GRAM-NEGATIVE BACILLI
THE ENTERICS:

Family Enterobacteriaceae:

Genus Escherichia & Genus Klebsiella

Objectives:
- Describe the morphology & physiology for E.coli & Klebsiella species.
- Determine the virulence factors for E.coli & Klebsiella species.
- Analyze the diseases & pathogenicity for E.coli & Klebsiella.
- Demonstrate the epidemiology and transmission.
- Outline the laboratory diagnosis.
- State the drug of choice and prophylaxis where regularly used.

Antigenic classification
- Depending on the cell surface structures that bind to specific antibodies (antigenic determinants). The Enterics have three major surface antigens, which differ slightly from one species to another.
- Somatic O antigen: this is the most external component of the lipopolysaccharide (LPS) of the gram-negative bacteria cell wall. O=Outer.
- Capsular K (capsule) antigen this is the capsule that covers the O antigen.
- Flagellaer H antigen: so only the motile bacteria that possess flagella have this antigen.
**Biochemical Classification:**

- Some of the important biochemical properties of the organisms, which can be measured in the lab, are:
  1. The ability to ferment lactose and convert it into gas and acid, which can be visualized by using dye that changes in color when the pH changes. Example: MacConkey agar. Escherichia coli and most of the enterobacteriaceae ferment lactose while Salmonella, Shigella and Pseudomonas aeruginosa do not.
  2. The production of H2S, ability to hydrolyze urea, liquefies gelatin, and decarboxylate specific amino acids.

**Pathogenesis:**

The enteric can produce 2 types of diseases:

- 1. Diarrhea with or without systemic invasion.
- 2. Various other infections such as urinary tract infection, pneumonia, bacteremia and sepsis, especially in debilitated hospitalized patients.

**Diarrhea:**

Generally diarrhea classifies to major categories:

- 1. Inflammatory diarrhea due to tissue invasion
- 2. Non-inflammatory diarrhea: no tissue invasion such as the diarrhea results from bacterial toxins, viral and heavy metal poisoning.

The clinical manifestation of diarrhea caused by enteric vary depending on the severity or depth of intestinal invasion.

- 1) No cell invasion: Watery diarrhea without systemic symptoms (such as fever) is the usual picture. Enterotoxigenic Escherichia coli and Vibrio cholera are examples.
- 2) Invasion of the intestinal epithelial cells: The cell death results in red blood cell leakage into the stool. Examples: Enteroinvasive Escherichia coli, Shigella, and Salmonella enteritidis.
- 3) Invasion of the lymph nodes and bloodstream: Examples: Salmonella typhi, Yersinia enterocolitica, and Campylobacter jejuni.
**FAMILY ENTEROBACTERIACEAE ESCHERICHIA COLI (E.COLI)**

- Escherichia coli is one of the normal flora of human intestinal tract. However, any genetic changes that resulting in transfer of virulence factors by conjugation with plasmid exchange, bacteriophages, and direct DNA insertion this will convert the non-virulence strain to virulent one in this manner, it can cause disease.

Nonpathogenic Escherichia coli + virulence factor = Disease

(Normal flora)

**STRUCTURE AND PHYSIOLOGY**

- *E. coli* is a gram negative bacilli, has fimbriae or pili that are important for adherence to host mucosal surfaces, and different strains of the organism may be motile or nonmotile.
Most strains can ferment lactose (that is, they are Lac+) in contrast to the major intestinal pathogens, *Salmonella* and *Shigella*, which cannot ferment lactose (that is, they are Lac-).

*E. coli* produces both acid and gas during fermentation of carbohydrates.

They are all facultative anaerobes.

Most strains are motile and not capsulated.

They all ferment glucose.

They all lack cytochrome oxidase (that is, they are oxidase negative).

**Typing strains is based** on differences in three structural antigens: O, H, and K. The O antigens (somatic or cell wall antigens) are found on the polysaccharide portion of the LPS. These antigens are heat stable and may be shared among different Enterobacteriaceae genera. O antigens are commonly used to serologically type many of the enteric gram-negative rods. The H antigens are associated with flagella, and, therefore, only flagellated (motile) Enterobacteriaceae such as *E. coli* have H antigen. The K antigens are located within the polysaccharide capsules. Among *E. coli* species, there are many serologically distinct O, H, and K antigens, and specific serotypes are associated with particular diseases. For example, a serotype of *E. coli* possessing O157 and H7 (designated O157:H7) causes a severe form of hemorrhagic colitis.

**Reservoir:**

- Human colon (normal flora); may colonize in vagina or urethra.
- Contaminated crops where human fecal fertilizer is used.
- Enterohemorrhagic strains: bovine feces.

**Transmission:**

- Endogenous.
- Fecal-oral.
- Maternal fecal flora.
- Enterohemorrhagic strains: bovine fecal contamination (raw or under cooked *beef*, milk, apple juice from fallen apples).
**Virulence factors**

Virulence factors include the following:

1. **Mucosal interaction:**
   - Mucosal adherence with pili (colonization factor).
   - Ability to invade intestinal epithelial cells.

2. **Exotoxin production:**
   - Heat-labile and stable toxin (LT and ST).
   - Shiga-like toxin (verotoxin).

3. **Endotoxin:** Lipid A portion of lipopolysaccharide (LPS).

4. **Iron-binding siderophore:** obtains iron from human transferrin or lactiferrin.

**Diseases**

Diseases caused by *Escherichia coli* in the presence of virulence factors include the following:

1. Diarrhea.
2. Urinary tract infection (MOST COMMON CAUSE OF UTI).
3. Neonatal meningitis (2ND MOST COMMON CAUSE).

**Escherichia coli Diarrhea**

*Escherichia coli* diarrhea may affect infants or adults. Infants worldwide are especially susceptible to *Escherichia coli* diarrhea, since they usually do not developed immunity yet.

Since fluids lost in the stool is often not adequately replaced, death from *Escherichia coli* diarrhea is usually due to dehydration.

About 5 million children die yearly from this infection.

*Escherichia coli is conceded as an important cause of* Traveler’s Diarrhea.

The severity of *Escherichia coli* diarrhea depends on which virulence factors the strain of *Escherichiacoli* possesses. These strains have been named based on their virulence factors and the different diarrheal diseases they cause.
### Strains of E. coli and disease they cause

<table>
<thead>
<tr>
<th>Strain of E. coli</th>
<th>Abbreviation</th>
<th>Syndrome</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterotoxigenic E. coli</td>
<td>ETEC</td>
<td>Watery diarrhea (traveler’s diarrhea)</td>
<td>Fecal/oral</td>
</tr>
<tr>
<td>Enteropathogenic E. coli</td>
<td>EPEC</td>
<td>Watery diarrhea of long duration, mostly in infants, often in developing countries</td>
<td>Fecal/oral (2nd most common infantile diarrhea)</td>
</tr>
<tr>
<td>Enterohemorrhagic E. coli (0157:H7)</td>
<td>EHEC (VTEC)</td>
<td>Bloody diarrhea; Hemorrhagic colitis and hemolytic uremic syndrome</td>
<td>Bovine Feces, Pitting</td>
</tr>
<tr>
<td>Enteroinvasive E. coli</td>
<td>EIEC</td>
<td>Bloody diarrhea</td>
<td>Fecal/oral</td>
</tr>
<tr>
<td>Enteroaggregative E. coli, (0104:H4)</td>
<td>EAEC</td>
<td>Persistent watery diarrhea in children and patients infected with HIV</td>
<td>Fecal/oral</td>
</tr>
<tr>
<td>Diffusely adherent E. coli</td>
<td>DAEC</td>
<td>Watery diarrhea</td>
<td>Fecal/oral</td>
</tr>
</tbody>
</table>

### Extraintestinal Infections

#### 1) Escherichia coli Urinary Tract Infections (UTI):
- The acquisition of a pili virulence factor allows Escherichia coli to travel up the urethra and infect the bladder (cystitis) and sometimes move further up to infect the kidney itself (pyelonephritis). Escherichia coli is the most common cause of urinary tract infections. Which usually occur in women and hospitalized patients with catheters in the urethra. Symptoms include burning on urination (dysuria), having to pee frequently (frequency), and a feeling of fullness over the bladder. Culture of greater than 100,000 (10⁵) colonies of bacteria from the urine establishes the diagnosis of a urinary tract infection.
2) **Escherichia coli Meningitis:**
- Capsulated strain of Escherichia coli is the second most common cause of neonatal meningitis (group B streptococcus is first). During the first month of life, the neonate is especially susceptible.

3) **Escherichia coli Sepsis**
- Escherichia coli is also the most common cause of gram-negative sepsis. This usually occurs in debilitated hospitalized patients. Septic shock due to the lipid A component of the LPS is usually the cause of death.

4) **Escherichia coli Pneumonia**
- Escherichia coli is a common cause of hospital-acquired pneumonia.

**METHODS FOR DIFFRENTIATING PATHOGENIC E.COLI FROM NORMAL FLORA**

- 1) Immunoassay looking for specific protein antigen (on or excreted from the bacterium).
- 2) Serotyping since certain serotypes are more often Pathogenic.
- 3) DNA probe for specific gene in a culture.
- 4) PCR for clinical specimen.
**Genus Klebsiella**

**Genus Features**
- Members of the genus Klebsiella are capsulated Gram-negative rods.
- They are non-motile but some strains express fimbriae.
- They may survive drying for months.
- They are facultative anaerobes.
- Strains can be differentiated by simple biochemical tests.

**Capsular stain showing large capsule around Klebsiella**
**Antigenic Structure**

- About 80 capsular K antigens are presently recognized.
- Types K1, K2, K3, K5 and K21 are particularly significant in human diseases.

Five different somatic O antigens occur in various combinations with the capsular antigens.

**Species of Medical Importance**

*Klebsiella pneumoniae*

*Distinguishing Features:*
- Gram-negative rods with large polysaccharide capsule.
- Mucoid, lactose-fermenting colonies on MacConkey agar.
- Oxidase negative.

*Reservoir:* human colon and upper respiratory tract (normal flora).

*Transmission:* endogenous.

**Pathogenesis (Virulence Factors)**

These include:
1) Complex capsule give protection against phagocytosis.
2) Endotoxin: causes fever, inflammation, and shock (septicemia).
3) Long-chain lipopolysaccharides (LPS) protects strains from the action of serum complement.
DISEASES CAUSED BY K. PNEUMONIAE

a) Pneumonia
- Community-acquired, most often in older males; most commonly in patients with either chronic lung disease, alcoholism, or diabetes (but this is not the most common cause of pneumonia in alcoholics; 
(S. pneumoniae is.)
- Endogenous; assumed to reach lungs by inhalation of respiratory droplets from upper respiratory tract.
- Frequent abscesses make it hard to treat with high fatality rate.
- Sputum is generally thick and bloody (currant jelly) but not foul smelling as in anaerobic aspiration pneumonia.

CHEST X-RAY OF PATIENT WITH K. PNEUMONIA
b) **Urinary tract infections-catheter-related** (nosocomial) from fecal contamination of catheters.

C) **Septicemia**: in immunocompromised patients may originate from bowel defects or invasion of IV lines.

**Laboratory diagnosis**

1) Specimens (site of infection e.g. urine, blood, sputum, pus...etc).
2) Staining: Gram's stain and Capsular stain.
3) Culture:37C0 24-48h.:
   a) Differential media:
      - MacConkey's agar (selective and differential media).
      - EMB (eosin methylene blue) contains special dye.
   b) Non differential medium: Blood agar.
4) Biochemical tests
   I. (IMViC) test.
   II. The API 20E system :(API= analytic profile index).
5) Motility test (at 37C0).
6) Serotyping: used for *E.coli* to determine the (0 Ag) and (H Ag), There are >150 (0 Ag), >50 (H Ag).
7) Antibiotic Sensitivity test: important as there is high percentage of antibiotic resistant strains.

**Prevention and treatment:**

Intestinal disease can best be prevented by care in selection, preparation, and consumption of food and water.

Maintenance of fluid and electrolyte balance is of primary importance in treatment.
Antibiotics may shorten duration of symptoms, but resistance is nevertheless widespread.

Extraintestinal diseases require antibiotic treatment. Antibiotic sensitivity testing of isolates is necessary to determine the appropriate choice of drugs.

**REFERENCES:**
- Baily & Scott diagnostic microbiology, 12th ed.

*The end*