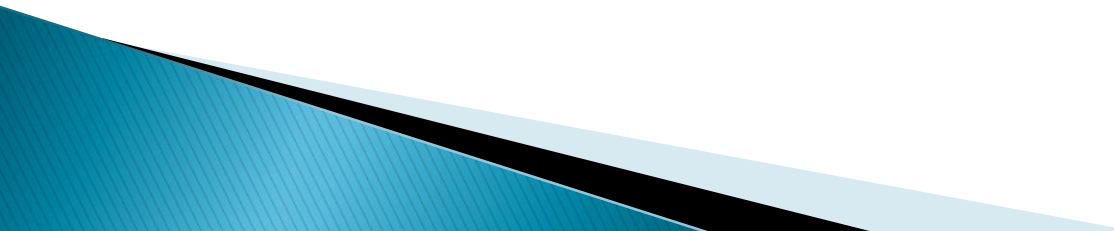


Normal Flora Of The GIT And Introduction To Infectious Diarrhea

Prof .Hanan Habib

Department of Pathology, College of Medicine,
KSU

Objectives

- 1- Know common normal flora of the GIT
 - 2- Understand the role of GIT normal flora in diseases.
 - 3-Recognise various types of acute diarrheal illnesses.
 - 4- Describe the epidemiology & host defenses in preventing GI infections.
 - 5-Explain the pathogenesis of *E.coli*, *Campylobacter* & *Yersinia* and their management
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Objectives

- 6-Discuss microbiological methods used for each bacterial agent causing diarrheal infection.
- 7-Describe the pathogens, risk factors, clinical presentation and prevention of food poisoning ,travelers and antibiotics associated diarrhea.

Introduction to Normal Flora

- ▶ Normal flora are microorganisms that are frequently found in various body sites in normal, healthy individuals.
- ▶ Constituents and number vary according to the age and physiologic status.
- ▶ Able to colonize and multiply under the existing condition of different body sites.
- ▶ Inhibit competing intruders.
- ▶ Have symbiotic relationship that benefit the host.
- ▶ Can cause disease in immunocompromized patients.

Normal Flora of the GIT

- ▶ **Oral cavity:** contain high number of flora which vary from site to site of the mouth.
- ▶ Saliva contain mixed flora : 10^8 organism /ml
- ▶ **Stomach :** empty stomach has no normal flora in health due to HCL and peptic enzymes
- ▶ **Small intestine :** very scanty except near colon
- ▶ **Colon of adults:** 10^{10} org/gm stool, >90% are *Bacteriodes* (anaerobic), 10% other bacteria.
- ▶ Direct effect of diet composition.

Normal Flora of the GIT

- ▶ **Mouth:** Viridans streptococci, *Neisseria* spp., *Moraxella*, *Peptostreptococcus*.
- ▶ **Nasopharynx :**
 - ▶ *Niesseria* spp., Viridans sterpt. *Moraxella*, *Peptostreptococcus*.
- ▶ **Stomach :** streptococci, *Peptosterptococcus*, others from mouth.
- ▶ **Small intestine:** scanty, variable
- ▶ **Colon of adults**
: *Bacteriodes*, *Fusobacterium*, *Bifidobacteria*, *Lactobacillus*, enterobacteria, *Clostridium*
- ▶ **Colon of Breastfeeding infants:** *Bifidobacterium*, *Lactobacillus*
- ▶ **Mouth:** *Candida albicans*
- ▶ **Nasopharynx:** *S.pneumoniae*, *N.meningitidis*, *H.influenzae*, *S.pyogenes*, *S.aureus*
- ▶ **Stomach:** none
- ▶ **Small intestine :** none
- ▶ **Colon of adults:** *B.fragilis*, *E.coli*, *Pseudomonas*, *Candida*, *Clostridium*
- ▶ **Colon of Breast feeding infants:** none

Normal flora (low virulence)

Potential pathogen (carrier)

Role of GIT Normal Flora in Disease

- ▶ Many are opportunistic pathogens. Example: perforation of the colon from ruptured diverticulum, feces enters into peritoneal cavity and cause peritonitis
- ▶ *Viridans* streptococci of oral cavity enters the blood and colonize damaged heart valves.
- ▶ Mouth flora play a role in dental caries.
- ▶ Compromised defense systems increase the opportunity for invasion.
- ▶ Death after lethal dose of radiation due to massive invasion of normal flora.

Role of Normal Flora in Diarrheal Diseases

- ▶ *E.coli* : the most common Enterobacteriaceae; a facultative flora of colon followed by *Klebsiella*, *Proteus* and *Enterobacter*.
- ▶ *Salmonella*, *Shigella* and *Yersinia* are NOT normal flora of the intestinal tract.
- ▶ Some strains of *E.coli* ,*Salmonella* ,*Shigella* and *Yersinia enterocolitica* are able to produce diseases in the intestinal tract.

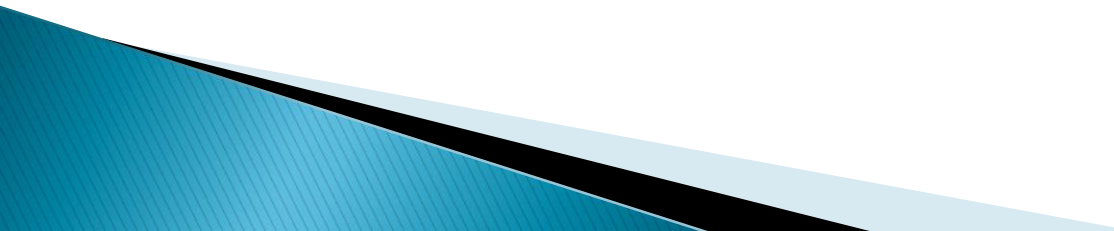
Intestinal Pathogens

- ▶ **Invasive and Cytotoxic strains** produce inflammatory diarrhea (**Dysentery**) with WBCs and /or blood in the stool.
- ▶ **Enterotoxin –producing strains** cause watery diarrhea with loss of fluid.
- ▶ Some produce **systemic illness** due to spread to multiple organs such as enteric (typhoid) fever.

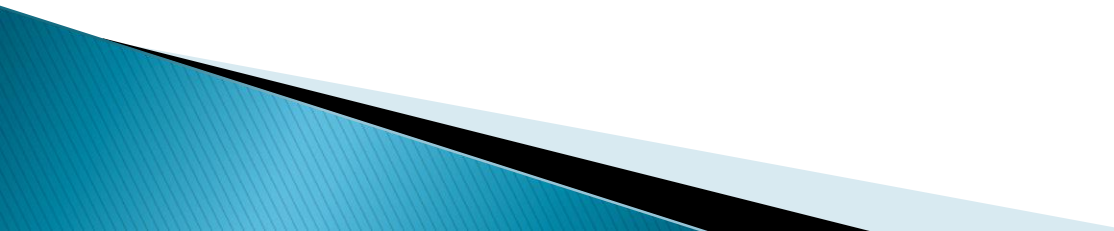
Acute Diarrheal Illnesses and Food Poisoning



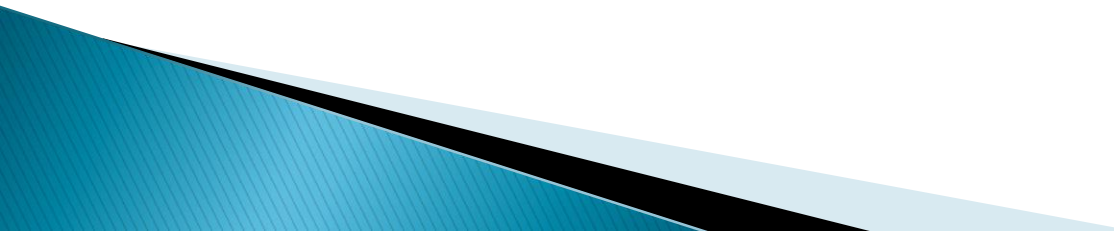
Introduction

- ❑ Acute diarrheal illness is one of the most common problems evaluated by clinicians.
 - ❑ A major cause of morbidity and mortality world wide.
 - ❑ Most of healthy people have mild illness but other might develop serious squeals so it is important to identify those individuals who require early treatment.
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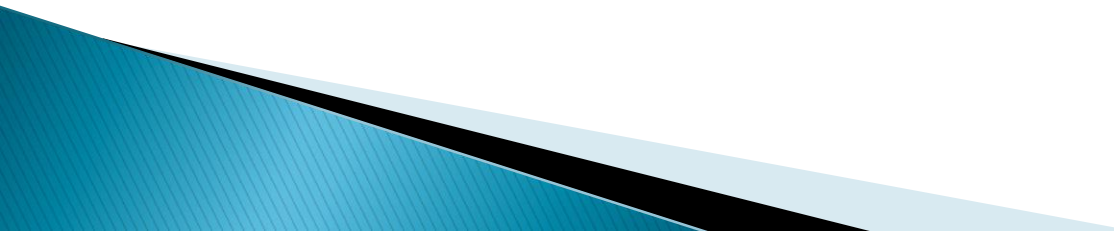
Definition of Diarrhea

- ❑ Stool weight in excess of 200 gm/day
 - ❑ Three or more loose or watery stools/day
 - ❑ Alteration in normal bowel movement characterized by decreased consistency and increased frequency
 - ❑ Less than 14 days in duration.
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Etiology

- ❑ **Viral:** 70–80% of infectious diarrhea in developed countries
 - ❑ **Bacterial:** 10–20% of infectious diarrhea but responsible for most cases of severe diarrhea
 - ❑ **Protozoan:** less than 10%.
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Epidemiology

- ❑ 1.2 – 1.9 episodes per person annually in the general population
 - ❑ 2.4 episodes per child <3 years old annually
 - ❑ 5 episodes per year for children <3 years old and in daycare
 - ❑ Seasonal peak in the winter.
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Classifications

- ❑ **Infectious Diarrhea:** Viral, Bacterial (eg. *Campylobacter*, *Shigella*, *Salmonella*, *Yersinia*, *Vibrio cholera* & *E.coli*).
- ❑ **Food Poisoning:** *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus* spp.
- ❑ **Traveler Diarrhea :** Enterotoxogenic *E.coli*.
- ❑ **Antibiotic Associated Diarrhea:** *Clostridium difficile*.

Risk Factors

- ❑ Food from restaurant
- ❑ Family member with gastrointestinal symptoms
- ❑ Recent travel to developing countries
- ❑ Patient underlying illness and medication, low stomach acidity, cyst, spores
- ❑ Abnormal peristalsis
- ❑ Low Immunoglobulin IgA.
- ❑ Antibiotics decrease the normal flora to less 10^{12}
- ❑ Median infective dose (**ID₅₀**)

Clinical Presentation and Pathogenic Mechanism I

Enterotoxin mediated

- ❑ Lack of pus in the stool (no gut invasion)
- ❑ Lack of fever
- ❑ Rapid onset performed toxin < 12 hour
- ❑ **Small intestine affected.**
- ❑ Vomiting non-bloody diarrhea, abdominal cramp.
- ❑ *Vibrio cholerae, Staphylococcus aureus, Clostridium perfringens and Bacillus cereus*
- ❑ Other viral and some parasitic infections.

Clinical Presentation and Pathogenic Mechanism II

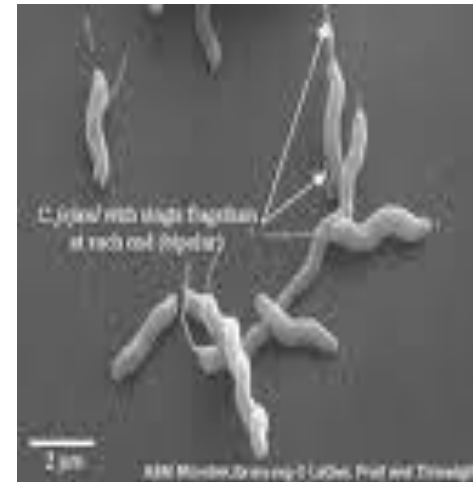
Invasive

- ❑ Pus and blood in the stool
- ❑ Fever due to inflammation
- ❑ *Shigella*, *Salmonella* spp., *Campylobacter*, some *E.coli* and *Entameba histolytica*
- ❑ Affect colonic mucosa
- ❑ Extension to lymph nodes
- ❑ Incubation period 1–3 days
- ❑ Dysentery syndrome—gross blood and mucous
- ❑ EHEC bloody diarrhea
- ❑ *Entameoba histolytica* 1–3 wk

Campylobacter

- ❑ Family : *Campylobacteraceae*
- ❑ Common species : *C.jejuni*, *C. coli*, *C fetus*.
- ❑ Epidemiology
- ❑ **Source:** dog , cat, birds, poultry ,water, milk, meat, person to person transmission can occur.

Campylobacter



Clinically

- ❑ Incubation period: 2–6 days
- ❑ Lower abdominal pain , watery or dysenteric diarrhea with pus and blood. fever in some patients . Nausea and vomiting are rare
- ❑ Self limiting after 2–6 Day
- ❑ Chronic carrier
- ❑ Guillain– Barrie’ syndrome and Reactive arthritis cases frequently preceded by *C.jejuni* infection.

Laboratory diagnosis and treatment

❑ Laboratory diagnosis:

- ❑ Transport media
- ❑ Culture on **CAMPYBAP** media contain antibiotics.
- ❑ Incubate in microaerophilic atmosphere (5%O₂ 10%CO₂ 85%N) at 42°C except *C.fetus* 37°C
- ❑ Identification :Gram stain/culture biochemical/Serology.

❑ Treatment:

- ❑ Only severe cases
- ❑ Erythromycin or Ciprofloxacin .

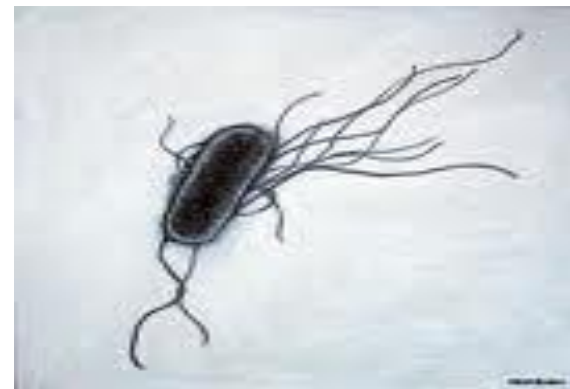
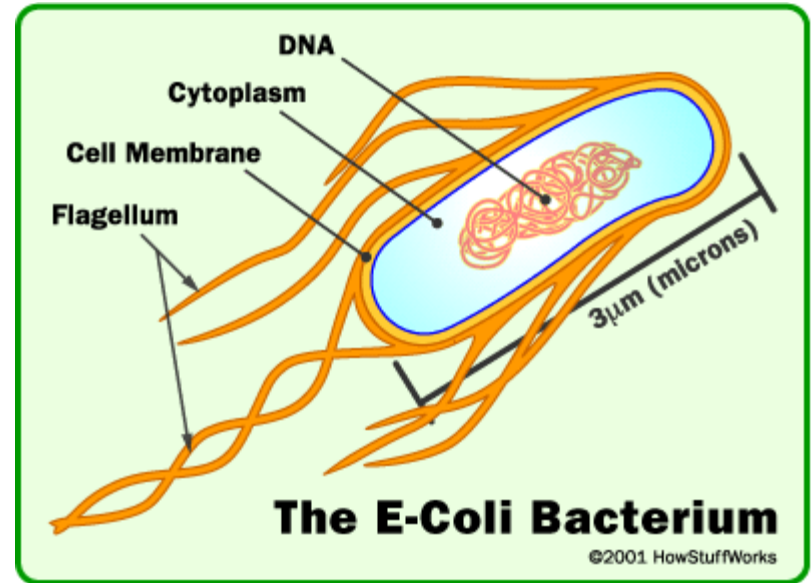
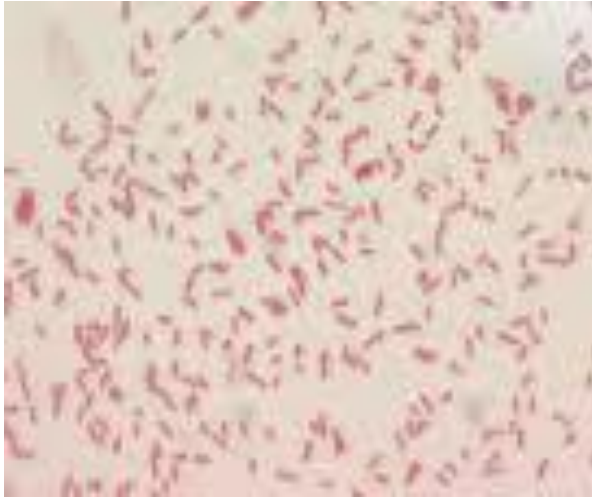
E. coli

- About 10 -15% of strains of *E. coli* associated with diarrhea.
- Based on virulence factors, clinical manifestation, epidemiology and different **O** and **H** serotype. There are **five** major categories of diarrheagenic *E. coli* .

Types of Diarrheagenic *E. coli* :

1. *Enterotoxigenic E. coli* (E T E C)
2. *Enteropathogenic E. coli* (E P E C)
3. *Enteroinvasive E. coli* (E I E C)
4. *Enterohaemorrhagic E. coli* (E H E C)
5. *Enteraggregative E.coli* (EAEC)

E. coli

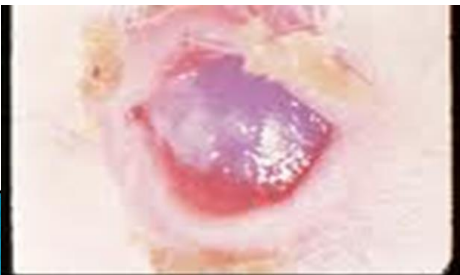


1. Enterotoxigenic *E.coli* (ETEC)

- ❑ Major cause of **Traveler's diarrhea** in infant and adult in developing countries from contaminated food and water.
- ❑ **It has high infective dose 10^6-10^{10}**
- ❑ Has heat-labile toxin (LT) and heat-stable toxin (ST) each has two fragment (A and B) . No invasion or inflammation.
- ❑ **LT** leads to accumulation of CGMP, which lead to hyper-secretion of fluid with no cellular injury
- ❑ Symptoms **watery diarrhea**, abdominal cramps and some time vomiting .
- ❑ Self limiting .No routine diagnostic method required.

2. Enteroinvasive *E.coli* (EIEC)

- ❑ Produce dysentery (Penetration, invasion and destruction). Common in children.
- ❑ Similar to *Shigella* spp. (non motile, LNF)
- ❑ Transmission :Fecal oral route .
- ❑ Fever, severe abdominal cramp, malaise and watery diarrhea
- ❑ Infective dose 10^6
- ❑ Diagnosis Sereny test and DNA probes.



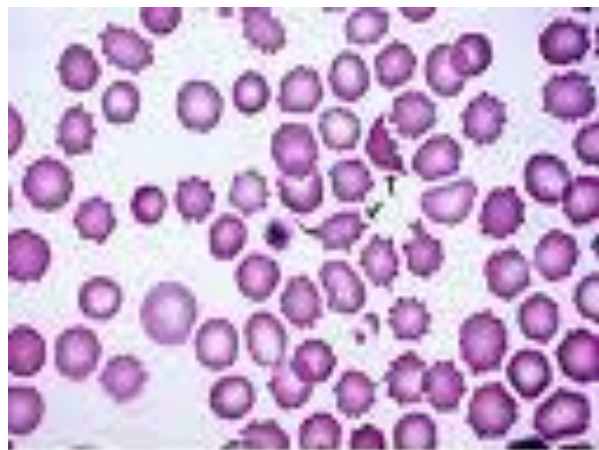
3-Enteropathogenic *E.coli* (*EPEC*)

- ❑ Cause infantile diarrhea (bottle fed infants)
- ❑ Disrupt microvilli and intestinal absorptive function.
- ❑ Outbreak in hospital nurseries and day care centers
- ❑ Low grade fever, malaise, vomiting and watery diarrhea
- ❑ mucous in stool but **no blood**.

4-Enterohemorrhagic *E.coli* (*EHEC*)

- ❑ **0157:H7** Hemorrhagic diarrhea, colitis and hemolytic uremic syndrome (**HUS**)=low Platelet count, hemolytic anemia and kidney failure
- ❑ Bloody diarrhea, low grade fever and stool has no leucocytes
- ❑ Fatal disease in young and elderly persons in nursing homes
- ❑ Undercooked hamburgers, unpasteurized dairy products, Apple cider, cookie dough
- ❑ **Cytotoxin =vertoxin I and vertoxin II Similar to Stx₁ (shiga-toxin I&II)**
- ❑ *E.coli* other than 0157:H7 can cause HUS.
- ❑ Diagnosis by culture on SMAC(*sorbitol MacConkey agar cefixime*), Vertoxin detection by immunological test or PCR.

HUS

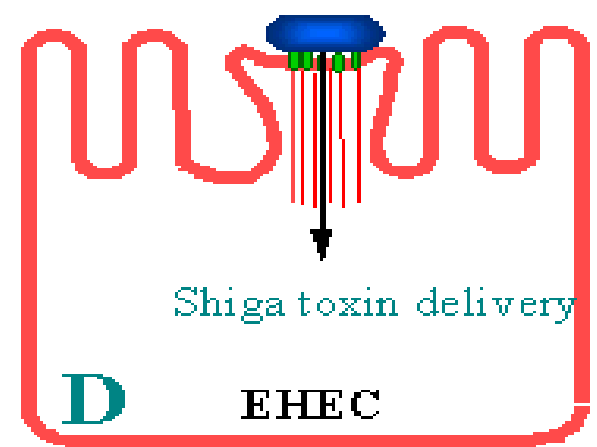
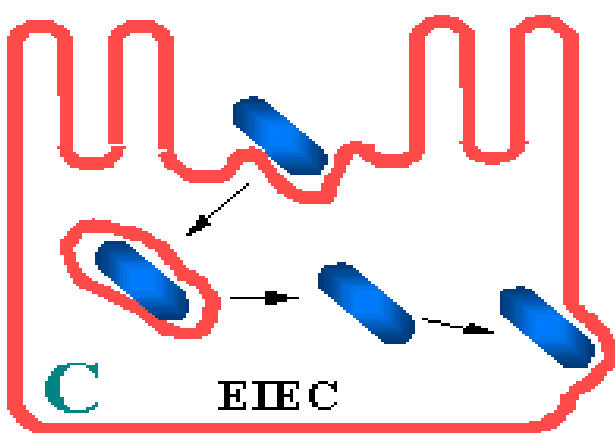
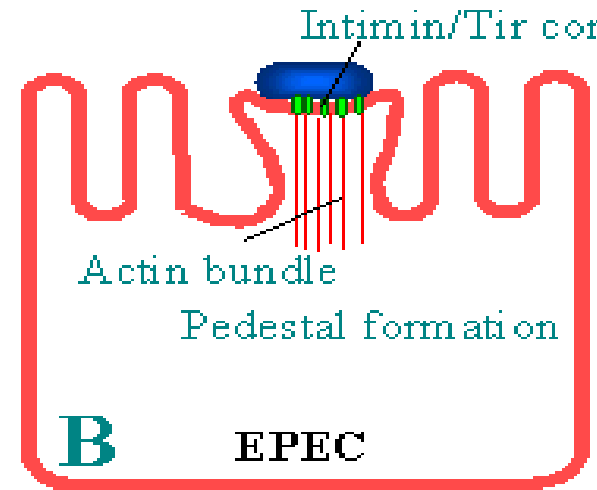
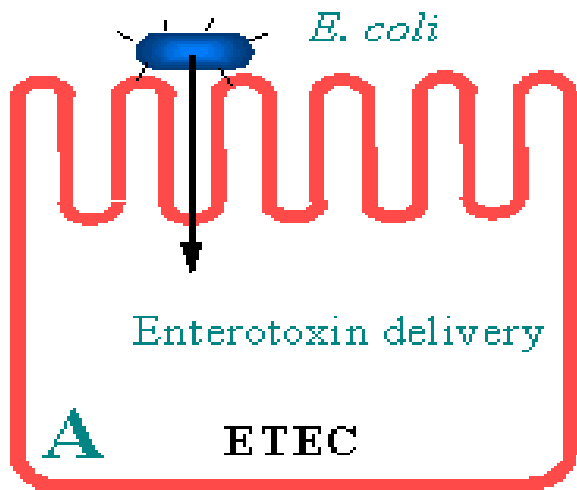


Source: Colman RA, Stiller ML, Felder RL, Wang B
Lumley J. Atlas of Hematology - 180 Color Plates and 1000
Diagrams & The Histochem Companion. DC, MD: Igiba; 2008.



5. Enteroaggregative *E.coli* (EAEC)

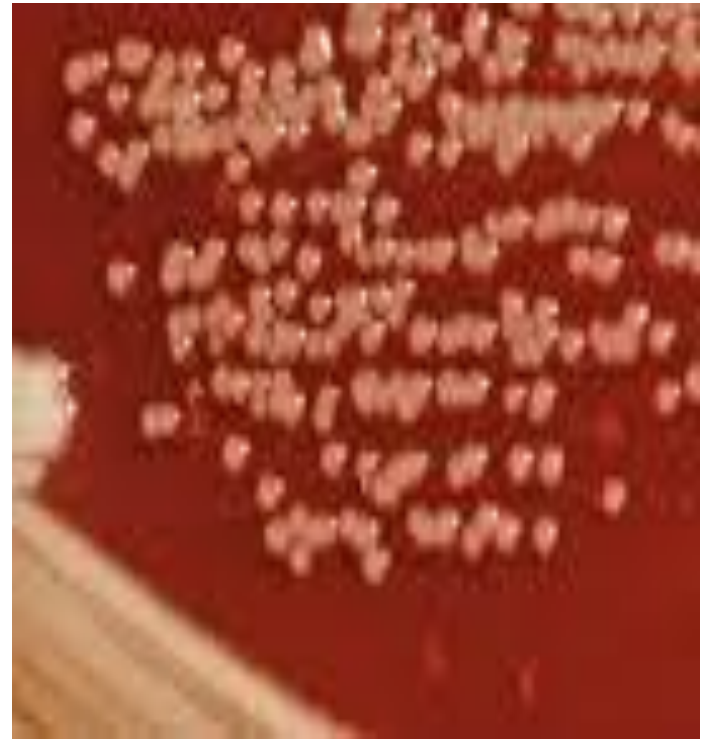
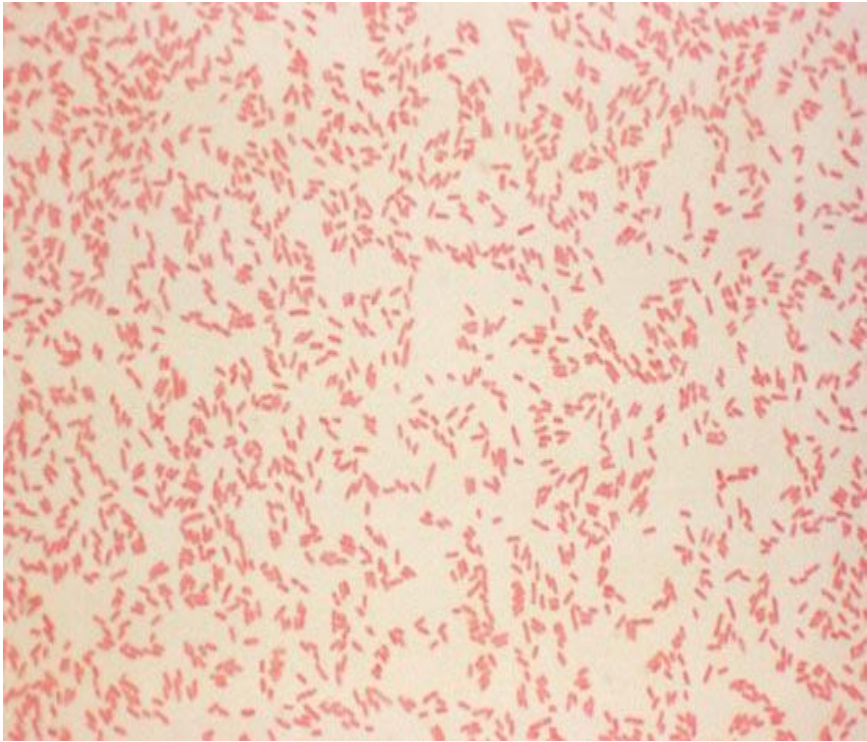
- ❑ Pediatric diarrheal disease
- ❑ Adhering to the surface of the intestinal mucosa. Produce aggregative stacked brick .
- ❑ Produce mucoid ,watery diarrhea, vomiting, dehydration and abdominal pain
- ❑ may resolve after two weeks or more .

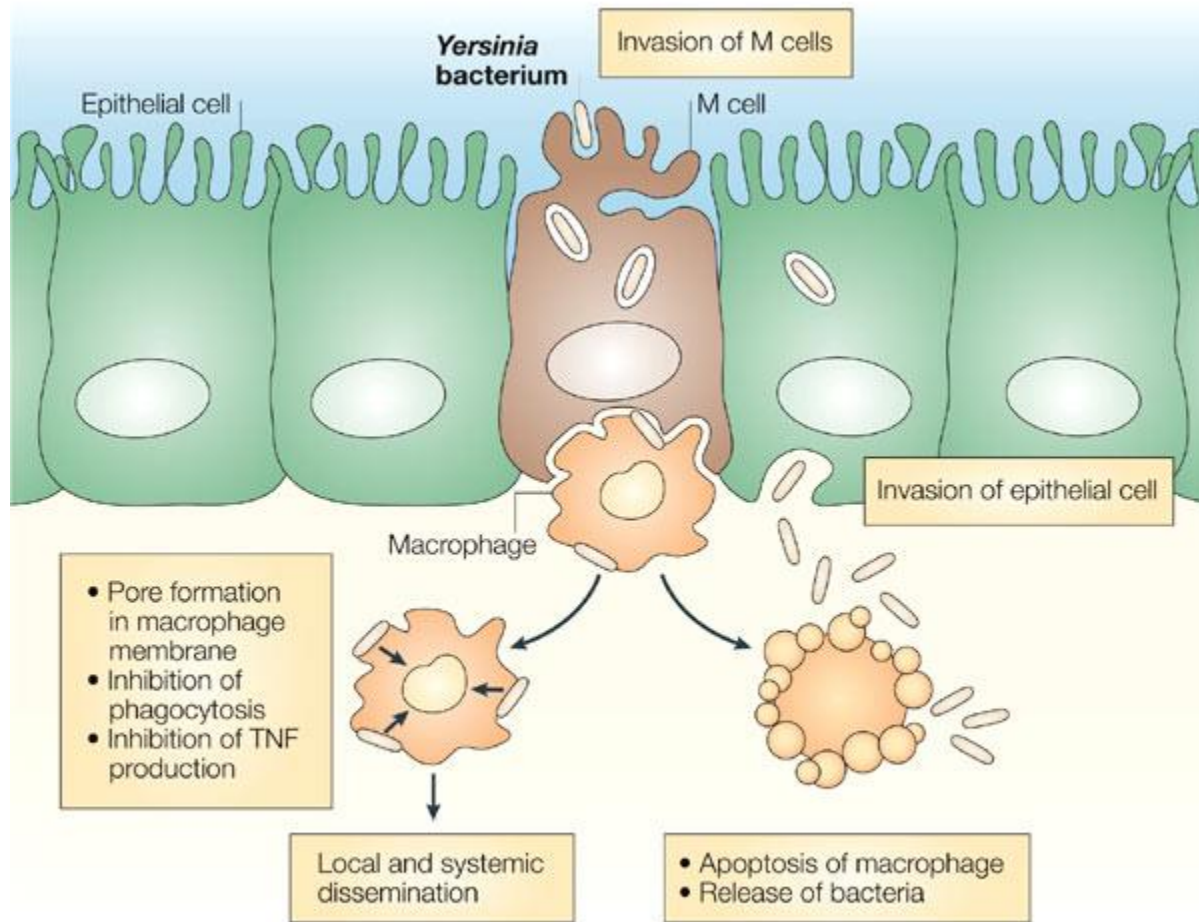


Yersinia enterocolitica

- ❑ Mesenteric lymphadenitis in children and septicemia in immunocompromised hosts
- ❑ Common in Europe, USA, Canada .Cat, dog, swine (chitterlings)
- ❑ Survive cold temperatures and associated with transfusion of packed red blood cells.
- ❑ **Presented with enteritis, arthritis and erythema nodosum**
- ❑ Generalize infection in adult and children 1–5 year, usually mild but in old children adult **mimic appendicitis**
- ❑ Growth at **25°–30°C** media Cefsulodin–Igrasan–Novobiocin

Yersinia enterocolitica





Clostridium difficile

- ❑ **Antibiotic associated diarrhea** (*ampicillin, cephalosporins & clindamycin*).
- ❑ Transmits from person to person via fecal-oral route
- ❑ **Cultured from inanimate hospital surfaces.**
- ❑ Disruption of the endogenous bacterial flora of the colon
- ❑ Produce **toxin A (enterotoxigenic & cytotoxic effects)** and **B (cytotoxic)** that can bind to surface epithelial cell receptors leading to inflammation mucosal injury and diarrhea.

Clostridium difficile spores and vegetative cells are ingested

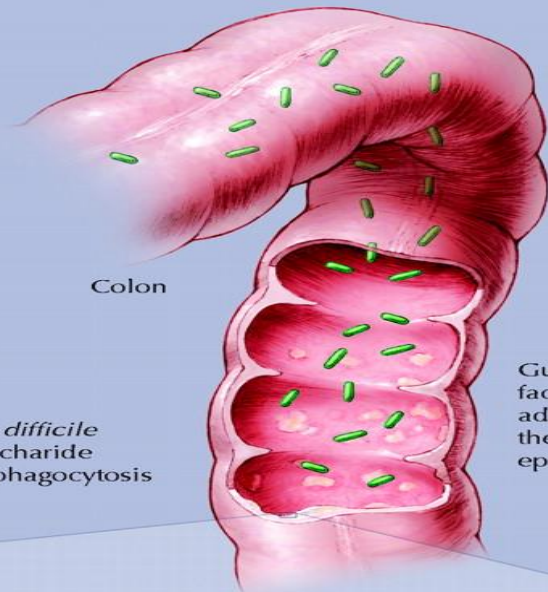
- Spores
- Vegetative cells

Most vegetative cells are killed in the stomach, but spores can survive the acid environment



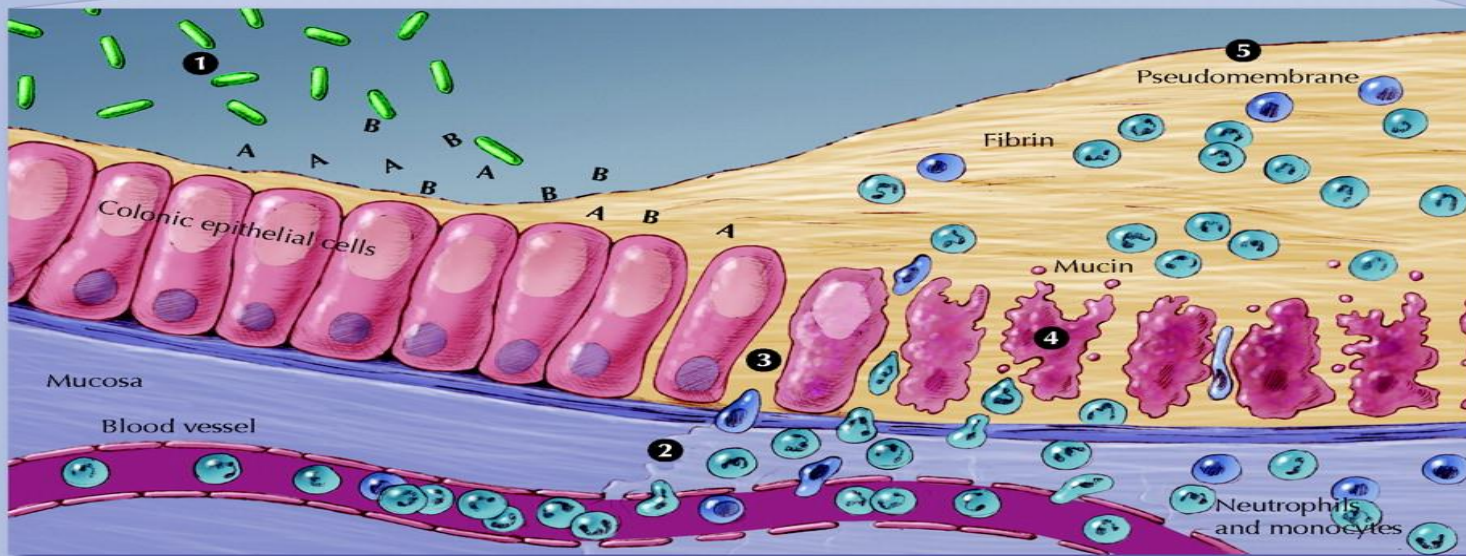
C. difficile spores germinate in the small bowel upon exposure to bile acids

Flagellae facilitate *C. difficile* movement; a polysaccharide capsule discourages phagocytosis



C. difficile multiplies in the colon

Gut mucosa facilitates adherence to the colonic epithelium



C. difficile vegetative cells produce toxins A and B and hydrolytic enzymes (1). Local production of toxins A and B leads to production of tumour necrosis factor-alpha and proinflammatory interleukins, increased vascular permeability, neutrophil and monocyte recruitment (2),

opening of epithelial cell junctions (3) and epithelial cell apoptosis (4). Local production of hydrolytic enzymes leads to connective tissue degradation, leading to colitis, pseudomembrane formation (5) and watery diarrhea.

C. difficile & pseudomembraneous colitis



Clostridium difficile

- ❑ Patient presents with fever, leukocytosis, abdominal pain and diarrhea
- ❑ **Pseudomembrane can result (neutrophils, fibrin, and cellular debris in the colonic mucosa) and toxic megacolon**
- ❑ **Diagnosis:** direct toxin detection from stool by enzyme immunoassay(EIA)
- ❑ **Treatment Metronidazole ± Vancomycin and supportive treatment**

Selected Clinical and Epidemiologic Characteristics of Typical Illness Caused By Common Foodborne Pathogens*

Pathogen	Typical Incubation Period	Duration	Typical Clinical Presentation	Assorted Foods
Bacterial				
<i>Salmonella</i> species	1-3 Days	4-7 Days	Gastroenteritis	Undercooked eggs or poultry, produce
<i>Campylobacter jejuni</i>	2-5 Days	2-10 Days	Gastroenteritis	Undercooked poultry, unpasteurized dairy products
<i>E. coli</i> , <i>Enterotoxigenic</i>	1-3 Days	3-7 Days	Gastroenteritis	Many foods
<i>Shigella</i> species	1-2 Days	4-7 Days	Gastroenteritis	Produce, egg salad

<i>Listeria monocytogenes</i>	2-6 weeks	Variable	Gastroenteritis, meningitis abortion	Deli meat , hotdogs, unpasteurized dairy products
<i>Bacillus cereus</i>	1-6 hour	<24 hour	Vomiting, Gastroenteritis	Fried rice, meats
<i>Clostridium botulinum</i>	12-72 hour	Days-months	Blurred vision , paralysis	Home-canned foods, fermented fish
<i>Staphylococcus aureus</i>	1-6 hour	1-2 Days	Gastroenteritis, particularly nausea	Meats, potato & pork, unpasteurized dairy products .
<i>Yersinia enterocolitica</i>	1-2 Days	1-3 weeks	Gastroenteritis, appendicitis-like syndrome	Undercooked pork , unpasteurized dairy products .

Lab diagnosis of diarrheal diseases

▶ Stool specimens:

Microscopy: for the presence of polymorphs or blood may help. Test for the presence of ova & parasites.

Culture :on selective media for *Salmonella*, *Shigella* & *Campylobacter*. Other organisms eg. *Vibrio cholerae*, EHEC or *Yersinia* if suspected.

Toxin assay: *C.difficile* toxins or Verotoxin if suspected.