Normal Flora Of The GIT And Introduction To Infectious Diarrhea

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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 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.

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 than 10^{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.

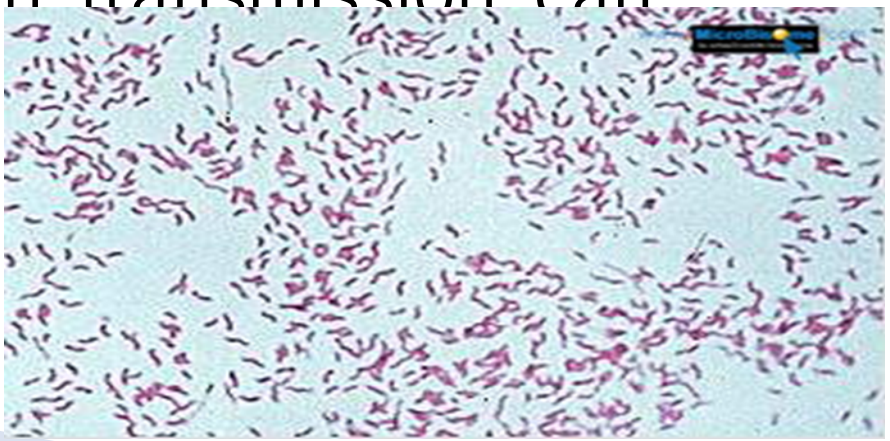
Clinical Presentation and Pathogenic Mechanism II

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 .
- world wide 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* 37°C
- Identification :Gram stain/culture / biochemical/Serology.

Treatment:

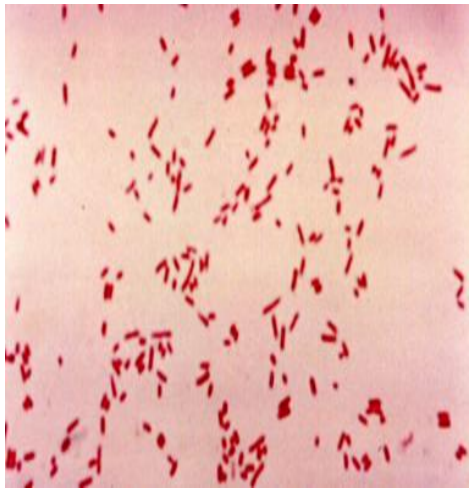
- Only severe cases
- Erythromycin or Ciprofloxacin .

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 serotype.

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.
- **It has 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 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).
- 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)*

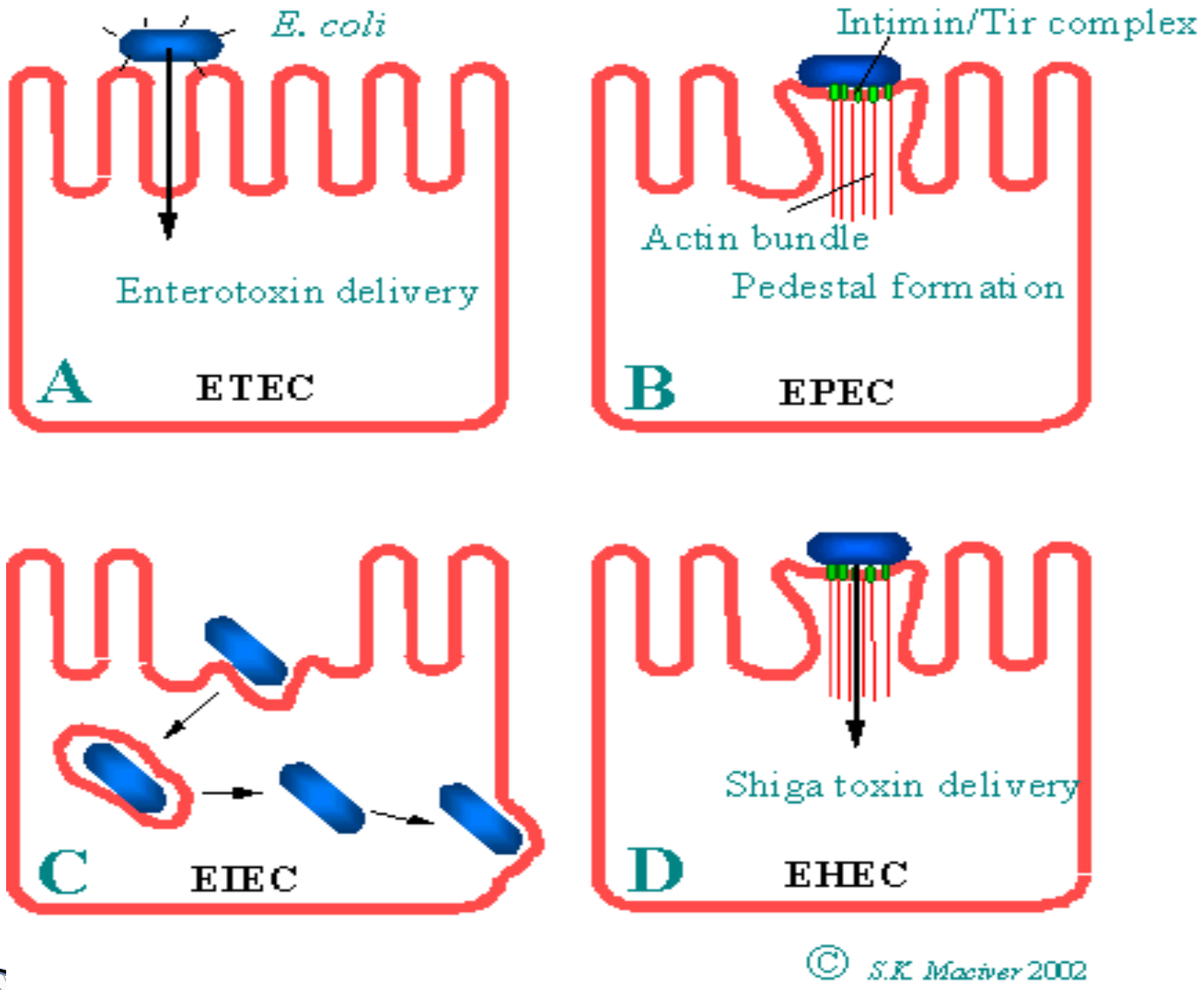
- **0157:H7** Hemorrhagic diarrhea, colitis and hemolytic uremic syndrome (**HUS**) manifested with low Platelet count, hemolytic anemia and kidney failure
- **Bloody diarrhea**, low grade fever and stool with no leucocytes
- Fatal disease in young and elderly persons in nursing homes
- Undercooked hamburgers, 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 0157: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 .


E. coli
Enterotoxin delivery
A
ETEC
Intimin/Tir complex
Actin bundle
Pedestal formation
B
EPEC
C
EIEC
Shiga toxin delivery
D
EHEC
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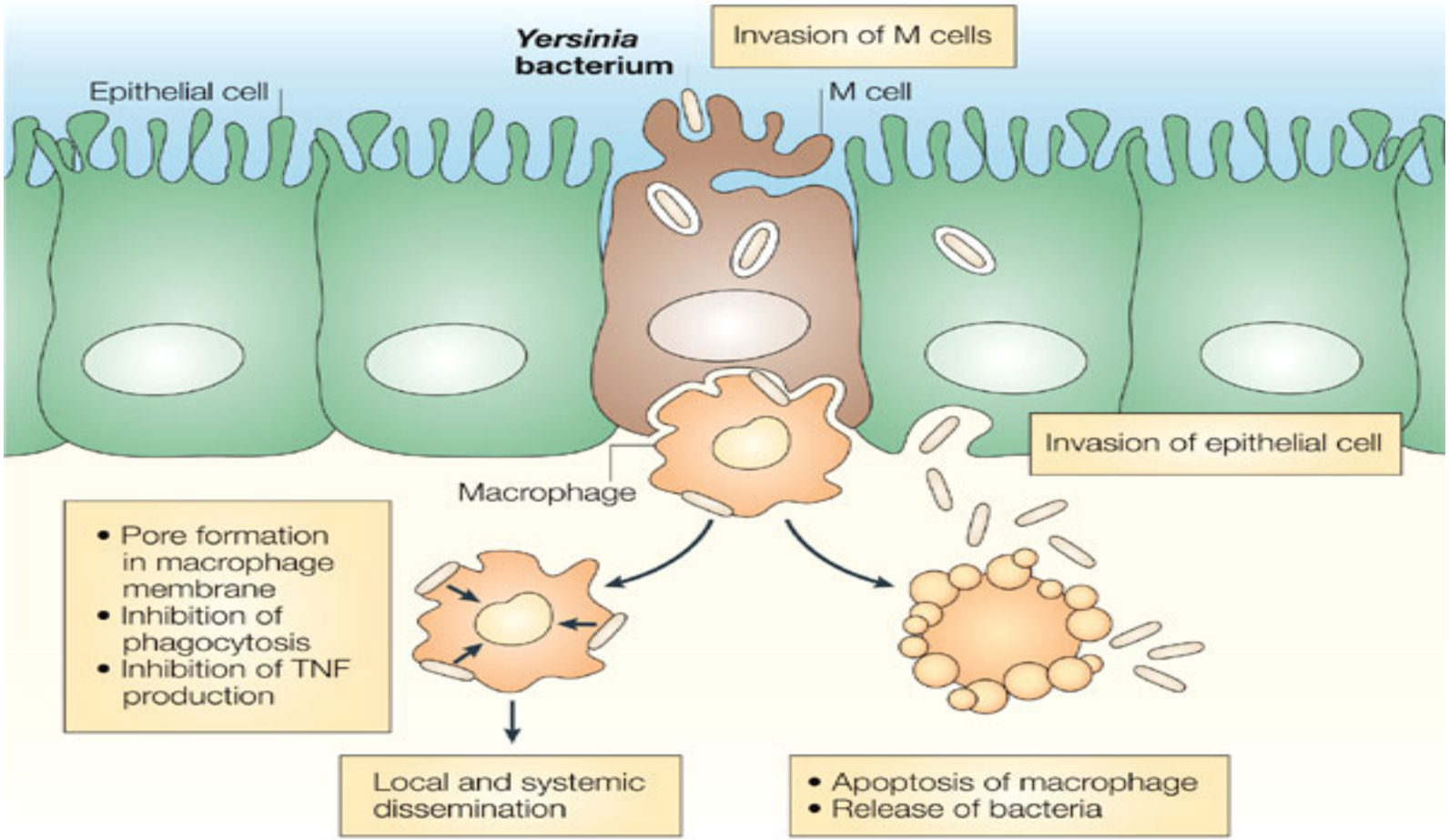
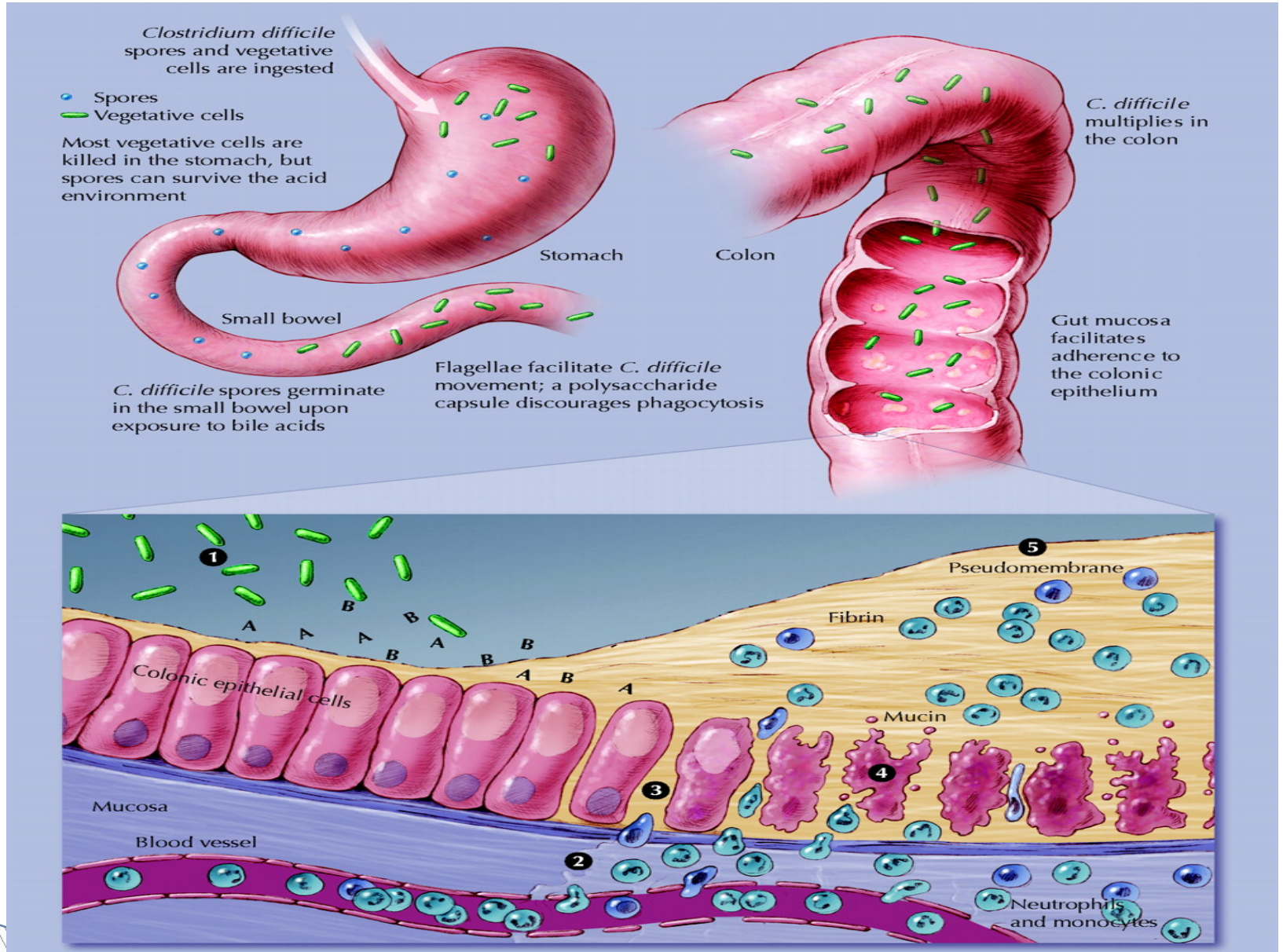
Yersinia enterocolitica

- 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°C, 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

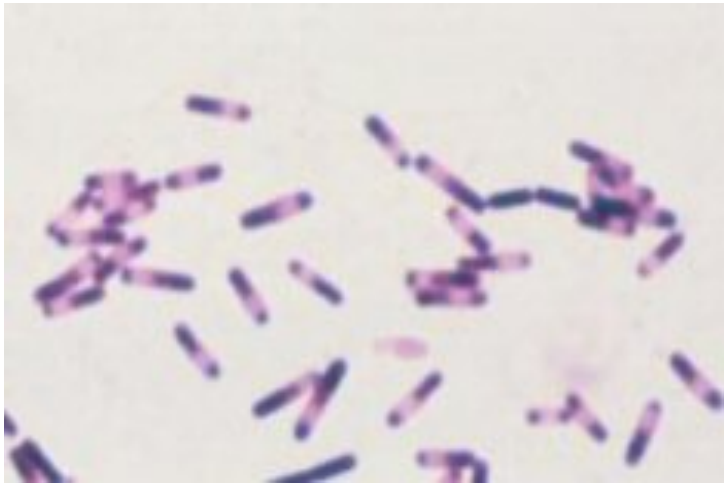
Clostridium difficile

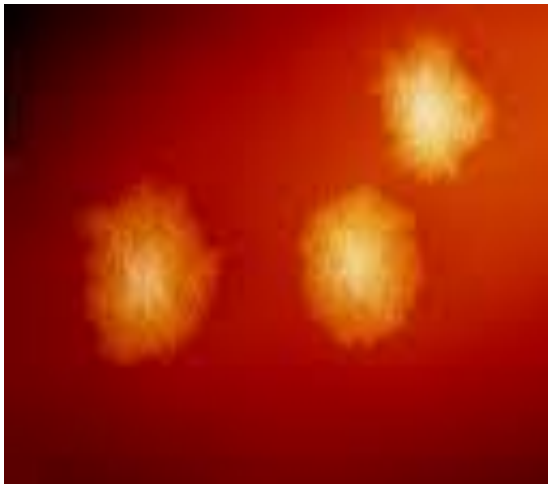
- **Antibiotic associated diarrhea** *(ampicillin, cephalosporins & clindamycin).* **Antibiotic used during the last 8 weeks (community acquired) or hospital stay for at least 3 days (hospital acquired).**
- Transmitted from person to person via fecal-oral route
- **Cultured from inanimate hospital surfaces.**
- 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.



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 **may help** .

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.

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  - 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