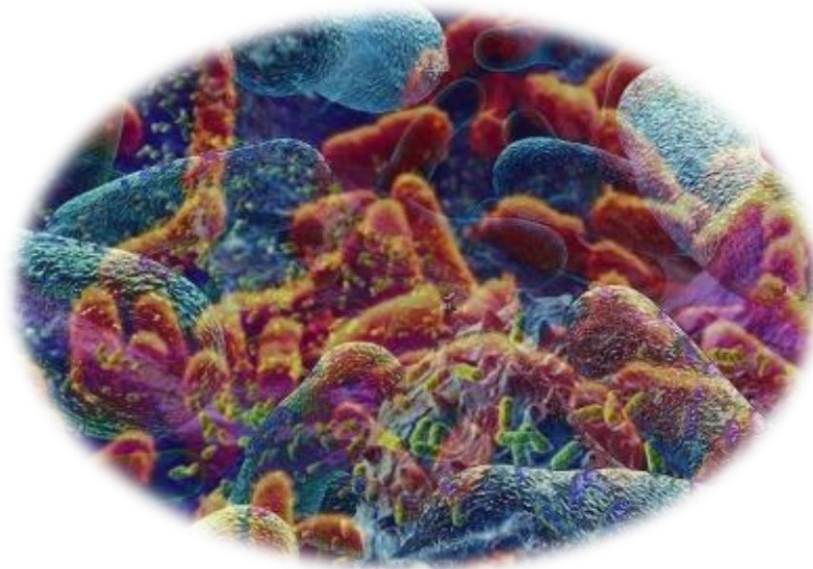# 431
Microbiology Team

Normal Flora & introduction to infectious diarrhea

GIT & HAEMATOLOGY BLOCK

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Normal Flora & introduction to infectious diarrhea

Background:

- Acute diarrheal illness is one of the most common problems evaluated by clinicians (the most common disease caused by infection of GIT).
- A major cause of morbidity and mortality worldwide.
- Most of healthy people have mild illness but others might develop serious squeals so it is important to identify those individuals who require early treatment.

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 ,diet and physiologic status.
- Able to colonize and multiply under the exiting condition of different body sites.
- **Inhibit competing intruders**.
- Have symbiotic relationship that benefits the host.
- **Can cause disease in immunocompromised patients.**

> Why do we have normal flora in our bodies ?
> To protect us from superinfections.

Normal flora in GIT:

- At birth, the gastrointestinal tract is sterile.
- Normal flora is then obtained through breastfeeding: (Bifidobacteria species (>90% of intestinal flora)) , and later by switching to cow's milk (Bifidobacteria joined by enteric, bacteroides, enterococci, lactobacilli and clostridia) . And finally solid food (Microflora similar to parents). The doctor didn't mention any of the above bacteria he only mentioned the line at the end of this page .
- The oral cavity contains very high number which varies from site to site of the mouth.
- Saliva contain mixed flora :10x8 organism /ml.
- Stomach : **very few** due to HCL (acidity) and peptic enzymes .
- Small intestine : **very scanty** except near colon.
- **Colon of adults:** 10x 10 org/gm stool, >90% are Bacteriodes (anaerobic), 10 % other bacteria.The Colon has the majority of Normal Flora in the GIT.
- The normal flora in the GIT **is mostly anaerobes**, Enterobacteriaceae , pseudomonas , viridians . E.coli could be found in very small amounts as normal flora in small intestine.

- 25-30% of fecal mass is bacteria.

Normal flora (low	Potential pathogen
▸ **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.infuenzae,S.pyogenes, S.aureus* ▸ **Stomach**: none ▸ **Small intestine** : none ▸ **Colon of adults**: *B.fragilis, E.coli, Psudomonas, Candida, Clostridium* ▸ **Colon of Breast feeding infants**: none

What the doctor stressed on here is **Neisseria**, and she said in general normal flora are bacterial species while the potential pathogen has specific pathogen's name.
e.g : Neisseria spp. is normal flora while Neisseria meningitides is a pathogen.

Role of GIT Normal Flora in Disease:

- Many are opportunistic pathogens, examples**, perforation of the colon from rupture causes feces to enter into peritoneal cavity and cause peritonitis**.
- Viridans strept. of oral cavity enters 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** is the most common Enterobacteriacae , a facultative flora of colon followed by Klebsiella, Proteus and Enterobacteria. (remember: the most common type of bacteria causing diarrhea are anaerobes while the most common enterobacteriaca is E.coli).
- 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. " *Salmonella* is an invasive intracellular

Intestinal Pathogens

- **Invasive and cytotoxic strains** produce inflammatory diarrhea (**dysentry**) with WBCs (pus) and /or blood in the stool. " Such as Shigella "
- **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

Definition of Diarrhea :

- Stool weight in excess of 200 gm/day , Three or more loose or watery stools/day.
- Characterized by decreased consistency and increased frequency.
- Less than 14 days in duration.

Etiology & Epidemiology :

- **Viruses are the most common cause of diarrhea 70-80 % and are more dangerous in children.**
- Followed by bacteria 10-20% (more dangerous in adults) . Less than 10% are caused by protozoa (self limiting"no need for treatment") , and it is rarely caused by Fungi.
- **Children under 3 years old are usually affected from the daycare**.
- Seasonal peak : in winter.

Risk factors :

1-Food from restaurants & Traveling	4-Low immunity
2-Family member with GI symptoms	5-Antibiotics
3-Abnormal Peristalsis	6-Decreased stomach acidity

Classification :

1) **Infectious Diarrhea :**
   - **Viral : ROTA Virus is the most common**
   - **Bacterial : Salmonella(most common) , Campylobacter** , Shigella, E.coli,Yersinea , Cholera
   - **Parasite : Entamoeba histolytica** , giardia
2) **Food Poisoning (enterotoxin mediated): Staphylococcus aureus , Clostridium Perfringes , Bacillus cereus**
3) **Traveler's diarrhea : EnteroToxogenic E. coli.**
4) **Antibiotic associated diarrhea : Clostridium Difficile.**

No vomiting in infectious diarrhea.

Unlike food poisoning (with vomiting) .

It is very important to know the clinical presentation and the causative organisms for each one , the incubation period is not important except for food poisoning (less than 12 hours) , the rest are ALL 1-3 days.

Clinical Presentation & Pathogenesis :

1) **Enterotoxin Mediated (eg : Food Poisoning)**
   Rapid onset , less than 12 hours .
   Watery diarrhea with No pus or blood in stool (no invasion)
   Vomiting , Mild abdominal discomfort (cramps)
   Affects **small intestine.**
   Same bacteria causing food poisoning + Vibreo cholero.
   Could be caused by viruses and some parasites .
   Self-limiting. (no need for antibiotic) .
2) **Invasive :**
   Incubation period : 1-3 days.
   Severe crampy lower abdominal pain (won't relive until patient passes stool)
   Dysentery (blood + mucous in stool)
   Pus in stool, Fever , Extension to lymph nodes
   Affects **colon**.
   It is caused by : **Shigella, Entamoeba histolytica**
   Entamoeba histolytica incubation period 1-3 weeks.

Rapid onset is because it is caused by toxins that will directly cause the diarrhea. (will not pass by regular mechanism of infection : attach , adhesion..etc.)

Toxin is heat stable. Will survive in food even if heated.

3) Bloody diarrhea :
Incubation period : 1-3 days
Causative organisms : Campylobacter , Salmonella , E.coli (EHEC) , Clostridium difficile (not always).

Shigella & Salmonella :

- **Salmonella enterica: the most common cause of food poisoning & diarrhea in Saudi.**
- Salmonella typhi transmitted through human faeces.
- Shigella causes local GI invasion , could cause bacteremia in immunocompromised.

Campylobacter :

- Common specious: **C. jejuni**
- Family: Campylobacteraceae
- Genus: arcobacter
- Source: dog , cat, birds, poultry (chicken) ,water, milk, meat, person to person can occur.
- **Spiral gram negative rods. (seagull shaped) .**
- Patient presents with **: Bloody diarrhea** , abdominal cramps ,nausea & vomiting.
- It is a **self limiting** disease after 2-6 days.
- However it is Resistance to Ciprofloxacin , Sensitive to Erythromycin or Tetracycline .
- Laboratory : transport media : Cary blair . Grow on Campy Bap agar.

> Campylobacter is a fastidious organism (need special media to grow) , Campy bap agar contains antibiotic (to prevent growth of normal flora) and contains low amount of Oxygen (microaerophilic organism) and it is heated to 42 degrees (heat resistance except for C. fetus.)

Yersinia enterocolitica :

- Common in USA & Canada & Europe.
- Could be isolated from cats , dogs and swine (pigs).
- **Causes mesenteric lymphadenitis** and pain in the right lower quadrant .It is **thought to be appendicitis.**
- Patient also presents with enteritis and could have arthritis and erythema nodosum.
- However in children between 1-5 years old it is usually mild.
- **Grows in CIN agar** (Cefsulodin-Igrasan-Novabiacin) and could grow in MacConkey agar but takes long time.
- Survive cold temperatures and is associated with transfusion of packed red blood cells.

> It is common in thanksgiving because they usually eat the pig's intestine (chitterlings) which contains the bacteria .

Clostridium difficile :

- **Antiobiotic associated diarrhea**. Especially ciprofloxacin.
- Transmited through fecal-oral route , have been cultured from animate hospital surfaces.
- It is normal flora in the colon but in small numbers . With excessive use of antibiotics lots of normal flora will be killed (but not Clostridium) which will cause it to multiply and produce toxins which will cause diarrhea and mucosal injury.
- Produces two toxins :
- **Toxin A : enterotoxin which causes diarrhea.**
- **Toxin B : cytotoxic which causes mucosal injury**.
- Patient presents with fever, leukocytosis, abdominal pain and foul smelling diarrhea.
- **It causes pseudomembrane colitis and toxic megacolon** which is fatal if not treated. It is sometimes associated with bloody diarrhea .
- Diagnosis, toxin detection by EIA
- Treatment : **Metronidazole** ± Vancomycin and supportive treatment.
- Clostridium forms spores which cause outbreaks(bathrooms in hospital) in elderly.
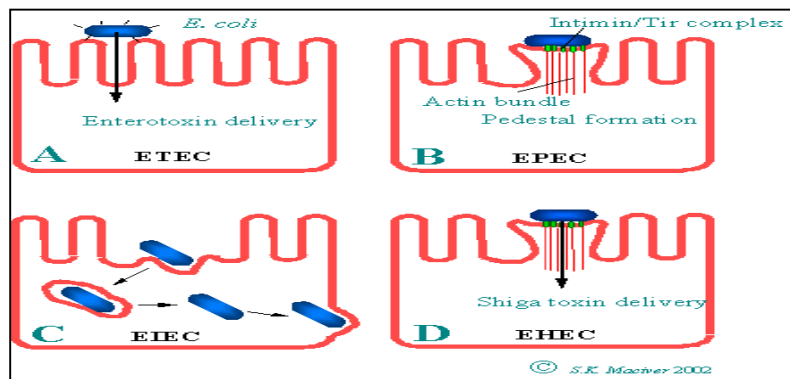
E.coli :

Only about 10 -15% of strains of E. coli associated with diarrhea.

Remember E.coli is Gram negative bacilli .

E. coli has 2 antigens , antigen O which is somatic and antigen H which is flagellar.

There are five major categories of diarrheagenic E.coli:

1. Enterotoxigenic E. coli (ETEC)	2. Enteropathogenic E. coli (EPEC)
3. Enteroinvasive E. coli (EIEC)	4. Enteroadherent E.coli (EAEC)
5. Enterohaemorrhagic E. coli (EHEC)	

1. **EnteroToxigenic E.coli : Traveler's diarrhea .**
- Affects people who travel from north America to South America and mexico 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 of CGMP, which lead to hyper secretion.
- Symptoms : watery diarrhea, abdominal cramps and sometimes vomiting .
- No routine diagnostic method.

2. **Enteropathogenic E. coli (EPEC):**
- Infantile diarrhea , could cause outbreak in hospital and daycare centers.
- Low grade fever, malaise, vomiting and diarrhea ,Stool mucous but no blood.

3. **Enteroinvasive E.coli (EIEC) :**
- Produce **dysentery.**

LNF : lactose none fermenting .

- **Similar to Shigella** (Non motile , LNF)
- Fever, severe abdominal cramp, malaise and watery diarrhea
- Fecal oral route , Infective dose10^6
- Diagnosis : **Sereny** test and DNA probes.

4. Enteroadherent E.coli (EAEC)
- Pediatric Diarrheal Disease
- Adhering to the surface of the intestinal mucosa
- Aggregative stacked brick
- Watery diarrhea, vomiting, dehydration and abdominal pain
- Two or more weeks

5. Interoheamorhhagic E.coli (EHEC) "the doctor has concentrated more on this subtype"
- E.coli O157H7 (the name of the serotype).
- **Raw beef** ,Undercooked hamburgers, unpasteurized dairy products, apple cider, cookie dough.
- **Hemorrhagic diarrhea**, colitis and hemolytic uremic syndrome (HUS) =↓Platelet count, hemolytic anemia and kidney failure
- **Bloody diarrhea**, low grade fever and stool has no leucocytes
- Fetal disease in young and elderly persons in nursing homes.
- Cytotoxin =vertoxin I and vertoxin II Similar to Stx_1 (shigotoxin I&II)
- E.coli other than O157H7 can cause HUS
- Diagnosis by culture on SMAC, MUG test , Vertoxin detection by immunological test or PCR.

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.**

About these 2 tables :

Dr. Fawzia said don't focus on the numbers but on the colored words of the last 2 columns .

Summary

- ✓ Viral infections are more common and more dangerous for children.
- ✓ Bacterial are more dangerous for adults.
- ✓ **NO treatment** for any kind of the diarrhea **except for cholera and shigella and antibiotic associated.**
- ✓ Food poisoning is <u>watery diarrhea</u> and caused by : Staphylococcus , Clostridium Perfringes , Bacillus cereus
- ✓ **Dysentery** is seen in infectious caused by **: Shigella , Entamoeba histolytica.**
- ✓ **Bloody diarrhea : Campylobacter , Salmonella , EHEC.**
- ✓ **EHEC causes BLOODY diarrhea.**

○ **E.coli:**

Pathogen	Infection
- Entero-toxigenic E. coli (ETEC).	Traveler's diarrhea
- Entero-pathogenic E. coli (EPEC).	Infentile diarrhea
- Entero-invasive E. coli (EIEC).	Dysentery (blood & mucus)
- Entero-haemorrhagic E. coli (EHEC).	Bloody diarrhea (very sever)
- Entero-adherent E.coli (EAEC).	Pediatric diarrheal disease (same as EPEC)

- ✓ **Salmonella** is the most common cause of diarrhea.
- ✓ **Campylobacter** most common organism causing **bloody diarrhea** it is cultured in **special agar** and has a **spiral rod** shape & it's **Gram Negative.**
- ✓ **Yersinia enterocolitica :** from pigs and mimics appendicitis.
- ✓ **Clostridium difficile:** antibiotic associated diarrhea "Pseudomembranous colitis". treated by : **Metronidazole**

Questions:

Which one of the following normal floras could lead to diarrheal disease ?

A. Salmonella
B. Shegilla
C. E.coli
D. Yersinia enterocolitica

A patient presets with bloody diarrhea, bacteria is grown in special agar shows " spiral gram negative rods" , what is the organism causing this disease.

A. Enterotoxic Ecoli
B. Clostridium perfrings
C. Vibrio cholera
D. Campylobacter Jejuni

A patient on ciprofloxacin came to dr.Muhammed clinic with symptoms of abdominal pain and foul smelling diarrhea, the laboratory result shows C.diffcile .. which of the following drugs is the best for treating such case?

A. Metronidazole
B. Erythromycin
C. Tetracycline
D. ciftriaxon

which of the the following organisms could lead to diarrhea associated with food poisoning?

A. Entameba histolytica
B. S.aureus
C. Shegilla
D. Rotavirus

answers: C - D - A - B