Enterobacteriacae

Assist. Prof.Dr.Aamer Al-chalaby

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Bacteria belonging to the family *Enterobacteriaceae*

1. Gram-negative rods up to 3 µm in length which ferment glucose and a wide range of other sugars.
2. Oxidase-negative. They are catalase positive.

3. The motile enterobacteria have peritrichous flagella.

4. The family contains more than 40 genera and over 180 species.

Enterobacteria can be arbitrarily grouped into three categories:-

Major pathogens,

Opportunistic pathogens.

Non-pathogens.

Usual habitat

- Bacteria belonging to the Enterobacteriaceae have a worldwide distribution.
- inhabit the intestinal tract of animals and humans and contaminate vegetation, soil and water.
- Some members of the family constitute part of the normal flora of the intestinal tract

Key point

- Gram-negative rods
- Growth on non-enriched media
- Oxidase-negative
- Facultative anaerobes, catalase-positive
- Most are motile by peritrichous flagella
- Ferment glucose, reduce nitrate to nitrite
- Enteric bacteria which tolerate bile salts in MacConkey agar cause a variety of clinical infections.

- **Major enteric and systemic pathogens: –**

-Escherichia coli

–Salmonella serotypes

–Yersinia species

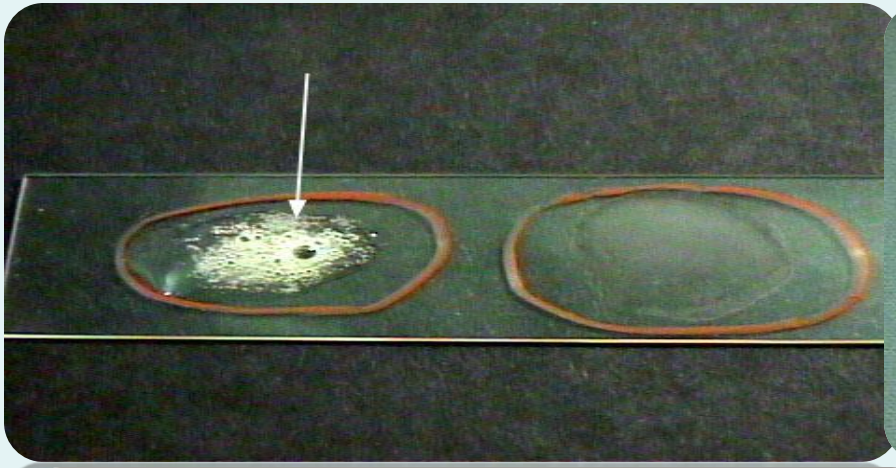
- **Opportunistic pathogens:**

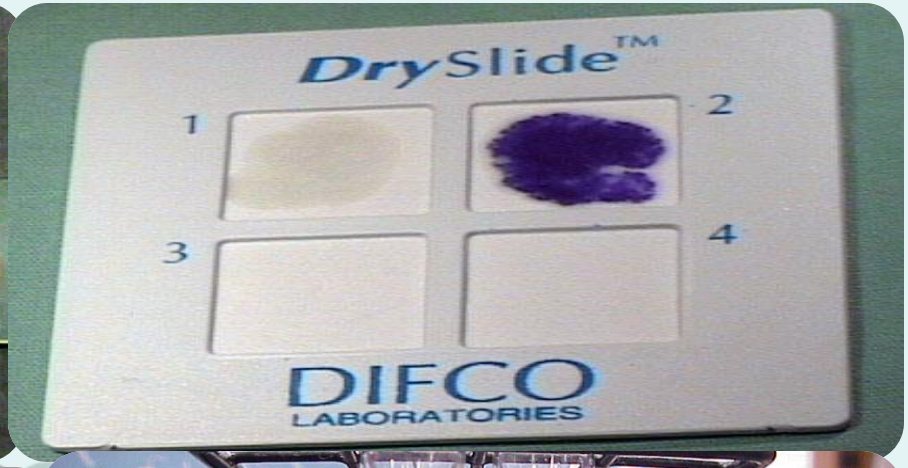
Proteus species

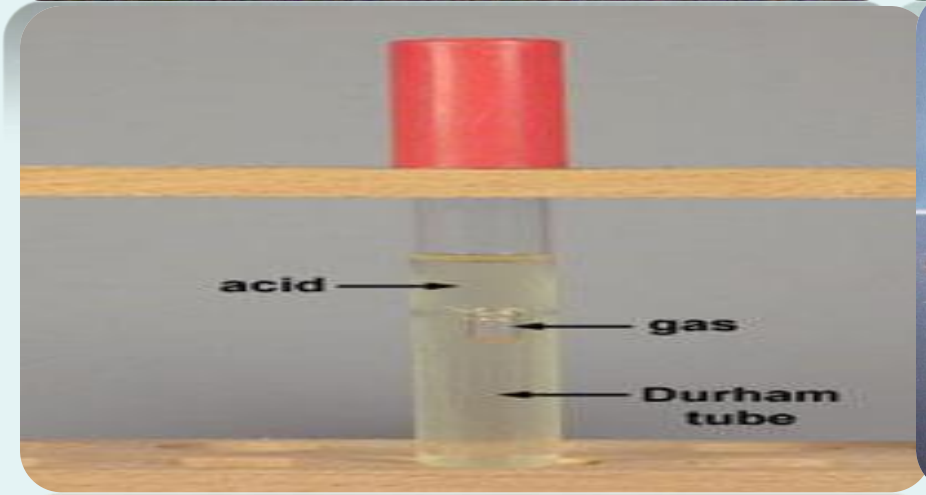
Klebsiella species

***Enterobacter species* like** *aerogenes, asburiae*

Catalase, glucose, nitrate +ve; oxidase -ve







Dfferentiation of the Enterobacteriaceae.

1. Lactose fermentation in MacConkey agar:

– The colonies of lactose fermenters and the surrounding medium are pink due to acid production from lactose.

- 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 number of commonly used media, are used to differentiate Salmonella from other entero-pathogens.

On BG agar(brilliant green), Salmonella colonies and the surrounding medium show a red alkaline reaction.

On XLD agar(xylose–lysine– deoxycholate) medium the colonies of most Salmonella serotypes are red (alkaline reaction) with black centers due to hydrogen sulphide (H2S) production.

On S.S. agar(salmonella-shegilla) the colonies of most salmonella brown with with black centers due to hydrogen sulphide (H2S) production.

3. A wide range of chromogenic agars have been developed for the easy identification and enumeration of *E. coli* in clinical samples and food specimens. (EMB) agar on which have a unique metallic sheen.

Lactose fermenter v/s non fermenter

4. Colonial morphology: –

- **Mucoid colonies are typical of Klebsiella and Enterobacter species while rare isolates of *E. coli* are mucoid.**

- **Proteus species produce characteristic swarming on non-inhibitory media such as blood agar.**

–***Serratia marcescens*** **is unique among the opportunistic pathogens in its ability to produce red pigment.**

5. Additional biochemical tests:

- Urease production distinguishes Proteus species from Salmonella species. Proteus species produce urease whereas Salmonella species do not.
- The IMVIC (indole production, methyl red test, Vogus-Proskauer test, citrate utilization) tests are a group of biochemical reactions used to differentiate *E. coli* from other lactose fermenters

Table 1 The clinical relevance, growth characteristics and biochemical reactions of members of the Enterobacteriaceae.

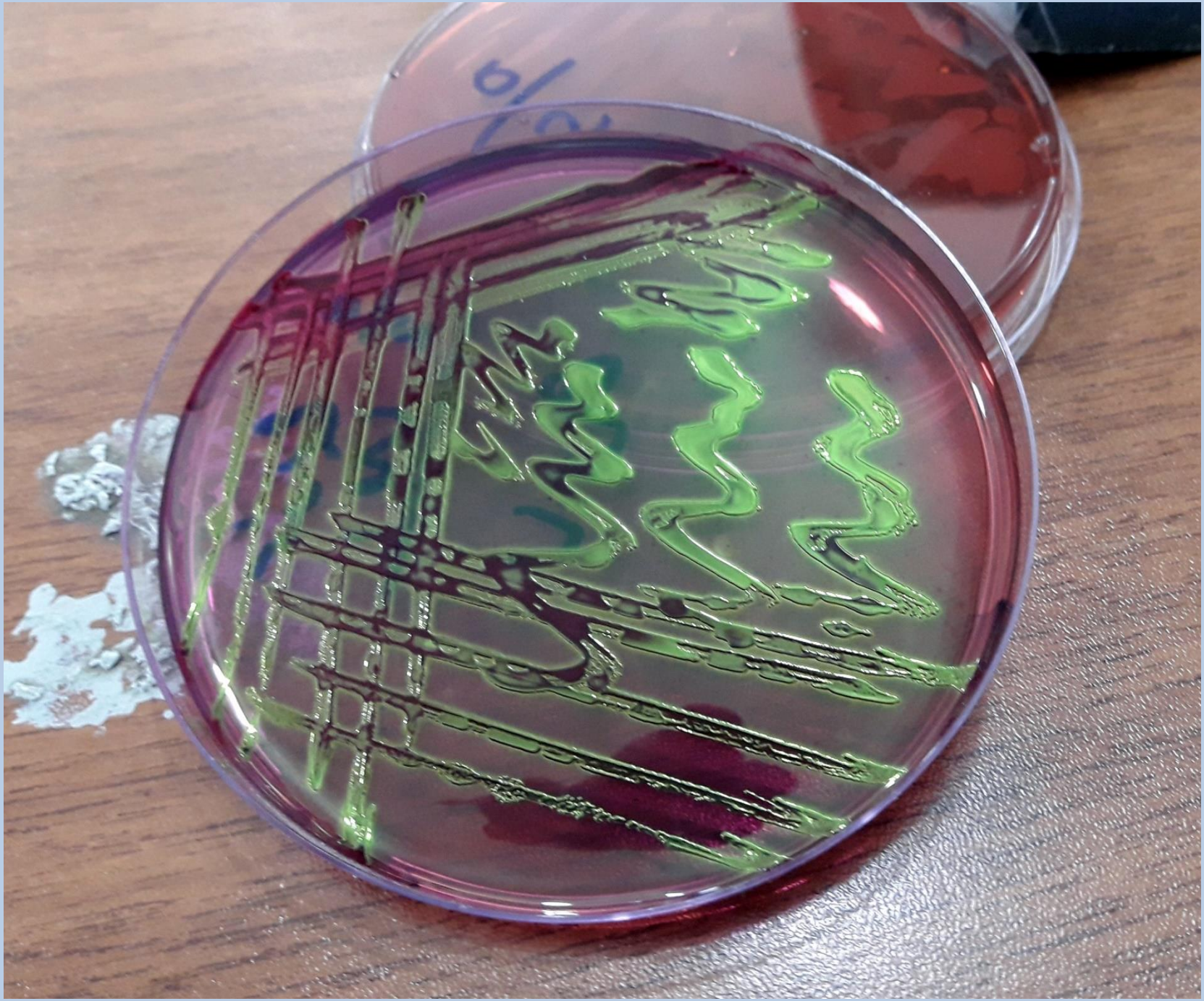
	Escherichia coli	*Salmonella* serotypes	*Yersinia* species	*Proteus* species	*Enterobacter aerogenes*	*Klebsiella pneumoniae*
Clinical importance	Major pathogen	Major pathogens	Major pathogens	Opportunistic pathogens	Opportunistic pathogen	Opportunistic pathogen
Cultural characteristics	Some strains haemolytic	–	–	Swarming growth[a]	Mucoid	Mucoid
Motility at 30°C	Motile	Motile	Motile[b]	Motile	Motile	Non-motile
Lactose fermentation	+	–	–	–	+	+
IMViC tests						
Indole production	+	–	v	±[c]	–	–
Methyl red test	+	+	+	+	–	–
Voges-Proskauer	–	–	–	v	+	+
Citrate utilization test	–	+	–	v	+	+
H_2S production in TSI agar	–	+	–	+	–	–
Lysine decarboxylase	+	+	–	–	+	+
Urease activity	–	–	+[b]	+	–	+

1.Escherichia coli

- ***Escherichia coli*** **is usually motile with peritrichous flagella and is often fimbriate.**

- **This lactose fermenter produces pink colonies on MacConkey agar and has characteristic biochemical reactions in IMVIC tests.**

- **Some strains produce colonies with a metallic sheen when grown on Eosin–methylene blue agar.**
- **Haemolytic activity on blood agar is a characteristic of certain strains of *E. coli*.**



Pathogenesis and pathogenicity

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

1. **Capsular polysaccharides**,
2. **Endotoxin, a lipopolysaccharide(LPS)**
3. **Fimbrial adhesins**
4. **The pathological effects of infection with pathogenic *E. coli,* other than those attributed to endotoxin, derive mainly from the production of enterotoxins,**
   shigatoxins or verotoxins
   cytotoxic necrotizing factors
5. **Alpha-hemolysin**
6. **Siderophores,**

TABLE 19-2 **Pathogenesis and Spectrum of Disease for Clinically Relevant *Enterobacteriaceae***

Organism	Virulence Factors	Spectrum of Disease and Infections
Escherichia coli (as a cause of extraintestinal infections)	Several, including endotoxin, capsule production, and pili that mediate attachment to host cells.	Urinary tract infections, bacteremia, neonatal meningitis, and nosocomial infections of other various body sites. Most common cause of gram-negative health care–associated infections.
Enterotoxigenic *E. coli* (ETEC)	Pili that permit gastrointestinal colonization. Heat-labile (LT) and heat-stable (ST) enterotoxins that mediate secretion of water and electrolytes into the bowel lumen.	Travelers and childhood diarrhea, characterized by profuse, watery stools. Transmitted by contaminated food and water.
Enteroinvasive *E. coli* (EIEC)	Virulence factors uncertain, but organism invades enterocytes lining the large intestine in a manner nearly identical to *Shigella* spp.	Dysentery (i.e., necrosis, ulceration, and inflammation of the large bowel); usually seen in young children living in areas of poor sanitation.
Enteropathogenic *E. coli* (EPEC)	Bundle-forming pilus, intimin, and other factors that mediate organism attachment to mucosal cells of the small bowel, resulting in changes in cell surface (i.e., loss of microvilli).	Diarrhea in infants in developing, low-income nations; can cause a chronic diarrhea.
Enterohemorrhagic *E. coli* (STEC)	Toxin similar to Shiga toxin produced by *Shigella dysenteriae*. Most frequently associated with certain serotypes, such as *E. coli* O157:H7.	Inflammation and bleeding of the mucosa of the large intestine (i.e., hemorrhagic colitis); can also lead to hemolytic uremic syndrome, resulting from toxin-mediated damage to kidneys. Transmitted by ingestion of undercooked ground beef or raw milk.
Enteroaggregative *E. coli* (EAEC)	Probably involves binding by pili, ST-like, and hemolysin-like toxins; actual pathogenic mechanism is unknown.	Watery diarrhea that in some cases can be prolonged. Mode of transmission is not well understood.
Shigella spp.	Several factors involved to mediate adherence and invasion of mucosal cells, escape from phagocytic vesicles, intercellular spread, and inflammation. Shiga toxin role in disease is uncertain, but it does have various effects on host cells.	Dysentery defined as acute inflammatory colitis and bloody diarrhea characterized by cramps; tenesmus, and bloody; mucoid stools. Infections with *Shigella sonnei* may produce watery diarrhea.
Salmonella serotypes	Several factors help protect organisms from stomach acids, promote attachment and phagocytosis by intestinal mucosal cells, allow survival in and destruction of phagocytes, and facilitate dissemination to other tissues.	Three general categories of infection are seen: • Gastroenteritis and diarrhea caused by a wide variety of serotypes that produce infections limited to the mucosa and submucosa of the gastrointestinal tract. *Salmonella* serotype Typhi and *Salmonella* serotype Enteritidis are the serotypes most commonly associated with *Salmonella* gastroenteritis in the United States. • Bacteremia and extraintestinal infections occur by spread from the gastrointestinal tract. These infections usually involve *Salmonella* serotype Choleraesuis or *Salmonella* serotype Dublin, although any serotype may cause these infections. • **Enteric fever** (typhoid fever or typhoid) is characterized by prolonged fever and multisystem involvement, including blood, lymph nodes, liver, and spleen. This life-threatening infection is most commonly caused by *Salmonella* serotype Typhi; *Salmonella* serotypes Paratyphi A, B, or C are rarely isolated in the United States.

Continued

• Identification criteria for isolates:

- On blood agar, colonies are greyish, round and shiny with a characteristic smell. Colonies may be haemolytic or non-haemolytic.
- On MacConkey agar, colonies are bright pink.
- IMVIC tests can be used for confirmation (Table.1)
- The colonies of some E. coli strains have a metallic sheen on EMB agar.
- A full biochemical profile may be necessary to identify isolates from coliform mastitis or cystitis.
- Some serotypes are found in association with certain disease conditions. Slide agglutination tests for O and H antigens are employed for serotype identification.

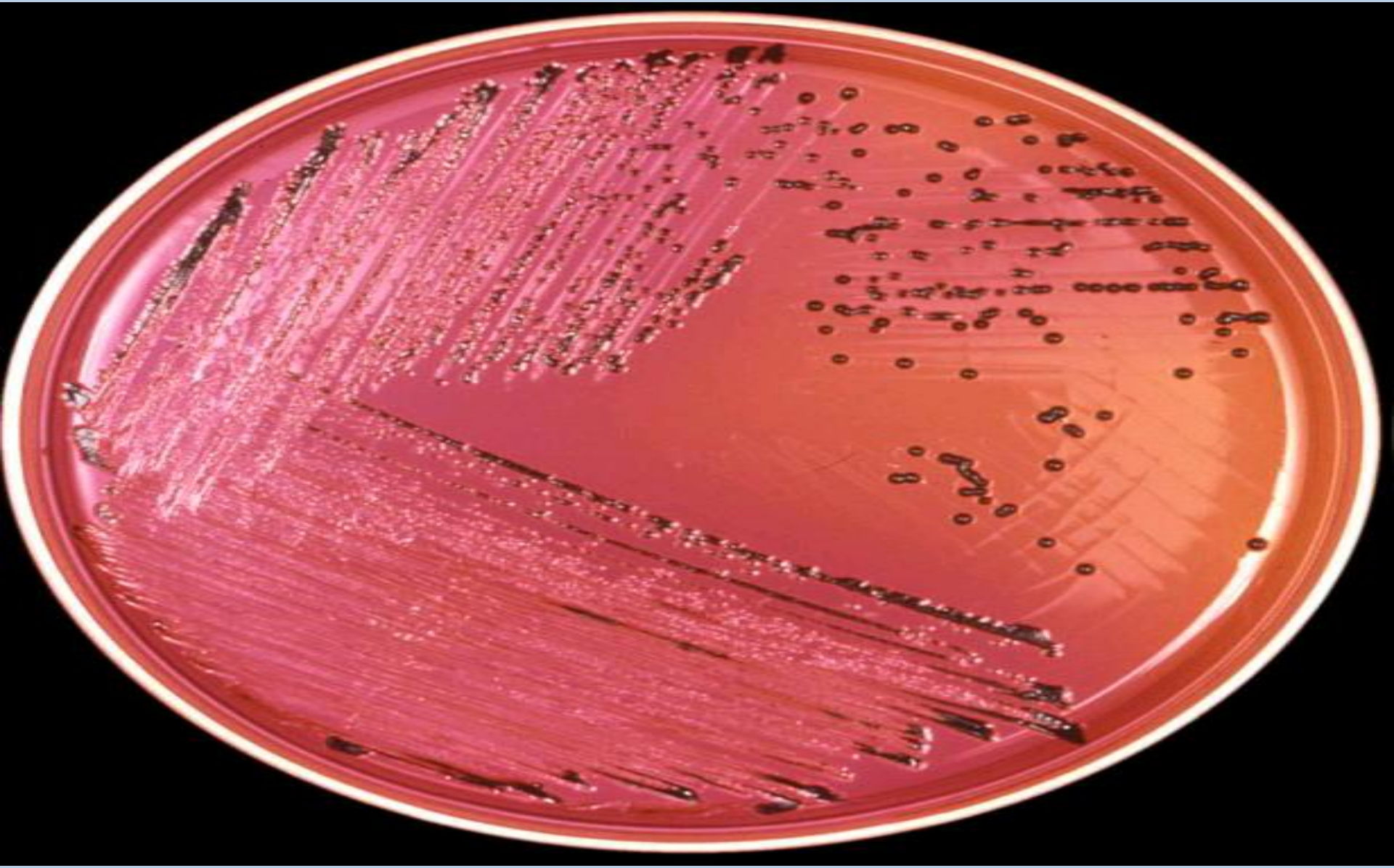
–For expression of fimbrial antigens, isolates should be sub cultured on Minca medium. Fimbrial antigens can be identified using ELISA or latex agglutination.

–PCR techniques using primers specific for genes encoding heat-labile and heat-stable enterotoxins may be used to identify enterotoxigenic strains of *E. coli*.

Salmonella spp.

- **Family: Enterobacteriaceae**
- **Gram-negative rods**
- **Motile except *Salmonella gallinarum* and *S. pullorum***
- **Aerobic and facultative anaerobic**
- **Catalase positive, Oxidase negative**
- **Fermentation glucose, manitol, maltose with acid and gas except *S. typhi* produce acid only, also non fermented lctose and sucrose.**
- **Non sporing, non capsulated**
- **Indol and proteus negative, methyl red positive, citrate positive except *S.typhi and S.paratyphi***

Salmonella grow on XLD agar



Salmonella (all serotypes) are facultative anaerobic, motile, gram-negative rods commonly isolated from the intestines of humans and animals.

Identification is primarily based on the ability of the organism to use citrate as the sole carbon source and lysine as a nitrogen source in combination with hydrogen sulfide (H_2S) production.

The genus comprises two primary species, *Salmonella enterica* (human pathogen) and *Salmonella bongori* (animal pathogen).

S. enterica is subdivided into six subspecies: subsp. *enterica,* subsp. *salamae,* subsp. *arizonae,* subsp. *diarizonae,* subsp. *houtenae,* and subsp. *indica.*

S. enterica subsp. *enterica* can be further divided into serotypes with unique virulence properties. Serotypes are differentiated based on the characterization of the heat-stable O antigen, included in the LPS; the heat-labile H antigen flagellar protein; and the heat-labile **Vi antigen,** capsular polysaccharide.

Salmonella serotypes	Several factors help protect organisms from stomach acids, promote attachment and phagocytosis by intestinal mucosal cells, allow survival in and destruction of phagocytes, and facilitate dissemination to other tissues.	Three general categories of infection are seen: • Gastroenteritis and diarrhea caused by a wide variety of serotypes that produce infections limited to the mucosa and submucosa of the gastrointestinal tract. *Salmonella* serotype Typhi and *Salmonella* serotype Enteritidis are the serotypes most commonly associated with *Salmonella* gastroenteritis in the United States. • Bacteremia and extraintestinal infections occur by spread from the gastrointestinal tract. These infections usually involve *Salmonella* serotype Choleraesuis or *Salmonella* serotype Dublin, although any serotype may cause these infections. • **Enteric fever** (typhoid fever or typhoid) is characterized by prolonged fever and multisystem involvement, including blood, lymph nodes, liver, and spleen. This life-threatening infection is most commonly caused by *Salmonella* serotype Typhi; *Salmonella* serotypes Paratyphi A, B, or C are rarely isolated in the United States.

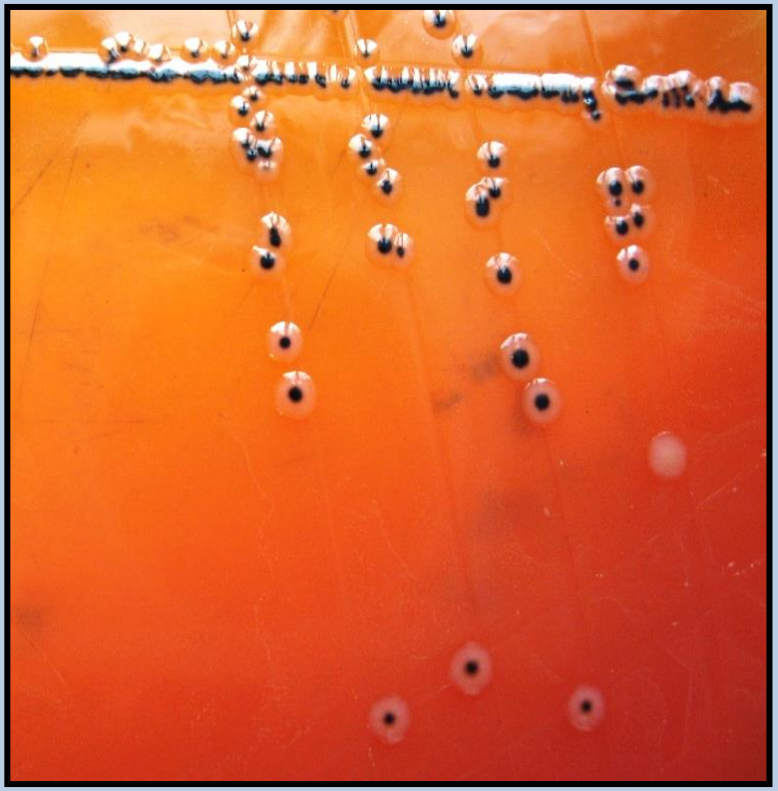
A DNA sequence–based method has been developed for molecular identification of DNA motifs in the flagella and O antigens.

The primary identifiable *Salmonella* serotypes are *Salmonella* serotype Typhi associated with a severe disease called **typhoid fever.** Diarrhea and vomiting are not associated with typhoid fever. The symptoms are often headache, abdominal cramping, constipation, and high fever. The patient may present with a rash and appear confused. Human carriers have been identified. The disease is transmitted person-to-person or through contaminated food and water.

A second serotype, *Salmonella* serotype Enteritidis, is associated with infections acquired from the ingestion of eggs or chicken.

Salmonella-associated gastroenteritis is typically accompanied by diarrhea, fever, and abdominal cramps.

Cases of gastroenteritis may cause extra intestinal infections such as bacteremia, urinary tract infection, or osteomyelitis. Transmission may be fecal-oral, person-to person, or contact with infected animals.



• Identification criteria for isolates:

1. **On brilliant green agar, colonies and medium are red indicating alkalinity. On XLD agar, colonies are red (alkaline) with a black centre, indicating H2S production.**

2. **Suspicious colonies, subcultured from the selective media into TSI agar, should be examined after incubation for 18 hours at 37°C to establish their biochemical identity as Salmonella , a biochemical profile using a battery of biochemical tests may allow definitive identification.**

3. **The isolates from the TSI agar slant are confirmed as Salmonella using commercially available antisera for O and H antigens in a slide agglutination test.**

4. • Serological tests such as ELISA and agglutination techniques are of greatest value. A rising antibody titer using paired serum samples is indicative of active infection.
5. • Molecular techniques have been developed for the identification of some serovars.

Opportunistic pathogens

This group of enterobacteria, which rarely cause enteric disease, are sometimes involved in localized opportunistic infections in diverse anatomical locations. Faecal contamination of the environment accounts for wide spread distribution of the organisms and contributes to the occurrence of opportunistic infection.

Predisposing factors include intercurrent infection, tissue devitalization and the inherent vulnerability of certain organs.

These opportunistic invaders have characteristics which may allow them to circumvent host defence mechanisms and colonize and survive in affected organs.

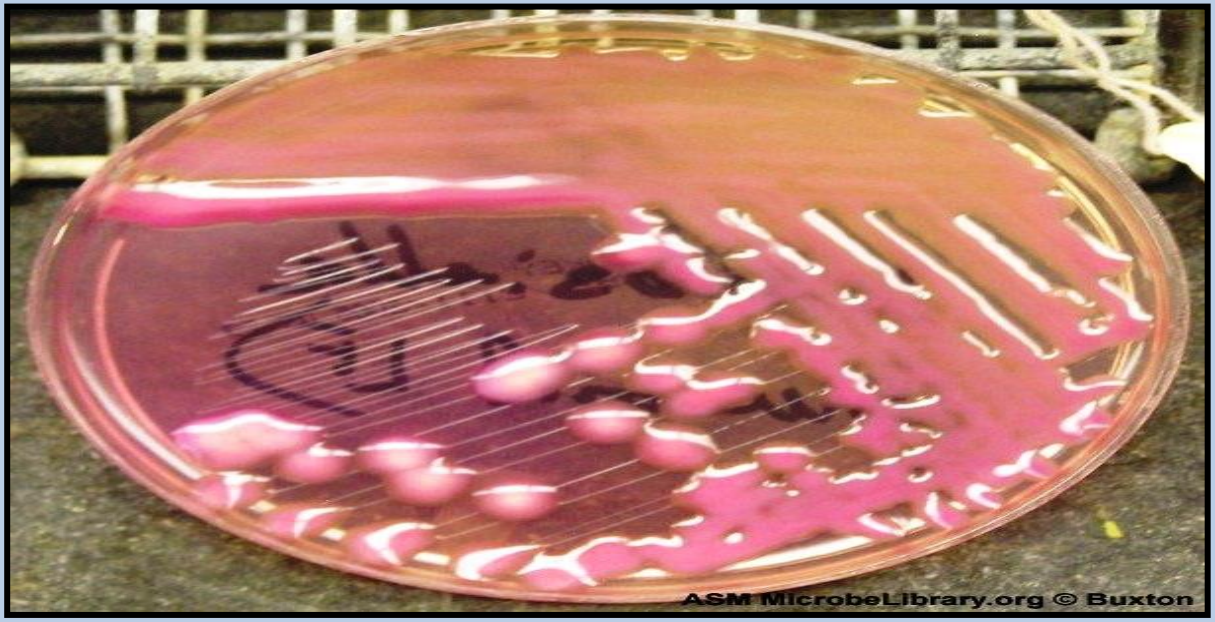
***Klebsiella spp*.**

(Klebsiella pneumoniae subsp. pneumoniae, Klebsiella rhinoscleromatis, Klebsiella oxytoca, Klebsiella ozaenae, and Klebsiella variicola) **are inhabitants of the nasopharynx and gastrointestinal tract..**

Klebsiella pneumoniae **and Enterobacter species produce abundant capsular material which may inhibit phagocytosis and enhance intracellular survival.**

Adhesins are of particular importance in those bacteria that colonize the lower urinary tract.

Siderophores produced by some opportunistic pathogens contribute to bacterial survival when the supply of available iron in tissues is limited.



Coloney morphology of Klebsiella on Maconkey agar

Some toxic effects of these opportunistic pathogens are attributable to release of endotoxin from dead bacteria, this can induce local and systemic changes which include inflammatory responses, pyrexia, endothelial damage and micro thrombosis.

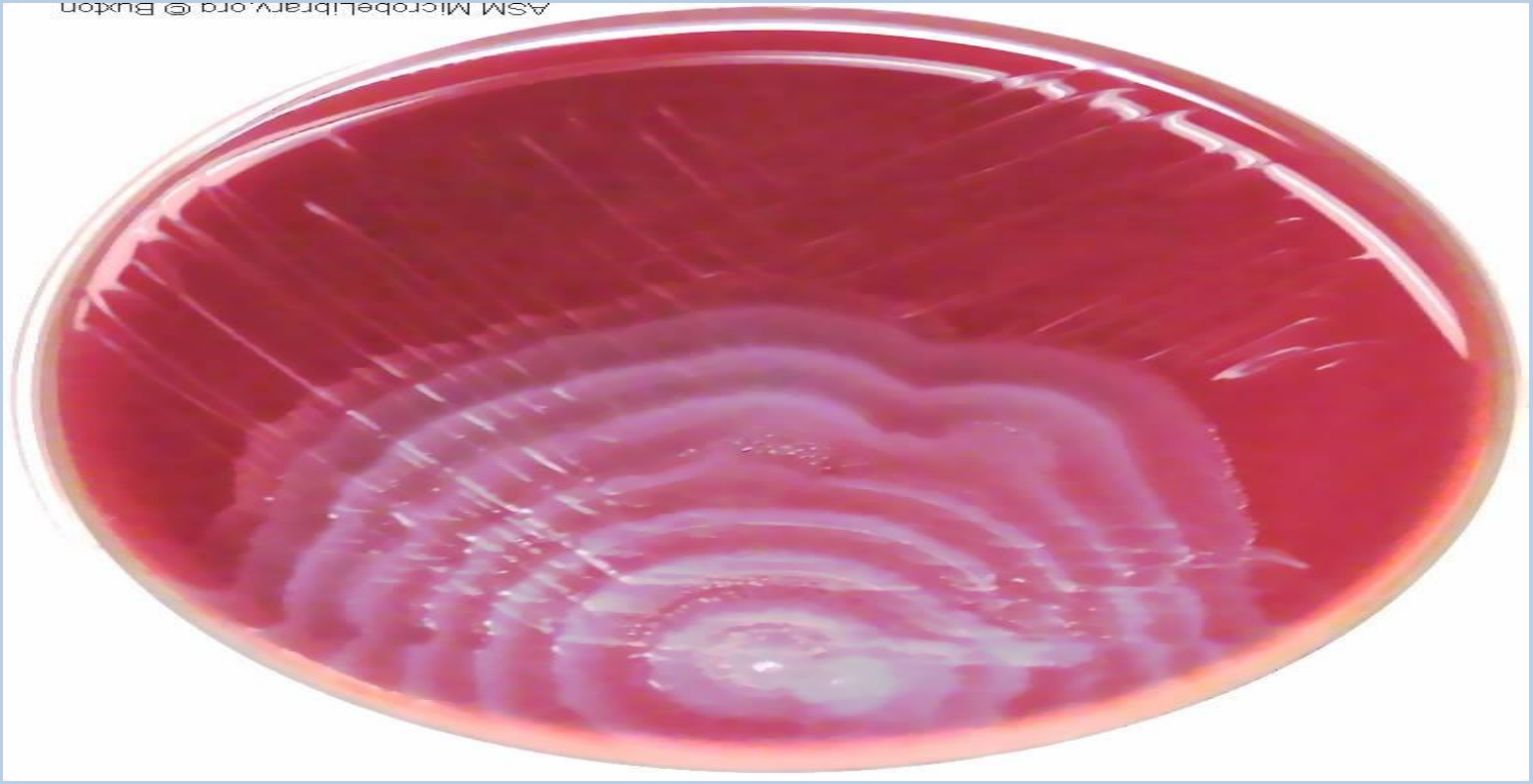
Causes some infections like liver abscesses, pneumonia, septicemia, and urinary tract infection.

***Proteus* spp**

The genera *Proteus (Proteus mirabilis, Proteus vulgaris, Proteus penneri)* are normal inhabitants of the gastrointestinal tract.

They are motile, non–lactose fermenters capable of deaminating phenylalanine. *Proteus* spp. are easily identified by their classic "swarming" appearance on culture media. However, some strains lack the swarming phenotype. *Proteus* has a distinct odor that is often referred to as a "chocolate cake" or "burnt chocolate" smell. However, for safety reasons, smelling plates is strongly discouraged in the clinical laboratory.

Because of its motility, the organism is often associated with urinary tract infections; however, it also has been isolated from wounds and ears. The organism has also been associated with diarrhea and sepsis.



Swarming phenomenon of proteus in blood agar

Shigella spp

Shigella spp. *(S. dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei)* are nonmotile; lysine decarboxylase–negative; citrate-, malonate-, and H2S-negative; non–lactose fermenting; gram-negative rods that grow well on MacConkey agar.

All strains ferment glucose without gas production, except a few strains (*S. flexneri* serotype 6 and *S. boydii* serotype 14). The four subgroups of *Shigella* spp. are *S. dysenteriae* (group A), *S. flexneri* (group B), *S. boydii* (group C), and *S. sonnei* (group D).

Each subgroup has several serotypes. Serotyping is based on the somatic LPS O antigen. *Shigella* and inactive *E. coli* strains are often very difficult to distinguish. After presumptive identification of a suspected *Shigella* species based on traditional biochemical methods, serotyping should be completed, especially in the case of *S. dysenteriae*.

- After presumptive identification of a suspected *Shigella* species based on traditional biochemical methods, serotyping should be completed, especially in the case of *S. dysenteriae.*
- Suspected strains of *Shigella* spp. that cannot be typed by serologic methods should be referred to a reference laboratory for further testing.
- Shigellosis may begin as watery diarrhea, fever, and abdominal cramps. Progressive infection then leads to dysentery with stools that contain blood or mucus. Lesions in the intestinal tract typically remain confined to the large intestine.
- Bloodstream infections are rare. All *Shigella* are capable of causing dysentery. However, *S. dysenteriae* produces the most severe forms of illness and may lead to hemolytic uremic syndrome (HUS).

Thank You

Dr. Aamer