MICROBIOLOGY

بسم الله الرحمن الرحيم

MID | Lecture 3

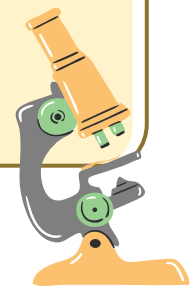
Enteric G- Rods (Pt.1)

﴿وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم﴾

اللهم استعملنا ولا تستبدلنا

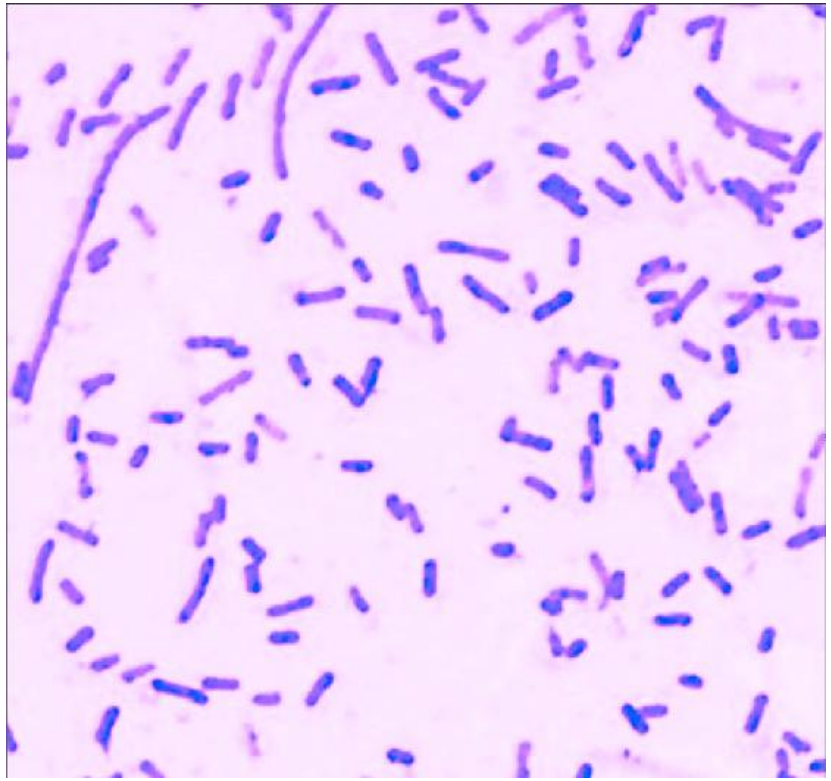
Written by: Mohammad Mahasneh

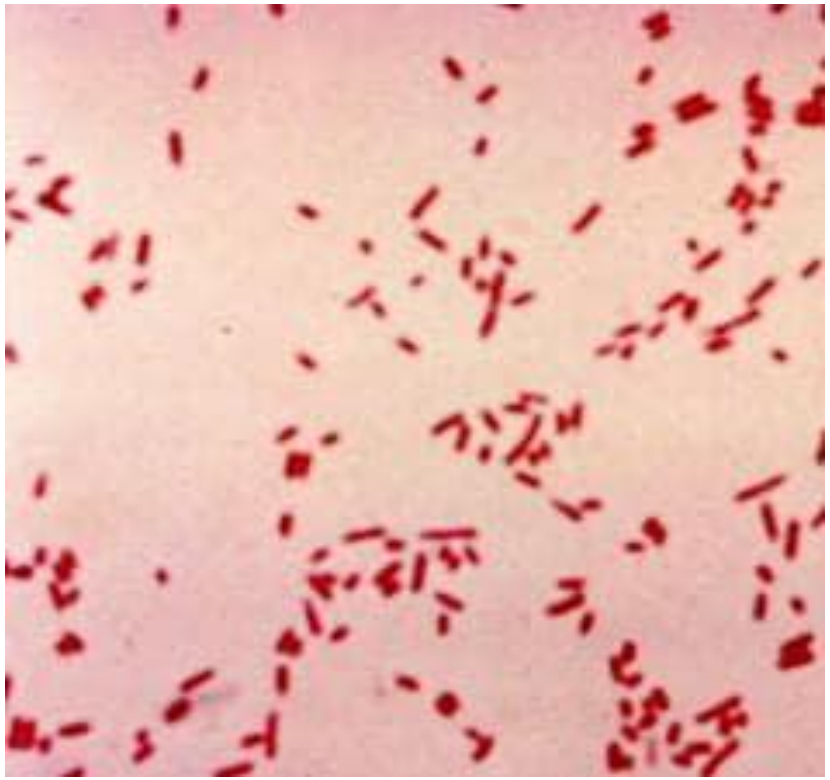
Muthanna Khalil



Which of the following images represents last lecture's content?

Click on the answer





Enteric Gram-Negative Rods (Enterobacteriaceae)

By: Nader Alaridah MD, PhD

Enterobacteriaceae, enteric bacteria & may also be called coliforms.

- large, heterogeneous group of gram-negative rods non-spore forming whose natural habitat is the intestinal tract of humans and animals, that's why they are called **coliforms** and has the prefix entero- in their family name.

  - Genera that belong to this family are considered responsible for many cases of **bacteremia**, **gastroenteritis**, **UTIs**, some exceptions will be discussed later.

- The family includes many genera (*Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus, Yersinia, Providencia, Citrobacter, Acinetobacter* and others).

  - As they are all **G- bacteria**, their cell wall has lipopolysaccharides, the innermost layer is composed of **lipid A** which is considered **an endotoxin**, the outermost one contain the **somatic O antigen**, while in the middle they all posses a common core polysaccharide called **e**nterobacterial **c**ommon **a**ntigen **(ECA)**.

Enterobacteriaceae, enteric bacteria & may also be called coliforms cont.

- Some enteric organisms, such as Escherichia coli, are part of the normal microbiota and incidentally cause disease, but others, the salmonellae and shigella, are regularly pathogenic for humans.

- Although **some genera are commensal** within the human GI tract, when they gain a new virulence feature or spread to a different anatomical location, they can establish a disease, such example is when ***E.coli*** gain a new virulence factor and cause gastroenteritis or gain access to the urinary tract and establish a UTI. **Some genera are always pathogenic** and are not part of the human normal flora such as ***salmonella and shigella***.

- **Virulence factors** could be gained by **transmissible plasmids**, **bacteriophages**, **pathogenicity islands**.

Enterobacteriaceae

- The most common group of gram-negative rods cultured in clinical laboratories. Along with staphylococci and streptococci are among the most common bacteria that cause disease.

Enterobacteriaceae share four common features:

I. Glucose fermenters
II. Catalase-positive
III. Oxidase-negative, this feature excludes pseudomonas from Enterobacteriaceae.
IV. They reduce nitrates to nitrites.

- They are either motile with peritrichous flagella or nonmotile.

  - Almost all genera are motile, examples are *E.coli* (except enteroinvasive strains of *E.coli*), *salmonella* and *proteus, n*on-motile genera include *shigella* and *klebsiella.*
  - *Yersinia* is an exception, at 37° they are non-motile, but at 25° they are motile.

Enterobacteriaceae cont.

- They grow aerobically and anaerobically (are facultative anaerobes). Eosin methylene blue EMB or MacConkey agar (differentiate lactose fermentation).
- Facultative anaerobes are ordinarily aerobes (respiration generates more ATP), but in the absence of oxygen, they go anaerobic (fermentation; with less ATP).
- They grow on peptone or meat extract media, grow well on MacConkey agar; ferment rather than oxidize glucose, often with gas production; are catalase positive and oxidase negative (except for Plesiomonas) and reduce nitrate to nitrite; and have a 39–59% G + C DNA content.

Antigenic Structure

- Heat-stable somatic O (lipopolysaccharide) antigens. are detected by bacterial agglutination. Antibodies to O antigens are predominantly IgM.

- Heat-labile K (capsular) antigens. large capsules consisting of polysaccharides (K antigens) covering the somatic (O or H) antigens can be identified by capsular swelling tests with specific antisera .

  - **Capsulated genera** such as some strains of ***E.coli*** *and* ***klebsiella*** has this **K antigen**, this antigen acts as an **antiphagocytic**.

Antigenic Structure cont.

- H (flagellar) antigens. agglutinate with anti-H antibodies, mainly IgG.
  - **Motile genera** posses this **H antigen**, they also could have sex pili that they get through bacteriophages and chromosomally-encoded fimbria.
- *Salmonella serotype Typhi*, the capsular antigens are called **Vi** antigens.
  - ***Salmonella typhi*** and ***paratyphi*** have a special capsular antigen called **Vi antigen**, it is considered a **virulence factor** as it facilitates the spread and the pathogenicity of the bacteria along with its **antiphagocytic activity**.
- Many gram-negative organisms produce Colicins (bacteriocins).
  - Bacteriocins are produced to reduce growth competition by other bacteria, ***E.coli*** specifically produce **colicins.**

One bacteria could have combination of hundreds or tens from each antigen, imagine how many possible serotypes are there for each bacteria species.

E coli–associated diarrheal diseases

- A member of the normal intestinal microbiota & in small numbers as part of the normal microbiota of the upper respiratory and genital tracts.

- These E coli 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.
  - ***E.coli*** are a major cause of **gastroenteritis** (our scope in this lecture), it is also responsible for **(20-30)% of sepsis cases**, it is associated with about **80% of UTIs**, it causes **meningitis** and **sepsis in neonates**, note that in the case of neonate's meningitis, as there is still no colonization of the bacteria in their GI tract, they get infected during delivery by the **exogenous *E.coli*** colonizing their mother's vaginal canal, while in **other cases** the infection is **endogenous** as it's the ***E.coli* in the normal flora** gaining virulence factor or changing their anatomical location as discussed previously.

E coli–associated diarrheal diseases cont.

- The small or large bowel epithelial cell adherence properties are encoded by genes on plasmids. Similarly, the toxins often are plasmid or phage mediated.
- Oxidase negative, lactose fermenters. Produce Green sheen colonies on EMB.

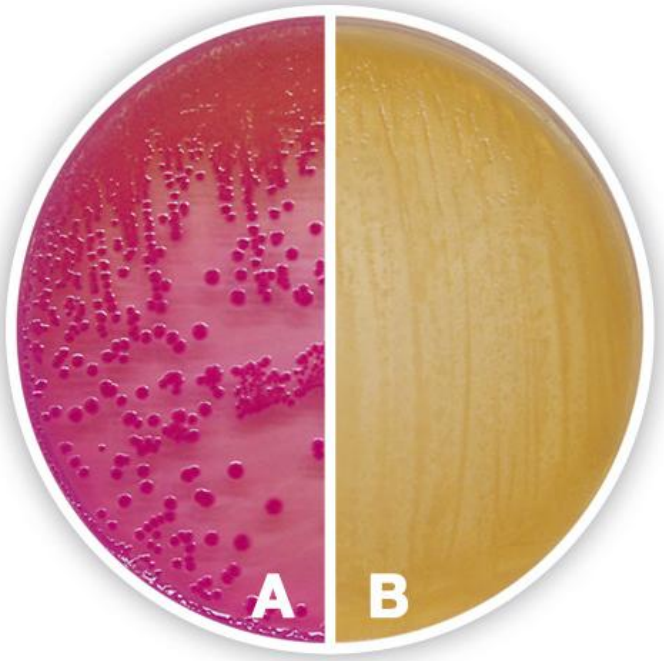
E.coli Laboratory Diagnostic Tests

One additional feature other than the four mentioned previously about E.coli is that it is lactose fermenting bacteria, this feature is of high medical importance in distinguishing *E.coli* from other non-lactose fermenting bacteria such as *salmonella* and *shigella.*

This Figure on the right shows MacConkey agar, it is both selective and differential media and its contents are:

- **Bile salts** and **crystal violet** to inhibit the growth of G+ bacteria and commensals, isolating G- bacteria.
- **Lactose** which is a fermentable carbohydrate.
- **Neutral red dye** that turns pink to red in acidic environment.

✓ **Lactose fermenting** bacteria colonies will appear on a background of **red to pink color**, notice the colonies of *Enterobacter* (similar color shown by *E.coli* colonies), while the **colorless** colonies indicates a **non-lactose fermenting** bacteria



A: Lactose fermenter
B: Non-lactose fermenter

E.coli Laboratory Diagnostic Tests cont.

This is an **E**osin **M**ethylene **B**lue (EMB) agar media, lactose fermenters that show pink colonies on MacConkey agar will show nucleated colonies with dark center, also a green sheen appearance is noticed.

Enteropathogenic E coli (EPEC) ➔ affects small bowel

- A major cause of infantile diarrhea, **associated with outbreaks of diarrhea in nurseries** especially in developing countries.
  - Also, they are considered the cause of more **than 30% of diarrhea cases** in **bottle fed infants**, they are less likely to get adults infected because adults need high inoculum of bacteria to get diseased, another cause is that they may have got immunized during their childhood.
- Pathogenicity requires two important factors, (attachment and effacement): the **bundle forming pilus encoded by a plasmid,** EPEC adherence factor (**EAF**) and the **chromosomal locus of enterocyte effacement (LEE**) **pathogenicity island** that promote the tight adherence characteristic of EPEC.
  - Note that EAF is a plasmid-encoded virulence factor while LEE is a chromosomal pathogenicity islands, these two factors must be present together to establish a disease.
- After attachment, there is loss of microvilli (effacement).
  - Attachment factor facilitate the attachment of the bacteria to the **enterocytes** of the **small intestine**, the effacement cause the **degeneration** of enterocytes **brush border** finally leading to **explosive watery diarrhea**.

EPEC clinical picture

- The result of EPEC infection in infants is severe, watery diarrhea; vomiting; and fever. Diarrheal stool often contains mucus but not blood.
  - No bloody diarrhea as it is watery not inflammatory, there are no RBCs or WBCs in the stool.
- It is usually self-limited but can be prolonged or chronic.
  - The **main management** for gastroenteritis is **fluid and electrolyte replacement therapy**, antibiotics may be used is chronic prolonged diarrhea that cause severe dehydration.
  - Make sure to know that for diarrheagenic strains of *E.coli,* antibiotics might help all conditions **except with one strain where it is contraindicated to use antibiotics**, discussed later.
- 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 the EPEC diarrhea can be shortened and the chronic diarrhea cured by antibiotic treatment.

Enterotoxigenic E coli (ETEC)

➔ **affects small bowel**

EPEC are commonly transmitted person-to-person, while ETEC are not that transmissible, yet it can cause outbreaks if a common food source is involved.

- A common cause of **"traveler's diarrhea"** and a very important cause of diarrhea in infants in developing countries.
- ETEC **colonization factors** (known as colonization factor antigens [CFAs]) specific for humans promote adherence of ETEC to epithelial cells of the small bowel.
  - **Colonization factor** are encoded on transmissible plasmids (same as EPEC adherence factor) will allow its growth and attachment to the small intestine mucosa, then it will start producing its toxins.

- It produces heat-stable toxin (ST) - (MW, 1500–4000), it has subtypes A and B, it activates guanylyl cyclase leading to increased local concentration of cyclic guanyl monophosphate cGMP. It also produces heat-labile toxin (LT), with subtypes 1 and 2, it activates adenylyl cyclase leading to increased local concentration of cyclic adenosine monophosphate cAMP.
  - Elevated levels of **cGMP** and **cAMP** is **hypersecretion** of fluid and electrolytes caused by increased chloride secretion into gut lumen which will attract sodium and water leading to **watery diarrhea**.

ETEC clinical picture

- Intense and prolonged hypersecretion of water & chlorides and inhibition of sodium reabsorption ⇔ increased osmolarity in the lumen attracts water ➔ watery diarrhea.

- The gut lumen is distended with fluid, hyper-motile and diarrhea ensue, 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.
  - **LT toxin** is **antigenic** and **immunogenic,** while **ST toxin** is **antigenic but not immunogenic**, people with specific antibodies for LT can be protected from traveler's diarrhea caused by ETEC

- 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.

Shiga toxin-producing E coli (STEC/EHEC) → affects large bowel

- Named for the cytotoxic toxins they produce. Linked to consumption of fresh products (e.g., lettuce, spinach, sprouts; if watered by contaminated sources) and of undercooked ground beef (hamburgers), as this strain is typically part of the normal flora of cattle.

  Another known cause in the US & Canada is apple cider (عصير تفاح بدون سكر مضاف) made from contaminated apples.

- There are at least two antigenic forms of the toxin referred to as Shiga-like toxin 1 and toxin 2 that affect the 28S subunit of the 60S ribosomal subunit ⇔ protein synthesis inhibitor.

  This toxin causes capillary **thrombosis**; RBCs passing by are lysed and become schistocytes (RBCs fragments). This causes hemolytic anemia and **bloody diarrhea.** STEC's effect is **purely toxin-mediated with no invasion.**

- STEC has been associated with hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome **HUS in 10-20% of cases**; a disease resulting in micro-angiopathic hemolytic anemia, acute renal failure (epithelial cells die) and thrombocytopenia.

  The toxin targets the kidneys due to that **Gb3 glycolipid receptor** is abundant on the **kidneys' epithelial cells**, mediating the effects above.

  Platelets are "over-recruited" to the kidneys → lower serum levels.

- Of the E coli serotypes that produce Shiga toxin, **usually a pediatric disease**, **O157:H7** is the most common and is the one that can be identified most readily in clinical specimens.

  Another serotype – O104:H4 – also causes bloody diarrhea, but it falls under enteroaggregative (discussed later).

STEC clinical picture

- Colonic edema and an **initial non-bloody secretory diarrhea** may develop into the STEC/EHEC/ **hallmark syndrome of grossly bloody diarrhea** (Significant abdominal pain and fecal leukocytes are common (70% of cases), **whereas fever is not**; absence of fever can incorrectly lead to consideration of noninfectious conditions (e.g., intussusception or ischemic bowel disease).

Red currant jelly stool
(study your patho!)

- 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. *Entamoeba histolytica* and CMV can cause bloody diarrhea as well.

(parasitology shows up again!)

Viruses are the most common cause of gastroenteritis regardless of age.

Apart from the 10-20% who develop HUS, the rest spontaneously resolve.
From the 10-20% who develop HUS, 30% suffer from chronic kidney failure, and up to 10% may die.

Knowing that STEC classically affects children, the quality of life being compromised at such a young age tells how dangerous this STEC/EHEC is.

STEC diagnosis and treatment

Sorbitol MacConkey agar can be used because STEC is non-sorbitol fermenter unlike most other *E.coli*'s.

- Tests for the detection of both Shiga toxins using commercially available enzyme immunoassays (EIAs) are done in many laboratories.

  They look for the most common serotype, namely **O157:H7.** Pediatricians in developed countries have this assay ready as a **point-of-care** test in their clinics.

THE DEFINITIVE DIAGNOSIS

- Other sensitive test methods include **(1) cell culture cytotoxin testing using Vero cells** (monkey kidney cells); STEC/EHEC are also called Vero-cytotoxic *E.coli* and (2) polymerase chain reaction for the direct detection of toxin genes directly from stool samples.

- 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 may increase the risk for HUS ➔ **contraindicated**.

General rule: Strong analgesics (opioids) and antimotility agents should NOT be given in such cases because they mask the pain, unnoticed deep invasion and perforation → peritonitis → sepsis → death.

Enteroinvasive E coli (EIEC) ➔ affects large bowel

- Produces a disease **very similar to shigellosis**. The disease occurs most commonly in children in developing countries and in travelers to these countries. Similar to Shigella, EIEC strains are **non-lactose** or **late lactose fermenters** and are **non-motile**.
- **Unlike shigella**, **EIEC require large inoculum** (10^8–10^{10} CFU).

- EIEC produce disease by invading intestinal mucosal epithelial cells.

  Like shigella, pathogenesis is by invasion → bloody diarrhea.
  Antibiotics can be prescribed here.

  Recall that antibiotics can be useful in all cases of *E.coli*-induced diarrhea **except** in case of STEC/EHEC (contraindicated).

Enteroaggregative E coli (EAEC) → affects small bowel

- Causes acute and **chronic diarrhea (>14 days in duration)** in persons in developing countries. These organisms also are the cause of foodborne illnesses in industrialized countries and have been associated with **traveler's diarrhea** and persistent diarrhea in **patients with HIV**.

  Also can cause **infantile diarrhea**.

- 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.

  Thus the name!

  There is another strain called **Diffuse Aggregative *E.coli*.**

- This group of diarrheagenic E coli is quite heterogeneous, and the exact pathogenic mechanisms are still not completely elucidated. Some strains of EAEC produce [(1)]**ST-like toxin (EAST),** others a [(2)]**plasmid-encoded enterotoxin** that produces cellular damage; a hemolysin and enterotoxin; they also can [(3)]have **pili** and **fimbriae → many ways for their pathogenesis**.

- Diagnosis can be suspected clinically but requires confirmation by tissue culture adhesion assays not readily available in most clinical laboratories.

The differential diagnosis of such clinical presentations depends on

- A practical approach to the evaluation of diarrhea is to **distinguish non-inflammatory from inflammatory cases**; the latter is suggested by grossly bloody or mucoid stool or a positive test for fecal leukocytes.

- ETEC, EPEC, and EAEC cause non-inflammatory (watery) diarrhea.

- **EIEC**, **STEC**, and EAEC cause inflammatory (bloody) diarrhea.

Due to microangiopathic hemolytic anemia (toxin-mediated)

Due to direct invasion

Treatment

- Treatment of gram-negative bacteremia and impending septic shock requires rapid **restoration of fluid and electrolyte balance**, institution of antimicrobial therapy if indicated (in **severe** forms of **non-STEC** cases), and treatment of disseminated intravascular coagulation.

- No single specific therapy is available. The sulfonamides (and TMP-SMX), ampicillin, cephalosporins (usually 3rd gen), fluoroquinolones, and aminoglycosides have marked antibacterial effects against the enterics, but **variation in susceptibility is great, and laboratory tests for antibiotic susceptibility are essential**.

- 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 be observed in regard to food and drink** in areas where environmental sanitation is poor and that early and brief treatment (eg, with ciprofloxacin or trimethoprim–sulfamethoxazole) be substituted for prophylaxis .

Control

Nevertheless, water chlorination kills *E.coli*

- The enteric bacteria establish themselves in the normal intestinal tract of both human and animals (contaminates soil & water) 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** (if > 4 CFU) or milk are accepted as proof of fecal contamination (by human microbiome) 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; see next slide).
- In preventing community acquired UTI’s, self hygiene practices are vital, especially when using public restrooms.

Closed Urinary Drainage System:

It means that once a urinary catheter (like a Foley catheter) is inserted into the bladder, it is connected to a sterile, *sealed* drainage bag through a closed tube. The system is **not open to the outside environment**, which helps **prevent bacteria from entering** and causing a urinary tract infection (UTI).

Without closed systems, Foley catheter usage, for example, can considerably increase the risk communication of UTI's iatrogenically.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	14	Note that EAF is a plasma encoded virulence factor while LEE is...	Note that EAF is a plasmid-encoded virulence factor while LEE is...
	20	Sorbitol MacConkey agar can be used because ETEC is non-sorbitol fermenter unlike most other *E.coli*'s.	Sorbitol MacConkey agar can be used because STEC is non-sorbitol fermenter unlike most other *E.coli*'s.
V1 → V2			

رسالة من الفريق العلمي:

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

الٓمٓ ﴿١﴾ ذَٰلِكَ ٱلْكِتَٰبُ لَا رَيْبَ فِيهِ هُدًى

لِّلْمُتَّقِينَ ﴿٢﴾ ٱلَّذِينَ يُؤْمِنُونَ بِٱلْغَيْبِ وَيُقِيمُونَ ٱلصَّلَوٰةَ

وَمِمَّا رَزَقْنَٰهُمْ يُنفِقُونَ ﴿٣﴾ وَٱلَّذِينَ يُؤْمِنُونَ بِمَآ أُنزِلَ

إِلَيْكَ وَمَآ أُنزِلَ مِن قَبْلِكَ وَبِٱلْءَاخِرَةِ هُمْ يُوقِنُونَ ﴿٤﴾

أُو۟لَٰٓئِكَ عَلَىٰ هُدًى مِّن رَّبِّهِمْ ۖ وَأُو۟لَٰٓئِكَ

هُمُ ٱلْمُفْلِحُونَ ﴿٥﴾