GIS 3

MICROBIOLOGY

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NOTE: • The bolded information throughout the sheet is what the professor focused on and is VERY IMPORTANT.

• The information surrounded by these brackets {} are points mentioned in the slides but were not discussed by the professor in the lecture.

Enterobacteriaceae

• Enterobacteriaceae are also known as **enteric bacteria and coliforms**.

- They are also called coliforms because they can be found in the colonic microbiota.

• **Gram negative bacilli**

• It's a large, heterogeneous group of gram-negative rods whose **natural habitat is the intestinal tract of humans and animals**. They can cause virtually all kinds of infections, but our focus is on GI infections.

• This family includes many genera, such as **Escherichia, Shigella, Salmonella, Yersinia,** Enterobacter, Klebsiella, Serratia, Proteus, and others.

- NOTE: in this system, we will talk about 4 of these genera (in bold) and in this lecture we will discuss the first two.

• Some enteric organisms, such as Escherichia coli, are part of the normal microbiota and <u>incidentally</u> cause disease, but others, such as salmonellae and shigellae, are <u>strictly pathogenic</u> for humans.

- E. coli of the microbiota can cause disease in one of **3 scenarios**:
    a) If they gain virulence factors through transmissible plasmids or phages.
    b) If they take advantage of lowered immunity (an opportunistic infection).
    c) If they get introduced into a sterile site (ex. blood, urine). This is the scenario in which E. Coli causes UTI by getting introduced into the urinary tract causing cystitis (inflammation of the bladder) and pyelonephritis (inflammation of the kidney).

•They are the **most common group of gram-negative rods cultured** in clinical laboratories.

• They are among the most common bacteria that cause disease along with staphylococci and streptococci.

• Common characteristics of <u>all</u> Enterobacteriaceae:

1- They are **all motile** with peritrichous flagella **EXCEPT** for Shigellae, Yersinia and Klebsiella. (peritrichous flagella = numerous flagella all around the cell)

2- They grow aerobically and anaerobically. (facultative anaerobes) (diagnostic feature from other gut commensals, such as the anaerobic Bacteroides found in the colon)
3- They **ferment**, rather than oxidize, **glucose**, often with gas production.
4- They are **oxidase negative** (except for Plesiomonas), **catalase positive**, and they can **reduce nitrate to nitrite**.
   - Being oxidase negative differentiates them from Pseudomonas.
5- They grow on peptone or meat extract media. Also, they grow well on MacConkey agar.
6- **{**Have a 39–59% (G + C) DNA content**}**.
7- They are either **lactose fermenters (E. coli) or non-lactose fermenters (shigellae and salmonella).**
8- Eosin methylene blue (**EMB) or MacConkey agar** differentiate lactose fermentation.
9- Have heat stable **LPS** (lipopolysaccharide). LPS is a principal component of the G-ve bacterial cell wall and is an endotoxin.

❖ Antigenic Structure of Enterobacteriaceae:

- They **ALL** possess **heat-stable somatic O (lipopolysaccharide) antigens**.
  - These antigens are detected by bacterial agglutination. Antibodies to O antigens are predominantly **IgM**.
  - Found in the outermost layer which has oligosaccharides (that's why it's called O antigen).
  - Another name for this antigen: Common enterobacterial core antigen.
- Enterobacteriaceae with a **capsule** possess **heat-labile K (capsular) antigens.**
  - **{**Large capsules consisting of polysaccharides (K antigens) covering the somatic (O or H) antigens.**}**
  - This antigen provides **resistance to serum killing but is NOT a virulence factor**. It can be identified by capsular swelling tests with specific antisera.
  - The **capsular antigen of Salmonella serotype Typhi** is called **Vi antigen** because it's not only resistant to serum killing but is also a virulence antigen.
- **Motile** Enterobacteriaceae possess **H (flagellar) antigens**.
  - **{**Agglutinate with anti-H antibodies, mainly IgG**}**.
- Many gram-negative organisms produce Colicins (**bacteriocins**).
  - Bacteriocins are peptide pore forming toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains (reducing the competition with other bacteria!)
  - Colicins are the bacteriocins produced by E. coli.

E. coli (1st genus):

• Main cause of **UTI** (uropathogen)

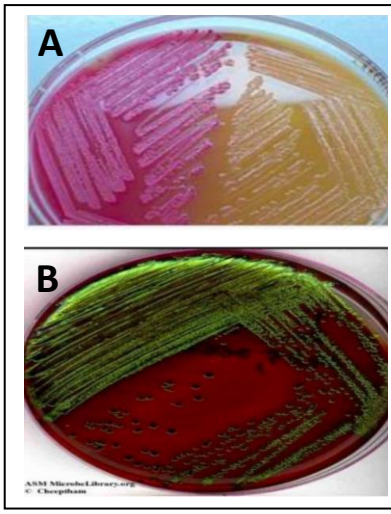
• A member of the **normal intestinal microbiota** in humans and animals. **{**It can also be found in small numbers in the normal microbiota of the upper respiratory and genital tracts**}**.

• They are classified by the characteristics of their virulence properties and each group causes disease by a different mechanism—at least five of which have been characterized.

• **{**The small or large bowel epithelial cell adherence properties are encoded by genes on plasmids. Similarly, the toxins often are plasmid or phage mediated**}**.

• They are **oxidase negative and lactose fermenters** (produce Green sheen colonies on EMB).

- **Eosin methylene blue (EMB) or MacConkey agar** have lactose and thus are used to differentiate lactose fermentation:



	Lactose fermenters	**Non-lactose fermenters**
MacConkey (pic A)	Pink	Colorless
EMB (pic B)	Black dots on colony (nucleated) (E. coli specifically forms **green sheen colonies**)	Colorless

❖ E coli-associated diarrheal diseases (cause gastroenteritis):

(NOTE: the professor focused on the main points to know about each classification and they are written in bold)

1-Enteropathogenic E coli (EPEC):

• **Enteropathogenic**

• A major cause of **infantile diarrhea** associated with **outbreaks of explosive diarrhea in nurseries,** especially in developing countries.

• Causes infantile diarrhea in the **bottle-fed population** (<2 years).

•Pathogenesis: It requires two important factors for **attachment and effacement**

- The bundle forming pilus is encoded by a plasmid. EPEC uses it for attachment to colonic mucosa. **(EPEC adherence factor (EAF))**

- The chromosomal locus of enterocyte effacement **(LEE)** pathogenicity island that promotes the tight adherence characteristic of EPEC.
- After attachment, there is **effacement (loss of microvilli, degeneration of epithelial cells of the intestine facing the lumen).**

• Patient to patient transmission is common in children (feco-oral as kids are unhygienic)

• It's unusual to occur in adults because of the high inoculum needed (but it can occur).

• Clinical picture: The result of EPEC infection in infants is severe, **watery diarrhea**, vomiting, and fever.

• Diarrheal stool often contains mucus but not blood.

• It is usually self-limited but can be prolonged or chronic.

- Hospitalization is necessary for patients with chronic diarrhea or dehydrated patients.

• **{**EPEC diarrhea has been associated with multiple specific serotypes of E coli; strains are identified by O antigen and occasionally by H antigen typing**}**.

• **{**The duration of EPEC diarrhea can be shortened and chronic diarrhea cured by antibiotic treatment**}**.

2-Enterotoxigenic E coli (ETEC):

• A common cause of "**traveler's diarrhea,**" especially in people travelling from developed to developing countries.

• Very important cause of diarrhea in infants in developing countries.

• Pathogenesis: **ETEC colonization factors** (known as colonization factor antigens [CFAs]). They are specific for humans and promote adherence of ETEC to epithelial cells of the small bowel.

• It produces toxins (hence the name!):

- **ST: Heat-stable** (MW, 1500–4000), activates guanylyl cyclase → increased local concentration of **cGMP**
- **LT: heat-labile** exotoxin, activates adenylyl cyclase → increased local concentration of **cAMP**
- Both toxins result in hypersecretion of fluids and electrolytes and poor absorption of sodium→ watery diarrhea.
- NOTE that **LT and colonization factors** are **immunogenic** (but the antibodies are short-lived).

• ETEC clinical picture:

- Intense and prolonged hypersecretion of water & chlorides and inhibition of sodium reabsorption.

- **{**The gut lumen is distended with fluid, hyper-motile and diarrhea ensues, lasting for several days**}**.
- LT is antigenic and **{**cross-reacts with the enterotoxin of Vibrio cholerae, identical mechanism of action**}**. LT stimulates the production of neutralizing antibodies in the serum of persons previously infected with enterotoxigenic E. coli.
- Persons residing in areas where such organisms are highly prevalent (eg, in some developing countries) are likely to possess antibodies and are less prone to develop diarrhea on re-exposure to the LT-producing E coli.

3-Shiga toxin-producing E coli (STEC/EHEC):

• Also called Verocytotoxin-producing E. coli (**VTEC**) and Enterohemorrhagic E. coli (**EHEC**).

- Vero cells are monkey kidney cells which are killed by the toxin.

• Named for the cytotoxic toxins they produce.

- Most common E. coli strain that causes gastroenteritis.
- Does not ferment sorbitol (unlike other E. coli) so we use Sorbitol MacConkey agar).

•Linked to consumption of of fresh products (e.g., **lettuce**, spinach, sprouts), **undercooked ground beef** (hamburgers), **apple cider**, and unpasteurized milk.

- Why? Because STEC can be found in the intestinal tract of cattle. The vegetables and apples can be contaminated by cattle feces.

• There are at least two antigenic forms of the toxin referred to as **Shiga-like toxin 1 and toxin 2** that affect **60S** ribosomal subunit (so it inhibits protein synthesis).

• STEC has been associated with hemorrhagic colitis.

• The **most common** of all the E coli serotypes that produce Shiga toxin is **O157:H7**. It also is the one that can be identified most readily in clinical specimens.

• STEC, EIEC and shigellae all cause bloody diarrhea, but **STEC is NOT INVASIVE** (unlike EIEC and shigellae) (very important). So, the pathogen remains attached to the lumen.

• **10% of patients infected with STEC develop HUS** (hemolytic-uremic syndrome). HUS is a disease resulting in micro-angiopathic hemolytic anemia, thrombocytopenia (low platelet count) and acute renal failure. Occurs frequently in children.

•STEC clinical picture:

- Colonic edema and an **initial non-bloody** secretory diarrhea may develop into the STEC/EHEC/ hallmark syndrome of grossly **bloody diarrhea** (contains RBCs, WBCs, some mucous).
- Why does it start as non-bloody diarrhea but then becomes bloody? The Shiga-like toxin causes occlusions in capillaries, so when RBCs transverse through→ they get hemolyzed (destructed) → causing bloody diarrhea

- Significant abdominal pain and fecal leukocytes are common (70% of cases), whereas **fever is not (not invasive= no fever). {**Note that absence of fever can incorrectly lead to consideration of noninfectious conditions (e.g., intussusception or ischemic bowel disease)**}**.

• **{**Occasionally, infections caused by C. difficile, Campylobacter, and Salmonella present in a similar fashion**}**.

•STEC/EHEC disease is usually **self-limited**, lasting 5–10 days.

• STEC is the **most common** cause of all the E coli-associated diarrheal diseases (gastroenteritis).

•STEC diagnosis and treatment:

- Tests for the detection of both Shiga toxins using commercially available enzyme immunoassays (**EIAs**) are done in many laboratories.
- **{**Other sensitive test methods include cell culture cytotoxin testing using Vero cells and polymerase chain reaction for the direct detection of toxin genes directly from stool samples**}**. There are also kits that test directly for the O157:H7 strain.
- Also, **Sorbitol MacConkey agar plate** is used for diagnosis because **EHEC is the only E. coli that doesn't ferment sorbitol** →so it won't grow on the plate and thus we can differentiate it from other E. coli strains.
- Many cases of hemorrhagic colitis and its associated complications can be prevented by thoroughly cooking ground beef and avoiding unpasteurized products such as apple cider.
- **Antibiotics are contraindicated because they increase the risk for HUS.**
- Anti-motility drugs (reduce motility of colon) and opioids are also **contraindicated**.

NOTE: In general, if we suspect **gastroenteritis → then opioids are contraindicated**. Why? Opioids are analgesic and thus will mask the acute symptoms of surgical emergencies such as ischemic bowel disease → causing colon perforation in the abdomen→ sepsis → septicemia→ septic shock.

4-Enteroinvasive E coli (EIEC):

• It's **INVASIVE** (so it causes **bloody diarrhea** and fever).

• Produces a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries.

• Note that E. coli are lactose fermenters **EXCEPT EIEC**. **Similar to Shigella**, EIEC strains are **non-lactose or late lactose fermenters** and are nonmotile.

• **Unlike shigella**, EIEC require **large inoculum** (10^8–10^10 CFU) to establish infection.

• EIEC produce disease by **invading intestinal mucosal epithelial cells**.

5-Enteroaggregative E coli (EAEC):

• Causes acute and **chronic diarrhea (diarrhea lasts more than 14 days)** in persons in developing countries.

• These organisms also are the cause of foodborne illnesses in industrialized countries

• Have been associated with traveler's diarrhea and **persistent diarrhea in patients with HIV**.

• They are characterized by their specific patterns of adherence to human cells. The organisms exhibit a diffuse or "**stacked-brick" pattern** of adherence to small intestine epithelial cells (that's why we call them entero**aggregative**).

• This group of diarrheagenic E. coli is quite heterogeneous, and the exact pathogenic mechanisms are **still not completely elucidated**. Some strains of EAEC produce **ST (Shiga toxin)-like toxin** (EAST). **{**Others produce a plasmid-encoded enterotoxin that produces cellular damage (hemolysin and enterotoxin)**}**.

• **{**Diagnosis can be suspected clinically but requires confirmation by tissue culture adhesion assays, which are not readily available in most clinical laboratories**}**.

Approach:

• A practical approach to the evaluation of diarrhea is to **distinguish non-inflammatory (non-bloody) from inflammatory diarrhea (bloody).**

• Inflammatory diarrhea is suggested by grossly bloody or mucoid stool or a positive test for fecal leukocytes.

• **ETEC, EPEC, and EAEC cause non-inflammatory diarrhea.**

• **EIEC, STEC cause inflammatory diarrhea**

Treatment:

• Treatment of gram-negative bacteremia and impending septic shock requires rapid restoration of fluid and electrolyte balance, institution of antimicrobial therapy, and treatment of disseminated intravascular coagulation.

• No single specific therapy is available. The sulfonamides, ampicillin, cephalosporins, fluoroquinolones, and aminoglycosides have marked antibacterial effects against the enterics, but variation in susceptibility is great, and laboratory tests for antibiotic susceptibility are essential.

• Trimethoprim-sulfamethoxazole, known in Jordan as **cotrimoxazole,** is also used.

• **Multiple drug resistance** is common and is under the control of transmissible plasmids.

Prevention:

• Various means have been proposed for the prevention of **traveler's diarrhea**, including daily ingestion of **bismuth subsalicylate suspension** (bismuth subsalicylate can inactivate E. coli enterotoxin in vitro) and regular doses of tetracyclines or other antimicrobial drugs for limited periods.

• Because none of these methods are entirely successful or lacking in adverse effects, caution should be observed in regard to food and drinks in areas where environmental sanitation is poor and that early and brief treatment (eg, with ciprofloxacin or trimethoprim–sulfamethoxazole) be substituted for prophylaxis.

• People should pay more attention to sanitation, hygiene, sterilization and avoidance of eating undercooked food.

Control:

• **{**The enteric bacteria establish themselves in the normal intestinal tract within a few days after birth and from then on constitute a main portion of the normal aerobic (facultative anaerobic) microbial flora**}**.

• E. coli is the **prototype**. **{**Enterics found in water or milk are accepted as proof of fecal contamination from sewage or other sources. Control measures are not feasible as far as the normal endogenous flora is concerned**}**.

• **{**Enteropathogenic E. coli serotypes should be controlled like salmonellae. Some of the enterics constitute a major problem in hospital infection. It is particularly important to recognize that many enteric bacteria are "opportunists" that cause illness when they are introduced into debilitated patients. Within hospitals or other institutions, these bacteria commonly are transmitted by personnel, instruments, or parenteral medications**}**.

• Their control depends on handwashing, rigorous asepsis, sterilization of equipment, disinfection, restraint in intravenous therapy, and strict precautions in keeping the urinary tract sterile (i.e., closed drainage).

Shigellae (2nd genus):

• The natural habitat of shigellae is limited to the intestinal tracts of humans and other primates, where they produce **shigellosis (also called bacillary dysentery).**

• Shigellae are slender **gram-negative rods.**

• They form **coccobacillary colonies** in young cultures.

• Shigellae are facultative anaerobes but grow best aerobically.

• **{**Convex, circular, transparent colonies with intact edges reach a diameter of about 2 mm in 24 hours**}**.

• All shigellae ferment glucose. They do not ferment lactose, but Shigella sonnei can. The inability to ferment lactose distinguishes shigellae on differential media.

• They are **non-motile**

- This characteristic **differentiates Shigellae from salmonella and E. coli**
- So, they **don't have H-antigen**

• They are **non-lactose fermenters**

- So, they produce colorless colonies in EMB

• They **do NOT produce H2S** (hydrogen sulfide gas)

- This characteristic **differentiates them from salmonella**

❖ Epidemiology:

• Man and certain primates are the only host (it's a **strictly human pathogen**)

• Age: Any age but commonly **under 5 or 10 y/o** (it's mainly **disease of children)**

• It occurs in warm months, **temperate** climates and **rainy seasons** in tropical countries.

• **{**Asymptomatic infection in endemic areas**}**.

• In **industrialized/ developed** countries, the **most common serotype is S. sonnei** with S. flexneri second.

• In **developing** countries, the **most common serotype is S. flexneri**

• Transmission: **feco-oral route, person to person, toilet seat, door handles, contaminated food and water supply, and a vector causing outbreaks (flies)**

- Mnemonic: Think of **4 Fs→ F**ood, **F**eces, **F**lies, **F**ingers
- As we can see from the routes of transmission, it's **highly communicable**

❖ Etiology:

• **Low numbers** are required to cause disease: **10-1000**.

• **Have O-antigens** since they are a genus of Enterobacteriaceae.

•The genus shigellae is subdivided into 4 species (A, B, C and D) according to their biochemical reaction and antigenic composition:

- **Group A Shigella Dysenteriae:** It's the most important and **classical** species. **{**It has 12 Serotypes**}** and **produces type 1 shiga toxin continuously**. It is the **most severe with the highest mortality rate**. However, it's **not common**.
- **Group B Shigella flexneri: {**8 serotypes and mild disease**}.** (most common in developing countries)
- **Group C Shigella boydii: {**18 serotypes**}**.

- **Group D Shigella sonnei: {**single and intermediately severe disease**}**. (most common in developed countries)
- Also, groups B, C, and D may produce the toxin if infected by a phage with the gene

❖ Pathogenesis:

• **{**Shigella infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare**}.**

• **{**The infective dose is on the order of less than 103 organisms (it usually is 105–108 for salmonellae and vibrios)**}**.

• The **essential pathologic process is invasion** of the mucosal epithelial cells (e.g. M cells) **{**by induced phagocytosis, escape from the phagocytic vacuole, multiplication and spread within the epithelial cell cytoplasm, and passage to adjacent cells**}**.

- **The MAIN pathogenesis of shigellae is invasion. The Shiga toxin produced is synergistic to the process but is NOT the main factor.**

• **{**Micro abscesses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding, and formation of "pseudomembrane" on the ulcerated area. This consists of fibrin, leukocytes, cell debris, a necrotic mucous membrane, and bacteria. As the process subsides, granulation tissue fills the ulcers, and scar tissue forms**}**.

❖ Toxins:

1) **Endotoxin (LPS, since it's gram -ve)**

• Upon autolysis, all shigellae release their toxic lipopolysaccharide. This endotoxin probably contributes to the irritation of the bowel wall.

2) Shigella Dysenteriae **Exotoxin**

• S dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that is **neurotoxic, cytotoxic and enterotoxic.**

- Acting as an enterotoxin, it produces diarrhea as does the E. coli Shiga-like toxin, perhaps by the same mechanism (inhibiting protein synthesis).
- Acting as a "neurotoxin," this material may contribute to the extreme severity and fatal nature of S. dysenteriae infections and to the central nervous system reactions observed in them (ie, meningismus, coma). **In infected neonates with symptoms of fever and bloody diarrhea, it may cause febrile convulsions (a differential of shigellosis) and eventually seizures.**

• **{**The toxic activity is distinct from the invasive property of shigellae in dysentery. The two may act in sequence, the toxin producing an early nonbloody, voluminous diarrhea

and the invasion of the large intestine, resulting in later dysentery with blood and pus in stools.**}**

❖ Clinical Findings:

• After a short incubation period (1–2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea. The diarrhea has been attributed to an exotoxin acting in the small intestine. A day or so later, as the infection involves the ileum and colon, the number of stools increases; they are less liquid but often contain mucus and blood.

• **{**Each bowel movement is accompanied by straining and tenesmus (rectal spasms), with resulting lower abdominal pain.**}**

• In more than half of adult cases, fever and diarrhea subside spontaneously in 2–5 days. However, in children and elderly adults, loss of water and electrolytes may lead to dehydration, acidosis, and even death. The illness caused by S. dysenteriae may be particularly severe.

• On recovery, most persons shed dysentery bacilli for only a short period, but a few remain chronic intestinal carriers and may have recurrent bouts of the disease. Upon recovery from the infection, most persons develop circulating antibodies to shigellae, but these do not protect against reinfection.

❖ Diagnostic Laboratory Tests:

A. Specimens:

- Specimens include fresh stool, mucus flecks, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically.

B. Culture

- The materials are streaked on differential media.
- MacConkey or EMB → appear colorless
- On **selective** media for shigellae **(Hektoen enteric agar or Salmonella –Shigella agar)**, which suppress other Enterobacteriaceae and gram-positive organisms.

C. Serology

- **Serology is NOT used to diagnose Shigella infections**. Why? Because there are many **false positives** since healthy people often have agglutinins against several Shigella species.
- **{**However, serial determinations of antibody titers may show a rise in specific antibody**}**.

❖ Treatment

• **{**Ciprofloxacin, ampicillin, doxycycline, and trimethoprim–sulfamethoxazole are most commonly inhibitory for Shigella isolates and can suppress acute clinical attacks of dysentery and shorten the duration of symptoms**}**.

• **They may fail to eradicate the organisms from the intestinal tract**.

- Therefore, the infection **persists** in shigellosis even though **humans are the only reservoir**.
- So, we call these people **convalescent carriers → they shed the pathogenic bacteria in their feces even though they are healthy. They should NOT work in food and plants handling.**

• **Multiple drug resistance** can be transmitted by plasmids, and resistant infections are widespread.

• Many cases are self-limited.

• **Opioids should be avoided** in Shigella dysentery (because a surgical emergency is part of the differential diagnosis).

❖ Prevention, and Control:

• **{**IgA antibodies in the gut may be important in limiting reinfection**}**.

• **{**Serum antibodies to somatic Shigella antigens are IgM**}**.

• Shigellae are transmitted by "food, fingers, feces, and flies" from person to person.

• Because humans are the main recognized host of pathogenic shigellae, control efforts must be directed at eliminating the organisms from this reservoir by:

1- Sanitary control of water, food, and milk.
2- Sewage disposal and fly control.
3- Isolation of patients and disinfection of excreta.
4- Detection of subclinical cases and carriers, particularly food handlers.
5- Antibiotic treatment of infected individuals.
6- Avoidance of undercooked food.

GOOD LUCK