Lecture 1+2+3 - Enteric gram-negetive rods (*enterobacteriaceae*)
Enteric bacteria or coliform

❖ General Characteristics:

- A large heterogeneous group of G-ve rods (non-spore forming), natural habitat is the G.I.T. of humans and animals, motile with peritrichous flagella or non-motile, aerobes and facultative anaerobes, ferment not oxidize carbohydrate, catalase +ve, oxidase-ve.
- It most common cultured in laboratory, includes more than 25genera & 110 spp., only 20-25 spp. are clinically significant. **the most common are :**

1) **Escherichia coli** (part of intestinal normal flora) cause disease incidentally.
2) **Klebsiella-Enterobacter-Serratia** group.
3) **Proteus-Morganella-Providencia** group.
4) **Citrobacter** (2,3,4,are as intestinal normal flora and incidentally cause disease but less than E.coli).
5) **Shigella**
6) **Salmonella** (Both **Shigella & Salmonella** are regularly pathogenic for humans)
7) **Other Enterobac.:Yersinia, Edwardsiella, Ewingella, Hafnia, Cedecea, Kluyvera**

❖ Enteric bacteria produce a variety of toxins and other virulence factors and enzymes, include:

1) **LPS (endotoxin)** have pathophysiological effects: fever, leukopenia, hypotension, hypoglycemia, activation the complement cascade, and disseminated intravascular coagulation (DIC).
2) Most of G-ve rods produce **exotoxins** such as **enterotoxins** and these toxins, has 2 types:

A. **heat-labile exotoxin (LT Exotoxin):** under genetic control transmissible plasmid .
LT exo. Contains 2 subunits (A&B): subunit B binds toGmI ganglioside at the brush border of epithelial cells of the small intestine and facilitates the entry of subunit A, which activates adenylyl cyclase → increase the concentration of cAMP and → hypersecration of sodium and lead to the diarrhea.

B. **Heat-stable enterotoxin (ST Enterotoxin):** activates guanylyl cyclase in enteric epithelial cells and stimulate fluid secretion and lead to the diarrhea.

3) **R-factor (R plasmid) & colonization or adherence factors.**
4) **Bactriocins (Colicins):** Virus-like bactericidal substances are produced by certain strains of bacteria against other strains of the same or closely related spp.; their production is controlled by plasmid . It can be used for typing of bacteria because bacteriocin-producing strains are resistant to their own bacteriocin .

❖ Antigenic structure

1) **antigen (Somatic Ag):**
   - Side chain of the cell wall LPS, consist of polysacchride heat-stable.
   - Eneric bac. are classified by more than 150 O–Ags. Antibodies to these Ags. are IgM.
2) **K–Ag. (Capsular Ag):** external to O–Ags. on some bacteria, heat-labile polysacchride or proteins , more than 100 K-Ags. Associated with virulence, *Salmonella typhi* → capsular Ag.(Vi Ag.).
3) **H- Ag. (Flagellar Ag):** a protein heat-labile or alcohols, more than 50 H – Ags., Abs. to H-Ags. are IgG.

❖ Diseases caused by enteric bacteria:

Generally as normal flora in intestine or upper R.T.,pathogenic only when they reach tissues (U.T.,biliary T., other abdominal sites,lungs,bone, meninges,prostatic G.) ,and may cause bacteremia.
Either hospital- or community- acquired infection

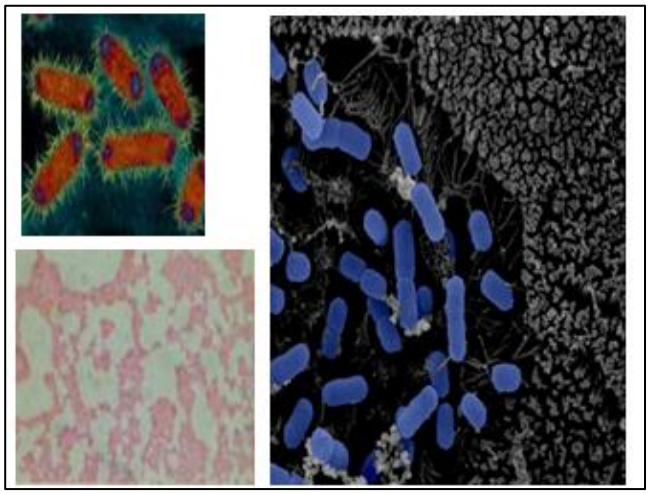
1) Escherichia coli

General Characteristics:

- Lactose fermented (pink colonies→ MacConkey`s agar), green metallic sheen colonies on EMB agar.
- Fermentative for mannitol & glucose with gas production.
- Hemolysis on blood agar, only when isolated from urine (UTIs)

Pathogenesis:

Depends on the site of infection, cannot differentiated by symptoms from other bacteria.
-The main infections are:



1) **UTIs (urinary tract infections):**

- *E.coli* is the most common of UTIs.(90% in young women).The symptoms includes: urinary frequency, dysuria , hematuria , pyuria , & flank pain with upper UTIs.
- UTIs can result in bacteremia with clinical sings of sepsis.
- **Nephropathogenic *E.coli*** (have specific O-Ags types & produce hemolysin).
- Pyelonephritis (have a specific types of pilus, P pilus).

2) **Diarrheal disease:**

Classified according to their virulence factors to :

A. **EPEC (Enteropathogenic *E.coli*):** diarrhea in infants & outbreaks diarrhea in nurseries in developing countries .
Virulence factors**:** chromosomally mediated factors cause tight adherence of EPEC to the mucosal cells of the small intestine, entry to these cells →watery diarrhea (self-limited or chronic), can treated by antibiotics. EPEC have specific serotypes of O&H Ags.

B. **ETEC (Enterotoxigenic *E.coli*):** traveler`s diarrhea & infants diarrhea in developing countries.
Virulence factors: colonization factors adherence it to epithelial cells of small intestine, some strains produce LT exotoxin others ST enterotoxin & some produce both of them.

C. **STEC (Shiga toxin producing *E.coli*) or EHEC (Enterohemorrhagic *E.coli*):** has been associated with-
   1. hemorrhagic colitis (a severe form of diarrhea)
   2. hemolytic uremic syndrome (a disease resulting in acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia)
   - (*E.coli* O157:H7 strain).There are at least 2 antigenic forms of the shiga-like toxin (shiga-like toxin -1 & -2).

D. **EIEC (Enteroinvasive *E.coli*):**
diarrhea in children in developing countries & traveler`s to these countries (invading intestinal mucosal cells).

E. **EAEC (Enteroaggregative *E.coli*):**
acute & chronic diarrhea in developing countries & as a food – borne illness in industrialized countries .Suggested that it adheres to the intestinal mucosa & elaborates enterotoxin & cytotoxin →mucosal damage →secretion mucous & secretory diarrhea .

3) **Sepsis:**

When host defenses are inadequate, *E.coli* may reach the bloodstream & cause sepsis in newborns & as a secondary to UTI in adults.

4) **Meningitis:** *E.coli* & group B streptococci are the leading causes of meningitis in infant (neonatal meningitis).75% of *E.coli* from meningitis cases have K1 Ag.
***NOTE:** the presence of *E.coli* in the water (colony count above 4/dLin drinking water) unacceptable feacal contamination, killed by chlorination of water.

2- Klebsiella-Enterobacter-Serratia Group

General Characteristics:

K.: lactose Fermentaion rapidly, viscous (mucoid) colonies because it have a large capsule, non-motile.
E.: lactose F. rapidly, raised colonies (small capsule), motile
S.: lactose F. slowly, may be pigmented colonies ,motile.

Pathogenesis:

K. pneumoniae: in 5% of normal persons (in R. T. & feces). It cause 1% of bacterial pneumonia (extensive hemorrhagic necrotizing consolidation of the lung), occasionally cause UTI & bacteremia with focal lesions in debilitated patients .
K. pneumoniae* subsp. *Ozaenae: hospital-acquired infection (upper R.T.) ,isolated from the nasal mucosa, a fetid, progressive atrophy of mucous membrans.
K. pneumoniae* subsp. *rhinoscleroderma: cause rhinoscleroma (destructive granulomatous disease of the nose & pharynx).
K. granulomatis (formerly *Calymmatobacterium granulomatis*) causes genital ulcerative disease.
E. aerogenes: free-living in GIT , opportunistic cause UTIs & sepsis .
S. marcescens: a common opportunistic pathogen in hospitalized patients, cause pneumonia, bacteremia & endocarditis .Can be treated by 3ed–generation of cephalosporins .

3- Proteus-Morganella-Providencia Group

General characteristics:

- **Proteus**: non-lactose F.,very active motile (peritrichous flagella)→swarming on blood agar ,urease +, susceptible to antimicrobial drugs (penicillins).
- **Morganella**: non-lactose F., motile, urease + .
- **Providencia**: lactose F. slowly or not , urease .

Pathogenesis:

Proteus: UTIs, bacteremia, pneumonia & focal lesions in debilitated patients .

- **P. mirabilis**: UTIs & other infections .
- **P.vulgaris**: nosocomial infection .
- The rapid motility of **Proteus** help to it invasion of the U.T., & production of urease resulting rapid hydrolysis of urea with liberation of ammonia (urine become alkaline)& promoting stone formation.
- Diagnosis by Weil-Felix test .

Morganella morganii: nosocomial pathogen .
Providencia (P. rettgeri ,P. alcalifaciens ,P.stuartii): normal intestinal flora ,all cause UTIs & other infections , resistant to antimicrobial therapy .

4- Citrobacter:

Lactose F. very slowly or not, motile, cause UTIs.

❖ **Diagnostic test for Enteric bacteria**

- **Specimens:** urine, blood, pus, C.S.F., sputum, others.
- **Culture:** on both blood agar & differential media.
- **Serological tests:** agglutination with specific antisera .
- Variation in bacterial susceptibility is great, so antibiotic sensitivity are essential .No single drug is available .
- Sulfonamides, ampicillin, cephalosporins, fluoroquinolons & aminoglycosides .

❖ **Prevention & control**

Depends on hand washing , rigorous asepsis , sterilization of equipments , disinfections , strict precautions in I.V. therapy & keeping U.T. catheters sterile .

Prophylaxis: using ciprofluxacin or trimethprim-sulfamethaxzole .

Prevention of traveler`s diarrhea, daily ingestion of bismuth subsalicylate suspension .

EPEC serotypes controlled by orally vaccines (a virulent mutant strain) or injection of killed bacterial suspension

Salmonella-Arizona group

Pathogenic by the oral route, transmitted from animals & animals products to humans & cause enteritis (enterocolitis), systemic infection & enteric fever.

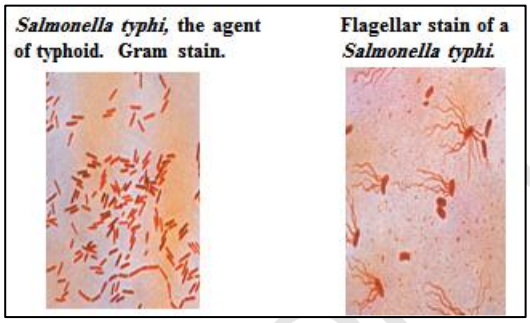
Morphology:

- Vary in length, motile with peritrichous flagellae. They survive freezing in water for long periods & resistant to certain chemicals (brilliant green, Na-tetrathionate & Na-deoxycholate) that inhibit other enteric bacteria, so such compounds useful for isolation salmonellae from feces.
- Aerobic or facultative anaerobic, grow in pH (6-8) & 15-41 C° → produce large, smooth & circular colonies (2-3 mm in diameter).
- On MacConkey's & deoxycholate-citrate agars → Pale colonies (non-lactose fermented). Ferment glucose, mannitol, mannose (with acid & with or without gas); produce H_2S (Black precipitate on TSI agar).

Antigenic Structure

1) **H (flagellar) Ag:** heat-labile protein & highly antigenic.
2) **O (somatic) Ag:** heat-stable polysaccharide (integral part of LPS).
3) **Vi (capsular or surface) Ag:** heat-labile, related to virulence

Variation may occurs by lose H Ag (become non-motile), lose of O Ag (change from smooth to rough colony form) & lose of Vi Ag partially or completely.



Classification

- 4 serotypes(group1)causes enteric fever (primarily infective for humans):
  ***Salmonella* Paratyphi A**(serogroup A)
  ***S.* Paratyphi B** (serogroup B)
  ***S.* Typhi and *S.* Enteritidis** (serogroup D)
  ***S.* Choleraesuis** (serogroup C_1)
- Currently, the genus *Salmonella* is devided into 2 species:

***1. S.entterica* (5 subsp.):**

1.= = subsp.*enterica* (subsp.I)
2.= = subsp.*salamae* (subsp.II)
3.= = subsp.*arizonae* (subsp.IIIa),and subsp.*diarizonae* (subsp.IIIb)
4.= = subsp.*houtenae* (subsp.IV)
5.= = subsp.*indica* (subsp.VI)

***2. S.bongori* (subsp.V)**

Most human illness is caused by subsp.I strains, rarely by IIIa, IIIb, & others, which found in cold-blooded animals.

Pathogenesis & Clinical Findings

- The majorities of salmonellae are pathogenic in animals (poultry, pigs, rodents, cattle & others)- → the reservoir for human infection. The bacteria enter via the oral route by contaminated food or drink → produce 3 main types of diseases (enteric or typhoid fever, bacteremia or septicemia or systemic infection & enteritis or enterocolitis).
- In typhoid fever: ingested ***S.Typhi*** reach the small intestine →------ enter the lymphatics & bloodstream, the blood carries them to many organs including the intestine →------ bacteria multiply in intestinal lymphoid tissue & excreted in stools. The lesions are hyperplasia & necrosis of lymphoid tissues (e.g., Peyer's patches), hepatitis, focal necrosis of liver & inflammation of the gall bladder, periosteum, lungs & other organs.

	Enteric or Typhoid fever	Bacteremia or Septicemia	Enterocolitis
Incubation Period	7-20 days	Variable	8-48 hours
Onset	Insidious	Abrupt	Abrupt
Fever	Gradual then high plateau with typhoidal state	Rapid rise then spiking (septic) temperature	Usually low
Duration of disease	Several weeks	Variable	2-5 days
Blood culture	+ve in 1-2 weeks of disease	+ve during high fever	-ve
Stool culture	+ve in 2nd week on -ve in earlier	Infrequently +ve	+ve soon after inset & after clinical recovery
Symptoms& clinical findings	Fever,headache,malaise, constipation, bardycardia,myalgia,spleen & liver enlarged, intestinal hemorrhage & Perforation→ bloody diarrhea (mortality rate 10-15%)	Following oral infection early invasion of the blood-stream,with focal lesions in lungs,bones,meninges,etc.without gastrointestinal symptoms	The most common of salmonellae infections. After ingestion (8-48hr) there is nausea, headache,vomiting & profuse diarrhea, inflammation lesions in small & large intestine (bacteremia 2-4%)

Diagnostic Lab. Tests:

- **Specimens:** blood & stool (urine rare).
- **Culture:**

1) Differential media (MacConkey's, EMB, Deoxycholate, Bismuth sulfite agar).
2) Selective media (SS, XLD, Hektoen enteric agar).
3) Enrichment media usually for stool (Selenite F broth or tetrathionate broth, incubation (1-2days) →- plated on differential & selective media.
4) Biochemical reaction patterns (TSI agar→------ black precipitate).

- **Serological tests:**

1) Agglutination test: serotyping for unknown culture + commercial kit, known sera (anti-O Ags for serogroups salmonellae A, B, C_1, C_2, D & E).
2) Widal test (tube dilution agglutination): determination of antibody titer in patient serum. The result as following:
   a) High titer O (≥ 1:160)- → Active infection.
   b) High titer H (≥ 1:160)- → Passive infection or past immunization.
   c) High titer Vi (in some carriers).

Immunity:

- Secretory IgA may prevent attachment of salmonellae to intestinal epithelium.
- Circulating Abs to O & Vi are related to resistance of infection →-relapses may occur in 2-3 weeks after recovery in spite of Abs-→ reinfection milder than the 1st infection.

Treatment:

- Enteric fever & bacteremia require antimicrobial therapy but enterocolitis do not, because the clinical symptoms & excretion of the salmonellae may be prolonged by antimicrobial therapy.
- In severe diarrhea, replacement of fluids & electrolytes is essential.
- Therapy: Ampicillin, trimethoprim-sulfomethaxzole or 3ed-generation cephalosporine. In most carriers, the organisms persist in the gall bladder (if gallstones are present) &in the biliary tract. Chronic carriers cured by ampicillin, but in most cases cholecystectomy must be + drug treatment.

Epidemiology:

- **Carriers:** After manifest or subclinical infection, some individuals continue to harbor salmonellae in their tissues for variable length of time (convalescent carriers or healthy permanent carriers). 3% of survivors of typhoid become permanent carriers, harboring the organisms in the gallbladder, biliary tract, or rarely, the intestine or urinary tract.
- The feces of persons who have unsuspected subclinical disease or carriers are a more important source, So food handlers are shedding organisms. The contamination of the following sources is important:

1) Water.
2) Milk & other dairy products (ice cream, cheese & custard
3) Shellfish.
4) Dried or frozen eggs.
5) Meat & meat products (poultry) or contaminated with feces by rodents or humans.
6) Recreational drugs (Marijuana).
7) Household pest (dogs, cats, turtles, etc.).
8) Animal dyes (carmine) used in drugs, food & cosmetics.

Prevention & Control:

1) Sanitary measures must be taken to prevent contamination of food & water.
2) Infected poultry, meats & eggs must be thoroughly cooked
3) Carriers must not be allowed to work as food handlers.
4) Strict hygienic precautions.

5) Vaccination:

a) 2 injections of acetone-killed ***S. Typhi*** followed by a booster injection some months later→------ partial resistance.

b) Oral administration of a live avirulent mutant ***S.Typhi*** strain.

Shigellae (shigella)

Natural habitat is the intestinal tract of humans and primates, causes bacillary dysentery.

Morphology & identification:

- Slender G-ve rods, coccobacillary in young culture, facultative anaerobes, non-motile.
- Convex, circular, transparent colonies, non-lactose F.(except ***S.sonnei***), mannitol fermenters(except ***S. dysenteriae***).
- Antigenic structure: somatic O-Ag (LPS), more than 40 serotypes (share with other enteric bacilli).
- **Classification:** on biochemical & antigenic structure, pathogenic spp. are: ***S.dysenteriae, S.flexneri, S.boydii, S.sonnei.***

Pathogenesis:

The infective dose is 10^3organisms(10^5-10^8 for salmonellae & vibrios), it is limited to the GIT, invasion of the mucosal epithelial cells by inducing phagocytosis, escape from the phagocytic vacuole, multiplication & spread within the cytoplasm then passage to adjacent cells. Bloodstream invasion is rare. **Microabscesses** formation in the wall of the large intestine & terminal ileum lead to necrosis, superficial ulceration, bleeding, & formation of **pseudomembrane** (consists of fibrin, leucocytes, cell debris, a necrotic mucous membrane, & bacteria). **Granulation tissue** fills the ulcers and scar tissue forms. Blood & pus found in stools.

Toxins:

1) **Endotoxin:** Causes irritation of the bowel wall.
2) **Shiga toxin** (exotoxin): produced by ***S.dysenteriae*** type 1(Shiga bacillus) heat-labile, antigenic protein acting as verotoxin of ***E.coli*** → inhibit sugars & amino acids absorption in the small intestine & acting as neurotoxin → ----- CNS reactions, meningismus & comma → ----- severity & fatal infection of ***S.dysenteriae*** .

Clinical Findings:

After short incubation period (1-2 days)→ sudden abdominal pain, fever & watery diarrhea. When infection involves the ileum & colon → the No. of stools increases (less liquid but contain mucus & blood).
In children & elderly, loss of water & electrolytes-→ dehydration, acidosis & death.
More than 50% of adult cases, fever & diarrhea subside spontaneously in 2-5days, few patients-→chronic intestinal carrier & recurrent of diseases.

Diagnostic Lab. Test:

Specimens: Fresh stool, mucus flecks & rectal swabs→ smear & culture.
Smear: Direct microscopic exam. Of stool-→ large No. of leukocytes & RBC's.
Culture: Using differential media (MacConkey's agar, EMB agar) & selective media (SS agar, Hektoen enteric agar).

- **Serology:** Normal persons have Abs against ***Shigella*** spp. → not used for diagnosis.

Immunity:

Serum IgM (anti-O shigellae Ags) → not protect against shigellae infection.

Treatment:

- Multiple drug resistance can be transmitted by R-factor so resistant infections are wide spread.
- Ciprofloxacin, Ampicillin, Tetracycline, Trim.-sulfamethasone & Chloramphenicol.

Epidemiology:

S. dysenteriae spread widely.
-Shigellae infections occur in children under 10 years, transmitted from person-person by food, fingers, feces & flies.

Prevention & Control:

Eliminating shigellae from reservoirs by:

1) Sanitary control of water, foods & milk; sewage disposal &fly control.
2) Isolation of patients & disinfecting of excreta.
3) Detection of subclinical cases & carriers especially food handlers.
Antibiotic treatment of infected individuals.