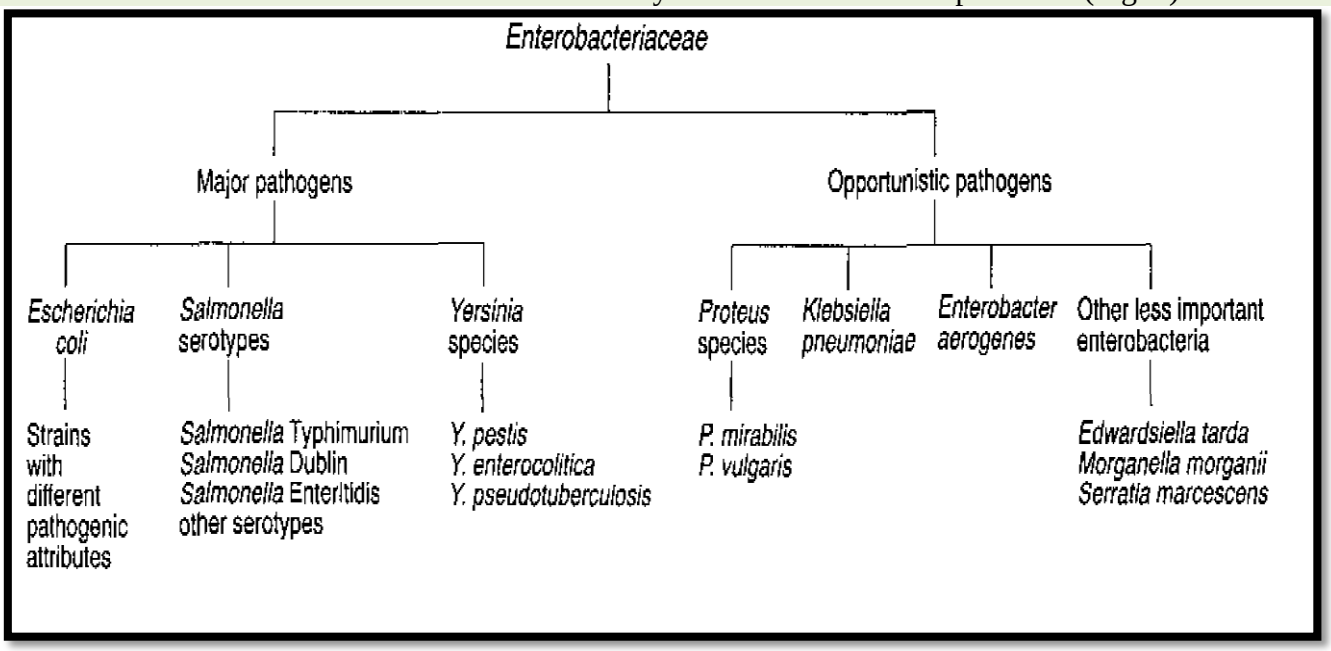

Fig. 1: Members of the *Enterobacteriaceae* of veterinary importance.

Usual habitat

Bacteria belonging to the *Enterobacteriaceae* have a worldwide distribution, inhabit the intestinal tract of animals and man and contaminate vegetation, soil and water.

Differentiation of the *Enterobacteriaceae*

Gram-negative rods which are oxidase-negative, facultative anaerobes and grow on MacConkey agar are presumed to be members of the *Enterobacteriaceae.* Few enterobacteria, apart from some strains of ***E. coli,*** produce haemolysis on blood agar.

1- Lactose fermentation in MacConkey agar.
   a- The colonies of lactose fermenters and the surrounding medium are pink due to acid production from lactose.
   b- The colonies of non-lactose fermenters and the surrounding medium have a pale appearance and are alkaline due to utilization of peptones in the medium.
2- Reactions on selective/indicator media:
   a- A number of media, commonly including brilliant green (BG) agar and xy1ose-lysine-deoxycholate (XLD) agar are used to differentiate salmonellae from other enteropathogens. On BG agar, salmonella colonies and the surrounding medium show a red alkaline reaction. On XLD medium the colonies of most salmonella serotypes are red (alkaline reaction) with black centers due to hydrogen sulfide (H2S) production.
   b- Eosin-methylene blue (EMB) agar is used for identifying *E. coli.* The colonies of some isolates have a metallic sheen, a feature unique to E. *coli.*
3- Colonial morphology:
   a- Mucoid colonies are typical of *Klebsiella* and *Enterobacter* species while rare isolates of *E. coli* are mucoid.
   b- Proteus species produce characteristic swarming on non-inhibitory media, such as blood agar.
   c- *Serratia marcescens* is unique among the opportunistic pathogens in its ability to produce red pigment.
4- Reactions in triple sugar iron **(TSI)** agar:

Members of the *Enterobacteriaceae* isolated on *BG* or XLD media can be differentiated by their reactions in TSI. Triple sugar iron agar contains 0.1% glucose, 1% lactose and 1% sucrose and chemicals to indicate H2S production. Phenol red is used as an indicator for pH change (red at pH 8.2, yellow at pH 6.4). A black precipitate of ferrous sulfide is indicative of H2S production. An inoculum from a single isolated colony of the organism under test is stab-inoculated with a straight wire into the butt of the TSI agar and the slant surface is inoculated. The loosely capped tube is incubated for 38 hours at **37°C.** The reactions in this rnedium of the more important members of the *Enterobacteriaceae* are presented in (Table 2).

5- Biochemical tests
6- Serotyping of *E. coli, Salmonella and Yersinia* species:

Slide agglutination tests with antisera are used to detect *0* (somatic) and H (flagellar) antigens in all three species and sometimes detection of K (capsular) antigens is carried out.

7- Molecular techniques, usually based on nucleic acid analyses, are used in reference laboratories for differentiating enterobacteria.

Table 1: The clinical relevance, growth characteristics and biochemical reactions of members of the *Enterobacteriaceae* which are of veterinary importance.

	Escherichia coli	***Salmonella* serotypes**	***Yersinia* species**	***Proteus* spccies**	***Enterobacter aerogenes***	***Klebsiella pneumoniae***
Clinical importance	Clinical Major	Clinical Major	Clinical Major	Opportunistic Pathogens	Opportunistic pathogens	Opportunistic pathogens
Cultural characteristics	Some strains haemolytic	-	-	**Swarming** Growth	Mucoid	Mucoid
Motility at 30°C	Motile	Motile	Motile	Motile	Motile	Non Motile
Lactose fermentation	+	-	-	-	+	+
IMViC tests						
Indole production	+	-	V	+-	-	
Methyl red test	+	+	+	+	-	-
Voges- Proskauer	-	-	-	V	+	+
Citrate utilization test	-	+	-	V	+	+
H2S production in TSI agar	-	+	-	+	-	-
Lysine decarboxylase	+	+	-	-	+	+
Urease activity	-	-	+ **except *Y. pestis***	+	-	+

Table 2: Reactions of the *Enterobacteriaceae* of veterinary importance in triple **sugar iron (TSI) agar.**

Species	pH change		*H2s* production
	Slant	Butt	
Salmonella serotypes	Red	Yellow	+
Proteus mirabilis	Red	Yellow	+
P. vulgaris	Yellow	Yellow	+
Escherichia coli	Yellow	Yellow	-
Yersinia enteroculitica	Yellow	Yellow	-
Y. pseudotuberculosis and *Y.pestis*	Red	Yellow	-
Enterobacter aerogenes	Yellow	Yellow	-
Klebsiella pneumoniae	Yellow	Yellow	-

Escherichia coli

General characteristics:-

1- *Escherichia coli* are usually motile with peritrichous flagella and often fimbriate (fig 2).
2- Lactose fermenter produces pink colonies on MacConkey agar and has characteristic biochemical reactions in IMViC and HUM tests.
3- Some strains produce colonies with a metallic sheen when grown on eosin-methylene blue agar.
4- Haemolytic activity on blood agar is a characteristic of certain strains of *E. coli.*

Fig. 2: Morphology of *E. coli*

Antigenic structure:-

1- Somatic antigens (O):- is lipopolysaccharide in nature and located at the surface of the cell wall **.**the specificity of these antigens is determined by carbohydrate side chains.
2- The flagellar (H):-antigens are protein in nature.
3- Capsular antigens (K) are composed of polysaccharides.
4- Proteinaceous fimbria (F): antigens act as adhesions facilitating attachment to mucosal surfaces.

Pathogenesis and pathogenicity:-

The virulence factors of pathogenic strains of *E. coli* include capsules, endotoxin, and structures responsible for colonization, enterotoxins and other secreted substances

1- **Capsular polysaccharides**: - which are produced by some *E. coli* strains, interfere with the phagocytic uptake of these organisms. Capsular material, which is weakly antigenic, also interferes with the antibacterial effectiveness of the complement system.

2- **Endotoxin, a lipopolysaccharide (LPS):-** component of the cell wall of Gram-negative organisms is released on death of the bacteria. It is composed of a lipid A moiety, core polysaccharide and specific side chains. The role of LPS in disease production includes pyrogenic activity, endothelial damage leading to disseminated intravascular coagulation, and endotoxic shock. These effects are of greatest significance in septicaemic disease.

3- **Fimbrial adhesions**:- which are present on many enterotoxigenic strains of *E. coli* allow attachment to mucosal surfaces in the small intestine and in the lower urinary tract. Firm attachment to the mucosa facilitates colonization by diminishing the expulsive effects of peristalsis and the flushing effect of urine.

4- The pathological effects of infection with pathogenic *E. coli,* other than those attributed to endotoxin; derive mainly from the production of enterotoxins, verotoxins or cytotoxic necrotizing factors. Enterotoxins which affect only the functional activity of enterocytes, verotoxins and cytotoxic necrotizing factors can produce demonstrable cell damage at their sites of action.

Two types of enterotoxins, heat labile (LT) and heat stable (ST) have been identified.

5- **Verotoxins (VT)**:- are similar structurally, functionally and antigenically to the Shiga toxin of *Shigella dysenteriae.* These toxins are heat-labile and lethal for cultured Vero cells. Verotoxigenic *E. coli* (VTEC) colonizing the intestines can damage enterocytes and, when verotoxin is absorbed into the blood stream, it exerts a deleterious effect on endothelial cells in relatively defined anatomical locations such as the central nervous system in pigs. Verotoxins inhibit protein synthesis in eukaryotic cells but the relatively greater degree of damage induced in certain tissues may relate to differences in receptors for these toxins. Vascular damage can lead to oedema, haemorrhage and thrombosis. The verotoxin VT2e is implicated in oedema disease of pigs.

6- **Two types of cytotoxic necrotizing factors,** CNF l and CNF 2, have been demonstrated in extracts of strains of E. ***coli*** isolated from cases of diarrhoea, septicaemia and urinary tract infections in animals and man. It is known that CNF l is encoded chromosomally whereas CNF 2 is encoded by a transmissible plasmid known as Vir.

7- Alpha-haemolysin, although often a useful marker for virulence in certain strains of *E. coli does* not appear to contribute directly to their virulence but is closely linked with the expression of other virulence factors. Haemolysin production is often a feature of strains of *E. coli* isolated from pigs with oedema disease and diarrhoea. It has been suggested that the action of a-haemolysin may increase the availability of iron for invading organisms.

8- Siderophores, iron-binding molecules such as aerobactin and enterobactin, are synthesized by certain pathogenic strains of *E. coli.* When available iron levels in the tissues are low, these iron-binding molecules may contribute to bacterial survival.

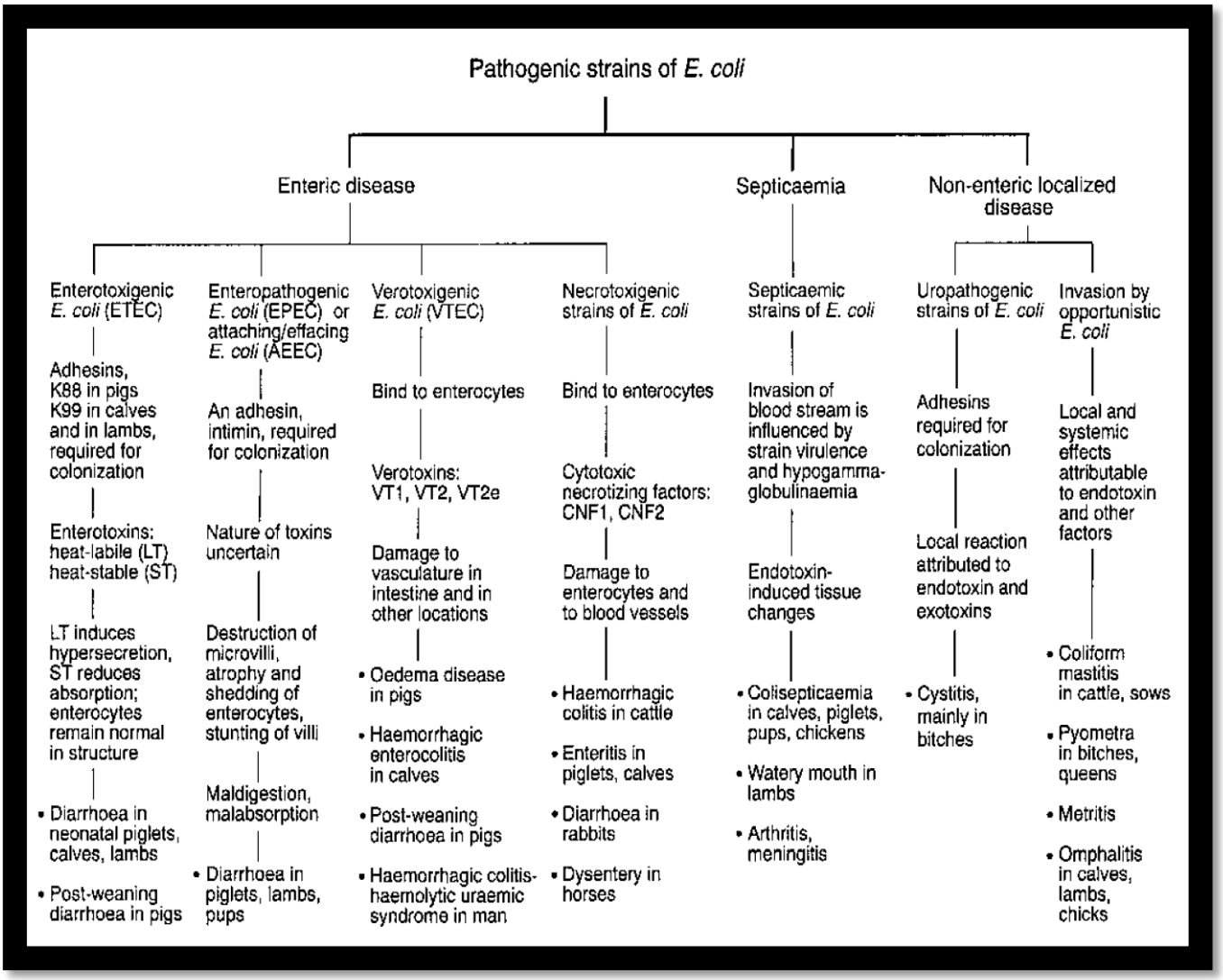

Fig. 3: An outline of the pathogenic strains of *Escherichia coli*, their virulence factors and **diseases** which they produce

Factors which may predispose young farm animals to infection with pathogenic *Escherichia coli* strains

1- Insufficient or no colostral immunity.
2- Overcrowding and poor hygiene, facilitating increased transmission of organisms.
3- Normal flora of neonates not fully established.
4- Naive immune system in neonates.
5- Receptors for ETEC adhesins are present only during first week of life in calves.
6- Pigs retain receptors for some adhesins past weaning age (post-weaning diarrhoea)
7- Digestive tract of young pigs equipped only for easily digested foods.
8- Accumulation of undigested and unabsorbed nutrients encourages replication of *E. coli*
9- Stress factors such as cold ambient temperatures and frequent mixing of animals.

Clinical infections:-

1-Enteric colibacillosis:-

Enteric colibacillosis affects primarily new-born calves, lambs and piglets.

a- Oral infection with a pathogenic strain of *E.* coli, colonization of the intestine and toxin production is prerequisites for the development of this condition.

b- Enterotoxigenic strains (ETEC), possessing fimbrial adhesins such as K88 and K99, are of particular importance in neonatal diarrhoea. These strains colonize the distal small intestine by attaching to receptors which are present on the enterocytes of neonates. They produce enterotoxins (LT and STa) which stimulate hyper secretory diarrhoea and interfere with fluid absorption without major morphologically-detectable damage to enterocytes. In contrast, necrosis of enterocytes with stunting and fusion of villi are features of enteric colibacillosis caused by strains of attaching effacing *E. coli* (AEEC), which colonize the lower small intestine and the colon. These strains induce diarrhoea through mal digestion and malabsorption of nutrients in the small intestine and by reducing the absorptive capacity of the colonic mucosa.

c- Diarrhoea develops within the first few days of birth. Faecal consistency is somewhat variable. In some cases faeces are profuse and watery; in others they are pasty, white or yellowish and rancid. This rancid faecal material may accumulate on the tail and hind limbs.

d- Depression becomes marked as dehydration and acidosis develop.

Fig. 4: Enteric colibacillosis

2- Colisepticaemia:-

Systemic infections with *E. coli* are relatively frequent in calves, lambs and poultry. Septicaemic strains of E. *coli* have special attributes for resisting host defense mechanisms. They invade the blood stream following infection of the intestines, lungs or umbilical tissues**.**

Septicaemic spread throughout the body commonly occurs in calves with low levels of maternally-derived antibodies, and the severity of the disease corresponds to the degree of hypogammaglobulinaemia. Colisepticaemia often presents as an acute fatal disease with many of the clinical signs attributable to the action of endotoxin. Pyrexia, depression, weakness and tachycardia, with or without diarrhoea, are early signs of the disease. Hypothermia and prostration precede death which may occur within 24 hours. Meningitis and pneumonia are commonly encountered in affected calves and lambs. Post-septicaemic localization in the joints of calves and lambs results in arthritis with swelling, pain, lameness and stiff gait.

Watery mouth occurs in lambs up to 3 days of age and has been associated with systemic invasion by *E. coli* .It is characterized by severe depression, loss of appetite, profuse salivation and abdominal distension. The condition is encountered in lambs born in confined lambing areas. Morbidity rates may exceed 20% and mortality in affected lambs is high, many dying within 24hours of clinical onset. Death is attributable to endotoxic shock.

In poultry, airsacculitis and pericarditis may develop following septicaernia. Coligranuloma (Hjarre's disease) is characterized by chronic inflammatory changes which are encountered at postmortem in laying hens and resemble tuberculous lesions.

3- Oedema disease of pigs.

Oedema disease is a toxemia which usually occurs 1 to 2 weeks after weaning in rapidly-growing pigs. The aetiology of the disease is complex with nutritional and environmental changes and other stress factors contributing to its development. A limited number of haemolytic *E. coli* serotypes have been isolated from the intestinal tract in cases of the disease. These non-invasive strains replicate in the tract and produce a verotoxin (VT2e) which is absorbed into the blood stream and damages endothelial cells with consequent perivascular oedema.

The onset of oedema disease is sudden with some animals found dead without showing clinical signs. Characteristic signs include posterior paresis, muscular tremors and oedema of the eyelids and the front of the face. The squeal may be hoarse due to laryngeal oedema. The faeces are usually firm. Flaccid paralysis precedes death which typically occurs within 36 hours of the onset of clinical signs. Animals which recover frequently have residual neurological dysfunction.

4-Coliform mastitis.

Infection of the mammary glands of cows and sows by members of the *Enterobacteriaceae***,** including *E. coli***,** occurs opportunistically. In dairy cows, the source of infection is faecal contamination of the skin of the mammary gland and relaxation of the teat sphincter following milking increases vulnerability to infection. Cows with low somatic cell counts are particularly susceptible to infection. No specific serotypes of *E. coli* have been linked with this form of mastitis. The acute form of the disease is characterized by endotoxaemia and can be life-threatening. Peracute disease may be fatal in 24 to 48 hours. Affected animals are severely depressed with drooping ears and sunken eyes**.** Mammary secretions are watery and contain white flecks.

Fig. 6: Coliform mastitis in domestic animals.

5-Urogenital tract infections:-

Opportunistic ascending infections of the urinary tract by certain uropathogenic strains of *E. coli* result in cystitis. These strains possess virulence factors such as fimbriae which facilitate mucosal colonization. Invasion of hyperplastic endometrium by opportunistic strains of *E. coli* is a critical factor in the pathogenesis of canine pyometra. Prostatitis in dogs is also associated with invasion of opportunistic *E. coli* strains.

Diagnostic:-

1- Clinical signs and the duration of illness may suggest the type of infection and the category of disease.

2- Specimens include faecal samples from animals with enteric disease, tissue specimens from cases of septicaemia, mastitic milk, samples of mid-stream urine and cervical swabs from suspected cases of pyometra or metritis.

a- Specimens cultured on blood and MacConkey agar are incubated aerobically at 37 hr for 24 to 48 hours (Fig. 7).

b- On blood agar the colonics are greyish, round and shiny with a characteristic smell. Colonies may be hernolytic or non-haemolytic.

c- On MacConkey agar colonies are bright pink.

d- IMViC tests can be used for confirmation

e- The colonies of some *E. coli* strains have a metallic sheen on EMB agar.

f- A full biochemical profile may be necessary to identify isolates from coliform mastitis or cystitis.

g- Some serotypes are found in association with certain disease conditions. Slide agglutination tests for O and H antigens are employed for serotype identification.

2- Enterotoxins in the small intestine can be detected, using methods employing monoclonal antibodies. Some of these reagents are available commercially.

3- Fimbrial antigens can be identified using ELISA or latex agglutination

4- DNA probes specific for genes encoding heat-labile and heat-stable enterotoxins may be used to identify enterotoxigenic strains of ***E. coli.***

8-The toxins produced by verotoxigenic and necrotoxigenic strains can be detected by Vero cell assay

9-Molecular methods based on the detection of genes encoding toxins are also used.

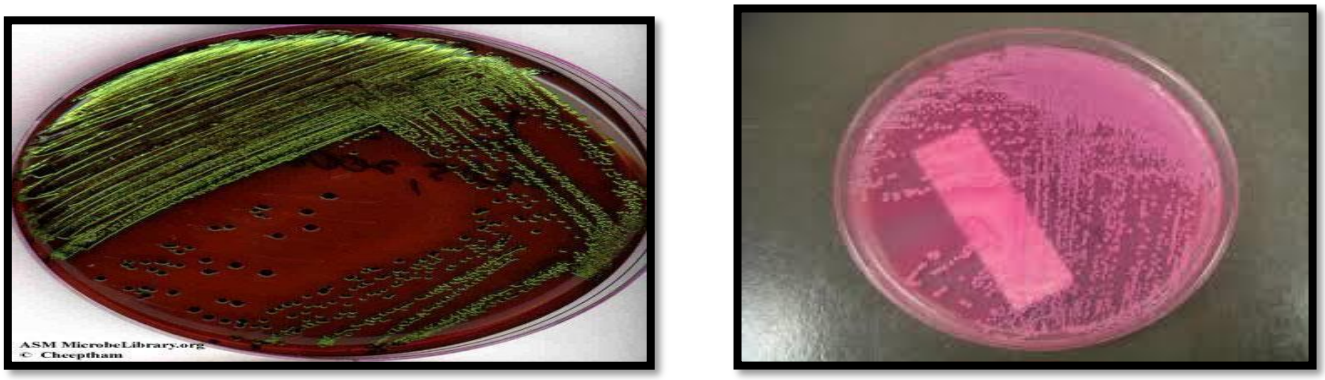

Fig. 7: Cultural characteristic *E. coli*