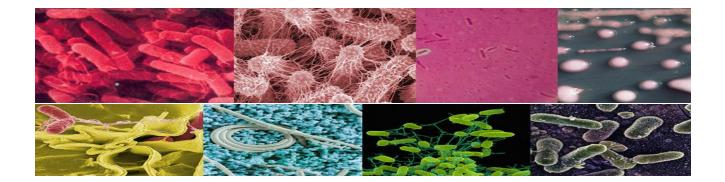
Medical Bacteriology-Lecture 17

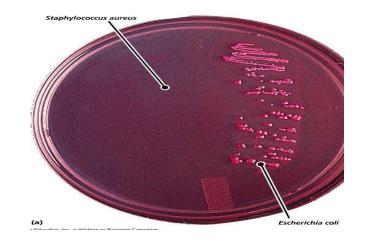
Oxidase negative Gram negative Rods

Enterobacteriaece coliforms (enterobacilli)



Comprises the following bacterial groups Oxidase negative Enterobacteriaceae

- a. Lactose-fermenters
- Escherichia spp.
- Klebsiella spp.
- Enterobacter spp.
- Citrobacter spp.
- b. Non-lactose fermenters
- Salmonella spp.
- Shigella spp.
- Proteus spp

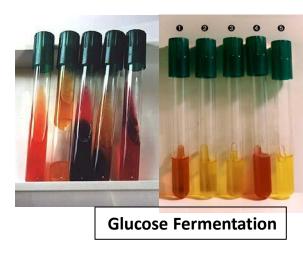


Pathogenic Enterobacteriaceae are often classified into three groups

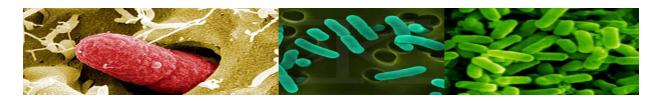
- Coliforms, rapidly ferment lactose, part of the normal microbiota, may be opportunistic
- pathogens. Examples: Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia
- Presence of coliforms in water is indicate of impure water and of poor sewage treatment (*i.e.* one of the indicators of fecal pollution of water: *E. coli*)
- Non coliform opportunists, do not ferment lactose
- True pathogens

Enterobacteriaece

- Gram-negative, rods, non-spore forming, facultative anaerobic bacteria.
- largest group of human pathogens
- Motile or non motile
- Found as normal flora in intestinal tract of humans and animals, soil, water.
- Grow a wide range of temperature in ordinary media. can diagnosis on selective and differential media
- Most are reduce nitrates to nitrites
- All ferment glucose with acid production.
- Oxidase negative. Catalase positive
- Release endotoxin from their cell wall. Some release exotoxin.
- Most of them possessed three types of antigens:
- H antigen- Found in the flagella. (Possessed by motile enterobacteriaceae).
- K antigen- Capsular polysaccharide. may be associated with virulence
- **O antigen-** Outer membrane lipopolysaccharide. Found in lipid A in the bacterial cell wall (fever, vasodilatation, inflammation, shock, and blood clots within blood vessels)



Escherichia coli



- The most common and important of the coliforms (found in 100% of human intestines)- live in the intestinal tracts of animals in health and disease
- **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
- Urinary tract infection A- Intestine \rightarrow Lymphatic \rightarrow Blood \rightarrow Kidneys
 - **B** Urethra \rightarrow Bladder \rightarrow Kidneys

- Diarrhea
- To cause diarrhea it must: Return from large intestine to small intestine

-Posses: - Pili plasmid coded
 Somatic antigen -Invasive
 Enterotoxins: Heat labile (LT) - Ribosylate adenylcyclase
 Heat stable (ST) - Ribosylate guanylcyclase

- Motile
- Over **700** antigenic types (serotypes) of *E. coli* are recognized based on **O, H, and K antigens**.
- Lactose-fermenting mucoid colonies on macconkey
- some strains are hemolytic on blood agar
- Produce indole

• **Pathogenic E. coli:** 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)

- causes outbreaks of **self-limiting infant diarrhea-** also cause **severe diarrhea in adults**
- 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, causes shigellosis-like dysentery in children in developing countries and traveler's diarrhea to these countries
- 3. Enterotoxigenic E.coli (ETEC)
- Colonization factor promote adherence to epithelial cells of small intestine followed by release of enterotoxin which causes toxin-mediated watery diarrhea in infants and young adults.
- 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 haemorrhagic colitis (severe form of diarrhea), and hemolytic uremic syndrome characterized by acute renal failure, hemolytic anemia and low platelet count
- 5. Enteroaggressive E.coli (EAEC)
- Adhere to human intestinal mucosal cells and produce ST-like toxin and hemolysin, causes acute and chronic diarrhea in persons in developing countries
- Produce **food-borne illness** in developed countries

Summary of the Virulence Determinants of Pathogenic E. coli

Adhesins fimbriae Intimin (non-fimbrial adhesin) **EPEC** adherence factor Invasins hemolysin Shigella-like "invasins" for intracellular invasion and spread Motility/chemotaxis flagella Toxins LT toxin ST toxin Shiga toxin cytotoxins endotoxin (LPS) Antiphagocytic surface properties capsules K antigens LPS Defense against serum bactericidal reactions LPS K antigens Defense against immune responses capsules K antigens LPS antigenic variation Genetic attributes genetic exchange by transduction and conjugation plasmids R factors and drug resistance plasmids toxin and other virulence plasmids siderophores and siderophore iron uptake systems pathogenicity islands



Lactose fermintor E. coli colonies

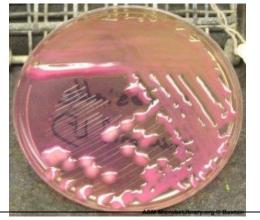


E. Coli colonies growing on EMB agar media

Klebsiella

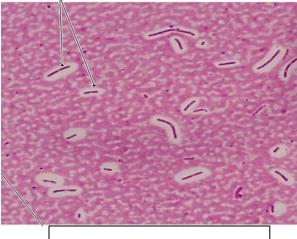
Non-motile, lactose-fermenting, **capsulated**, large gram-negative rods Found in the digestive and respiratory systems of humans and animals

- Can cause opportunistic infections- hospital acquired (nosocomial)
- No water borne disease ever associated with Klebsiella in drinking water
- Main species of medical importancce:
- K. pneumoniae Pneumoniae
- K. rhinoscleromatis= rhinoscleroma
- *K. ozenae* = ozena
- *K. pneumoniae:* most commonly isolated pathogenic species
- It causes: **Pneumonia-** Urinary tract infection- Septicemia and meningitis (especially in neonates)- Wound infection.
- It is found as a commensal in the intestinal tract, and also found in moist environment in hospitals.
- It is an important nosocomial pathogen.
- Produce a capsule that protect the bacteria from phagocytosis (mucoid colonies)
- More than 80 serotypes of *K. pneumoniae* recognized
- Treatment: Based on sensitivity testing



K. pneumoniae colonies on MacConkey agar media

Capsules



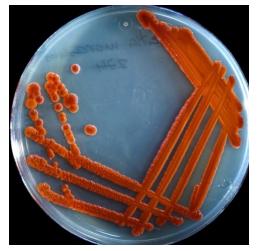
K. pneumoniae capsule

Serratia

Produce a red pigment when grown at room temperature

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

- Serratia marcescens
- Serratia rubidaea
- Serratia liquifaciens
- Serratia odorifera



Red pigment of *Serratia marcescens* colonies grown at room tempreture

Enterobacter

- gram-negative lactose fermenting- motile rods
- Found in soil, water, vegetable, sewage, the digestive tracts of animals and humans
- opportunistic pathogens- nosocomial infections of immunocompromised patients
- 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
- Causes urinary tract infection, wound infection and septicaemia in immunocompromised.

Citrobacter

- Gram-negative lactose fermenting- motile rods
- opprtunistic 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 any body sites, particularly, the urinary tract.

Morganella

- Morganella morganii
- Gram negative rods
- catalase positive
- cause diarrhea in infant

Salmonella

- Most isolates are motile- Non-lactose fermenting , H2S producing colonies
- It grows on simple media- It never ferment sucrose
- live in the intestinal tracts most salmonella infections in humans are from food contaminated with animal feces or Poultry and eggs.
- Species of medical importance are: *S. typhi* (typhoid fever)(the only host is human)
 - S. paratyphi

S. enteritidis (gastroenteritis)

- Salmonellosis (Enteric fever) or Typhoid: caused by S. typhi and S. paratyphi, transmitted by fecal contaminated food and drinks (bacteria invasion of the bloodstream)
- Incubation period: 10-14 days- Bacteria can pass through the small intestines into the bloodstream and into the liver, spleen, bone marrow, and gall bladder
- Infectious dose: High
- Reduced gastric acidity- Disrupted intestinal microbial flora- Compromised local intestinal immunity
- (fever, headache, malaise, chills, enlargement of liver and spleen, skin rashes).
- Paratyphoid fever is milder than typhoid fever
- Complications: Intestinal perforation- Lower gastrointestinal bleeding- Dissemination to different body organs including meanings and brain- Mortality rate: Untreated cases:10-15%-Treated cases:< 1%
- 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

Ingestion of *S. typhi* ↓ Penetration of epithelial lining (Incubation period 5-14 days) ↓ Invasion of lymphatic tissue in *small intestine* ↓ Multiplication in macrophages (Vi and O antigen) In intestinal lymphatic tissue (Peyers patches). Ulceration of peyers patches (Role of endotoxin). Stool cultures positive

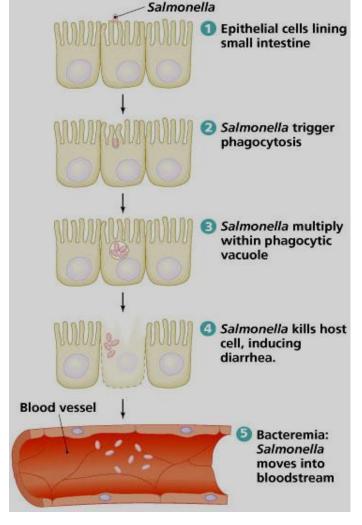
Draining lymph nodes Further growth and multiplication ↓ Invasion of blood stream

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Generalized septicemic infection (spread)

1- Gall bladder **2-** liver **3-** bone marrow **4-** spleen (hyperplasia – splenomegaly) **5-**pyelonephritis – urine cultures positive (2nd and 3rd wks) **6-** Lungs (bronchitis and/or pneumoni)a

 sputum cultures positive 7- Rose spots (small spots hemorrhages on skin)





Non ferment Lactose, H₂S positive colonies of *S. enterica* on Deoxycholate Citrate Agar

Shigella

Gram-negative non-motile rods. Non-lactose fermenting colonies

• Primarily a parasite of the digestive tract of humans

Medical importance spesiec are: *S. dysenteriae* (more serous) *S. flexneri*

- Shigellosis (bacillary dysentery) is caused by S. dysenteriae and S. flexneri
- found in human large intestines as pathogen
- Route of infection is fecal-oral route
- Inoculum dose: 103 organisms (small)
- Produce a diarrhea-inducing enterotoxin
- Toxins: Endotoxin: irritate the bowel wall

Exotoxin: act as Enterotoxin, cytotoxic and neurotoxin (Shiga toxin, also called the verotoxin)

Shiga toxin is produced by *S. dysenteriae*. shiga toxin include dysentery, hemorrhagic colitis, and hemolytic uremic syndrome.

- shigellosis (bacillary dysentery) characterized by sudden bloody mucoid diarrhea, severe abdominal cramps with frequent painful passage of low-volume stools containing blood and mucus, tenesmus, fever, generalized muscle ache and weakness
- Complication: Dehydration

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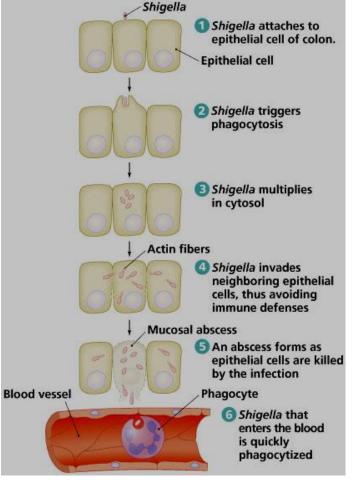
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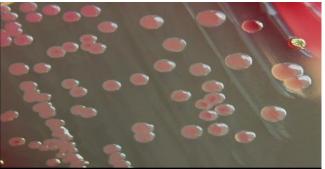
- Electrolyte and acid-base disturbance
- High prevalence: Poor sanitation
 - Poor personal hygiene
- Polluted water supply
- Young children are frequently affected.

Mechanism of pathogenesis

Ingestion of *Shigella* Large intestine (colon) Invasion, penetration of epithelial cells Intracellular multiplication (focus of infection) Deleterious effects of endotoxin and enterotoxin can lead to ulcerative colitis. Intracellular location provides some protection against host defenses. Diarrhea, loss of H2O and electrolytes. Inflammatory response PMN'S phagocytes killed Extension to supportive tissue (lamina mucosa)

Multiplication in Peyers patches (lymphatic tissue) Antigenic stimulation IgA formation and recovery in 2-7 days





Shigella flexneri on Endo agar (non ferement lactose)

Proteus

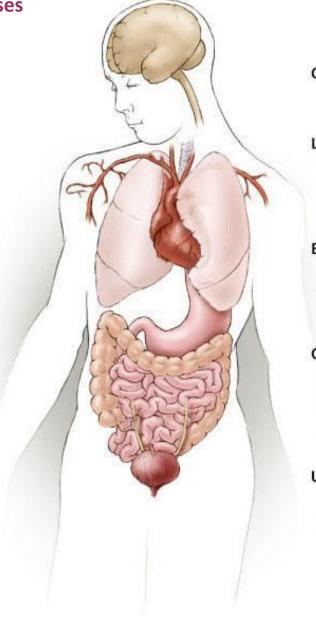
- Gram-negative, motile, non-capsulated- non-lactose fermenting
- found in the intestinal tract of humans and animals, soil, sewage and water.
- The characteristic feature of Proteus in culture is "swarming" growth over the surface of the agar media
- Ditching of culture media prevents spread of proteus species
- Urease positive
- Species of medical importance: *P. mirabilis* (Indole negative)

P. vulgaris (Indole positive)

- **Proteus mirabilis** is one of the common species of Enterobacteriaceae isolated in clinical pathogen in urinary tract infections nosocomial infections-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



Central nervous system Escherichia

Lower respiratory tract Klebsiella Enterobacter Escherichia

- Bloodstream Escherichia Klebsiella Enterobacter
- Gastrointestinal tract Salmonella Shigella Escherichia Yersinia
- Urinary tract Escherichia Proteus Klebsiella Morganella

Yersinia



- Short, pleomorphic microaerophilic or facultative anaerobic gram negative coccbacillus- non motlie
- three species of facultative intracellular bacteria that are pathogenic for humans

Y. pestis (Pneumonic, bubonic and septicemic plague)
Y. pseudotuberculosis
Y. enterocolitica

These are primarily animal pathogens, and humans are accidental hosts for infection.

Bubonic Plaque (black Plaque): bites- characterized by high fever- Enlarged lymph nodes, painful lymph nodes called buboes- death rate (55%).

Pneumonic Plaque: rapidly- infection of the lungs- High mortality rate (95%) during 24-36hrs- painful in muscles- high fever- enlarged liver and spleen- bloody sputum

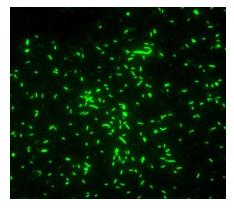
Inoculum dose: 108-109 organism

Incubation period (IP) =5-10 days

Diagnosis and treatment must be rapid due to the fast progression and deadliness of the plague

Phage typing

Flourescent antibody



Y. pestis, Direct Fluorescent Antibody Stain

Mechanism of pathogenesis

Dogs and cats
Rodent→ Rodent
\downarrow Fleas \downarrow
$\downarrow \downarrow \downarrow$
Septicemic plague $\leftarrow \leftarrow \leftarrow$ Man
\downarrow \downarrow 1-6 days
\checkmark Small pustule (or no local lesion)
\downarrow \downarrow
↓ Phagocytosis
 ↓ Small pustule (or no local lesion) ↓ ↓ ↓ Phagocytosis ↓ Bacteria survive, macrophage killed, ↓ Cal+ determinant or VWa+ ↓ ↓
\downarrow Cal+ determinant or VWa+
\downarrow \downarrow
Enlarged lymph nodes (buboes)- Bubonic plague
\mathbf{V}
Lymphatic system
 ↓ Lymphatic system ↓ ↓
→ Septicemia (endotoxin, Schwartzman reaction)
\downarrow \downarrow \downarrow
Pneumonia (Pneumonic plague) Meningitis

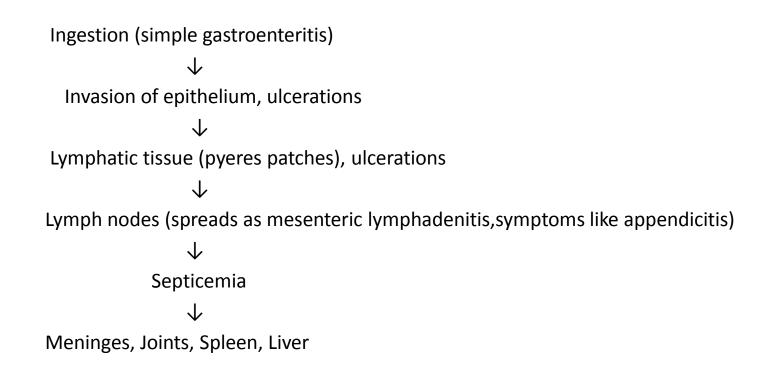
Virulence Factors

- ٠
- Antigenic change at 37c antiphagocytic capsule, protein (V), and Lipoprotein (W). Hemorrhagic lesions Hemolysin, coagulase, fibrinolysin. ٠
- ٠
- ٠

Yersinia enterocolitica and Yersinia pseudotuberculosis

- Non-lactose fermenting- gram negative rods
- Urease positive- Oxidase negative
- motile, the flagella are produced during growth at 22 but not at 37c
- *Y. enterocolitica*: Human infection occurs by contaminated food and drinks from domestic animals or rodents
- Causes inflammation of the intestinal tract
- **Gastroenteritis:** diarrhea, fever, abdominal pain
- antibiotic therapy recommended
- More virulent than Y. pseudotuberculosis
- *Y. pseudotuberculosis:* Human infection results from ingestion of food and drinks contaminated by animal feces

Y. pseudotuberculosis: Mechanism of pathogenesis



Y. enterocolitica: Mechanism of pathogenesis

similar with Y. pseudotuberculosis plus (more likely than in Y. pseudotuberculosis)

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Local skin lesion

↓

Red, spreading necrosis

↓

Local lymph nodes

↓

Lymphatic system

↓

Septicemia

↓

See Y. pseudotuberculosis
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More prevalent than pseudotuberculosis, more likely cause infection on the skin and to cause food poisoning

Review Questions

- Why Salmonella typhi needs a large numbers of cells to initiate their infections?
- Write the characteristic of diarrhea for the following diseases: (cholera- salmonella foodborne infectionshigella 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?
- Found in 100 % of human intestines-live in the intestinal tracts of animals in health and disease- some strains are hemolytic on blood agar, indole positive, what is the bacteria?
- *Escherichia coli* can infect kidney by two ways. What are they?
- *E. coli* has a five pathogenic strains, what they are and explain them? Give two examples of strains that causes traveler's diarrhea?
- Most of enterobacteriaceae possessed three types of antigens. discus?
- Give three example of lactose fermintor- non lactose fermintor- true pathogens of enterobacteriaceae?

Cont. Review Questions

- Has a major antipahocytic polycaccharide capsule, No water borne disease ever associated with drinking water, hospital acquired- cause opportunistic infections, what is the bacteria, give the two species of medical important and their diseases?
- The enterotoxin *Citrobacter freundii* is similar *with* other bacterial enterotoxin, what is it?
- Give an example of disease causes by *Morganella morganii*?
- Compare between Salmonella and Shigella?
- What is the causative agent of the following diseases: Typhoid fever- Plaque-bacillary dysentery?
- What do you know about shigellosis toxins?
- What is the major characteristic of *proteus*, how can stop its swarming, its two medical importance species, two examples of diseases (urinary tract infections- wound infections), what is the major virulence factor?
- Plague is a deadly infections that is caused by *Yersinia pestis*. Primarily carried by rodents and spread to humans via fleas or rodent. The Plague can be bubonic plaque, or pneumonic plaque. Explain? Give three examples of its virulencfactors?
- Compare between Pseudomonas and Enterobacteriaceae?