

Medical Bacteriology – Lecture 16

Oxidase negative Gram Negative Rods

Enterobacteriaceae

Enterobacteriaceae

- **Gram-negative rods**
- **Oxidase negative**
- largest group of human pathogens
- **Found as normal flora in intestinal tract of humans and animals, environmental sites (soil, water and plants).**
- non-spore forming
- aerobic, facultative anaerobic bacteria.
- Motile or non-motile
- Grow in wide range of temperature on ordinary media.
- Can diagnosis on selective and differential media
- **Most are reduce nitrates to nitrites**
- **All ferment glucose with strong acid production and often gas.**
- Catalase positive
- **Release endotoxin from their cell wall.**
- **Some release exotoxin.**
- Wide biochemical and antigenic heterogeneity

- **Modes of infections;** contaminated food and water (*Salmionella*, *shigella*, *E. coli*) , endogenous (UTI, abdominal abbesses), abnormal host colonization (nosocomial pneumonia), transfer between debilitated patients, insect (flea) vector (unique for *y. pestis*)

- **Types of infection disease;**
 - intestinal** (diarrheal) infections
 - extraintestinal** UTI (primarily cystitis
 - respiratory (nosocomial pneumonia)
 - wound (surgical wound infection)
 - blood stream (gram negative bacteremia)
 - central nerves system (neonatal meningitides)

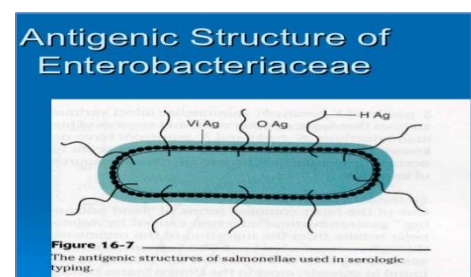
Most of them possessed three types of antigens:

- H antigen-** Found in the flagella. (Possessed by motile Enterobacteriaceae).
- K antigen-** Capsular polysaccharide (acidic polysaccharide antigen), may be associated with virulence inhibited phagocytosis.
- O antigen-** Outer membrane lipopolysaccharide. Found in lipid A of outer membrane; causes (fever, vasodilatation, inflammation, shock, and blood clots within blood vessels)

Classification based on lactose fermenter

•**a. Lactose-fermenters** such as

- *Escherichia spp.*
- *Klebsiella spp.*



- *Enterobacter spp.*
- *Citrobacter spp.*

• **b. Non-lactose fermenters** such as

- *Salmonella spp.*
- *Shigella spp.*
- *Proteus spp.*

Pathogenic Enterobacteriaceae are also classified into

• **Coliforms**

rapidly ferment lactose

part of the normal microbiota

may be opportunistic

Examples: *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*

• Presence of coliforms in water is indicate of impure water and of poor sewage treatment (*i.e.* one of the indicators of fecal pollution of water: *E. coli*)

• **Non coliform opportunists**

do not ferment lactose

- True pathogens

Escherichia coli

- **The most common normal inhabitant of large intestine**
- **found in 100% of human colon** (live in host intestinal tracts of health and disease)
- **Motile**
- **Not all *E. coli* strains are pathogenic**
- **Many pathogenic strains have polysaccharide capsule**
- **Lactose-fermenting mucoid colonies** on MacConkey
- some strains are hemolytic on blood agar
- **Produce indole**
- some strains causes **Gastroenteritis**; is the most common disease associated with *E. coli* (enteropathogenic, enterotoxigenic and enteroinvasive strains)
- Often mediated by exotoxins that produce the symptoms associated with gastroenteritis
- Most common cause of **non-nosocomial urinary tract infections**- Wound infections, Neonatal septicemia and **meningitis (Capsule)**- Dysentery, diarrhea of infants, diarrhea of travelers, pneumonia, endocarditis
- ***E. coli* is the most common cause of UTI in young women, UTI may leads to bacteremia and sepsis.**
- Over 700 antigenic types (serotypes) of *E. coli* are recognized based on O, H, and K antigens.

Virulence factors

1) **Surface O antigens** (endotoxic activity, protect bacteria from phagocytosis and complement effects) – **K antigen** (affords protection against phagocytosis and antibacterial factors in normal serum)

2) **Fimbriae** (found in small numbers, mediate mannose resistant hem agglutinin, act as virulence factor)

3) **Toxins**; *E. coli* produce two types of toxins (hemolysis and enterotoxins)

Enterotoxins;

Heat labile (LT); increase Ribosylate adenylcyclase, resemble cholera toxin in structure & action

Heat stable (ST) – increase Ribosylate guanylcyclase

Verotoxin- shiga like toxin

Urinary tract infection

- A- Intestine → Lymphatic → Blood → Kidneys
- B- Urethra → Bladder → Kidneys

Diarrhea

To cause diarrhea it must:

Return from large intestine to small intestine

Possess:

Pili plasmid coded

Somatic antigen -Invasive

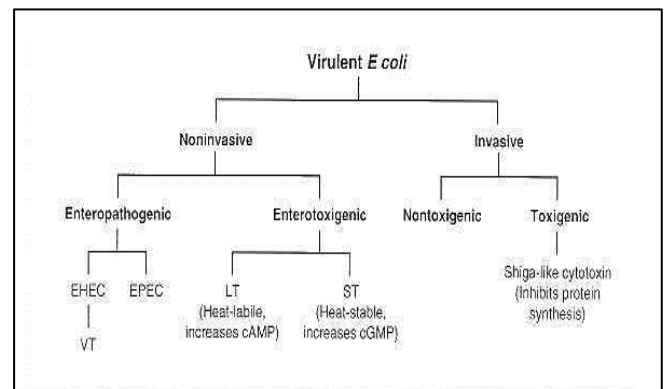
Enterotoxins: Heat labile (LT), Ribosylate adenylcyclase

Heat stable (ST), Ribosylate guanylcyclase

Diarrhea causing *E. coli* strains : As a pathogen, *E. coli* is known for its ability to cause intestinal diseases. Five classes (strains) of *E. coli* that cause diarrheal diseases:

1. Enteropathogenic *E. coli* (EPEC)

- Institutional Outbreak, sporadic diarrhea
- causes **self-limiting diarrhea** in infants and children, can be chronic
- also cause **severe diarrhea in adults**
- **do not produce enterotoxins**
- **Non- invasive**
- antibiotic treatment shorten the duration of illness and diarrhea



2. Enteroinvasive *E. coli* (EIEC)

- Non-motile
- **non-lactose fermenting *E. coli***
- invade the mucosa of the colon
- resemble shigella, **causes shigellosis-like dysentery in children in developing countries**

- causes traveler's diarrhea to these countries

3. Enterotoxigenic *E.coli* (ETEC)

- Colonization factor or fimbria promote **adherence to epithelial cells of small intestine** followed by release of **enterotoxin** which **causes toxin-mediated mild watery diarrhea in infants and young adults** to fatal disease indistinguishable from cholera.

Enterotoxin causes movement of water and ions from the tissues to bowel resulting in watery diarrheas

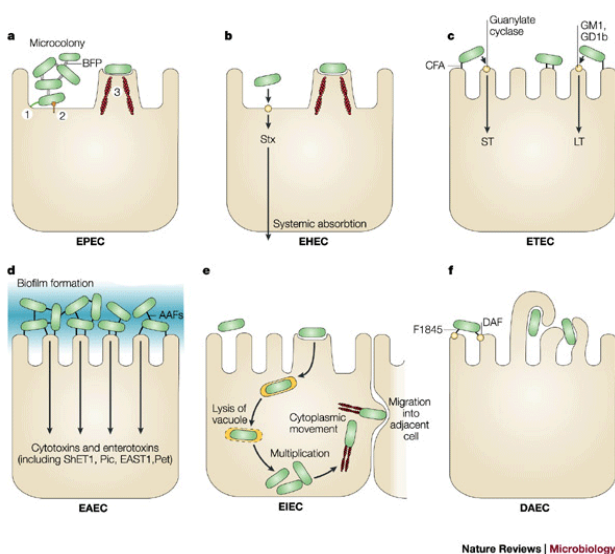
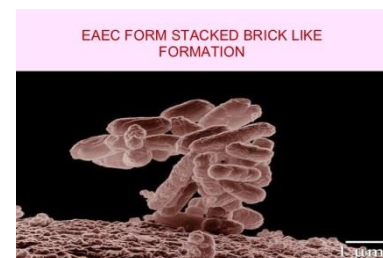
- It is an important cause of **traveler's diarrhea**
- Antibiotic prophylaxis can be effective but may increase drug resistance.

4. Entero haemorrhagic *E.coli* (EHEC)

- **Cytotoxic verotoxin** producing *E.coli* serotype O157:H7 **causes** mild diarrhea to **haemorrhagic colitis (severe form of diarrhea)** and hemorrhagic uremic syndrome in young children and elderly.
- hemolytic uremic syndrome characterized by acute renal failure, hemolytic anemia and low platelet count.
- may occur sporadically or as outbreaks of food poisoning

5. Enteroaggressive *E.coli* (EAEC)

- Adhere to human intestinal mucosal cells and **produce heat stable ST-like enterotoxin toxin and hemolysin**
- **Appear aggregated stacked brick formation**
- **causes acute and chronic diarrhea** in developing countries
- associated with persistent diarrhea
- **Produce food-borne illness** in developed countries



The categories of diarrhoeagenic *E. coli* each have unique features in their interaction with host cells. a) EPEC adhere to small bowel enterocytes, but destroy the normal microvillar architecture, inducing the characteristic attaching and effacing lesion. Cytoskeletal derangements are accompanied by an inflammatory response and diarrhea. b) EHEC induce the attaching and effacing lesion, but in the colon. The distinguishing feature of EHEC is the elaboration of Shiga toxin, systemic absorption of which leads to potentially life-threatening complications. c) ETEC adhere to small bowel enterocytes and induce watery diarrhea by the secretion of heat-labile (LT) and/or heat-stable (ST) enterotoxins. d) EAEC adheres to small and large bowel epithelia in a thick biofilm and elaborates secretory enterotoxins and cytotoxins. e) EIEC invades the colonic epithelial cell, lyses the phagosome and moves through the cell by nucleating actin microfilaments. The bacteria might move laterally through the epithelium by direct cell-to-cell spread or might exit and re-enter the baso-lateral plasma membrane.

Klebsiella

Large gram-negative rods

Non-motile

lactose-fermenting

capsulated

Found in the digestive and respiratory systems of humans and animals

Can cause **opportunistic infections- hospital acquired (nosocomial)**

No water borne disease ever associated with *Klebsiella* in drinking water

Main species of medical importance:

- *K. pneumoniae* = **Pneumoniae**
- *K. rhinoscleromatis* = rhinoscleroma
- *K. ozenae* = ozena

K. pneumoniae

- most commonly isolated pathogenic species
- causes: **Pneumonia** (serious disease with high case fatality)- Urinary tract infection- Septicemia and meningitis (especially in neonates)- Wound infection. Rarely diarrhea.
- **It is found as a commensal in the intestinal tract, also found in moist environment in hospitals.**
- It is an important **nosocomial pathogen.**
- Produce a **capsule** (mucoid colonies); **major virulence factor** that protect the bacteria from phagocytosis
- More than 80 serotypes of *K. pneumoniae* recognized
- Urease positive

Treatment: Based on sensitivity testing

Serratia

- Produce a **red pigment when grown at room temperature**
- **Can grow on catheters, in saline solutions, and other hospital supplies**
- Can cause life-threatening **opportunistic infections** in the urinary and respiratory tracts of immunocompromised patients
- Doesn't ferment lactose on MacConkey agar media
- Difficult to treat due to **resistance to various antimicrobial drugs**

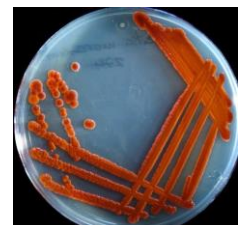
Main species of medical importance:

Serratia marcescens

Serratia rubidaea

Serratia liquifaciens

Serratia odorifera



Red pigment of *S. marcescens* colonies grown at room temperature

Enterobacter

- **lactose fermenting**
- motile
- capsulated
- Found in soil, water, vegetable, sewage, the digestive tracts of animals and humans
- **opportunistic pathogens- nosocomial infections**
- Causes urinary tract infection, pneumonia, wound infection, septicaemia in immunocompromised
- Difficult to treat due to **resistance to various antimicrobial drugs**
- Medical important species is *Enterobacter aerogens*.
- It produces mucoid colony resembling *klebsiella* on MacConkey Agar
- Positive to citrate test

Citrobacter

- **lactose fermenting**
- motile
- **opportunistic pathogen.**
- Medical important species is *Citrobacter freundii*.
- associated with urinary tract infection, wound infection and septicaemia in immunocompromised
- *Citrobacter freundii*; Enterotoxigenic (the enterotoxin is similar to the ST enterotoxin of *E. coli*).
- *Citrobacter diversus*; Neonatal meningitis and brain abscesses, neonatal septicemia.
- *Citrobacter amalonaticus*; Opportunistic pathogens can infect anybody sites, particularly, the urinary tract.

Salmonella

- Most isolates are **motile**
 - **Non-lactose fermenting**
 - **H₂S producing colonies**
 - It grows on simple media, it never ferment sucrose
 - **Attack small intestine**
 - **most salmonella infections in humans are from food contaminated with animal feces or Poultry and eggs.**
 - **invasion of the bloodstream, bacteria can invade small intestine and reach the lymph nodes**
 - **Virulence factors:** fimbria (adherence)- enterotoxins
- **Species of medical importance are:**
- S. typhi* (typhoid fever); the only host is human
 - S. paratyphi*
 - S. enteritidis* (gastroenteritis)- salmonella non-typhi

Salmonellosis (Enteric fever) or Typhoid:

- caused by *S. typhi* and *S. paratyphi*
- transmitted by fecal contaminated food and drinks
- **Infectious dose: High**
- **Incubation period: 10-14 days**
- **Goes to Peyer's patches**
- **prevents phagolysosome fusion in macrophages**
- **Bacteria can pass through the small intestines into the bloodstream and into body organs**
- Reduced gastric acidity- Disrupted intestinal microbial flora- Compromised local intestinal immunity

Progress of Typhoid fever:

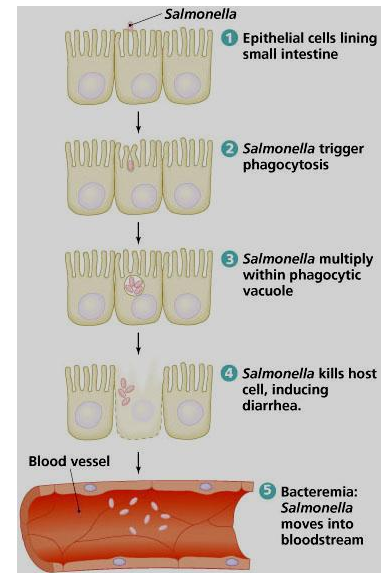
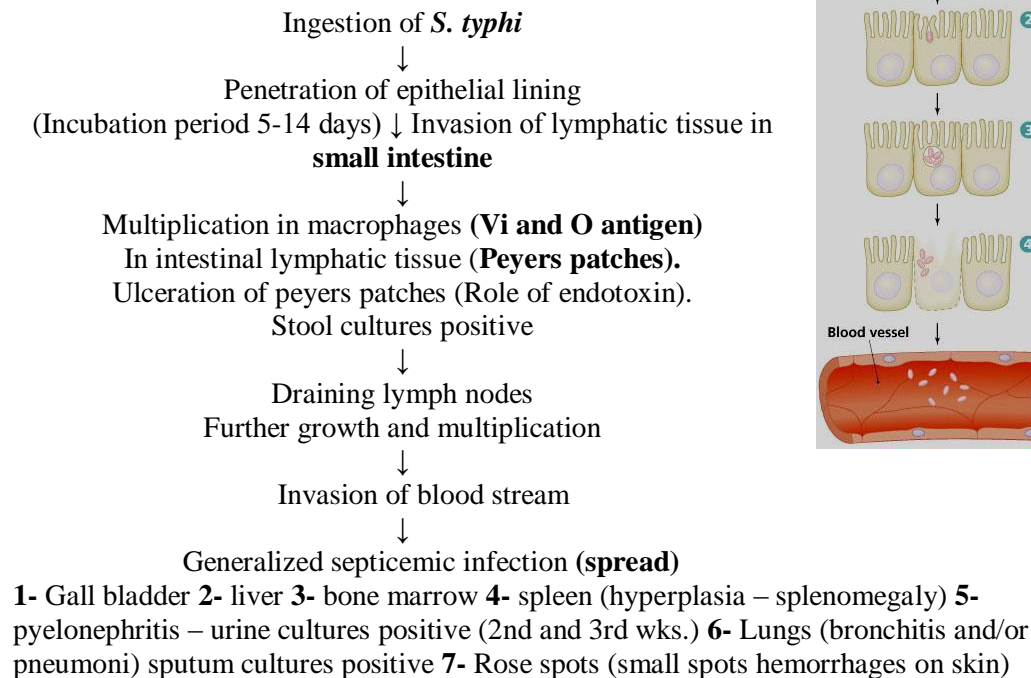
- **First week;** fever, malaise, headache, chills, then constipation, lymph node →
Blood stream, liver, spleen and bone marrow.
- Engulfment of salmonella by phagocytes (multiple intracellularly)
- Released into blood stream again that can lead to high fever (blood culture positive)
- **2nd and 3rd week;** sustained fever, prolonged bacteremia
- Invade gallbladder and Peyer's patches rose spots 2nd week of fever
- Biliary tract
- Organism isolated from stool in large number
- **Complications:**
- **Intestinal perforation- Lower gastrointestinal bleeding- Dissemination to different body organs including meninges and brain.**
- **Mortality rate:** Untreated cases:10-15%- **Treated cases:**< 1%

Paratyphoid fever is milder than typhoid fever

Gastroenteritis

- caused by *S. enteritidis* and *S. typhimurium*
- **foodborne infection/intoxication**
- **(initial watery diarrhea, later bloody mucoid diarrhea associated with abdominal pain and tenesmus).**

Mechanism of pathogenesis



Shigella

- non-motile
- Non-lactose fermenting
- Non very invasive, localized (cannot reach the blood)
- Primarily a parasite of the digestive tract of humans
- All species of Shigella have O antigens and lack H antigen

Medical importance species are:

S. dysenteriae (more serous), produce Shiga toxin
S. flexneri

Shigellosis (bacillary dysentery)

- caused by *S. dysenteriae* and *S. flexneri*
- found in human **large intestines** as pathogen
- Route of infection is fecal-oral route
- **Inoculum dose: 10^3 organisms (small)**
- **Produce a diarrhea-inducing enterotoxin**
- **Shigellosis;** characterized by sudden bloody mucoid diarrhea, severe abdominal cramps with frequent painful passage of low-volume stools containing blood and mucus and WBC, tenesmus, high fever, generalized muscle ache and weakness
- Penetrate epithelial cells lead to local inflammation, shedding of intestinal lining and ulcer formation
- Invade and survive in macrophages

- **Toxins:** **Endotoxin:** irritate the bowel wall

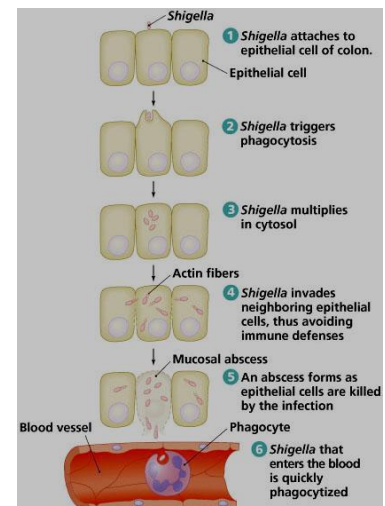
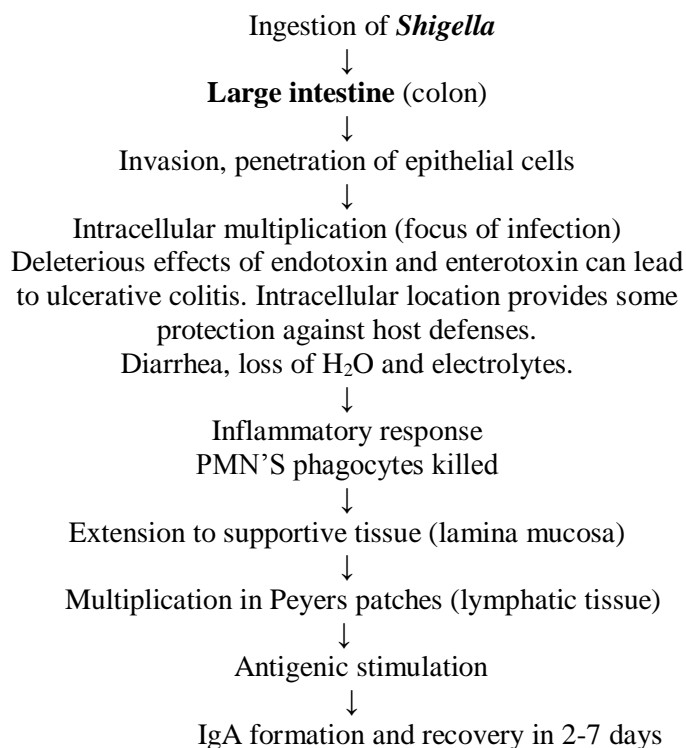
Exotoxin: act as **Enterotoxin, cytotoxic and neurotoxin (Shiga toxin, also called the verotoxin)**, because its cytotoxic effect on Vero cells, a cell line derived from African green monkey kidney cells.

Shiga toxin include dysentery, hemorrhagic colitis, and hemolytic uremic syndrome.

- **Complication:** Dehydration
Electrolyte and acid-base disturbance

High prevalence: Poor sanitation
Poor personal hygiene
Polluted water supply
Young children are frequently affected.

Mechanism of pathogenesis



Proteus

- **motile**
- non-capsulated
- **non-lactose fermenting**
- **putrefactive (fish) odor**
- found in the intestinal tract of humans and animals, soil, sewage and water.



- The characteristic feature of *Proteus* in culture is “swarming” growth over the surface of the agar media (nutrient agar and blood agar)
- Ditching of culture media prevents spread of proteus species
- Urease positive

- Species of medical importance: *P. mirabilis* (Indole negative)

P. vulgaris (Indole positive)

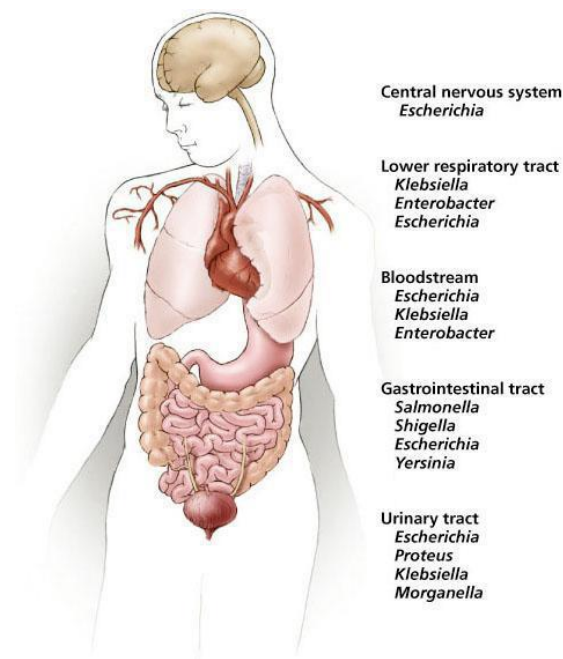
Proteus mirabilis

- one of the common species of Enterobacteriaceae isolated in clinical pathogen in urinary tract infections
- nosocomial infections
- pneumonia
- Septicemia
- Abdominal and wound infection
- Secondary invader of ulcer, burn

P. vulgaris

- Important nosocomial pathogen
- Isolated in wound infection and urinary tract infection

Enterobacteriaceae Diseases



Review Questions

What is major characteristics of Enterobacteriaceae ?

Why *Salmonella typhi* needs a large numbers of cells to initiate their infections?

Write the characteristic of diarrhea for the following diseases: (Salmonella foodborne infection- shigella dysentery)

E. coli is part of the normal flora of the human intestine, but it can cause diarrhea. Explain?

Give three examples of *E. coli* infections? (**non nosocomial urinary tract infection, meningitis, septicemia**)

What is the distinct pigment produced by *Serratia* at room temperature?

What is major characteristics of *E.coli*, *Kebsiella*?

E. coli has a five pathogenic strains, what they are and explain them?

Which *E. coli* strains causes traveler's diarrhea?

Most of Enterobacteriaceae possessed three types of antigens. discuss?

Give three example of lactose fermentor- non lactose fermentor- true pathogens of Enterobacteriaceae?

The enterotoxin *Citrobacter freundii* is similar with other bacterial enterotoxin, what is it?

Compare between Salmonella and Shigella (both of similarities, differences)?

What is the causative agent of the following diseases: Typhoid fever- bacillary dysentery?

What do you know about shigellosis toxins?

What is the major characteristic of *proteus*, how can inhibited its swarming?

What is the major virulence factor of *K. pneumoniae*?

Compare between Pseudomonas and Enterobacteriaceae?

Give three examples of; Opportunistic pathogens, nosocomial bacteria?