

## Lecture-9

# Gram-negative Rods and Gram-negative Cocci

Prof. Dr. Nada Khazal K. Hindi





## table show the Major pathogen and Representative Diseases

Major pathogens	Representative Diseases
<i>Escherichia coli</i>	Urinary tract infection, travelers diarrhea, neonatal meningitis
<i>Shigella</i>	Dysentery diarrhea
<i>Salmonella,</i>	Typhoid fever, Enterocolitis
<i>Klebsiella pneumoniae</i>	Have polysaccharide capsule, Pneumonia, Urinary tract infection, colonize hospitalized patients
<i>Enterobacter</i>	Have polysaccharide capsule Pneumonia, Urinary tract infection colonize hospitalized patients
<i>Proteus</i>	produce swarming, Kidney stones, Urinary tract infection ( nosocomial UTIs)
<i>Serratia marcescens</i>	Pneumonia, respiratory tracts and Urinary tract infection
<i>Helicobacter</i>	gastric ulcers
<i>Campylobacter</i>	food poisoning
<i>Yersinia pestis</i>	Plague, Enterocolitis, mesenteric adenitis
<i>Providencia, Morganella</i>	Urinary tract infection ( nosocomial UTIs)
<i>Citrobacter</i>	UTI, pneumonias, and intraabdominal abscesses
<i>Haemophilus influenzae</i> ( <i>Pfeiffer's bacillus</i> )	influenza pandemic, It is responsible for a wide range of localized and invasive infections, in infants and children, including pneumonia, meningitis

# Enterobacteriaceae

Enterobacteriaceae is a large family of Gram-negative bacteria found primarily in colon of human and animals many as part of the normal flora.

## General properties of members

1. found in the gastrointestinal (GI), facultative anaerobes, non spore forming, some spp. has capsulate, some motile.
2. catalase positive
3. Reduce nitrates to nitrite. Major spp and related disease in following table.

## Pathogenesis

They contain lipopolysaccharide (LPS) in their cell wall, which is both antigenic and a potential virulence factor (endotoxin). In addition several exotoxins are produce by *E. coli* and *V. cholerae*, these exotoxins are called **enterotoxines that activate adenylate cyclase** within the cells of the small intestine, causing diarrhea.





**Antigenic structure:** Three surface antigens:

1. cell wall antigen or somatic Ag (O Ag).
2. the flagellar antigen (H Ag).
3. the capsular or antigen or (K Ag) polysaccharide Ag in encapsulated bacteria.

## **Diagnosis of Enterobacteriaceae**

**-culture:** Blood agar, eosine methylen blue (EMB), and

**-Macconkey (selective and differential media)**, it is **differential media** to detection of lactose fermentation on macconkey agar; **lactose fermenter such as *E. coli*, *Enterobacter* and *Klebsiella* forms pink colonies**, while non lactose fermenter such as *Shigella*, *Salmonella*, *Proteus*, *Providencia*, *Morganella*, and *Serratia* forms pale or colorless colonies.

Macconkey **is selective media** which suppressing Gram+ bacteria due to contain crystal violet.

**-*Proteus* and *Salmonella* produce H<sub>2</sub>S from sulfur-containing amino acids.**

**- oxidase positive : *Neisseria*, *Vibrio* and *Pseudomonas*.**

**- *H. pylori*, *Proteus* and *Klebsiella* produce urease**

**-Serological** test depending on the presence or absence of anti-H or anti-K or anti-O antibodies.

**- molecular techniques** by using PCR specific for selected bacterial genes.

# MacConky

selective  
media

growing G-ve

inhibit G+ve

due to contain crystal violet  
which act on peptidoglycan

differential  
media

lactose fermenter  
(Pink)

- ① E coli <sup>casule</sup> - +
  - ② Klebsiella ⊕
  - ③ Enterobacter ⊕
- only 3

nonlactose  
fermenter  
(Pale)

- ① Salmonella
- ② Shigella
- ③ Serrat

**Table-1 Key Characteristics to differentiate some group of Enterobacteriaceae:**  
 (According to baily and Scotts' Diagnostic Microbiology) للاطلاع

<u>Bacteria</u>	<u>E. coli</u>	<u>Shigella sonnei</u>	<u>Salmonella typhi</u>	<u>Klebsiella pneumoniae</u>	<u>Klebsiella oxytoca</u>	<u>Proteus vulgaris</u>	<u>Proteus mirabilis</u>	<u>Morganella morganii</u>
<u>Test</u>								
Indole	+	-	-	-	-	+	-	+
Methyl Red (MR)	+	+	+	V	-(v)	+	+	+
VogesProskauer (VP)	-	-	-	+	+	-	V	-
Simmons' Citrate	-	-	-	+	+	-(v)	+(v)	-
Hydrogen Sulfide (H <sub>2</sub> S)	-	-	+w	-	-	+	+	-
Urea	-	-	-	+	+	+	+	+
Motility	V	-	+	-	-	+	+	V
Gas from D- glucose	+	-	-	+	+	+	+	+
Lactose	+	-	-	+	+	-	-	-



<i>GENERA</i>	<i>Voges Proskauer (VP)</i>	<i>Phenylalanine Deaminase (PDA)</i>
<i>Klebsiella</i>	Positive (+ve)	Negative (-ve)
<i>Enterobacter</i>	+ve	(-ve)
<i>Serratia</i>	+ve	(-ve)
<i>Proteus</i>	- ve	(+ve)
<i>Morganella</i>	-ve	(+ve)
<i>Providencia</i>	-ve	(+ve)
<i>Escherichia</i>	-ve	(-ve)
<i>Shigella</i>	-ve	(-ve)
<i>Salmonella</i>	-ve	(-ve)
<i>Citrobacter</i>	-ve	(-ve)
<i>Yersinia</i>	-ve	(-ve)

# Growth of *Enterobacteriaceae* on SS agar



- A. *Klebsiella pneumoniae*
- B. *Escherichia coli*
- C. *Salmonella* sp.
- D. *Proteus mirabilis*
- E. *Ps. aeruginosa*

→ Both are lactose fermenters

→ Both *Salmonella* sp. & *Proteus* product H<sub>2</sub>S

→ *Pseudomonas* colonies are nearly colorless



Dr.T.V.Rao



# 1. *E. coli*

**Diseases** : Urinary tract infection, travelers diarrhea, neonatal meningitis, and sepsis. Nosocomial infection

These include sepsis/bacteremia, endotoxic shock, and pneumonia. **Habitat**: human colon, vagina, urethra.

From the urethra, it ascends and causes UTI acquired during birth neonatal meningitis and by fecal –oral route in diarrhea.

## pathogenesis

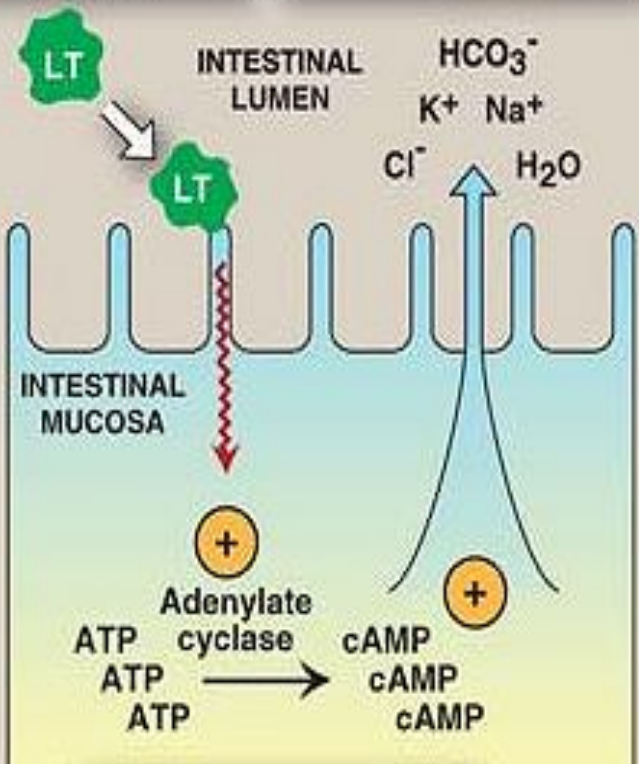
1. **Endotoxin** in cell wall cause septic shock.
2. Also **enterotoxin (heat labile toxin LT)** which stimulates adenylate cyclase by ADP-ribosylation resulting in the increasing in cyclic AMP(cAMP) that causes out flow of chloride ions and water resulting in diarrhea.
3. **enterotoxin (heat stable ST)** causes diarrhea by stimulating guanylate cyclase.
4. **vero toxin (Shiga- like toxin)** is an enterotoxin, it causes bloody diarrhea and hemolytic uremic syndrome associated with eating undercooked meat. Verotoxin inhibits the proteins synthesis. Following table shows strains of *E.coli* and disease.

**Virulence factors**: enterotoxin, pili for attachment, and capsule that suppress phagocytosis.



**1** LT binds to a receptor and activates adenylate cyclase.

**3** Elevated levels of cAMP cause active secretion of ions and water.



**2** Adenylate cyclase produces elevated levels of cAMP.

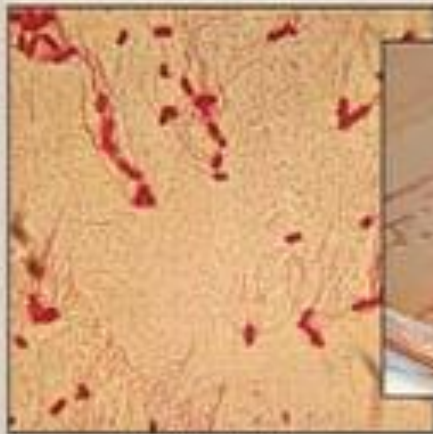


*E. coli*  
(Gram stain)



MacConkey agar

- **Gram-negative**
- Short rods
- Facultative anaerobe
- Ferments glucose
- Most strains ferment lactose
- Catalase-positive
- Oxidase-negative
- Culture on MacConkey agar



*Salmonella typhi*



*Salmonella* species  
on MacConkey agar



STRAIN	ABBREVIATION	SYNDROME	THERÁPY <sup>1</sup>
Enterotoxigenic <i>E. coli</i>	ETEC	Watery diarrhea	Antibiotics may be useful. <sup>2</sup>
Enteropathogenic <i>E. coli</i>	EPEC	Watery diarrhea of long duration, mostly in infants, often in developing countries	Antibiotics may be useful. <sup>2</sup>
Enterohemorrhagic <i>E. coli</i>	EHEC	Bloody diarrhea; Hemorrhagic colitis and hemolytic uremic syndrome (HUS)	Avoid antibiotics because of the possible risk of potentiating HUS.
Enteroinvasive <i>E. coli</i>	EIEC	Bloody diarrhea	Rehydration and correction of electrolyte abnormalities.
Enteroadherent <i>E. coli</i>	EAEC	Persistent watery diarrhea in children and patients infected with HIV	Rehydration and correction of electrolyte abnormalities.

## Prevention & Treatment

Eating cooked meat and drinking boiled water, Doxycycline may be prevent traveler diarrhea.

Maintenance of fluid and electrolyte balance is primary importance in treatment.

Prevention of UTI involves limiting the frequency and duration of urinary catheters.

Prevention of sepsis involves removing or switching sites of I.V. catheters.

There is no vaccine against *E. coli* infection.

# *Salmonella typhi*

**Disease:** Typhoid fever.

**Habitat** human colon only.

**Transmission** by fecal-oral route.

## **Pathogenesis**

*Salmonella* invade epithelial cells of the small intestine and reticuloendothelial cells system (liver and spleen). Endotoxins cause fever. Disease may remain localized or become systemic, sometimes with disseminated foci. The organisms are facultative, intracellular parasites that survive in phagocytic cells

## **Clinical significance**

*Salmonella* infection can cause both intestinal and extra intestinal diseases.

**Gastroenteritis:** This localized disease (also called salmonellosis) is caused by *S. enteritidis* and *S. typhimurium* (non typhoidal salmonella). It is characterized by nausea, vomiting, and diarrhea (usually non bloody), which develop generally within 48 hours of ingesting contaminated food or water. Fever and abdominal cramping are common.





**Enteric (typhoid) fever:** This is a severe, life-threatening systemic illness, characterized by fever and frequently abdominal symptoms. It is caused *S. typhi*. Nonspecific symptoms may include chills, sweats, headache, anorexia, weakness, sore throat, cough, myalgia, and either diarrhea or constipation. A small percentage of patients become chronic carriers.

**Other sites of *Salmonella* infection:** *Salmonella* can also cause abdominal infections (often of the hepatobiliary tract and spleen), osteomyelitis, bacteremia, septic arthritis.

## **Treatment and prevention**

For enteric fever, appropriate antibiotics include beta-lactams and fluoroquinolones. Prevention of salmonella infection is accomplished by proper sewage disposal, correct handling of food, and good personal hygiene.

# *Shigella dysenteriae*

**Habitat** human colon only.

**Transmission** by fecal-oral route.

*Shigella* species cause shigellosis or dysentery enterocolitis (bacillary dysentery) a human intestinal disease that occurs most commonly among young children

## **Pathogenesis and clinical significance**

*Shigella* invade and destroy the mucosa of the large intestine. Infection rarely penetrates to deeper layers of the intestine. *Shigella* has exotoxin (Shiga toxin), enterotoxic and cytotoxic. *Shigella* cause classic bacillary dysentery, characterized by diarrhea with blood, mucus, and painful abdominal cramping.

## **Treatment and prevention**

ciprofloxacin or azithromycin can reduce the duration of illness. Protection of the water and food supply, and personal hygiene are crucial for preventing shigella infections.





# *Campylobacter jejuni*

*C. jejuni* are curved, spiral, or S-shaped organisms that microscopically resemble vibrios. Motile by single, polar flagellum



## Pathogenesis and clinical significance

Campylobacter may cause both intestinal and extraintestinal disease, **causes food poisoning**. Symptoms (abdominal cramping and diarrhea, which may or may not be bloody).

*C. jejuni* is a cause of **traveler's diarrhea**.

# *Pseudomonas*

*P. aeruginosa*, the primary human pathogen, is widely distributed in nature (soil, water, plants, and animals). It may colonize healthy humans without causing disease, it is also a significant opportunistic pathogen and a major cause of nosocomial(hospital-acquired) infections. It is motile by polar flagella, catalase and oxidase positive, produce many Exo-pigment and obligatory aerobic. Nutritional requirements are minimal, can grow on a wide variety of organic substrates. This explains why the organism is responsible for many nosocomial infections. **It is multidrug resistance**

## **Pathogenesis & clinical significance**

*P. aeruginosa* produces numerous toxins and extracellular products that promote local invasion and dissemination of the organism. These may cause keratitis and endophthalmitis, external otitis or swimmer's ear wound sepsis, UTI, pneumonia or cystic fibrosis, GTI, CNS; meningitis and brain abscesses. it can cause severe hospital-acquired infections, especially in immunocompromised hosts; it is often antibiotic resistant.



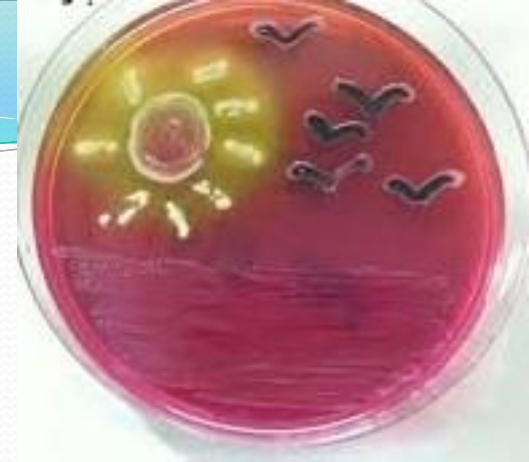


# *Vibrio cholerae*

Members of the genus *Vibrio* are short, curved, rod-shaped organisms.. They are rapidly motile by means of a single polar flagellum. O and H antigens are both present, but only O antigens are useful in distinguishing strains of vibrio that cause **epidemics**. which cause gastroenteritis and extraintestinal infections.

## **Pathogenesis**

Following ingestion, *V. cholerae* infects the small intestine. Adhesion factors are important for colonization and virulence. The organism is noninvasive, and causes disease through the action of an enterotoxin that initiates an outpouring of fluid. Cholera toxin is protein bound Gs protein. Gs protein activates adenylate cyclase, which produces elevated levels of intracellular cAMP. This, in turn, causes an outflowing of ions and water to the lumen of the intestine. `



## **Clinical significance**

cholera is characterized by massive loss of fluid and electrolytes from the body. After an incubation period ranging from hours to a few days, profuse watery diarrhea (rice-water stools) begins. Untreated, death from severe dehydration causing hypovolemic shock may occur in hours to days. Patients with suspected cholera need to be treated prior to laboratory confirmation, because death by dehydration can occur within hours.

## **Laboratory identification**

*V. cholerae* grows on s blood and MacConkey agars. Thiosulfate citrate bile salts sucrose (TCBS) medium can enhance isolation. The organism is oxidase-positive

**Treatment and prevention:** Replacement of fluids and electrolytes is crucial in preventing shock. doxycycline can shorten the duration of diarrhea and excretion of the organism.



# *Helicobacter*

Helicobacter are curved or spiral organisms. motile by multiple polar flagella. *H. pylori* is microaerophilic, and produces urease. It causes acute gastritis and duodenal and gastric ulcers. *H. pylori* are unusual in their ability to colonize the stomach, where low pH normally protects against bacterial infection.



## **Pathogenesis & Clinical significance**

Transmission of *H. pylori* is thought to be from person to person; the organism has not been isolated from food or water. Untreated, infections tend to be chronic, even lifelong. *H. pylori* colonizes gastric mucosal (epithelial) cells in the stomach, and duodenum or esophagus only. The organism survives in the mucous layer that coats the epithelium, and causes chronic inflammation of the mucosa. the organism is non-invasive. Initial infection with *H. pylori* causes acute gastritis. Urease released by *H. pylori* produces ammonia ions that neutralize stomach acid in the vicinity of the organism, favoring bacterial multiplication.

## **Laboratory identification**

Noninvasive diagnostic tests include serologic tests (ELISA for serum antibodies to *H. pylori*). Invasive tests involve gastric biopsy specimens obtained by endoscopy. *H. pylori* can be detected in such specimens histologically, by culture, or by a test for urease.



# *Neisseria*

Gram– cocci in pairs (diplococci), catalase and oxidase positive, non motile, non hemolytic. The genus contains at least 30 spp. Two important spp are pathogenic for human; *N. gonorrhoeae* (gonococcus), Diplococci in kidney shape and *N. meningitidis* (meningococcus), Diplococci in spherical shape, encapsulated.

## *N. gonorrhoeae* (gonococcus)

**Transmission** *N. gonorrhoeae* causes gonorrhoea only in human. It is transmitted in horizontal by sexually from person to person.



# Pathogenesis and clinical finding

## Venereal disease (VD)

**Genital: In male** are characterized by urethritis with yellow and creamy pus (purulent discharge) and painful urination with accompanied by dysuria, sometime leading to urethral stricture and infertility.

**In female**, the primary infection is in endocervix and extends to urethra and vagina, causing a purulent vaginal discharge and cervicitis. It may then progress to causing salpingitis, which can result in sterility or ectopic pregnancy.

## Extra genital

Proctitis (infection of rectum), stomatitis, infection of conjunctiva of newborn (ophthalmia neonatorum) is acquired from infected birth canal of mother. To prevent the infection instillation of tetracycline or erythromycin into conjunctiva sac of newborn.



## **Lab Diagnosis**

microscopic: gram- stain smear of pus reveal many intracellular diplococci.

Cultured on Thayer-Martin medium, incubated under 5-10% CO<sub>2</sub>.

Serologic test: ELISA, CFT.

## **Control**

Antibiotics: penicillin G, sulfonamide, ciprofloxacin.  
No effective vaccine is available

## ***N. meningitidis* (meningococcus)**

**Transmission:** the natural reservoir is human and transmitted by airborne droplets to other person.

### **Pathogenicity and clinical feature**

The humans are natural host in nasopharynx and causes *meningitides* only in human. the nasopharynx is portal entry. The organisms attach to epithelial cells of nasopharynx aid of pili, may be enter the bloodstream and spread to specific sites such as meninges, joints, disseminated through the body or it spread may be through sheath of olfactory nerve to meninges. The symptoms of meningococcal meningitis are fever, headache, stiff neck and increased level of PMNs in CSF.

### **Lab Diagnosis**

microscopic: gram- stain smear of pus reveal many intracellular diplococci in PMN.

Cultured on Thayer-Martin medium, & chocolate agar incubated under 5-10% CO<sub>2</sub>.

Serologic test: latex agglutination test.

**Control** :Antibiotics: penicillin G, sulfonamide, ciprofloxacin.  
immunization Vaccines are used.



## *Bordetella*

*B. pertussis* and *B. parapertussis* are the human

pathogens . The former causes the disease

pertussis (**whooping cough**). Whooping

cough is a highly contagious disease and a

significant cause of morbidity and mortality worldwide. They are small, aerobic, encapsulated, coccobacilli that grow singly or in pairs.

### **Pathogenesis & Clinical significance**

Transmission of *Bordetella* is via droplets spread by coughing, , disease is most common children (ages one to five). *B. pertussis* binds to ciliated epithelium in the upper respiratory tract. The bacteria produce of toxins and other virulence factors that interfere with ciliary activity, eventually causing death of these cells. The incubation period for pertussis ranges from 1 to 3 weeks. The disease can be divided into two phases: **catarrhal and paroxysmal**.



**Catarrhal phase:** This phase nonspecific symptoms (rhinorrhea, mild conjunctival infection, mild fever, and then progresses to include a dry, nonproductive cough).

**Paroxysmal phase:** With worsening of the cough, the paroxysmal phase begins. The term whooping cough derives from the paroxysms of coughing followed by inspires rapidly. Large amounts of mucus may be produced.

## **Treatment & Prevention**

Erythromycin is the drug of choice for infections. Pertussis vaccine is available that has had a significant effect on lowering the incidence of whooping cough. It contains proteins purified from *B. pertussis*, and is formulated in combination with diphtheria and tetanus toxoids (DPT). To protect infants who are at greatest risk of life-threatening *B. pertussis* disease, immunization is generally initiated when the infant is two months old.



# Legionella



Legionella are facultative intracellular parasites and fastidious, with a particular requirement for L-cysteine. In nature, Legionella cells are unencapsulated, relatively slender rods. Legionella cause respiratory tract infections. There are two distinctly different presentations: **Legionnaires' disease (LD): This is an atypical, acute lobar pneumonia** and **Pontiac fever: This is an influenza-like illness.**



## Brucella

*Brucella* are primarily pathogens of animals.

They are aerobic, facultative, intracellular parasites that can survive and multiply within host phagocytes. *Brucella* are unencapsulated, coccobacilli arranged singly or in pairs. Lipopolysaccharide and cell wall Ag are the major virulence factor. ***B. abortus* causes brucellosis (undulant or Malta fever)** is a **zoonosis** (a disease of animals may be transmitted to humans under natural conditions).

## *Pasteruella multocida*

*Pasteurella* primarily colonize mammals and birds, pasteurella infections are **considered zoonosis**, which can cause either disease or asymptomatic infections. Pasteurellae are coccobacilli or rods that often **exhibit bipolar staining**. Virulence factors include capsule and endotoxin. Pasteurellae **are cases of acute, painful cellulitis , Soft tissue infections**



## Anaerobic Gram-Negative Rods (Bacteroides)

*Bacteroides* are anaerobic organisms slender rods or coccobacilli. Their polysaccharide capsule is an important virulence factor, resistance to phagocytosis. If it is introduced into the abdominal cavity, *B. fragilis* causes **peritonitis, abdominal abscesses, and bacteremia**.



Zoonosis infection :*Pasteruella, Brucella and Bacillus*





\* Oxidase (+)

- ① Pseudomonas
- ② vibrio
- ③ Nisseria

\* Urease production

- ① H. pylori
- ② proteus
- ③ Klebsiella

H<sub>2</sub>S production  
on TSI, K<sub>2</sub> Black color

- ① Salmonella
- ② proteus

Non-ferromial infect

- ① Staph.
- ② Pseudo
- ③ Enterobacter

chromatingranules



- ① C. Diphtheria
- ② Yersinia
- ③ Pasturella

SS agar

- ① Salmonella
- ② Shigella

Zoonosis disease

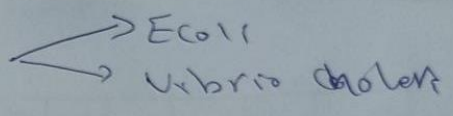
- ① Brucella
- ② Pasturella
- ③ Bacillus anthracis

Bacterin causes food poisoning

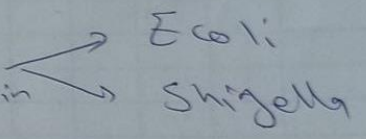
- ① Staph.
- ② B. cereus
- ③ C. botulinum
- ④ Campylobacter



\* enterotoxin heat labile LT  
causes watery diarrhea



\* enterotoxin verotoxin shiga like toxin  
causes bloody diarrhea

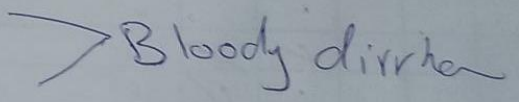


exotoxin act on inhibit the protein synthesis  
produce by Bacteria

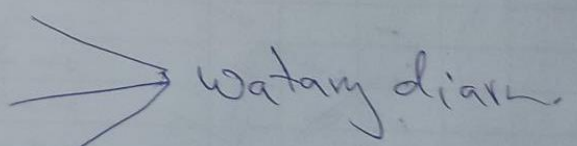
- ① Shigella
- ② C. Diphtheria

Strain of E coli

① EHEC  
② EIEC



③ EPEC  
④ ETEC  
⑤ EAEC



Exo toxin of Bacillus

PA + EF + LT = Necrosis + Edema  
Death all

PA + LT = Necrosis

PA + EF = Edema

LT + EF = NO change

Neisseria

~ gonorrhoeae

① diplococci  
Kidney shape

② non capsulated

~ meningitidis

① diplococci  
spherical shape

② capsulated