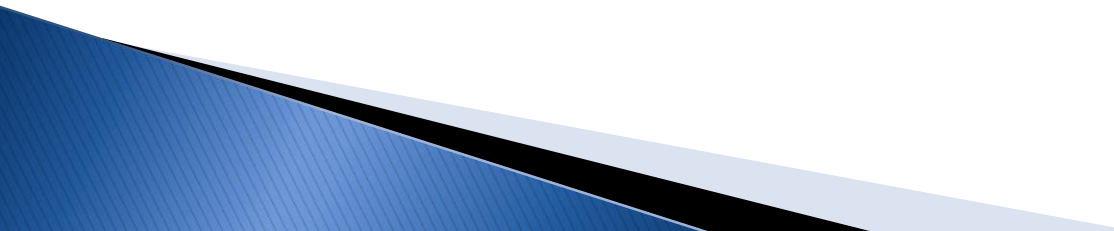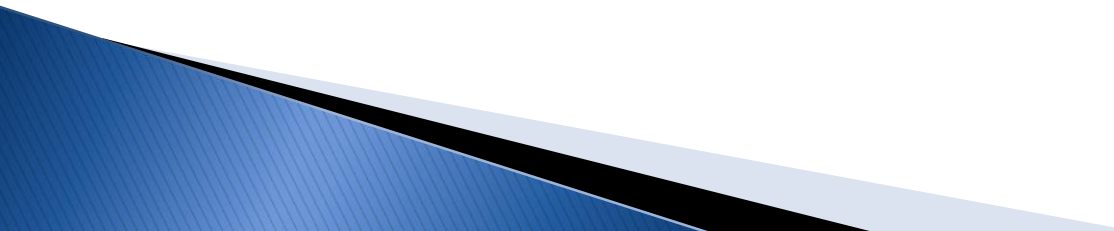# Normal Flora Of The GIT And Introduction To Infectious Diarrhea

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Microbiology Unit

Objectives

- Recall the common normal flora of the GIT
- Understand the role of the normal flora of the GIT in diseases.
- Describe the epidemiology, risk factors & host defenses in preventing GI infections.
- Describe various types of acute diarrheal illnesses, the pathogens that cause them, their clinical presentation, pathogenic mechanism and prevention.

Objectives

▸ Explain the pathogenesis of *E.coli, Campylobacter, Yersinea & Clostridium difficile* and their management.

▸ Discuss microbiological methods used for the diagnosis of common bacterial agents causing diarrheal infection.

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 exiting 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**: contains 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** :
- *Neisseria* spp., Viridans sterpt. *Moraxella, Peptostreptococcus*.
- **Stomach** : Streptococci, *Peptosterptococcus*, others from mouth.
- **Small intestine**: scanty, variable
- **Colon**: *Bacteriodes, Fusobacterium, Eubacterium, Lactobacillus,* Enterobacteriaceae, *Clostridium, Enterococcus*

- **Mouth**: *Candida albicans*
- **Nasopharynx**: *S.pneumoniae, N.meningitidis, H.infuenzae, S.pyogenes, S.aureus*
- **Stomach**: none
- **Small intestine** : none
- **Colon**: *B.fragilis, E.coli, Pseudomonas, Candida, Clostridium (C. perfringens, C. difficile)*

Normal flora (low virulence)	Potential pathogen (carrier)

Role of GIT Normal Flora in Disease

- Many are opportunistic pathogens , eg. perforation of the colon from ruptured diverticulum, feces enter 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 *Enterobacteriacae;* 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 cause diseases in the intestinal tract.
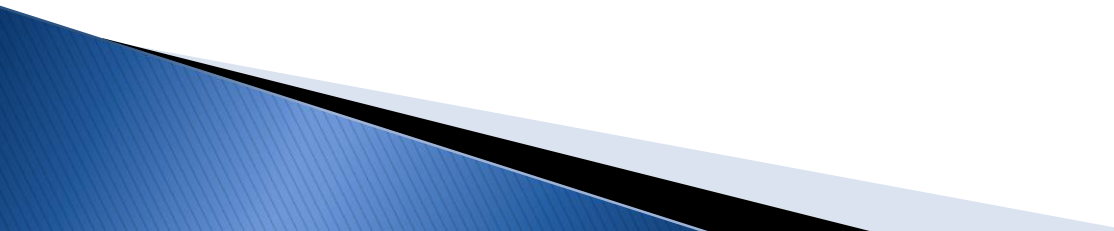
Intestinal Pathogens

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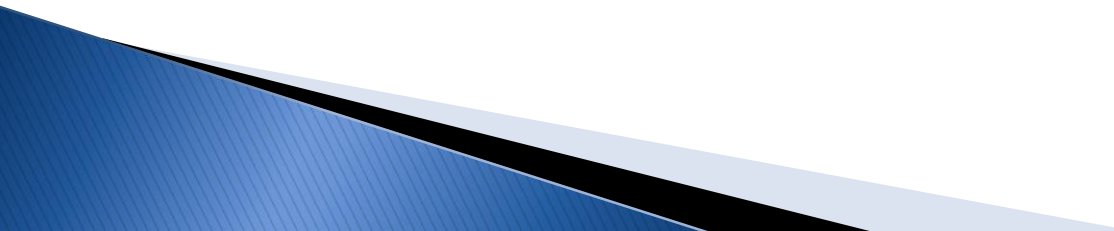- Some produce **systemic illness** due to spread to multiple organs such as enteric (typhoid) fever.
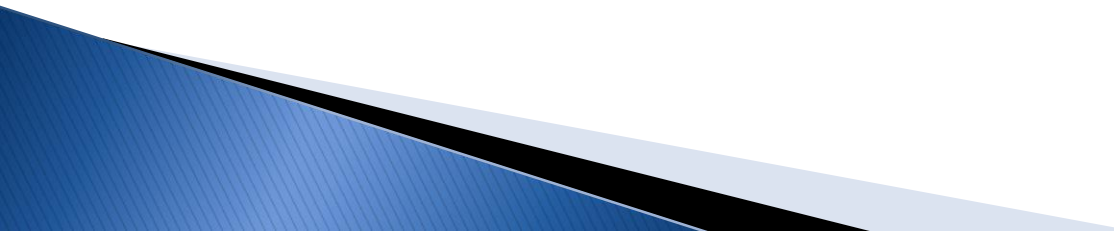
Acute Diarrheal Illnesses and Food Poisoning

Introduction

- Acute diarrheal illness is one of the most common problems evaluated by clinicians.

- A major cause of morbidity and mortality worldwide.

- 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.
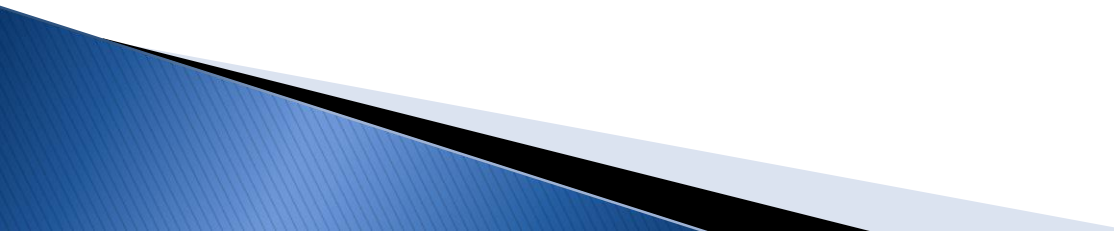
Definition of diarrhea

- Stool weight in excess of 200 gm/day, or 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.

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%.

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.

Classifications

- **Infectious Diarrhea:** caused by Viral or Bacterial infections (eg. *Campylobcator, Shigella, Salmonella, Yersinia, Vibrio cholerae & E.coli*).

- **Food Poisoning**: caused by *Staphylococcus aureus, Clostridium perfringens, Bacillus* spp.

- **Traveler Diarrhea** : caused by Enterotoxigenic *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 A (IgA).
- Antibiotics decrease the normal flora to less than10^{12}
- Median infective dose (ID$_{50}$)

Clinical Presentation & Pathogenic Mechanism I

Enterotoxin mediated
- Lack of pus in the stool (no gut invasion)
- No fever
- Some have rapid onset ($<$12 hour if due to preformed toxin ingestion)
- **Small intestine affected.**
- Vomiting, non-bloody diarrhea, abdominal cramps.
- *Vibreo cholerae, Staphylococcus aureus, Clostridium perfringens and Bacillus cereus*
- Some viral and 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 *Entameoba 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

- Gram negative curved (spiral or S-shape) bacilli .
- worldwide infection ,more common among children
- Common species : *C.jejuni, C. coli, C fetus*.
- **Source**: dog , cat, birds, poultry ,water, milk, meat, person to person transmission can occur.
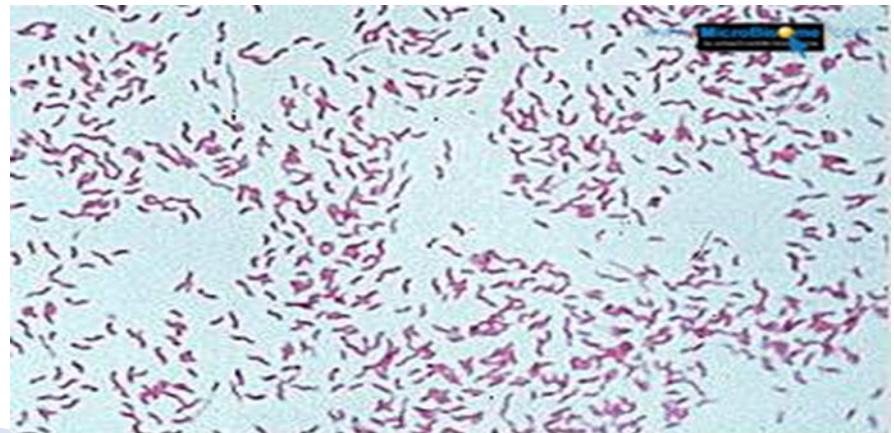
Clinical presentation-*Campylobacter*

- 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 days.
- Chronic carrier & outbreaks uncommon.
- May lead to autoimmune disease like *Guillain-Barrie'* syndrome and extra-intestinal infections eg. reactive arthritis ,bacteremia ,lung infection and others frequently preceded by *C.jejuni* infection.

Laboratory diagnosis and treatment

Lab diagnosis
- Use transport media
- Culture on CAMPY BAP media containing antibiotics.
- Incubate in **microaerophilic** atmosphere ($5\%O_2$ $10\%CO_2$ $85\%N$) at **42°C** except *C.fetus at* 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. Other strains associated with extra-intestinal diseases (septicemia, meningitis & UTI).
- ❑ Based on virulence factors, clinical manifestation, epidemiology and different O and H serotypes.

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. Enteroaggregative E.coli   (EAEC)

1. Enterotoxigenic *E.coli* (ETEC)

- Major cause of Traveler's diarrhea in infant and adult in developing countries due to consumption of contaminated food and water.
- **Has a high infective dose 10^6-10^{10}**
- Produce 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 cAMP, which leads to hypersecretion 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).
- Mainly seen 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

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**
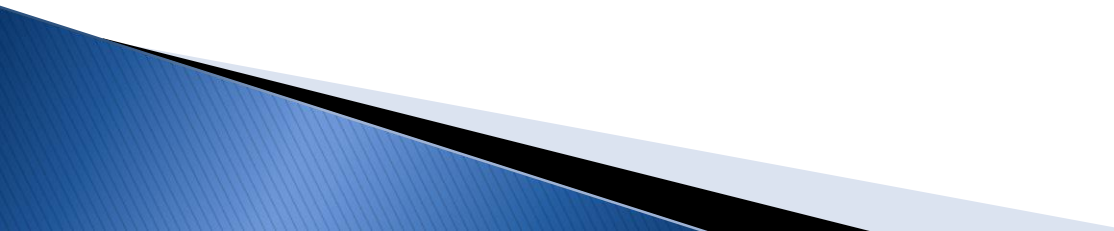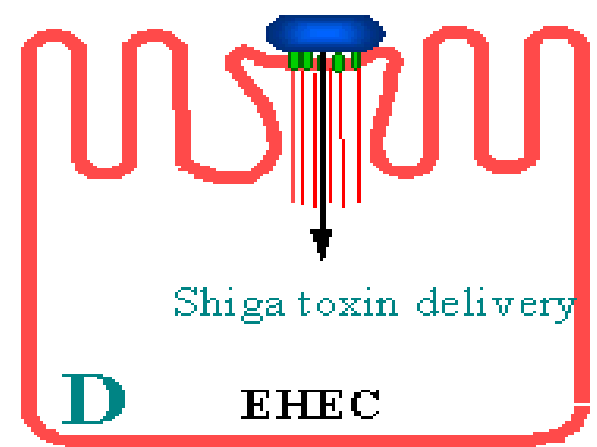- mucous in stool but no blood.

4-Enterohemorrhagic *E.coli (EHEC)*
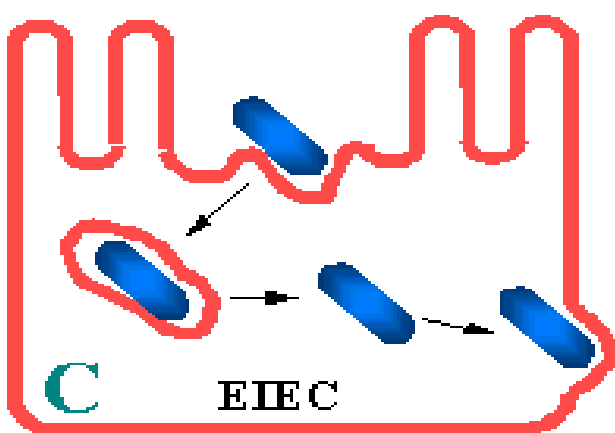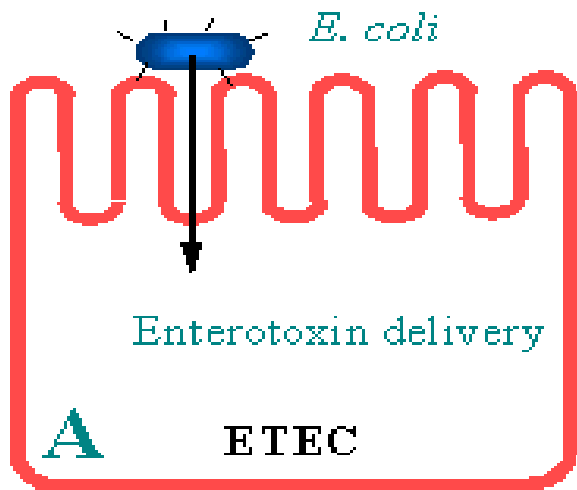
- **O157:H7** Hemorrhagic diarrhea, colitis and hemolytic uremic syndrome (**HUS**) manifested with low platelet count, hemolytic anemia and kidney failure

- **Bloody diarrhea**, low grade fever and stool with no leucocytes

- Fatal disease in young and elderly persons in nursing homes

- Food implicated :undercooked hamburgers, unpasteurized dairy products, Apple cider, cookie dough

4-Enterohemorrhagic *E.coli* (EHEC)

❑ **Cytotoxin : Shiga-toxin I & II (verotoxin I and verotoxin II)** (Similar to toxin produced by *Shigella dysenteriae*)

❑ *E.coli* other than O157:H7 can cause HUS.

❑ Diagnosis by culture on SMAC*(sorbitol MacConkey agar)*, Vertoxin detection by immunological test or nucleic acid testing (NAT).

❑ **Management** of HUS required.
❑ Antimicrobial therapy not recommended .

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 .

A — ETEC
E. coli — Enterotoxin delivery

B — EPEC
Intimin/Tir complex — Actin bundle — Pedestal formation

C — EIEC

D — EHEC
Shiga toxin delivery

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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**
- Generalized infection in adult and children 1-5 year, usually mild but in old children and adult mimic appendicitis
- Growth at 25°-30℃, media: Cefsulodin-Irgasan-Novobiocin (CIN media)

Yersinia bacterium

Invasion of M cells

Epithelial cell

M cell

Invasion of epithelial cell

Macrophage

- Pore formation in macrophage membrane
- Inhibition of phagocytosis
- Inhibition of TNF production

Local and systemic dissemination

- Apoptosis of macrophage
- Release of bacteria

Nature Reviews | Immunology
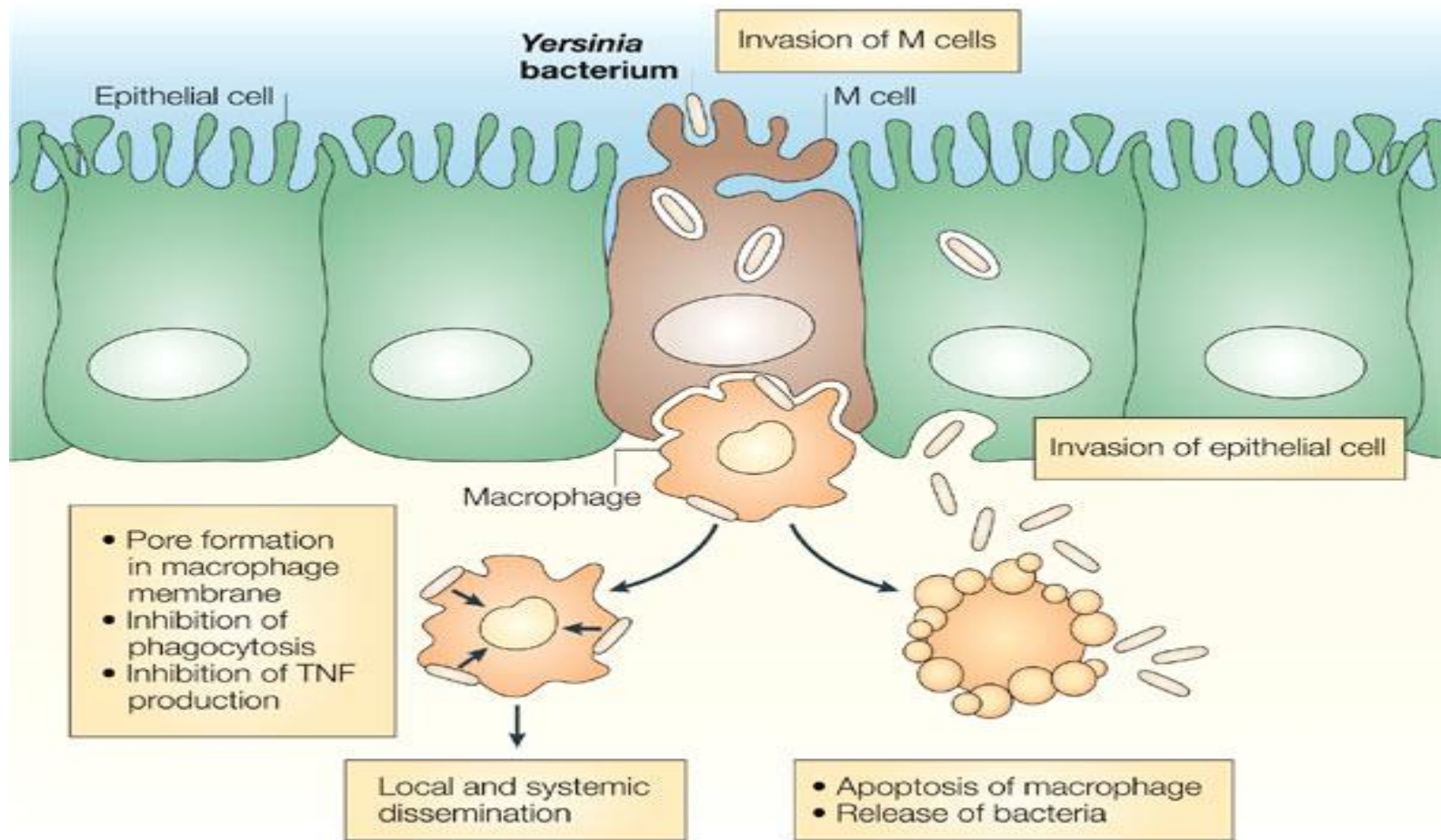
Clostridium difficile

- **Antibiotic associated diarrhea** *(ampicillin, cephalosporins & clindamycin).* Antibiotic used during the last 8 weeks (community acquired) or hospital stay for at least 3 days (hospital acquired).

- Transmitted 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 (enterotoxic & cytotoxic effects)** and **B (cytotoxic)** that can bind to surface epithelial cell receptors leading to inflammation ,mucosal injury and diarrhea.

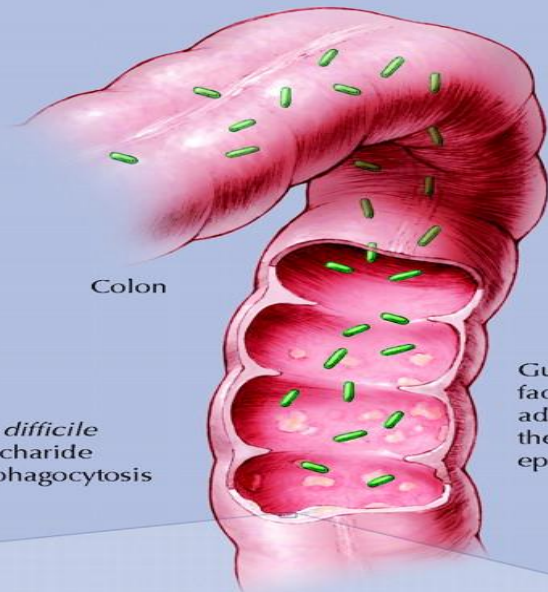Clostridium difficile spores and vegetative cells are ingested

- Spores
- Vegetative cells

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

Stomach

Colon

C. difficile multiplies in the colon

Small bowel

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

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

Gut mucosa facilitates adherence to the colonic epithelium

Pseudomembrane

Fibrin

Mucin

Colonic epithelial cells

Mucosa

Blood vessel

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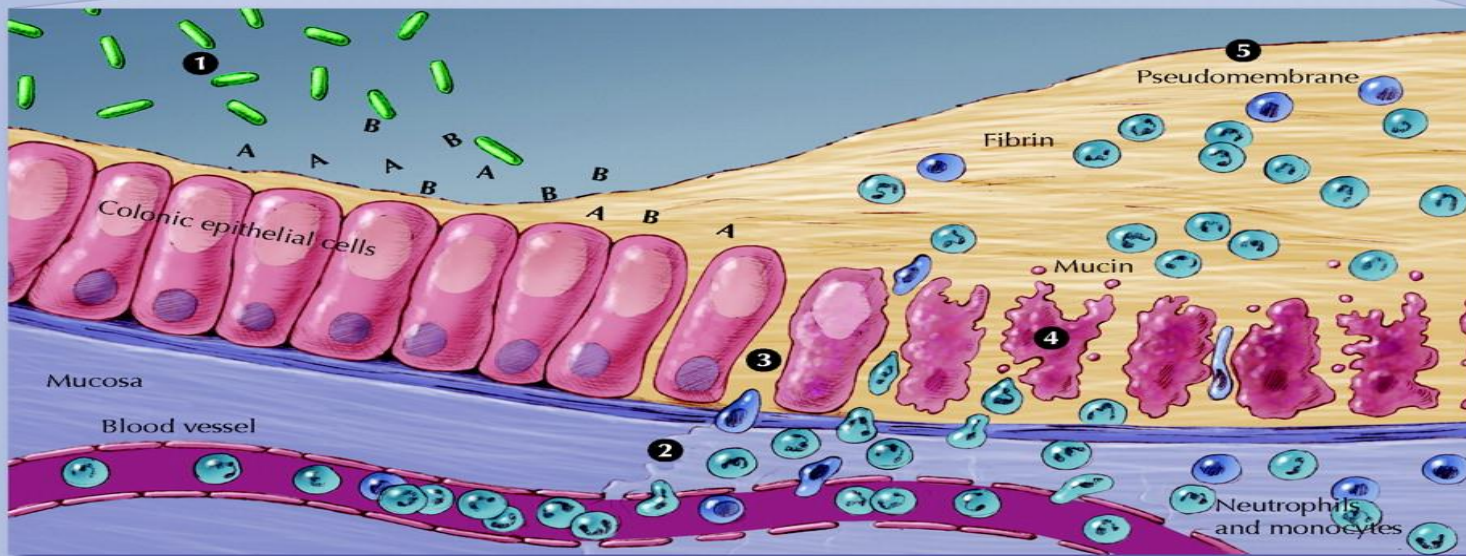
C. difficile vegetative cells produce toxins A and B and hydrolytic enzymes (**1**). Local production of toxins A and B leads to production of tumour necrosis factor-alpha and proinflammatory interleukins, increased vascular permeability, neutrophil and monocyte recruitment (**2**), opening of epithelial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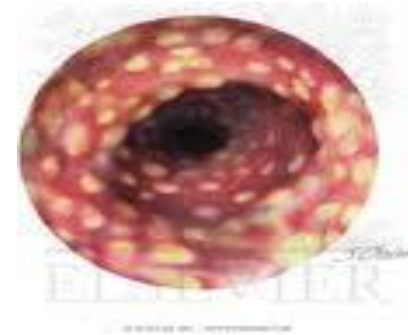
Clostridium difficile

- 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), or nucleic acid testing (NAT).
- Treatment Metronidazole ± oral 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				
Salmonella species	**1-3** Days	4-**7** Days	Gastroenteritis	Undercooked **eggs or poultry,** produce
Campylobacter jejuni	2-5 Days	2-10 Days	Gastroenteritis	Undercooked **poultry**, unpasteurized **dairy** products
E. coli, Enterotoxigenic	**1-3** Days	3-**7** Days	Gastroenteritis	Many foods
Shigella species	1-2 Days	4-**7** Days	Gastroenteritis	Produce, egg salad

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

Lab diagnosis of diarrheal diseases due to bacterial causes

▸ **Stool specimen**:

Microscopy: for the presence of polymorphs or blood <u>may help</u> .

Culture :on selective media for *Salmonella, Shigella & Campylobacter*.

Culture for *Vibreo cholerae*, *EHEC* or *Yersinia* if suspected.

Toxin assay: if *C.difficile* toxins is suspected.

REFERENCES

- Ryan, Kenneth J. Sherris Medical Microbiology, Latest Edition. McGraw-Hill Education.
  - Intestinal flora, part of chapter 1
  - Enteric infections and food poisoning, part of the chapter on Infectious Diseases: Syndromes and Etiologies
  - *Clostridium difficile*, part of chapter 29
  - *Campylobacter*, part of chapter 32
  - *E. coli & Yersinia enterocolitica*, part of chapter 33