Enteric Gram-Negative Rods (Enterobacteriaceae)

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Enterobacteriaceae, enteric bacteria & may also be called coliforms.

- 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, are part of the normal microbiota and incidentally cause disease, but others, the salmonellae and shigellae, are regularly pathogenic for humans.

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.

- They are either motile with peritrichous flagella or nonmotile.

- 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 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 .

- H (flagellar) antigens. agglutinate with anti-H antibodies, mainly IgG .

- Salmonella serotype Typhi, the capsular antigens are called **Vi** antigens.

- Many gram-negative organisms produce Colicins (bacteriocins).

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.

Enteropathogenic E coli (EPEC)

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

- 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.

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

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.

- ETEC colonization factors (known as colonization factor antigens [CFAs]) specific for humans promote adherence of ETEC to epithelial cells of the small bowel.

- It produces a ST - (MW, 1500–4000), activates guanylyl cyclase -and heat-labile exotoxin (LT)- where it activates adenylyl cyclase - . Leading to increased local concentration of cyclic Guanyl and Adenosine monophosphate cGMP, cAMP respectively.

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.

- 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)

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

- 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.

- 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.

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

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).

- 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.

- 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.

- 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.

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 non-motile. Unlike shigella , EIEC require large inoculum (10^8–10^{10} CFU).

- EIEC produce disease by invading intestinal mucosal epithelial cells.

Enteroaggregative E coli (EAEC)

- 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.

- 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.

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

- 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 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.

- 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 (ie, closed drainage).

Shigellosis (Bacillary dysentery)

- The natural habitat of shigellae is limited to the intestinal tracts of humans and other primates, where they produce bacillary dysentery.

- Shigellae are slender gram-negative rods; coccobacillary forms occur 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. With the exception of Shigella sonnei, they do not ferment lactose. The inability to ferment lactose distinguishes shigellae on differential media

- Non-motile,non-lactose fermenters,do not produse H2S, and produce a colorless colonies in EMB.

Epidemiology

- Man and certain primates are the only host.

- Age: any age but commonly under 5 y/o.

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

- Asymptomatic infection in endemic areas.

- In industrialized countries , S.sonnei is most common with S.flex second.

- Transmission: feco-oral route, person to person, toilet seat, door handles, contaminated food and water supply and a vector causing outbreaks: flies maybe .

Etiology

- The genus shigella is subdivided into 4 species (A,B,C and D) according to their biochemical reaction and antigenic composition . Low number are required to cause disease : 10-1000.

- Group A Shigella Dysenteriae 12 Serotypes, most imp. type 1 shiga, most sever disease.

- Group B Shigella flexneri 8 serotypes mild disease.

- Group C Shigella boydii 18 serotypes.

- Group D Shigella sonnei single , intermediately sever disease .

Pathogenesis

- Shigella infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare. Shigellae are highly communicable; the infective dose is on the order of less than 10^3 organisms (it usually is 10^5–10^8 for salmonellae and vibrios).

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

- 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

- A. Endotoxin

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

- B. 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.

- In humans, 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).

- 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 (eg, MacConkey or EMB agar) and on selective media (Hektoen enteric agar or Salmonella –Shigella agar), which suppress other Enterobacteriaceae and gram-positive organisms.

- C. Serology

- Normal persons often have agglutinins against several Shigella species. However, serial determinations of antibody titers may show a rise in specific antibody. Serology is **not used** to diagnose Shigella infections .

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.

- 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.

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; sewage disposal and fly control; (2) isolation of patients and disinfection of excreta; (3) detection of subclinical cases and carriers, particularly food handlers; and (4) antibiotic treatment of infected individuals.

The Salmonella-group

- Salmonellae are often pathogenic for humans or animals when acquired by the oral route.

- They are transmitted from animals and animal products to humans, where they cause enteric fever ,gastro- enteritis and systemic infection.

- Most isolates are motile with peritrichous flagella. They almost never ferment lactose or sucrose. They form acid and sometimes gas from glucose and mannose. They usually produce H2S.

- They survive freezing in water for long periods. Salmonellae are resistant to certain chemicals (eg, brilliant green, sodium tetrathionate, sodium deoxycholate) that inhibit other enteric bacteria; such compounds are therefore useful for inclusion in media to isolate salmonellae from feces.

- Salmonellae are named by genus (Salmonella), species (enterica), and subspecies (e.g., typhi or enteritidis) .

Subspecies o f Medical Importance

- S. enterica subsp. Typhi.

- S. enterica subsp. Enteritidis

- S. enterica subsp. Typhimurium

- S. enterica subsp. Choleraesuis

- S. enterica subsp. Paratyphi

- S. enterica subsp. Dublin

The "Enteric Fevers" (Typhoid Fever)

- Four serotypes of salmonellae that cause enteric fever can be identified in the clinical laboratory by biochemical and serologic tests. These serotypes should be routinely identified because of their clinical significance.

- Salmonella Paratyphi A (serogroup A), Salmonella Paratyphi B (serogroup B), Salmonella Choleraesuis (serogroup C1), and S Typhi (serogroup D).

- Salmonella serotypes Enteritidis and Typhimurium are the two most common serotypes reported in developed world.

Epidemiology

- Typhoid fever is sever systemic disease.

- Incidence differ significantly developing vs developed counties 0.2-4 cases to up to 500 /10^5 population.

- Humans are the natural reservoir. The feces of persons who have unsuspected subclinical disease or are carriers are a more important source of contamination than frank clinical cases that are promptly isolated, such as when carriers working as food handlers are "shedding" organisms.

- Many animals, including cattle, rodents, and fowl, are naturally infected with a variety of salmonellae and have the bacteria in their tissues (meat), excreta, or eggs.

- Food ,water contaminated with human faeces, vertical transmission (trans- placental).

Pathogenesis

- The vast majority of salmonellae, however, are chiefly pathogenic in animals that constitute the reservoir for human infection; these include poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others

- Stomach acidity and normal intestinal microbiota are important determinants of susceptibility.

- The salmonella invades peyer paches and transported to other intestinal L.N. where they multiply in Mononuclear cells to mesenteric L.N. to blood through thoracic duct (transient bacteraemia).

- Circulating organism reach reticule-endothelial cells in liver ,spleen and bone marrow and circulating endo -toxin cause prolonged fever.

- Inflame mucosa and lymphatics .Necrosis and sloughing of overlaying epithelium producing ulcer that may bleed. Ulcers heal without scarring.

- Cell mediated immunity is important

Clinical manifestations

- Incubation 7-14 days. Onset is insidious.

- 1st week

- Fever malaise ,anorexia myalgia headache, abdominal pain ,diarrhoea early and later constipation.

- Temp. increase in a stepwise fashion become unremitting and high (a high platuea).

- 2nd week

- High fever, fatigue, cough ,epistaxis. abdominal symptoms more sever, rose spots and rash.

- 3-4 weeks

- If no complications, symptoms & signs gradually resolve.

- In the pre-antibiotic era, the chief complications of enteric fever were intestinal hemorrhage and perforation, and the mortality rate was 10–15%.

Enterocolitis

- This is the most common manifestation of salmonella infection.

- In the United States, S Typhimurium and Salmonella Enteritidis are prominent, but enterocolitis can be caused by any of the more than 1400 group I serotypes of salmonellae.

- Eight to 48 hours after ingestion of salmonellae, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stools. Low-grade fever is common, but the episode usually resolves in 2–3 days. Inflammatory lesions of the small and large intestine are present.

- Bacteremia is rare (2–4%) except in immunodeficient persons.

- Blood culture results are usually negative, but stool culture results are positive for salmonellae and may remain positive for several weeks after clinical recovery.

Bacteremia with Focal Lesions

- This is associated commonly with S choleraesuis but may be caused by any salmonella serotype. After oral infection, there is early invasion of the bloodstream (with possible focal lesions in lungs, bones, meninges, and so on), but intestinal manifestations are often absent.

- Blood culture results are positive.

Diagnostic Laboratory Tests

- A. Specimens

- culture : positive in Blood, Bone marrow, Stool & Urine culture results may be positive after the second week.

- In enteric fevers, the stools yield positive results from the second or third week on; in enterocolitis, the stools yield positive results during the first week. A positive culture of duodenal drainage establishes the presence of salmonellae in the biliary tract in carriers.

B. Bacteriologic culturing for Isolation of Salmonellae

1. Enrichment cultures— The specimen (usually stool) also is put into selenite F or tetrathionate broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae.

2. Differential and Selective medium cultures—EMB, MacConkey, or deoxycholate medium. salmonella-shigella (SS) agar, Hektoen enteric agar and xylose-lysine decarboxylase (XLD) agar.

3. Final identification— Suspect colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera.

C. Serologic Methods

- 1. Agglutination test— In this test, known sera and unknown culture are mixed on a slide. Clumping, when it occurs, can be observed within a few minutes. This test is particularly useful for rapid preliminary identification of cultures. There are commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C1 , C2 ,D, and E.

Serologic Methods

- 2. Tube dilution agglutination test (Widal test)—

- Serum agglutinins rise sharply during the second and third weeks of S Typhi infection. The Widal test to detect these antibodies against the O and H antigens has been in use for decades.

- At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titer.

- Serial dilutions of unknown sera are tested against antigens from representative salmonellae. False-positive and false-negative results occur. The interpretive criteria when single serum specimens are tested vary, but a titer against the O antigen of greater than **1:320** and against the H antigen of greater than **1:640** is considered positive.

- High titer of antibody to the Vi antigen occurs in some carriers. Alternatives to the Widal test include rapid colorimetric and EIA methods.

- Results of serologic tests for Salmonella infection cannot be relied upon to establish a definitive diagnosis of typhoid fever and are most often used in resource poor areas of the world where blood cultures are not readily available .

Immunity

- Infections with S Typhi or Salmonella Paratyphi usually confer a certain degree of immunity.

- Reinfection may occur but is often milder than the first infection. Circulating antibodies to O and Vi are related to resistance to infection and disease. However, relapses may occur in 2–3 weeks after recovery despite antibodies.

- Secretory IgA antibodies may prevent attachment of salmonellae to intestinal epithelium.

- Persons with S/S hemoglobin (sickle cell disease) are exceedingly susceptible to Salmonella infections, particularly osteomyelitis. Persons with A/S hemoglobin (sickle cell trait) may be more susceptible than normal individuals (those with A/A hemoglobin).

Treatment

- Although enteric fevers and bacteremias with focal lesions require antimicrobial treatment, the vast majority of cases of enterocolitis do not.

- Antimicrobial treatment of Salmonella enteritis in neonates is important. In enterocolitis, clinical symptoms and excretion of the salmonellae may be prolonged by antimicrobial therapy. In severe diarrhea, replacement of fluids and electrolytes is essential.

- Antimicrobial therapy of invasive Salmonella infections is with fluoroquinolones, ampicillin, trimethoprim–sulfamethoxazole, or a third-generation cephalosporin.

- Multiple drug resistance transmitted genetically by plasmids among enteric bacteria is a problem in Salmonella infections.

- Susceptibility testing is an important adjunct to selecting a proper antibiotic. In most carriers, the organisms persist in the gallbladder (particularly if gallstones are present) and in the biliary tract. Some chronic carriers have been cured by ampicillin alone, but in most cases cholecystectomy must be combined with drug treatment.

Prevention and Control

- Three percent of survivors of typhoid become healthy permanent carriers, harboring the organisms in the gallbladder; biliary tract; or, rarely, the intestine or urinary tract.

- Sanitary measures must be taken to prevent contamination of food and water by rodents or other animals that excrete salmonellae.

- Infected poultry, meats, and eggs must be thoroughly cooked.

- Carriers must not be allowed to work as food handlers and should observe strict hygienic precautions.

- Two typhoid vaccines are currently available : an oral live, attenuated vaccine and a Vi capsular polysaccharide vaccine for intramuscular use.

- Vaccination is recommended for travelers to endemic regions, especially if the traveler visits rural areas or small villages where food choices are limited, efficacy of 50–80%.

The End