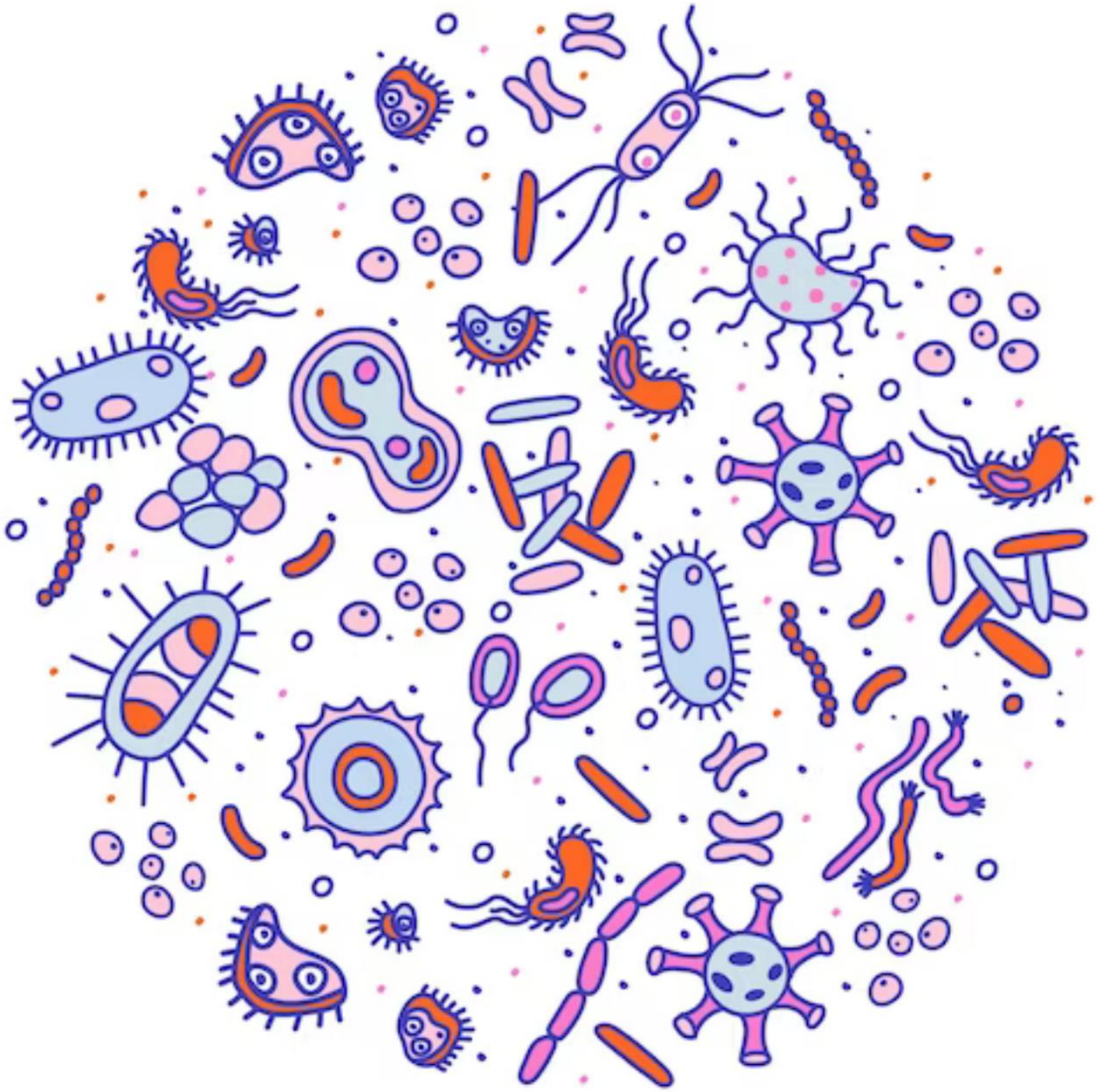
Microbiology

Sheet n. 2



Writer:
- Ibrahem Al-Shawabkeh
- Suhaib Zaiter

Corrector:
- Ibrahem Al-Shawabkeh
- Suhaib Zaiter

Clostridium difficile infection (CDI)

Quick recap:

CLOSTRIDIUM SPECIES are G+ spore forming bacilli.

EPIDEMIOLOGY

- **Ubiquitous in the environment and colonizes the intestine of 50% of healthy neonates and 4% of healthy adults,** its number decreases gradually with aging.

- **A major cause of healthcare-associated infection; patients taking antibiotics, e.g. cephalosporins** (2nd generation)**, clindamycin,** Fluoroquinolone and cancer treatments **are at increased risk of developing C. difficile antibiotic associated diarrhea.**

- **This is due to suppression of the normal bowel flora and subsequent overgrowth of C. difficile. Infection may be endogenous or exogenous (through ingestion of environmental spores).**

PATHOGENESISEPIDEMIOLOGY

- Toxin- mediated diseases

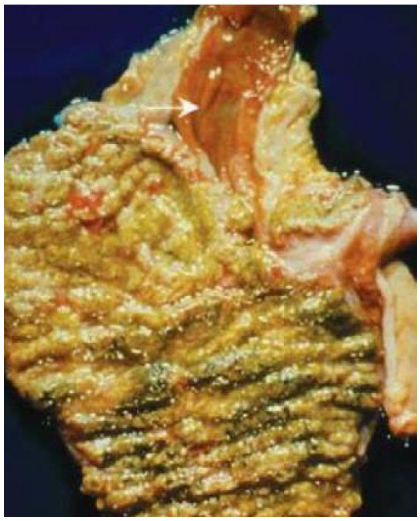
- **Produces two major toxins: Toxin A (enterotoxin,** which is chemoattractant of PMNs, so it causes hyper-cytokines at the site of infection) **and Toxin B (cytotoxin,** its MOA through ADP-Ribosylation of GTP, which controls actin cytoskeleton of infected cell. End up with loss of enterocytes.) Mainly, but there are many toxins that are mediated in pathogenesis.

- **Toxin A induces cytokine production with hypersecretion of fluid** (watery diarrhea).

- **Toxin B induces depolymerization of actin with loss of cytoskeleton. Adhesion factor and hyaluronidase production are also associated virulence factors.**

- **Hypervirulent, hypertoxin producing strains now recognized (e.g., ribotype 027, 078).**

DISEASE



- **Antibiotic associated diarrhea,**
- **Mild to moderate.**
- **Pseudomembranous colitis (PMC,** elevated yellow-white plaques on the colon and filled with micro abscesses, fibrin, platelets and dead cells), **fulminant colitis** (the infected patients have more chance to get toxic meglocolon).
- **Severe forms**

DIAGNOSIS

- It is diagnosed by exclusion.
- **The diagnosis of CDI is based on a combination of clinical criteria:**

- **(1) diarrhea (≥3 unformed stools per 24 h for ≥2 days) with no other recognized cause plus,**
- **(2) toxin A or B detected in the stool (e.g. ELISA, latex agglutination, and polymerase chain reaction (PCR)) or culture of C. difficile on selective agar** (CCFA).
- **(3) pseudomembranes seen in the colon.**
- **PMC is a more advanced form of CDI and is visualized at endoscopy in only ~50% of patients with diarrhea who have a positive stool culture and toxin assay for C. difficile.**

- Diarrhea is typically diagnosed based on frequency and consistency. Following this initial assessment, healthcare providers may investigate further to determine toxin. Diagnostic methods may include laboratory tests such as PCR or Test agglutination.

TREATMENT AND PREVENTION

- when patient use broad spectrum antibiotics, many bacteria will suppress which give a chance for C. difficile to establish infection.

- **Discontinue other antibiotics therapy.**

• Oral administration of vancomycin (in severe cases, (it is only situation to get vancomycin orally)) **or metronidazole** (mild cases, Orally) **is recommended for CDI treatment.**

- Flagyl is the first-line drug here.
- If there's vancomycin-resistance, Fidaxomicin is administered.
- Last choice is fecal transplantation to restore normal microbiota (Orally or Enema (إبر شرجية

• Caution in overprescribing broad-spectrum antibiotics (limited-spectrum drugs should be considered first).

• In the nursing home setting, patients who are symptomatic should be isolated.

• Autoclave bed pans (treatment kills spores).

Enteric Gram-Negative Rods (Enterobacteriaceae), enteric bacteria & may also be called coliforms.

- Coliforms means colon microbiota.

- Non-Spore former.

- Enterobacterial common antigen (ECA) is a family-specific surface antigen shared by all members of the Enterobacteriaceae.

- **large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals.**
- **The family includes many genera (Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus, and others).**

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

- Under certain conditions, commensal bacteria can become opportunistic pathogens and cause infections, such as:

1. Immunocompromised Host

2. Disruption of Normal Microbiota

3. Changes in the host environment or migration to a different site, as seen with E. coli, a coliform bacterium capable of causing cystitis or pyelonephritis if it moves to the urethra.

4. Impairment of mucosal barrier function.

5. Acquisition of virulence factors via bacteriophages or transmissible plasmids.

ENTEROBACTERIACEAE

- E.coli is responsible to 70%-80% of UTI, 30% of bacteremia infections, and gastroenteritis and many diseases outside GIT.
- E. coli primarily causes indigenous infections, except for neonatal meningitis and gastroenteritis, which are exogenous.

• 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.

• They are either motile with peritrichous flagella(majority) or nonmotile (salmonellae, shigella, and Yersinia).

• They grow aerobically and anaerobically (are facultative anaerobes). Eosin methylene blue EMB or MacConkey agar (differentiate lactose fermentation).

• 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 pseudomonas"distinctive for pseudomonas") and reduce nitrate to nitrite; and have a 39–59% G + C DNA content.

ANTIGENIC STRUCTURE

• Heat-stable somatic O (LPS, Oligosaccharide, Outermost layer) 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), protective against phagocytosis and serum killing. covering the somatic (O or H) antigens can be identified by capsular swelling tests with specific antisera.**

- **H (flagellar) antigens (presenting in motile bacteria). agglutinate with anti-H antibodies, mainly IgG.**

- **Salmonella serotype Typhi, the capsular antigens are called Vi (Virulence factor) antigens.**

- **Many gram-negative organisms produce Colicins (bacteriocins) which is** peptides produced from E.Coli itself against other E.coli strains.

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.**

- **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 (**Eosin methylene blue**)**

- MacConkey agar contains bile salts to prevent commensal G+ to grow.

+ve ->lactose fermenter-> convert neutral dye its pinkish color(E.g. E.coli)

-ve -> non lactose fermenter-> colorless (E.g., Salmonellae and shigella, and Yersinia)

-EMB: E.Coli appears as dark nucleated colony with black center.

In GIT we are going to talk about diarrheagenic E.coli strains which causes gastroenteritis.

ENTEROPATHOGENIC E COLI (EPEC)

• **A major cause of infantile (<1 year) diarrhea, associated with outbreaks of diarrhea in nurseries(حضانات) especially in developing countries (no need of high infectious dose to cause outbreaks)**

- Also, it could affect bottle feeding children. الي برضعوا بالرضّاعة
- Person to person transmission is very highly probable in EPEC.
- In adult high infectious dose is required to cause outbreaks.

• Pathogenicity (in small intestine) requires two important factors, (attachment and effacement) A&E: attachment by the bundle forming pilus encoded by a plasmid, EPEC adherence factor (EAF) and the chromosomal locus of enterocyte effacement (LEE) cause effacement, pathogenicity island that promote the tight adherence characteristic of EPEC.

- The most important in pathogenicity is attachment and effacement.
- Effacement: shorting and thinning especially in the department of Obstetrics and Gynecology.

• After attachment, there is loss of microvilli (effacement).

- The epithelial cells in the intestine have a brush border (villus), which undergo shortening and thinning in EPEC infection.
- What is the outcome? Watery diarrhea (hypersecretion of fluids and electrolytes)
- The majority resolve spontaneously, while some cases may become chronic, and others require hospitalization and fluid replacement due to signs of dehydration.
- EPEC infection can be controlled by antibiotics administration.

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.

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

• 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)

• A common cause of "traveler's diarrhea" and a very important cause of diarrhea in infants in developing countries(Mainly it is related to traveler diarrhea).
There is no outbreak, and person to person transmission is unlikely.

• ETEC needs colonization factors to establish infection → colonization factors (known as colonization factor antigens [CFAs]) specific for humans promote

adherence of ETEC to epithelial cells of the small bowel. (Pathogen also happen in the small intestine)

• It produces a ST (heat stable) - (MW, 1500–4000), activates guanylyl cyclase -and heat-labile exotoxin (LT)- where it activates adenylyl cyclase. Leading to increased local concentration of cGMP, cAMP respectively, which will lead to watery diarrhea.

- 2 types of ST toxin (A and B) A the only one that affects humans.
- 2 types of LT toxin (1 and 2) 1 the only one that affects humans.

Click me to see Indian food

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 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.

- ST is antigenic but not immunogenic while LT antigenic and immunogenic.
- Keep in mind, immunogenic pathogens can initiate an immune response, while antigenic pathogens are recognized by the immune system.
- That's why people who got affected by LT enterotoxin E. coli they might develop short live immunity.
- Some kind of immunity in the form of secretory immunoglobulin type A as well as immunoglobulin against colonization factor.
- This explains why someone coming from the West to India, for example, might infect and cause disease while locals don't, as travelers are susceptible due to their lack of previous exposure to the heat-labile enterotoxin which leading to short-lived immunity.

• 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)

Has three different names:

1)STEC/EHEC: refers to E. coli strains capable of producing Shiga toxins.

2) -producing E. coli (VTEC): is another term for STEC/EHEC, highlighting their ability to produce Verocytotoxins (Shiga toxins), it can be diagnosed

3)Enterohemorrhagic Escherichia coli (EHEC): specifically refers to STEC strains associated with causing hemorrhagic colitis and HUS in humans. (Affects large intestine)

. Bloody diarrhea is typically associated with pathogen invasion, but EHEC differs in that it doesn't invade tissues.

• **Named for the cytotoxic toxins they produce. Linked to consumption of fresh products (e.g., lettuce, spinach, sprouts) and of undercooked ground beef (hamburgers).**

- The first one that cause bloody diarrhea but there is no invasion of mucosa and submucosa.
- Bloody diarrhea is caused by hemolytic anemia in affected patients, making children under 5 years more susceptible to developing Hemolytic Uremic Syndrome (HUS).

• **There are at least two antigenic forms of the toxin referred to as Shiga-like toxin 1 and toxin 2** (more virulent than 1) **that affect 60S ribosomal subunit.**

- The homology between Shiga like toxin 1 and toxins produced by Shigella dysenteriae almost 100% while between 2 and Shigella dysenteriae is less than 80%

• **STEC has been associated with hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome HUS; a disease resulting in micro-angiopathic hemolytic anemia, acute renal failure, and thrombocytopenia.**

Shiga toxins bind to specific receptors called Gb3 (globotriaosylceramide) on vascular endothelial cells and glomerular cells in the kidneys. This binding leading to microthrombosis. Which can obstruct blood flow, leading to tissue damage and organ dysfunction, particularly in the kidneys, the destruction of red blood cells as a result of microthrombosis can contribute to hemolytic anemia and thrombocytopenia. Those RBCs are excreted with feces as bloody diarrhea.

-The majority of HUS cases recover, 5-10% are fatal, and approximately 30% may develop chronic renal failure.

•**Of the E coli serotypes that produce Shiga toxin, O157:H7, is the most common and can be identified most readily in clinical specimens.**

- Shiga toxin, O157:H7,O104:H4 cause outbreaks
- feco-oral transmission is linked to consumption undercooked beef, which contains E. coli as part of cattle's normal gut flora, It has a high mortality rate.

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).

- No fever because there is no invasion of mucosa and submucosa.
- Intussusception: intestine slides into each other , similar to the way a telescope collapses. (patients have currant jelly stool)
- Abdominal cramps, so you don't give these patients antimotility agents.
- Administering strong painkillers is contraindicated because they can induce sleep in the patient, delaying their ability to perceive symptoms of intussusception.
- Antibiotics are also contraindicated because they can induce stress on E. coli, leading to increased production of Shiga toxin.

• 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 DIAGNOSIS AND TREATMENT

• Tests for the detection of both Shiga toxins using commercially available enzyme immunoassays (EIAs) are done in many laboratories. (Available to detect theses serotype (O157:H7 and O104:H4)

• Other sensitive test methods include cell culture cytotoxin testing using Vero cells and polymerase chain reaction(PCR) **for the direct detection of toxin genes directly from stool samples.**

Vero cell cytotoxin assay is sensitive methods, using Vero cells to detect the presence of E. coli. In this test, Vero cells, a type of monkey kidney cells, are cultured and exposed to samples suspected of containing E. coli. If E. coli is present and produces cytotoxins, it causes damage to the Vero cells, which can be observed microscopically. This method is highly sensitive and can detect even small amounts of E. coli toxins.

- We said that E. coli strains are lactose fermenter, if we replace lactose by sorbitol all E. coli species will ferment sorbitol except this, so we call it sorbitol MacConkey negative.

• 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.** (So, antibiotics are contraindicated in these patients)

ENTEROINVASIVE E COLI (EIEC)

• **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 nonmotile. Unlike shigella , EIEC require large inoculum (10^8–10^{10} CFU).** (High infectious dose)

- Pathogenesis occurs in large intestine.
- **-EAEC is similar to shigella which is the prototype bacterium that causes invasive diseases in the intestines.**
- **EIEC need high infectious dose to cause infection in comparison with shigella.**

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

- Causing bloody diarrhea and fever.

ENTEROAGGREGATIVE E COLI (EAEC)

- Difficult to diagnose because there is no single biomarker, but you have to make exclusion of another E.coli strains.

• **Causes acute and chronic diarrhea (>14 days in duration) in persons in developing countries,** immunocompromised people**. 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.**

• **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.**

• **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-**

like toxin (EAST), others a plasmid-encoded enterotoxin that produces cellular damage; a hemolysin and enterotoxin. (that's why we call them enteroaggregative).

- Since it can produce Shiga like toxin, people who got affected with this type may develop HUS syndrome (but not like STEC/EHEC)
- Pathogenesis isn't well understood

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

APPROACH

- Enterotoxigenic, Enteropathogenic, and Enteroaggregative → in small intestine, mostly watery diarrhea.
- Enteroinvasive, Enterohemorrhagic → large intestine, cause bloody diarrhea.

• 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 diarrhea.

• EIEC, STEC/EHEC cause inflammatory diarrhea.

- When we say inflammatory→ WBCs
- When we say bloody → RBCs
- WBCs, RBCs, and mucus+ → dysentery (bloody diarrhea with electrolyte imbalance)

TREATMENT

- Main cornerstone in management of gastroenteritis patients (regardless of the infectious cause) is fluid and electrolytes replacement, then antibiotics.

• 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.

34:00

• 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.

• 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

• 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).