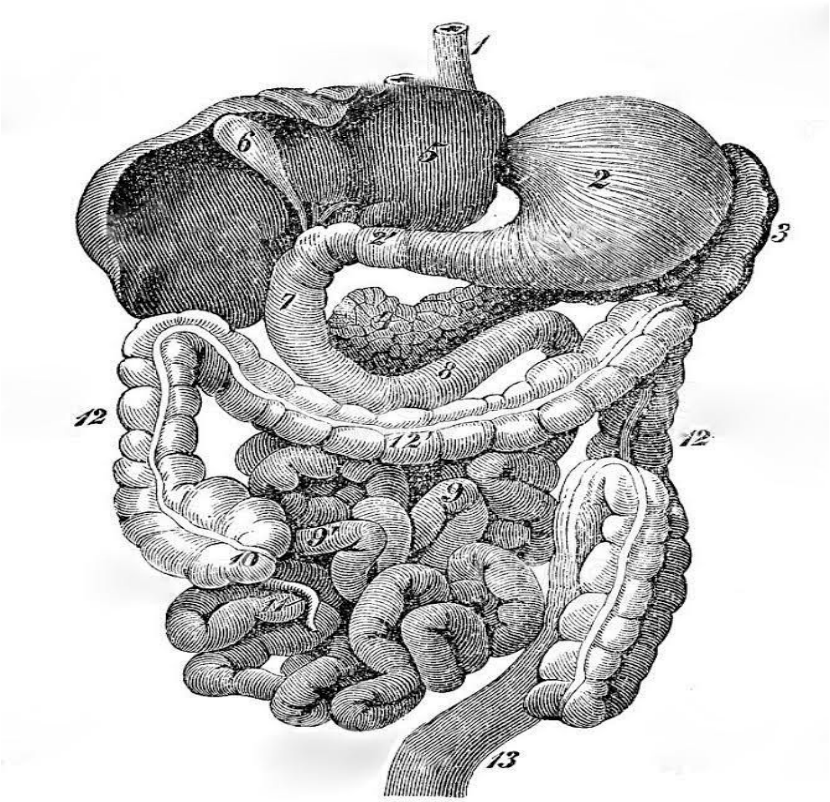
Microbiology

435's Teamwork
GastroIntestinal & Nutrition Block



- Kindly check our Editing File before studying the document.
- Please contact the team leaders for any suggestion, question or correction.
- Pay attention to the statements highlighted in red.
- Extra explanations are added for your understanding in grey.
- **Footnotes color code:** General | Females | Males.
- **color code:** Female's notes | Male's notes.

Revised by

خولة العماري & هشام الغفيلي

Normal flora and introduction to infectious diarrhea

Resources: Sherris Medical Microbiology, LIR Microbiology,...

Learning Objectives:

By the end of this lecture, you should be able to :

1. Define and recognize the various types of acute diarrheal illness
2. Describe the epidemiology the host defenses in preventing the gastrointestinal infection
3. Explain pathogenesis by which Escherichia coli campylobacter and yersinia and their management
4. Discuss the microbiological methods used for diagnosis of each of the bacterial agents including microscopy, selective media for maximal recovery
5. Describe the pathogens, risk factors, clinical presentation and prevention of food poisoning travelers and antibiotic associated diarrhea.
6. Name the etiological agents causing food poisoning and their clinical presentation

Normal flora - Gastrointestinal tract

Ecology[1]: اماكن عيش البكتيريا

- Birth→ **sterile**
- **Breast-fed Bifidobacteria species** (>90% of intestinal flora)
- Switch to cow's milk: Bifidobacteria joined by **Enteric, bacteroides, enterococci, lactobacilli and clostridia**
- Switch to solid food/ Microflora similar to parents
- Primarily anaerobic[2]:Facultative aerobes deplete oxygen
- Adult excretes $3x10^{13}$ bacteria/day→25%-35% of fecal mass = bacteria

GI ecology→Varies كل لما نزلنا تحت كلما كثرت البكتيريا

Esophagus: Microbes associated with saliva and food	**Stomach:** Low pH limits population numbers (10 bacteria/ml)
Small intestine **Proximal small intestine** (duodenum and jejunum): Sparse (<10^3 bacteria/ml fluid) due to acid from stomach, bile and pancreatic secretions **Distal small intestine** (ileum): Increases (10^8 bacteria/ml) due to pH change In adult-bacteria per gram: Duodenum →10^3-10^6 Jejunum and ileum→10^5-10^8	**Large intestine** 10^9-10^11/ml >350 species (E.coli = 0.1% of total population) In adult-bacteria per gram: Cecum and transverse colon →10^8-10^{10} sigmoid colon and rectum →10^{11}

[1] The branch of biology that deals with the relations of organisms to one another and to their physical surroundings.علم البيئة
[2] In the normal adult colon, 96–99% of the resident bacterial flora consists of anaerobes

Infectious diarrhea

Background:

- Most of the Diarrhea is caused mainly by viruses
- Diarrhea can be watery, bloody or mucus
- Diarrhea with mucus and blood we call it "dysentery" you should know it, it's serious and dangerous it can lead to complication and it's infectious, other types aren't that serious unless it's in high amounts and high frequencies (it usually leads to dehydration)
- Immunocompromised patients develop severe diarrhea from unusual and uncommon organisms
- People on antacids are more susceptible for developing diarrhea
- 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.

Definitions	Etiology	Epidemiology	Risk Factors	Diagnostic approach
-Stool weight in excess of 200 gm/day - 3 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	- 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%	1.4 million deaths in study in 2010 - 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	- Food from restaurant - Family member with Gastrointestinal symptoms - Recent travel to developing countries - Patient underlying illness and mediation (↓Stomach acidity cyst, spores) - Abnormal peristalsis - Low IgA - Antibiotics decrease the normal flora to less 10^{12} - Median infective dose (ID50)[3]	**-Duration** Chronic vs acute **-Symptoms** :Fever, bloody, weight loss and dehydration **-Risk factor**: Travel , immunocompromised , diet, medications, outbreak -Fever and blood do culture Watery no fever **-Symptomatic** treatment Hospital acquire think about *C.difficile* **-Chronic diarrhea** think about Protozoa (giardia, crypto, cyclo, microsporidia, MAC Other malabsorption, lactase deficiency. Or bacterial overgrowth

[3] that amount of pathogenic microorganisms that will produce demonstrable infection in 50per cent of the test subjects.

Classifications			
Infectious diarrhea: viral Bacterial(organism.Campylobacter, Shigella, Salmonella,Yersinia, Cholera and E.coli)	**Antibiotic associated diarrhea:** Clostridium difficile.	**Traveler diarrhea:** Enterotoxigenic E-coli IP >1 day last 3	**Food poisoning:** A STORY: أخوانك جابوا بيتزا و أكلوا و تذكروك بعد ساعتين و حطوها لك بالثلاجة جيت انت بعد فترة جيعان طلعت الأكل من الثلاجة وطبعاً سخنت بالمايكروايف و بكذا على أساس انه الحرارة قتلت كل الباكتيريا لكن بعد ساعة و نص .. تقيأت ؛ ليش؟ انت سخنت الأكل كيف جاك تسمم؟ لأن The toxin was performed in the room temperature and it's heat stable (so there's no difference if you killed the bacteria with heat; the toxin is there already) and this is what we call it food poisoning and it is a poison of the Autonomic nervous system(**Enterotoxin mediated)**. ^Remember what causes the manifestation of the disease is NOT the bacteria itself but its toxins What are the bacteria that can cause food poisoning? THREE: **Staphylococcus, Clostridium perfringens, Bacillus** In Buffet (البوفيه) some people get sick and not others and it could be for different reasons may be because someone ate the salad (which is contaminated) while others didn't, or some have lower acidity in their stomach, Sometime people who came late they get the food poisoning because the toxins will be produced because the temperature comes down, So we can say it depends on the type of food he eats, the time and the person himself

Don't worry about the incubation periods all what you need to remember that all of them need 1-3 days but only the food poisoning is 1-18h, So all the infectious diarrhea takes more than day but only food poisoning less than a day (most likely within 6 hours) that start with the stomach (causing vomiting) and then will cause diarrhea, OK? So simple; but remember this exception please and you will be done Listeria monocytogenes takes (2-3) or (2-6) weeks and Endameba histolytica takes 1-3 weeks

Clinical Presentation and Pathogenic Mechanism

Non-invasion

- Enterotoxin mediated
- Lack of pus in the stool (no invasion)
- Lack of fever
- Rapid onset preformed toxin<12 hr
- Small intestine
- causes watery -rarely bloody- diarrhea,nonbloody diarrhea,Vomiting, abdominal cramp.
- Non-invasive infectious can be caused by Salmonella, Campylobacter, Yersinia, Listeria and Cholera
- ***Vibrio cholerae***, *Staphylococcus aureus, Clostridium perfringens* and *Bacillus cereus*

Other viral and some parasitic infection

- The source of Listeria monocytogenes is unpasteurized milk; cheese (basically cows' products), it is very serious in children it can cause brain abscess or meningitis, and also for pregnant lady it can cause abortion

Invasion

that means it has cytotoxicity that kills the cells leading to **bloody mucus diarrhea;** they're called invasive NOT because they enter the blood but they damage the mucosa of the intestine (causing destruction of the cells leading to the presence of blood in the stool)

You should know that the MOST INVASIVE are two organisms only either Shigella, or Endameba Histolytica and they (ALWAYS) produce blood and mucus (bloody mucus diarrhea)^that's why they're called invasive

- Pus and blood in the stool
- Fever due to inflammation
- ***Shigella*, and Endameba histolytica**
- Affect colonic mucosal surface of the bowel
- Sometime, some *E-coli* EHEC , *Yersinia* and *Clostridium difficile*
- Extension to lymph nodes
- Incubation period 1-3 days
- E.histolytica 1-3 wk
- Dysentery syndrome- gross blood and mucous

I want you to know the enterotoxigenic and neurotoxigenic:
Neurotoxigenic is food poisoning (staph. Aureus, Clostridium perfringens and bacillus cereus)
how enterotoxins cause diarrhea?
In short you just need to know that there is heat stable and heat labile, they go and enter the cell and then the active subunit work on cAMP and then affect the channels and prevent absorption of Cl leading to diarrhea

TOXIN TYPE	TOXIN-PRODUCING BACTERIA	TOXIN NAME, IF RELEVANT
Neurotoxin Central autonomic Nervous system Food poisoning	*Staphylococcus aureus* →	Enterotoxin B
	Bacillus cereus →	Emetic toxin
	Clostridium botulinum →	Botulinum toxin
Enterotoxin	*Vibrio cholerae* →	Cholera toxin
	Enterotoxigenic *Escherichia coli* →	Heat-labile toxin, heat-stable toxin
	Clostridium perfringens →	Enterotoxin
Cytotoxin	*Shigella dysenteriae* type I →	Shiga toxin
	Enterohemorrhagic *E. coli* →	Shiga toxins 1 and 2 Globotriaosylceramide (Gb3) 23 S r RNA in 60 Subunit
	Vibrio parahaemolyticus →	Thermostable direct hemolysin
	Campylobacter jejuni →	Cytolethal distending toxin
	Clostridium difficile →	Toxin A, toxin B
	Clostridium perfringens →	Alpha toxin
	E. histolytica →	protozoal phospholipase A and pore-forming peptides

Shigella and *Salmonella*

will be discussed in a separate lecture with more details(u can skip it)

- Salmonella enterica is the **common** cause of food poisoning in Saudi Arabia.

Salmonella we have to types
(1) that is transported through humans (typhi and paratyphi) the
(2) through the reptile (الزواحف) and snakes

- Salmonella typhi transmitted through human faeces.
- Shigella causes local Gastrointestinal invasion and bacteremia less common in normal host
- Shigella is in human ONLY and its infectious dose is too low that means (few organisms are capable of causing the infection) like 10 organisms are enough whereas cholera it needs 100 thousands in order to have the infection

Campylobacter

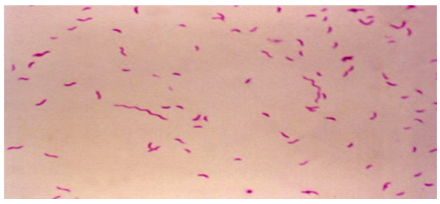
- Family Campylobacteraceae
- Genus arcobacter
- Source: poultry, birds, dog , cat, →water, milk, meat, person to person can occurs عن طريق الدجاج و منتجاتها زي البيض
- campylobacter is Seagull shaped (مثل شكل طائر النورس) they need specific high temperature to grow (42ºC) It's the only bacteria that grows at 42-degree different temperature that they will not grow

Clinically

- IP(incubation period) 2-6 days
- Abdominal cramp, bloody diarrhea , nausea and vomiting are rare
- Self limiting 2-6 Day
- Chronic carrier
- GB and Reactive arthritis

Laboratory diagnosis self limiting in the majority of cases NO need for Lab investigation

- Transport media Cary Blair
- CAMPY BAP contain antibiotics
- Incubate in 5%O2 10%CO2 85%N at 42°C except *C.fetus* 37°C
- Gram stain/culture biochemical/Serology



Treatment

Ciprofloxacin, Erythromycin or Tetracycline

Clostridium difficile

- Antibiotic associated **diarrhea**
- Transmit from person to person via Fecal-Oral route
- Have been cultured from in animate hospital surfaces
- Disruption of the indigenous bacterial flora of the colon
- Produce toxin A and B that can bind to surface epithelial cell receptors leading to inflammation mucosal injury and diarrhea
- 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, toxin detection by EIA
- Treatment Metronidazole ± Vancomycin and supportive treatment

E.Coli

- E.coli is a normal flora of GIT but sometimes they have somatic and flagellar antigens that make some of them toxigenic and can cause different types of diarrhea (like traveler and enterohemorrhagic)
- Based on virulence factors, clinical manifestation, epidemiology and different O(somatic antigen) and H (Flagellar antigen)serotype.
- Only about 10 -15% of strains of E. coli associated with diarrhea.
- There are five major categories of diarrheagenic E.coli:

Entero**toxigenic** E.coli **(ETEC)**	- Enterotoxigenic E. coli is same as the salmonella and food poisoning and they have heat stable and heat labile toxins -Major cause of traveler's diarrhea in infant and adult in developing countries from contaminated food and water - It has ↑infective dose 10^6-10^10 - Has heat-labile toxin (LT) and heat-stable toxin (ST) each has two fragment (A and B) LT leads to accumulation [4]of CGMP, which lead to hyper secretion - Symptoms watery diarrhea, abdominal cramps and some time vomiting - No routine diagnostic method.
Entero**invasive** E.coli **(EIEC)**	- Produce dysentery (Penetration, invasion and distraction) - Similar to Shigella spp (Non motile, LNF)Enteroinvasive E-coli same as Shigella - Fecal oral route - Fever, severe abdominal cramp, malaise and watery diarrhea - Infective dose: 10^6 - Diagnosis Sereny test and DNA probes.[5]
Prof ali alsomily said it is very very imp **Enterohemorrhagic E.coli (EHEC)** أخطرنوع	Entero hemorrhagic E.Coli is found in the beaf المشكلة مش في البييف نفسه لكن الأبقار لما يجوا يذبحوها في المصلخ يكون الجي إي تي حق الحيوان مفتوح و بالتالي هذا الي يسبب العدوى فلما تجي تاكل البييف يكون مصاب و ملوث بالعدوى بسبب هالشيء و خصوصاً لما ما يطبخ أو ما يستوي بشكل كامل لذلك نسميه Hamburger's disease It's very common during the summer because of the BBQ trips The problem with this, is not the bloody diarrhea but it can cause renal failure because they obstruct the blood flow to the kidneys ... and can be missed easily because they present first with bloody diarrhea only and after days it causes hemolytic uremic syndrome - O157H7 and Non O157H7 [6]Hemorrhagic diarrhea, colitis and hemolytic uremic syndrome (HUS)=↓Platelet count, hemolytic anemia and kidney failure - Undercooked hamburgers, unpasteurized dairy products, apple cider, cookie dough - Bloody diarrhea, low grade fever and stool has no leucocytes - Fetal disease in young and elderly persons in nursing homes - Cytotoxin = verotoxin I and verotoxin II Similar to Stx1 (shigotonin I&II) - E.coli other than 0157H7 can cause HUS - Diagnosis by culture on SMAC, MUG test , Verotoxin detection by immunological test or PCR

Enteropathogenic and Enteroadherent E-coli are NOT important
Prof alsomily said (you won't be asked about it)

[4] Without invasion or inflammation
[5] Self limiting no need to investigation
[6] there are many strains of EHEC that can cause hemorrhagic diarrhea but These the most common cause of hemorrhagic diarrhea

Yersinia enterocolitica

Yersinia enterocolitica is present in the GIT of the pork (that's why it's more common during the thanksgiving), rarely can cause diarrhea but usually comes with Mesenteric lymphadenitis (affecting the lymph node of the ileocecal junction) causing pain in the right lower quadrant (MIMICS APPENDICITIS)

- Mesenteric lymphadenitis in children and septicemia in immunocompromised hosts
- Common in Europe, USA, Canada and 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 1'C adult children 1-5 yrs usually mild but in old children adult mimic appendicitis
- Growth at 25°c-30°c media Cefsulodin-Igrasan-Novabiacin (CIN)

جدول مهم جدا للصميلي ونبه عنه أثناء المحاضرة وشرح عليه، وهو تقريبا يلخص الثلاث محاضرات
(Normal flora+Cholera+shigella & salmonella)

Type	Organism	Incubation Period	Source Risk factors	Clinical	Diagnosis	Treatment
Infectious diarrhea Invasive Dysentery Blood+mucous	Shigella gram-negative rods	1-3 Days	Human	Small amount of stool with blood and mucous and lower abdominal pain (trismus)	Culture on selective media	Ciprofloxacin
	Entamoeba histolytica	1-3 wks	Contaminated Food with human excreta		Microscopy	Metronidazole
	EIEC gram-negative rods	1-3 Days	Contaminated Food with human excreta		Culture and toxin detection	
Infectious diarrhea Non-invasive Watery +/-blood	Salmonella gram-negative rods	1-3 Days	S.typhi and S.para Human Others reptiles and snakes	Watery +/-Blood	S.typhi and S.paratyphoid Others watery diarrhea	Ampicillin or ciprofloxacin or metronidazole or trimethoprim sulfa
	campylobacter jejuni small, curved, gram-negative rods		poultry	Watery+/-Blood	Special media at 42 °C at microaerophilic condition	Erythromycin
	EHEC		beef	Watery then bloody diarrhea Renal failure	Culture and toxin detection	

Infectious diarrhea Non-invasive Watery +/-blood	Vibrio cholera Comma-shape gram-negative rods	1-3 Days	Salt water	Rice water		Ciprofloxacin Doxycycline
	Yersinia Gram Neg bacilli		Pork intestine	Pseudo appendicitis	Cold enrichment 25-30°C CIN (Yersinia)	Gentamycin
	Listeria Monocytogenes Gram pos bacilli	2-3 wks	unpasteurized dairy products	Meningitis in neonate and old Abortion		Ampicillin
Food Poisoning Watery (preformed toxin)	Staphylococcus aureus	1-6 hour	Contaminated food from human flora	Vomiting then watery diarrhea	Culture and toxin detection	Observation and supportive treatment
	Clostridium perfringens	8 to 16 hours	Food contaminated with Soil			
	bacillus cereus spore forming aerobic Gram pos bacilli	8 to 16 hours	8 to 16 hours			
Travelers	ETEC	1-3 Days	Travel	Watery diarrhea	Culture Toxin detection	ciprofloxacin
Antibiotics Associated	Clostridium Difficile	1-3 Days	Antibiotics use patientà patient	Bloody diarrhea Toxin A and B	Toxin detection PCR	Metronidazole +/- Vancomycin

Summary (شامل كل الي قاله بروف الصميلي خلال المحاضرة)

Infectious diarrhea

Most of the Diarrhea is caused mainly by viruse
Diarrhea can be watery, bloody or mucus
Diarrhea with mucus and blood we call it "dysentery" you should know it, it's serious and dangerous it can lead to complication and it's infectious, other types aren't that serious unless it's in high amounts and high frequencies (it usually leads to dehydration)
Immunocompromised patients develop severe diarrhea from unusual and uncommon organisms

People on antacids are more susceptible for developing diarrhea

Classifications

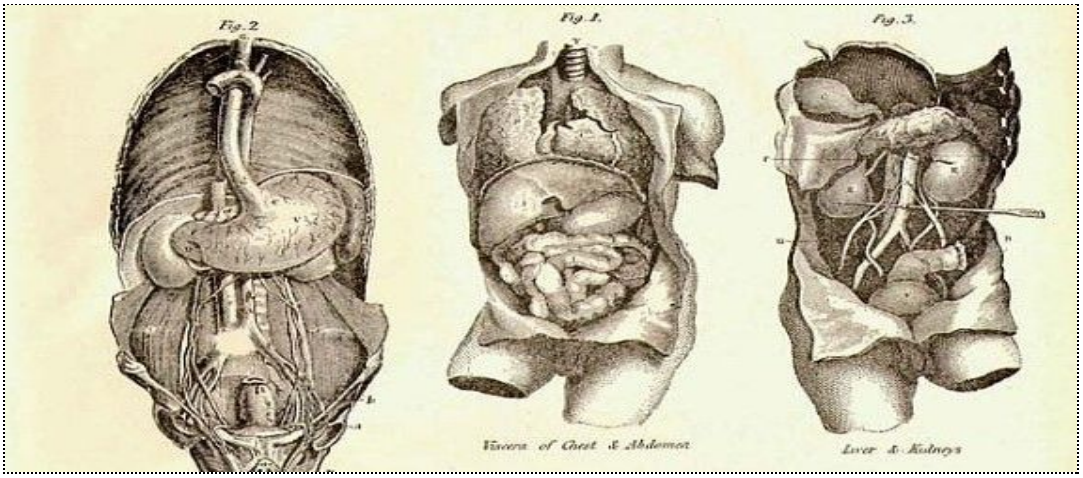
Infectious diarrhea: Viral most common Bacterial organism.(Campylobacter, Shigella, Salmonella,Yersinia, Cholera and E.coli)	**Antibiotic associated diarrhea:** Clostridium difficile	**Traveler diarrhea:** Enterotoxigenic E-coli	**Food poisoning:** Staphylococcus, Clostridium perfringens, Bacillus. -**Enterotoxin mediated:** these bacteria produce heat stable toxin that affect the Autonomic nervous system -I want you to know the enterotoxigenic and neurotoxigenic: Neurotoxigenic is food poisoning (staph. Aureus, Clostridium perfringens and bacillus cereus) how enterotoxins cause diarrhea? In short you just need to know that there is heat stable and heat labile, they go and enter the cell and then the active subunit work on cAMP and then affect the channels and prevent absorption of cl leading to diarrhea

Clinical Presentation and Pathogenic Mechanism

non-invasion	invasion
Enterotoxin mediated that causes watery -rarely bloody- diarrhea caused by Salmonella, Campylobacter, Yersinia, Listeria and Cholera	that means it has cytotoxicity that kills the cells leading to bloody mucus diarrhea; they're called invasive NOT because they enter the blood but they damage the mucosa of the intestine (causing destruction of the cells leading to the presence of blood in the stool) MOST INVASIVE are two organisms only either Shigella, or Endameba Histolytica and they (ALWAYS) produce blood and mucus (bloody mucus diarrhea)

Bacteria	Description
Shigella, Salmonella and Campylobacter	- Salmonella enterica is the common cause of food poisoning in Saudi Arabia. - Salmonella we have to types (1) that is transported through humans (typhi and paratyphi) the (2) through the reptile and snake - Shigella is in human ONLY and its infectious dose is too low that means (few organisms are capable of causing the infection) like 10 organisms are enough whereas cholera it needs 100 thousands in order to have the infection - campylobacter is Seagull shaped (مثل شكل طائر النورس) they need specific high temperature to grow (42°C) It's the only bacteria that grows at 42-degree different temperature that they will not grow Source: poultry, birds, dog , cat, →water, milk, meat, person to person can occurs
Yersinia enterocolitica	present in the GIT of the pork (that's why it's more common during the thanksgiving), rarely can cause diarrhea but usually comes with Mesenteric lymphadenitis (affecting the lymph node of the ileocecal junction) causing pain in the right lower quadrant (MIMICS APPENDICITIS)
Clostridium -difficile	- Antibiotic associated diarrhea - Have been cultured from in animate hospital surfaces - Disruption of the indigenous bacterial flora of the colon - Produce toxin A and B that can bind to surface epithelial cell receptors leading to inflammation mucosal injury and diarrhea - Pseudomembrane can result
E.Coli	- E.coli is a normal flora of GIT but sometimes they have somatic and flagellar antigens that make some of them toxigenic and can cause different types of diarrhea (like traveler and enterohemorrhagic) - Based on virulence factors, clinical manifestation, epidemiology and different O and H serotype. - There are five major categories of diarrheagenic E.coli: Enterotoxigenic E.coli (ETEC): traveler's diarrhea same as the salmonella and food poisoning and they have heat stable and heat labile toxins Enteroinvasive E.coli (EIEC):Produce dysentery & Similar to Shigella spp Enterohemorrhagic E.coli (EHEC):found in the beaf ,calledHamburger's disease ,lead to hemolytic anemia and kidney failure Enteroadherent E.coli (EAEC) Enteropathogenic E.coli (EPEC)
E. histolytica	comes from the contaminated food
Listeria monocytogenes	The source of Listeria is unpasteurized milk; cheese, it is very serious in children it can cause brain abscess or meningitis, and also for pregnant lady it can cause abortion

Incubation period
all of them need **1-3 days** but only the food poisoning is 1-18h, So all the infectious diarrhea takes more than day but only food poisoning less than a day (most likely within 6 hours) that start with the stomach (causing vomiting) and then will cause diarrhea, OK? So simple; but remember this exception please and you will be done Listeria monocytogenes takes (2-3) or (2-6) weeks and Endameba histolytica takes 1-3 weeks



إِنَّا كُلَّ شَيْءٍ خَلَقْنَاهُ بِقَدَرٍ ﴿٤٩﴾

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