Microbiology

Doctor 2017 | Medicine | JU

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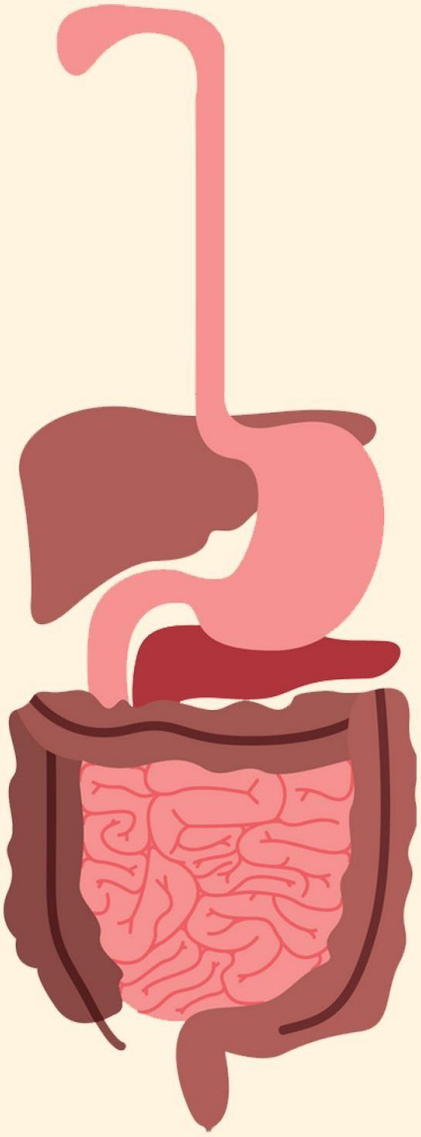


2nd system - GI

Note: focus is on GI diseases.

Outline:

Family: Enterobacteriaceae

Genus; (first 2 discussed in this lecture)

1-E. coli 2-Shigella 3-Salmonella 4-Yersinea

Enterobacteriaceae:

- Enteric bacteria & may also be called coliforms.
- Large, heterogeneous group of G-ve rods whose natural habitat is the intestinal tract of humans and animals.
- The **family** includes many **genus's** (**Escherichia**, **Shigella**, **Salmonella**, **Yersinia** Enterobacter, Klebsiella, Serratia, Proteus, and others),we will discuss 4
- Some enteric organisms, such as Escherichia coli, are part of the normal microbiota, they are mostly always harmless and only **incidentally** cause disease, but others, like salmonellae and shigella, are **always pathogenic** for humans. (Nonpathogenic E. coli strains can become pathogenic if they gain virulence factors by way of either plasmid or phage.)
- They are the most common group of G-ve rods cultured in clinical laboratories.
- They are among the most common bacteria that cause disease along with staphylococci and streptococci.

Common characteristics of all Enterobacteriaceae:

1. They are ubiquitous (can be found in water, soil & intestinal tract of animals and humans (part of intestinal flora)).
2. They are all **motile** with peritrichous flagella except for **Shigella**, **Yersinia** and **Klebsiella**.
3. They grow aerobically and anaerobically (are **facultative anaerobes**), a diagnostic feature from other gut commensals.
4. They have **a rapid growth rate** (e.g. E. coli double every 20 min)
5. **Ferment** rather than oxidize **glucose**, often with gas production
6. **Oxidase negative** (except for Plesiomonas) and **catalase positive** and reduce nitrate to nitrite.
7. They grow on peptone or meat extract media ,also grow well on MacConkey agar
8. Have a 39–59% (G + C) DNA content.
9. **They have two types of Pilli/fimbriae**

a) Common chromosomal mediated (attachment to surfaces)

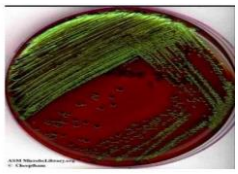
Note: focus is on GI diseases.

b) Plasmid mediated F or sex pilus (stabilizes mating bacteria during the process of **conjugation and transfer of genetic material).**

10. Are either lactose fermenters (E. coli) or non-lactose fermenters (shigella and salmonella)

11. Eosin methylene blue EMB or MacConkey agar (differentiate lactose fermentation)

	Lactose fermenters	Non-lactose fermenters
MacConkey	Pink	Colorless
EMB	Dark color ***E.coli specifically forms green sheen colonies***.	Colorless



12. Have heat stable **LPS** (lipopolysaccharide, principal component of G-ve bacterial cell wall /endotoxin), composed of 3 parts:

a) O (oligosaccharide), outermost somatic antigen, used for serologic classification of Enterobacteriaceae family.

b) Common enterobacterial antigen, core polysaccharide.

c) Lipid A, endotoxin activity of LPS.

13. **Antigenic structures used for serologic classification:**

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

b) **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.

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

d) Salmonella serotype Typhi, the capsular antigens are called **Vi antigens** instead.

e) Many G-ve organisms produce Colicins (bacteriocins).

Bacteriocins are pore forming toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain (e.g. Colicins are produced by E.coli)

Note: focus is on GI diseases.

14. Produce **Enterobactin** /Aerobactin, typical iron-chelating compound (siderophores), why is it needed? Iron in the body is always bound so to counterpart this binding these bacteria produce their own siderophores as they use iron for growth.
15. In general any gastroenteritis treatment is supportive by giving fluid and electrolyte replacement plus antimicrobials which are thought to shorten duration of treatment and reducing shedding ,(except in any shiga-toxin producing bacteria *,discussed later)*

End of common features of Enterobacteriaceae family

1st genus: E.coli

- Responsible for 80% of UTI (uropathogen), >30 -50% of septicemia, major cause of neonatal sepsis and meningitis, cause gastro-enteric and diarrheal disease (diarrheagenic).
- A member of the **normal intestinal microbiota**, (endogenous microbiota) & in small numbers as part of the normal microbiota of the upper respiratory and genital tract.
- That's why almost all infections are **endogenous** except in both neonatal meningitis and neonatal gastroenteritis because E.coli are still not resistant at the time so they gain access when passing though vaginal canal.
- **All E. coli ferment sorbitol except EHEC, diagnostic feature.**
- Have 5 different discovered virulence factors each causing disease in a different mechanism (2 examples bellow)

a) The small or large bowel epithelial cell **adherence** properties are encoded by genes on plasmids.
b) The **toxins** often are plasmid or phage mediated.

- Our focus is the **5 diarrheagenic strains** and their distinguishing features Note: In each diarrheal strain we will classify the diarrhea is inflammatory or noninflammatory diarrhea:
    a) Inflammatory: stool contains RBC, WBC, blood and mucus.
    b) Non inflammatory: watery diarrhea /secretory.

10 minutes

Note: focus is on GI diseases.

The five diarrheal strains of E.coli:

1. **Enteropathogenic E coli (EPEC)**

- A major cause of **infantile diarrhea** (<1 year old), associated with outbreaks of diarrhea in nurseries especially in developing countries,
- Unusual(but can occur) in adults as it has a **large** inoculum(10^8) · Affects bottle fed infants more than breast fed.
- Transmission by contaminated water and food (feco-oral)
- **Pathogenicity (formation of lesions)requires two important factors, (attachment and effacement)**

  1- Initial **attachment** facilitated by the bundle forming pilus encoded by a plasmid**, this factor is called, EPEC adherence factor (EAF)**

  2-after that: **effacement**, which is destruction and degeneration of brush border (microvilli) and this promotes the tight adherence characteristic of EPEC, this factor is encoded by **chromosomal locus of enterocyte effacement (LEE) pathogenicity island.**
- Onset **is rapid, few hours**
- The result of EPEC infection in infants is severe, **watery diarrhea**; vomiting;
- abdominal cramps, nausea and fever. Diarrheal stool often contains mucus but not blood.
- It is usually **self-limited** within few days but can be prolonged or chronic.
- Fluid replacement, (water and electrolyte) required as with all watery diarrhea to prevent dehydration.
- EPEC diarrhea has been associated with multiple specific serotypes of E coli; strains · 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.

2. **Enterotoxigenic E coli (ETEC)**

- A common cause of "**traveler's diarrhea**" and a very important cause of diarrhea in infants in developing countries (environmental sanitation is poor).
- Feco-oral route of infection (infected food or water) and is most commonly acquired from salads and marinated meats.
- Direct person-person spread is unusual because large infectious dose is needed.
- Pathogenesis: **colonization** and then produce toxin in the intestine.

Note: focus is on GI diseases.

- For colonization they use 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 2 types of **exotoxins**:
  - Heat stable (**ST**) (a&b) usually not immunogenic, activates guanylyl cyclase.
  - Heat-labile (**LT**) (1, 2&3) immunogenic, activates adenylyl cyclase.
- Increased local concentration of cyclic Guanyl and Adenosine monophosphate cGMP, cAMP respectively, thus blocking of Na+ reabsorption leading to hypersecretion of water and chlorides into the lumen by osmosis → **watery diarrhea**
  - Incubation: 1-2 days lasting for several days (3-5).
- **Self-limited**, No invasion, No inflammatory process.

Immunity against ETEC might develop, unknown for how long (factors contributing to this immunity are **LT** and **CF** factor). 20 minutes

3. STEC /EHEC/VTEC

- **STEC: Shiga toxin-producing E coli** , 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 Shiga toxin has **AB** configuration, binding of toxin to receptor with **B**inding subunit followed activation of toxic effect by **A**ctive subunit →inhibition of protein synthesis.
  (Shiga like toxin 1 is identical to the one produced by shigella genus).
- **EHEC: Entero-hemorrhagic E.Coli →bloody** inflammatory diarrhea, unlike EPEC and EHEC due to **inflammation** of **mucosa**
- **VTEC: Verotoxin-producing E.Coli,** as they produce immediate cytotoxic and cytopathic effect on vero cells (cell line monkey cells used in lab culture).
- The highest incidence of shiga-toxin producing E.coli infections is in children <5-10 yrs.
- Linked to consumption of fresh products (e.g., lettuce, spinach, sprouts) and of undercooked ground beef (hamburgers).
- **Not part of human Microbiota (unlike EPEC and ETEC), and main reservoir is gut microbiome of cattle** (drop apple phenomenon, picking up an apple without washing, contamination with feces of cattle on the ground).
- Direct person-person spread is common since the infectious dose is low.
- The lesions are in the **colon**, unlike EPEC and EHEC.

Note: focus is on GI diseases.

- Shiga-toxin causes capillary thrombosis and inflammation of the colon's mucosa leading to hemorrhagic colitis (severe form of diarrhea).
- **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).
- Also associated with hemolytic uremic syndrome **HUS.**
- We are **afraid** of the sequel **HUS, a disease resulting in micro-angiopathic hemolytic anemia, thrombocytopenia and acute renal failure**. (Thought to be caused by capillary congestion due to toxin production, which has receptors in the kidney and when the RBC's transverse through they are hemolyzed).
- Of the EHEC, **O157:H7** is the most common and is the one that can be identified most readily in clinical specimens. ▪ Diagnosis:

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

b) **Also, Sorbitol MacConkey agar plate is used since EHEC is the only E. coli that doesn't ferment sorbitol so won't grow on the plate, differentiate it from other E.coli strains.**

c) **Cell culture cytotoxin testing using Vero cells.**

d) **Polymerase chain reaction for the direct detection of toxin genes directly from stool samples**.

e) Treatment:

f) As mentioned before, **antimicrobials** and **antibiotics** are **CONTRAINDICATED** in **STEC** why? It's thought that any stressed or dying STEC will produce more toxin increasing chance of developing HUS.

g) **Opioids** and **anti-motility** agents (reduce motility of colon) are also contraindicated as they also mask the symptoms, the signs and the compilations. So just give fluid and electrolyte replacement and monitoring patient by frequent kidney function test and complete blood count.

h) Mostly self-limited without the sequel (HUS).

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.

Note: focus is on GI diseases.

4.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.**
- EIEC require **large inoculum** (10^8–10^{10} CFU), unlike **shigella** (<100 bacteria inoculum).
- **Pathogenesis**: **Invasion** of intestinal mucosal epithelial cells (O124, O143 are serotypes associated with invasion)
- Starts with watery diarrhea, when invasion occurs→ **inflammatory bloody diarrhea.**
- Accompanied by abdominal cramps, fever, nausea and vomiting.
- Opioids and anti-motility agents are contraindicated as with any invasive pathogenesis as they masks the disease.

4. Enteroaggregative E coli (EAEC)

- They are characterized by their specific patterns of **adherence** to human cells. The organisms exhibit a diffuse or "**stacked-brick**" pattern of adherence (agglutinate) to small intestine epithelial cells and sometimes to the colon.
- 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** (the commonest strain in HIV patients).
- 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.
- Clinical manifestation varies from watery to bloody diarrhea, inflammatory cells accompanied by fever and vomiting.

30 minutes

Summary:

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

EIEC, STEC cause inflammatory diarrhea (either due to production of a toxin or due to invasion).

Note that bloody diarrhea differential diagnosis is larger and include (Salmonella, Shigella)

Note: focus is on GI diseases.

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.

Treatment of E.coli infections

- Treatment of G-ve bacteremia and impending septic shock requires rapid **restoration of fluid and electrolyte balance**, institution of antimicrobial therapy (**except STEC**) and treatment of disseminated intravascular coagulation.
- No single specific therapy is available. The sulfonamides, ampicillin, cephalosporins, tetracycline (doxycycline) fluoroquinolones (and aminoglycosides have marked antibacterial effects against the enterics, but variation in susceptibility is great.
- Trimethoprim-sulfamethoxazole known in Jordan as cotrimoxazole is used and laboratory tests for antibiotic susceptibility are essential.
- Multiple drug resistance is common and is under the control of transmissible plasmids, ciprofloxacin, a fluoroquinolone, very commonly used and very high resistance against it.

Prevention of E.coli infections

- Avoid under-cooked food, and caution to be observed in regard to food and drink in areas where environmental sanitation is poor.
- Prophylaxis before treatment.
- To prevent travelers' diarrhea, daily ingestion of **bismuth subsalicylate suspension.** (bismuth subsalicylate **(also called pink dye)** can inactivate E.coli enterotoxin in vitro) and regular doses of tetracyclines (doxycycin) or other antimicrobial drugs for limited periods.

Control of E.Coli infections

- 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, especially with UTI's. 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, it can also colonize genital tract after being transmitted through sexual intercourse.

- Their control depends on handwashing, rigorous asepsis, sterilization of equipment, disinfection, restraint in intravenous therapy, strict precautions in keeping the urinary tract sterile (ie, closed drainage also during urinary tract catheterization).

2nd genus: Shigella (some repetition of characteristics)

- Disease: **shigellosis** also called **bacillary dysentery**.

- Shigella are slender G-ve rods of the family Enterobacteriaceae.
- Coccobacillary forms occur in young cultures, **facultative anaerobes** but grow best **aerobically.**
- Convex, circular, transparent colonies with intact edges reach a diameter of about 2 mm in 24 hours (short doubling time as previously mentioned in the common features).
- In contrast to E.coli, **they are non-motile, and they don't ferment lactose**. The inability to ferment lactose distinguishes shigella on differential media.
- **Don't produce H_2S and produce a colorless colonies in EMB.**
- **All shigella ferment glucose.** With the exception of **Shigella sonnei.**
- The natural habitat of shigella is **limited to the intestinal tracts of humans and other primates**, where they produce bacillary dysentery.
- **Shigella is resistant to stomach acidity.**
- Shigella infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare.
- Shigella are highly communicable; the infective dose is on the order of less than 10^3 organisms (small inoculum 10-1000)
- **Epidemiology**: Man and certain primates are the only host, and shigellosis is strictly a human disease with only a human reservoir.
- **Age**: any age but commonly under 5 y/o, adults less frequent, usually in **homosexual adults** and in adults who are in close contact with infected children.
- Most common cause of bloody diarrhea in children.
- It occurs in warm months, temperate climates and rainy seasons in tropical countries.
- **Transmission**: feco-oral route, person to person, toilet seat, door handles, contaminated food and water supply and a vector causing outbreaks: flies maybe.
- **Asymptomatic** infection in endemic areas.

Note: focus is on GI diseases.

- **Etiology**: The genus shigella is subdivided into **4** species (A, B, C and D) according to their biochemical reaction and antigenic composition.
- Group A Shigella **Dysenteriae** 12 Serotypes, most imp. type1 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.
- The most common cause of shigellosis in developed world is S.**sonnei,** and S.**flexanari** in Jordan and the developing world.
- **The cornerstone of Pathogenesis in shigella is invasion** of the mucosal epithelial cells (M cells) **after passing stomach acidity,** plus **shiga toxin production** which worsens symptoms and signs of the disease.
- Mechanism of invasion: trans cytosis induced by phagocytosis through the M cells (lack glycocalyx), escape endocytic vacuole, multiplication and spread within the epithelial cell cytoplasm, hijack actin and microtubule cytoskeleton to propel them to adjacent cells (to compensate non motility) and then invade adjacent cells horizontally, in contrasts to salmonella which invade vertically also remaining in endocytic vacuole: (M cells →lamina prop → lymh nodes →liver and spleen)
- 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 "pseudo membrane" 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.
- Note: in shigellosis the main pathogenesis is the invasion of shigella not the shigella toxin itself, so in this case antimicrobial therapy is given to the patient unlike STEC.
- **Toxins:**

A. **Endotoxin** Upon autolysis, all shigella 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 heatlabile exotoxin that is **neurotoxic**, **cytotoxic** and **enterotoxic**. As an enterotoxin, it produces **diarrhea** as does the E coli Shiga-like toxin**:** (A and B configuration), Gb3 receptor in intestine and renal tissue → protein synthesis inhibition→HUS. A "**neurotoxin**," because of **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 shigella in dysentery. Both may act in sequence, the toxin producing an early non-bloody, voluminous diarrhea and the invasion of the large intestine, resulting in later dysentery with blood and pus

  in stools. (Note that the toxin is responsible for the systemic manifestation of shigellosis).

- **Clinical Findings of shigellosis : (has 4 phases)**
- **1st phase:** 1st week: 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.
- **2nd phase**: 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** becoming→ **bloody diarrhea** , each bowel movement is accompanied by straining and tenesmus (rectal spasms), with resulting lower abdominal pain.
- **3rd phase**: 2nd week: (rose spots, erythema on trunk chest and back).
- **4th phase:** post infectious phase, might retain dormant bacteria specially patients with all bladder stones→asymptomatic carriers.
- **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 shigella, but these do not protect against reinfection.
- **Asymptomatic carriers**: those who retain dormant shigella in their body (gall bladder that's why cholecystectomy is required just like in salmonella) 40 minutes

<u>Diagnostic Laboratory Tests</u>

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) →colorless colonies and then subculture on 2 selective media (Hektoen enteric agar

or Salmonella –Shigella agar), which suppress other Enterobacteriaceae and G+ve organisms, will be non-motile and will not produce H_2S gas.

C. Serology: Normal persons often have agglutinins against several Shigella O antigens these antibodies give a false positive and that's why 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.

Note that here even though we have shiga toxin production in which antibiotics are contraindicated we still give them because of **invasion** (the more important pathogenic property).

They may fail to eradicate the organisms from the intestinal tract as there is multiple **drug resistance** (so. ampicillin→ciprofloxacin) can be transmitted by plasmids, and resistant infections are widespread.

Many cases are self-limited.

Opioids and Anti-motility agents should be avoided in Shigella dysentery, as it masks the symptoms, signs and complications (such as intestinal perforation) just like **EIEC**.

Prevention, and Control

- Short lived immunity
- IgA antibodies in the gut may be important in limiting reinfection Serum antibodies to somatic Shigella antigens are IgM.
- Shigella are transmitted by (the 4 F's) "food, fingers, feces, and flies" from person to person. Because humans are the main recognized host of pathogenic shigella, 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. 48 minutes

Good luck