Enteric Gram-Negative Rods (Enterobacteriaceae)

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Enterobacteriaceae, enteric bacteria & may also be called coliforms (part of normal colon microbiome).

- large, heterogeneous group of gram-negative rods non-spore formers whose natural habitat is the intestinal tract of humans and animals. Even in tap water, we periodically check for E. Coli composition (by human/animal fecal contamination and whether it is safe for consumption or not).

- The family includes many genera (Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus, Morganella and others).

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

- All share Enterobacterial common core antigen (ECA) in cell wall.

- Notes:
- Those which are part of the normal microbiome cause disease under one/combination of the following cases:

1. Decreased immune status (opportunistic infection in this case).
2. Introduced to other site (e.g. can be transported from perineal flora to urinary tract, causing UTI such as cystitis & pyelonephritis). Similarly, during abdominal surgeries, it can cause peritonitis. (Remember, in most cases, it is your own E. Coli).
3. At the same site (i.e. the colon), it gains new virulence factors through bacteriophages or transmissible plasmids.

Neonatal meningitis and enterocolitis are the only cases where the E. Coli infection could be exogeneous.

Enterobacteriaceae

- The most common group of gram-negative rods cultured in clinical laboratories, causes any disease you can think of (70-80% of UTI, >30% of bacteremia infections and sepsis/septicemia by G-ve bacteria, many gastroenteritis diseases and so on). Along with staphylococci and streptococci are among the most common bacteria that cause disease.

- They are either motile with peritrichous flagella (AKA H-antigen) (the majority) or nonmotile (Shigella, Klebsiella 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 Plesiomonas) and reduce nitrate to nitrite; and have a 39–59% G + C DNA content.

- Notes:
- So, all Enterobacteriaceae share common characteristics:

1. All have ECA
2. They are G-ve and, thus, have LPS in their composition.
3. Have ability to ferment glucose
4. Reduce nitrate to nitrite
5. Oxidase-negative, distinguishes them from Pseudomonas.
6. Catalase-positive.

Antigenic Structure

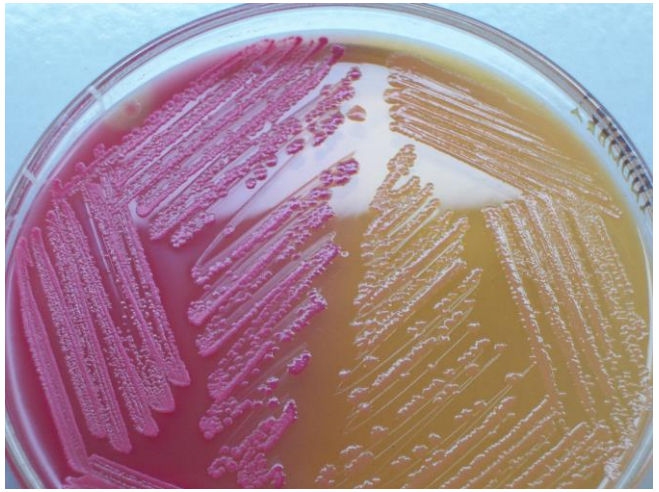
- Heat-stable somatic O (lipopolysaccharide) antigens. (outer membrane oligosaccharides) are detected by bacterial agglutination. Antibodies to O antigens are predominantly IgM.

- Heat-labile K (capsular) antigens (to prevent phagocytosis and serum killing), this is applied to Klebsiella and Enterobacter cloacae. 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 (motile Enterobacteriaceae, like E. Coli). agglutinate with anti-H antibodies, mainly IgG .
- Salmonella serotype Typhi (Causes Typhoid Fever), the capsular antigens are called **Vi** antigens. (called Vi because not only does resist serum killing but it also is a virulence factor).
- Many gram-negative organisms produce Colicins (bacteriocins). (peptides liberated from E. Coli against other strains of E. Coli to reduce competitons, called bacteriocins if against strains other than E. Coli. Many antibiotics have been derived from them).

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.

MacConkey Agar



EMB

- Notes:
- EMB & MacConkey Agar both have lactose. For example, MacConkey has bile and bile salts to prevent commensal and G+ve bacteria from growing. If growing strains have lactose-fermenting abilities, they turn neutral dyes to a pinkish color, while non-fermenters give colourless colonies.
- On EMB, non-fermenters (e.g. Shigella, Salmonella) give dark-nucleated colonies with black center, while fermenters (e.g. E. Coli) give green colour, also called green metallic sheen.
- Summary: Lactose-fermenters give pink and green colonies on MacConkey and EMB agars, respectively. Non-fermenters give colourless and dark-nucleated colonies on MacConkey and EMB agars, respectively.

Enteropathogenic E coli (EPEC)

- A major cause of infantile diarrhea (less than a year old), associated with outbreaks of diarrhea in nurseries (also affects bottle-fed infants, major cause of diarrhea) especially in developing countries. Outbreaks in nurseries → person-to-person transmission is very highly likely by EPEC. In adults, it needs high doses so it doesn't cause an outbreak, while for infants it is the opposite.

- Pathogenicity requires two important factors, (attachment and effacement) : the bundle forming pilus encoded by a plasmid**,** EPEC adherence factor (**EAF**) for attachment in small intestine 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. Those with signs of dehydration need hospitalization and fluid replacement/respiration.
- 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. no outbreak→ person-to-person transmission highly UNLIKELY. Most likely caused by vegetables or meat with marinated sauces contaminated with ETEC.
- 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 (has 2 types: A and B, A affects humans - (MW, 1500–4000), activates guanylyl cyclase -and heat-labile exotoxin (LT) (has 2 types: 1 and 2, 1 affects humans)- where it activates adenylyl cyclase - . Leading to increased local concentration of cyclic Guanyl and Adenosine monophosphate cGMP, cAMP respectively. Result: Increased secretion of fluid and electrolyte into gut

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 & immunogenic while ST is antigenic but not immunogenic (that's why people infected with LT might develop short-term immunity in the form of secretery IgA, as well as Ig against colonization factor, which explains why locals eating the same contaminated food would not be infected) 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), also called Very Cytotoxic E. Coli

- Named for the cytotoxic toxins they produce (Shigella-like toxin). Linked to consumption of of fresh products (e.g., lettuce, spinach, sprouts) and of undercooked ground beef (hamburgers). Because EHEC strains are part of the normal flora in cattles and can contaminate vegetables with their 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. Type 2 more severe than type 1. Homology between Shiga-like toxin 1 and the Shigella toxins produced by Shigella dysenteriae is almost 100%, Type 2 only 80%.
- STEC has been associated with hemorrhagic colitis, a severe form of diarrhea (bloody diarrhea, but without invasion, rather through elaboration of Shigella toxin), and with hemolytic uremic syndrome HUS (higher probability in children <5 years old); a disease resulting in micro-angiopathic hemolytic anemia, acute renal failure and thrombocytopenia.
- Of the E coli serotypes that produce Shiga toxin, O157:H7 (also O104:H4, both of which can cause outbreaks) is the most common and is the one that can be identified most readily in clinical specimens.

- Notes:
- The toxin binds to GP3 receptors in kidney glomeruli and the vascular endothelium, causing micro-thrombosis. RBCs cannot pass through that portion→ break down and excreted with feces. Thrombocytopenia because platelets try to seal the injured endothelium.
- EHEC is a nightmare for children because, while 50-60% recover spontaneously, 10% die and the remaining 30% have chronic renal failure, so they will spend the rest of their lives with kidney dialysis → poor quality of life.

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 (no invasion of mucosa and submucosa); absence of fever can incorrectly lead to consideration of noninfectious conditions (e.g., intussusception or ischemic bowel disease) which are emergency cases.

- 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.

- Notes:
- EHEC affects the large intestine.
- In EHEC, though they have abdominal cramps, we do not give them anti-motility agents (e.g. buscopan, Spasmopan®).
- Though they have severe pain, we do not give them strong painkillers, such as morphine, because the patient would become drowsy and sleep, so that, if they have intussusception, they would be awake and would have hemorrhage and perforation and even TSS because the bowel contents have been leaked to the peritoneum. So, if the patient were asleep, it would be discovered too late for surgeons to resect and anastomose the gut.
- Important note: Antibiotics might help in all E.Coli gastroenteritis infection except EHEC, it is contraindicated because it stresses EHEC to elaborate more toxins. It also increased the probability to develop HUS multiple folds.

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 (Vero cells die if STEC are present) and polymerase chain reaction for the direct detection of toxin genes directly from stool samples. Remember also all E.Coli ferment lactose, but even if we replace lactose with sorbitol, they all ferment it EXCEPT EHEC, 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. (apple cider because apples could fall from trees and become contaminated by cattle feces).
- Antibiotics may increase the risk for HUS. Contraindication

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) to cause disease. In shigellosis, a few hundred Shigella are enough to cause the disease.

- EIEC produce disease by invading intestinal mucosal epithelial cells. It is the prototype of invasive bacterial diseases.

- Like EHEC, EIEC affect the large intestine. They cause bloody diarrhea and fever.

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. hence the name enteroaggregative.

- 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. They also have pathogenicity island.

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

- Notes:
- Its diagnosis is difficult since it doesn’t have a single biomarker, so we have to exclude all other diarrheagenic E. Coli strains.
- Since they produce Shiga-like toxins (ST-like toxins), then they can also cause HUS, just like Shigella, but it is not as severe as those infected with Shigella or EHEC.

- 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. They cause watery diarrhea.
- EIEC, STEC cause inflammatory diarrhea. They cause bloody diarrhea.
- Inflammatory and non-inflammatory are interchangeable terms with bloody and watery diarrhea, respectively.
- Inflammatory = WBC, bloody = RBC. If WBC+RBCs found in mucus, they are called dysentery.
- Remember: diarrhea could be bloody even if blood cannot be seen grossly. We use test called occult blood test to confirm.

Treatment

- Treatment of gram-negative bacteremia and impending septic shock requires rapid restoration of fluid and electrolyte balance (main cornerstone in management of gastroenteritis regardless of cause), 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 (ETEC), 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). If there is no need to install catheter, it would be better for the patient to avoid E. Coli.

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 (catalase positive, oxidase negative); coccobacillary forms occur in young cultures (However, in late culture, they become bacilli, hence the name "Bacillary dysentery"). 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 (Dr. said they are late-lactose fermenters; they need more than 1 week to ferment lactose). The inability to ferment lactose distinguishes shigellae on differential media
- Non-motile (Salmonella are motile), non-lactose fermenters (another difference from Salmonella is that they ferment other carbohydrates), do not produce H2S (nor gas, while Salmonella do), and produce a colorless colonies in EMB (in MacConkey agar as well).
- Remember: always, Shigella is the prototype of invasive bacterial diseases in GI and causes inflammatory diarrhea (WBC + RBC + mucus = dysentery).

Epidemiology

- Man (exclusive human disease, humans are the reservoir) and certain primates are the only host.
- Age: any age (60-70% under 10y/o) but commonly under 5 y/o (exclusive paediatric disease). It can occur in older patients in contact with these children, in homosexuals. It causes outbreaks in schools and nurseries, as well as custodian institutions (e.g. jails)).
- It occurs in warm months, temperate climates and rainy seasons in tropical countries.
- Asymptomatic/very mild infection in endemic areas, especially developing countries (no bloody diarrhea as in sporadic cases).
- In industrialized countries , S.sonnei is most common with S.flex second. S. flex is the most common cause in developing countries.
- 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 severe disease.
- Group B Shigella flexneri 8 serotypes mild disease.
- Group C Shigella boydii 18 serotypes.
- Group D Shigella sonnei single , intermediately severe disease .
- All species produce Shiga toxin which is encoded by a bacteriophage (phage absent = no toxin production). Prime, fundamental and cardinal feature of Shigella infection is invasive disease of mucosa and submucosa. Thus, this invasion is responsible for the bloody diarrhea and not the toxins, they only act synergistically along with the invasion.

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 small intestinal epithelial cells (eg, M cells in MALT) by induced phagocytosis, escape from the phagocytic vacuole, multiplication and spread within the epithelial cell cytoplasm (another difference from Salmonella which stays in the endosome and replicates there), 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 micro-abscesses and “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 (remember they are G-ve)
- Upon autolysis, all shigellae release their toxic lipopolysaccharide, especially by lipid A. This endotoxin probably contributes to the irritation of the bowel wall.

B. Shigella Dysenteriae Exotoxin (most severe one). We have different molecular forms and different quantities, producing variable symptoms.

- S dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that is neurotoxic (only seen with Shigella, not STEC), cytotoxic (Remember, we said they can kill Vero cells and enterocytes as well) and enterotoxic (acts synergistically with invasion to produce bloody diarrhea and increased frequency of bowel movement.
- 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, seizures and convulsions at first (one of the DDx with diarrheal diseases) and, then, 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 (effect of invasion, synergized by toxin). However, they are rarely recovered from blood despite their neurologic effects.
- 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 (thus, fluid and electrolyte replacement is the main therapeutic approach and, then, antibiotics), 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 (1% of infected people) and shed Shigella, even if antibiotics were administered and may have recurrent bouts of the disease. Upon recovery from the infection, most persons develop circulating antibodies (only against corresponding (homologous) serotypes. In other words, against the type that they were infected with. So, they can be reinfected if this serotype gains new attributes by some new phages) to shigellae, but these do not protect against reinfection. (very short lived, 3-4 years only)

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 (definitive isolation and identification for Shigella and salmonella, as well)
- The materials are streaked on differential media (eg, MacConkey or EMB agar, circular, transparent, colourless, convex colonies) and, then, subculture on selective media (Hektoen enteric agar or Salmonella –Shigella agar), followed by final agglutination and confirmation of Shigella identification which suppress other Enterobacteriaceae and gram-positive organisms.
- C. Serology (mix antibodies in lab with Shigella from culture for final identification of genus, species and even serotypes)
- 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 and fluoroquinolones are most commonly inhibitory for Shigella isolates and can suppress acute clinical attacks of dysentery and shorten the duration of symptoms and, most importantly, prevent secondary transmission, especially through household contact. (While in EHEC antibiotics are not given, here, we do give them).

- They may fail to eradicate the organisms from the intestinal tract. (keep transmission cycle going)

- Multiple drug resistance can be transmitted by plasmids, and resistant infections are widespread. Many cases are self-limited.

- Opioids and antiperistalsis agents should be avoided in Shigella dysentery. (To prevent masking effect (something is happening inside the body but it is not apparent) and also, it increases the shedding period and, even, symptomology period).

Prevention, and Control

- IgA antibodies in the gut may be important in limiting reinfection (cornerstone in GI immunity)
- Serum antibodies to somatic Shigella O-antigens are IgM. (Short-lived immunity)
- Shigellae are transmitted by “food, fingers, feces, and flies” (the 4 F’s) from person to person (control measures should be directed at the 4 F’s). 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 G-ve, facultative anaerobes

- Salmonellae are often pathogenic for humans (chronic intestinal carriers, especially in biliary tree in people who have stones, 1-5%) or animals (major reservoir host, especially in poultry (intestinal tract of chickens. It is found in meat and dairy products, reptiles, birds and even rodents) when acquired by the oral route.
- They are transmitted from animals and animal products to humans, where they cause enteric (Typhoid) fever (most **severe** form of Salmonellosis, caused by typhoidal Salmonella, exclusive human disease, caused by chronic human carriers only who are the reservoir) ,gastro- enteritis (enterocolitis, most **common** form of Salmonellosis) and systemic infection (transient bacteremia and focal lesions in brain (meningitis), heart (endocarditis), lungs (pneumonia) and bone (osteomyelitis)).
- 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. (motility, H2S and gas formation are the main differences from Shigella)
- 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, infects humans and warm-blooded animals, and bongori, infects cold-blooded animals), and subspecies, also called subtype (e.g., typhi or enteritidis)

Subspecies o f Medical Importance

- S. enterica subsp. Typhi. (typhoidal Salmonella) cause Typhoid fever
- S. enterica subsp. Enteritidis cause Salmonella gastroenteritis/enterocolitis
- S. enterica subsp. Typhimurium cause Salmonella gastroenteritis/enterocolitis (Typhimurium and Enteritidis most common species isolated worldwide, including USA)
- S. enterica subsp. Choleraesuis common cause of bacteremia and focal lesions
- S. enterica subsp. Paratyphi (Typhoidal Salmonella) cause typhoid fever
- S. enterica subsp. Dublin cause gastroenteritis, commonly isolated in Europe

The “Enteric Fevers” (Typhoid Fever) systemic disease

we have typhoidal Salmonella and non-typhoidal Salmonella

- 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.

- Typhoidal Salmonella: Salmonella Paratyphi A (serogroup A), Salmonella Paratyphi B (serogroup B), Salmonella Choleraesuis (Dr. said here, it should be Paratyphi) (serogroup C1), and S Typhi (serogroup D).

- Salmonella serotypes Enteritidis and Typhimurium are the two most common serotypes reported in developed world. This sentence, also, shouldn’t be here as these serovars (serotypes) cause enterocolitis, not Typhoid fever.

Epidemiology

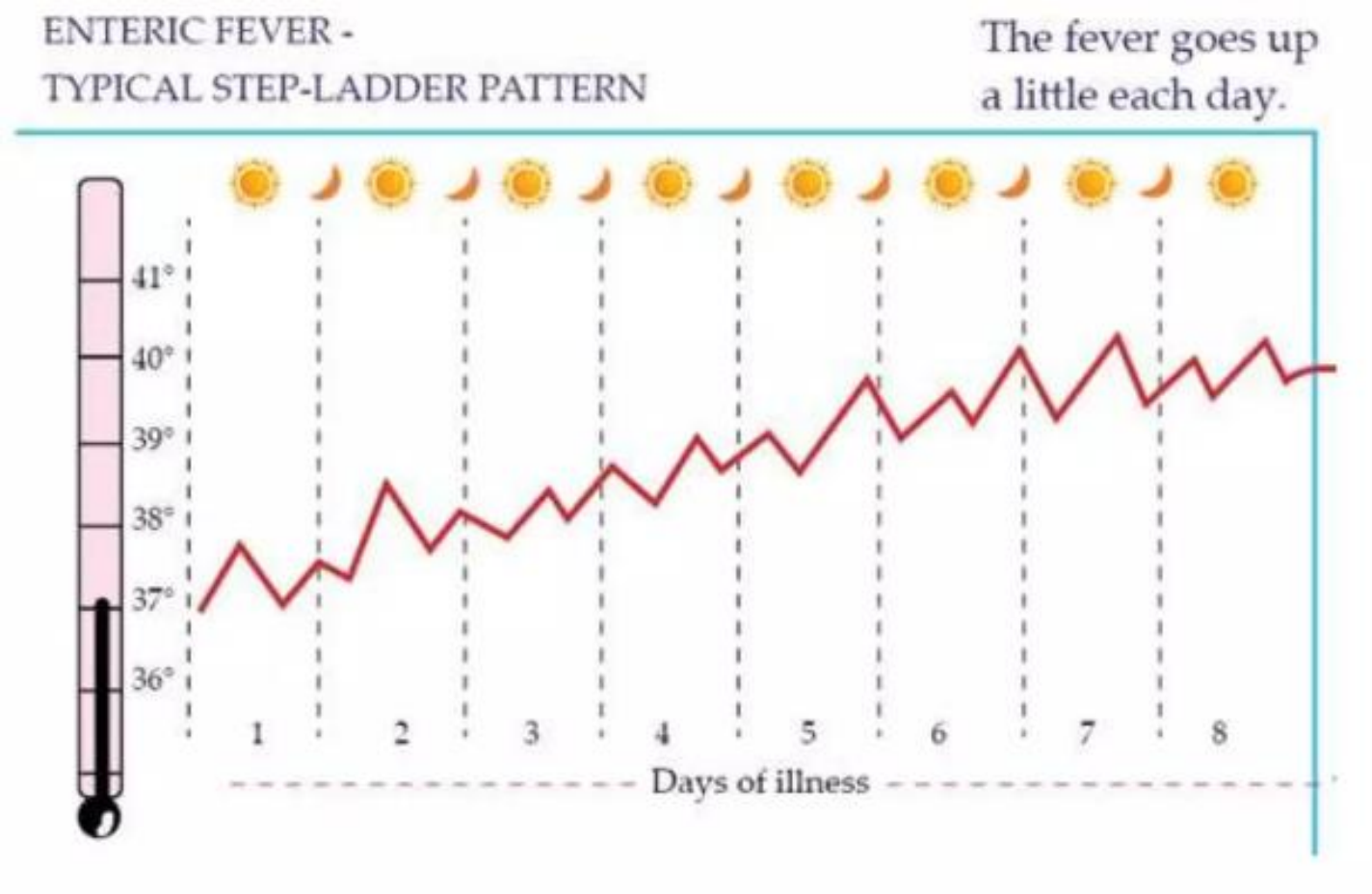
- Typhoid fever is sever systemic disease. (with antibiotics, death rates decreased significantly; pre-antibiotic era: 15-20%, post-antibiotic era: <1%).
- Incidence differ significantly developing vs developed counties 0.2-4 cases to up to 500 in developing countries /10^5 population. (around 0.5mil deaths yearly)
- 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 (feco-oral route), 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 (M cells), remain in endocytic vacuole, where they replicate, ingulfed by macrophages and transported to other intestinal L.N. through lymphatics and to bone marrow, liver and spleen through blood where they multiply in Mononuclear cells to mesenteric L.N. to blood through thoracic duct (transient bacteraemia). In Shigella, disease confined to intestinal tract.
- Circulating organism reach reticule-endothelial cells in liver ,spleen and bone marrow and circulating endo -toxin cause prolonged fever. (Chief complaint)
- Inflame mucosa and lymphatics .Necrosis and sloughing of overlaying epithelium producing bloody diarrhea 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. Most importantly, we have no GI symptoms.
- Temp. increase in a stepwise fashion become unremitting and high (a high platuea).
- 2nd week
- High fever, fatigue, cough ,epistaxis (lung involvement) . abdominal symptoms more sever (starts as watery diarrhea and then bloody, alternating bowel habits), rose spots (on trunk, ¼ of patients) 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%. Some others carry Salmonella as part of their normal tract microbiome, especially in people with cholelithiasis



The stepladder form of fever. Temperature increases then decreases below what the previous temperature was. Then, it increases to reach a temperature higher than before.

Enterocolitis/Gastroenteritis

- This is the most common manifestation of salmonella infection.

- In the United States, S Typhimurium and Salmonella Enteritidis are prominent (Dublin in Europe, FYI Dublin is the capital of Ireland which is in Europe), 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. Incubation period is 2-3 days, like Shigella. They cause superficial ulcerations and trigger inflammatory response and subside within 5-7 days. It is usually associated with poultry, as we said, and cutting boards infected with Salmonella.

- Bacteremia is rare (2–4%) except in immunodeficient persons (like cancer patients and congenitally immunodeficient patients)

- Blood culture results are usually negative, but stool culture results are positive for salmonellae (in typhoid fever, in the 1st 2 weeks, blood culture is +ve while stool culture is –ve) 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 transient invasion of the bloodstream (with possible focal lesions in lungs, bones (osteomyelitis, especially in children), meninges, and so on), but intestinal manifestations are often absent.
- Remember, Staph. Auerus is the most common cause of osteomyelitis, generally, and Salmonella is the most common cause in children and sickle-cell disease patients (careful, not those who carry the trait but, rather, those who are sick with it).
- Blood culture results are positive and –ve stool culture.

Diagnostic Laboratory Tests Bacterial isolation and identification is the definitive Salmonella diagnosis

- 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 to confirm chronic carrier trait but it requires sophisticated procedures and sophisticated labs to carry out

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 (differential cultures for non-lactose fermenters). salmonella-shigella (SS) agar, Hektoen enteric agar and xylose-lysine decarboxylase (XLD) agar. (selective agars)

3. Final identification of genus, species and subspecies— Suspect colonies from solid media are identified by biochemical reaction patterns and slide and tube agglutination tests with specific sera.

The numbers here indicate order of steps, not different choices for identification.

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 (from the patient's serum)against the O and H antigens and even capsular antigens (Vi antigen in S. Typhi) 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 (cross reactivity by antibodies against other GI infections (e.g. E. Coli, Shigella, Yersinia, Vibrio Cholera, etc., and if one was vaccinated) . 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. Short-lived, 1-2 years at max.
- 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).

- Note: all Salmonella species (those causing Typhoid fever, enterocolitis and bacteremia) can result in bacteremia but it is usually associated with Typhi, Paratyphi and Choleraesuis.

Treatment

- Although enteric fevers and bacteremias with focal lesions require antimicrobial treatment, the vast majority of cases of enterocolitis do not, just fluid and electrolyte replacement. Of course, there are exceptions (newborns, elderly, immunocompromised and severely dehydrated patients).
- 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. Remember that humans are the only reservoir and the only to be infected with Typhoid fever, so those chronic carriers keep the transmission cycle going.

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. Should also be careful with sauces and mayonnaises.
- Carriers must not be allowed to work as food handlers and should observe strict hygienic precautions.
- Two typhoid vaccines are currently available against Typhi and Paratyphi: an oral live, attenuated vaccine and a Vi capsular conjugated 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%. Lasts for <2 years.

The End

Good Luck!