E. Coli, Klebsiella

Dr. R.K. Kalyan
Professor
Microbiology
KGMU, Lko
Learning objectives

The students will be able to answer the following questions:

- Describe morphology and antigens
- Describe Pathogenesis & Clinical features
- Choose appropriate lab diagnosis and interpret the results
- Describe prevention and treatment
Enterobacteriaceae

- **Family Characters (General Properties)**
  - Gram-negative bacilli or cocobacilli
  - Non sporing, non acid fast
  - Aerobes and facultative anaerobes, Non fastidious
  - Ferment glucose to produce acid with or without gas
  - **Reduce nitrate to nitrite**
  - Catalase positive, **oxidase negative**
  - Motile with peritrichous flagella, or non-motile
  - Mostly commensals in human intestine - **Coliform bacilli**
## Classification: Oldest method based on their action on lactose

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lactose fermentation</th>
<th>Colonies on MacConkey agar</th>
<th>Examples</th>
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</thead>
<tbody>
<tr>
<td>Lactose fermenters (LF)- all are coliform bacilli</td>
<td>Ferment lactose producing acid</td>
<td>Produce pink colored colonies, (acid changes the color of neutral red indicator to pink)</td>
<td>Escherichia, Klebsiella Citrobacter</td>
</tr>
<tr>
<td>Non lactose fermenters (NLF)</td>
<td>Do not ferment lactose</td>
<td>Produce pale or colorless colonies</td>
<td>Salmonella, Shigella Proteus, Morganella, Providencia and Yersinia</td>
</tr>
<tr>
<td>Late lactose fermenters (LLF or previously called as paracolon bacilli)</td>
<td>Ferment lactose after 2-8 days of incubation</td>
<td>At 24 hrs incubation-produce pale or colorless colonies, After 2 days- produce pink color colonies</td>
<td>Shigella sonnei</td>
</tr>
</tbody>
</table>
Classification: Common morphological, biochemical and similar DNA base compositions. Bergey’s manual, Kauffmann, Edwards-Ewing

Ewing’s Classification: Family is classified into its major subdivisions, groups or tribe-genera-subgenera-species-types-biotypes, serotypes, bacteriophage types, colicin types

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Genus</th>
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<tbody>
<tr>
<td>Tribe I-Escherichieae</td>
<td>Escherichia, Shigella</td>
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<tr>
<td>Tribe II-Edwardsielleae</td>
<td>Edwardsiella</td>
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<tr>
<td>Tribe III-Salmonelleae</td>
<td>Salmonella</td>
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<tr>
<td>Tribe IV-Citrobactereae</td>
<td>Citrobacter</td>
</tr>
<tr>
<td>Tribe V- Klebsiellaeae</td>
<td>Klebsiella, Enterobacter, Hafnia, Serratia, Pantoea</td>
</tr>
<tr>
<td>Tribe VI-Proteeae</td>
<td>Proteus, Morgenella, Providencia</td>
</tr>
<tr>
<td>Tribe VII- Yersinieae</td>
<td>Yersinia</td>
</tr>
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</table>
ESCHERICHIA COLI

• Genus named after Escherich who first isolated the bacilli under the name *Bacterium coli commune* in 1885

• Most common aerobe harboured in gut of humans and animals

• Detection of the thermotolerant *E. coli* (survives at 44°C) in drinking water \( \rightarrow \) recent contamination with human or animal feces

• Other species are less important as human pathogens - *E. fergusonii, E. hermannii and E. vulneris*
Morphology

- E. coli is a GNB, 1-3µm x 0.4-0.7µm
- Most strains are motile by peritrichate flagellae
- Non-sporing and non-capsulated
Culture

- Aerobic and facultative anaerobe and grows on ordinary culture medium at 37°C (10-40°C) in 18-24 hrs

- MacConkey’s medium- pink, circular, moist, smooth, with entire margin, non mucoid colonies

- Some strains show β- haemolysis on BA media

- In liquid uniform turbidity
Biochemical Reaction

- They ferment most of the sugars (glucose, lactose, mannitol, maltose) with acid and gas.
- Typical strains do not ferment sucrose.
- Indole and methyl red (MR) reaction are positive but Voges-Proskauer (VP) and citrate utilisation tests are negative (IMVic++)
- Urease –ve, Gelatin not liquified, H2S not formed.
- No growth in KCN medium.
Virulence factors of *E. coli*

Two types of virulence factors of Esch. coli have identified

1. Surface antigens
   i. Somatic Ag (O)
   ii. Flagellar (H)
   iii. Capsular antigens (K)
   iv. Fimbrial antigen

2. Toxins: enterotoxin, haemolysin and verocytotoxin
Antigenic structure: Serotyping of E. coli based on presence of O, k, and H antigens detected by agglutination reactions

Somatic or O antigen:

- Most important virulence factor → endotoxic activity, Protects from phagocytosis and bactericidal effect of complement

  - Lipopolysaccharide (LPS) antigen of cell wall, heat-stable

  - Occasionally, it cross reacts with O antigens of other genera of enterobacteriaceae (Citrobacter, Salmonella, Shigella, and Yersinia)

- Early O serotypes - commensals of intestine- 1,2,3 etc

- Late O serotypes - diarrhea producing strains 26,55,111 etc

- More than 173 O seotypes
Virulence factors of *E. coli*

- **Flagellar or H antigen** (H from *Hauch*, meaning film of breath)
  - These are thermolabile and 75 H antigens
  - Heat labile, monophasic
  - Motility contributing to virulence

- **Capsular or K antigen**
  (K for Kapsel, German for capsule)
  - Polysaccharide capsular antigen present on the envelope or microcapsule
  - They cause ‘O’ inagglutinability by homologous antigen
  - Expressed by some strains only - neonatal meningitis, pyelonephritis and septicemia
  - Most strains of intestinal *E. coli* do not possess K Ags.
  - Encloses O antigen → inagglutinable by the O antiserum
  - 103 K antigens are described
  - Inhibits Phagocytosis
Virulence factors of *E. coli*

- **Fimbrial antigen (pilus)** - organ of adhesion
- Thermolabile proteins and heating the organisms at 100°C leads to detachment of fimbriae
- Type I fimbriae mediate adhesion of bacterium to cells that contains mannose residue
- Adhesions enhances bacterial pathogenicity - UTI
- **CFA (colonization factor antigen):** enterotoxigenic *E. Coli*
- **Mannose resistant fimbriae** (e.g. P, M, S, F1C and Dr fimbriae):
  - Hemagglutinate with RBCs that is not inhibited by mannose
  - Expressed by uropathogenic *E. coli* and *role in diarrhoeal ds.*
- **P fimbriae** bind specifically to the P blood group antigens present on human RBCs and uroepithelial cells
Antigenic types

- On the basis of O antigen, *E.coli* has been divided into a number of O groups.

- Each O group divided into subgroups on the basis of K antigens.

- Each of these subgroups includes strains with different H antigens.

- Thus antigenic pattern of a strain is recorded as the number of the particular antigen it carries.

- E.g O111:K58:H12.
Resistance

- *E. coli* is excreted in faeces of human and animals and contaminate soil and water

- It is killed by moist heat at 60°C usually within 30 minutes

- It can be killed by 0.5-1 part per million (ppm chlorine in water

- It can survive for several days in soil, water, dust and air
Toxins

1. Enterotoxins: produced by enterotoxigenic strains of E. coli (ETEC). diarrheagenic strains of E. coli
   - Heat labile toxin (LT) and heat stable toxin (ST) and verocytotoxin

2. Hemolysins: virulent strains of E. coli (especially pyelonephritis strains)
   - Can lyse erythrocytes of some species
   - A large proportion of E. coli recovered from extra-intestinal lesion of man

3. Verocytotoxin (VT)

4. Cytotoxic necrotizing factor 1 (CNF1) and secreted autotransporter toxin (SAT): Cytotoxic to bladder and kidney cells

- Siderophores (i.e. aerobactin)—Helps in iron uptake
1. Heat labile enterotoxin
LT (heat-labile toxin)

- **Produced by:** Enterotoxigenic *E. Coli*
- Plasmid coded, Resembles cholera toxin but less potent
- LT is composed of one enzymatically active polypeptide A (A for active) and 5 identical B (B for binding) subunits
- **Mechanism of action:**
  - **Subunit B:** Binds to GM1 ganglioside receptors on intestinal epithelium → A fragment is internalized and cleaved into A1 and A2 peptides
LT (heat-labile toxin)

- Fragment A:
- Fragment A2 helps in tethering A and B subunits together
- Fragment A1 - active fragment, causes ADP ribosylation of G protein → upregulates activity of adenylate cyclase → intracellular accumulation of cAMP → increased outflow of water and electrolytes into the gut lumen → diarrhea

Detection of LT:
- Toxin detection: latex agglutination, ELISA
- Molecular methods: PCR detecting gene coding for LT
ST (heat-stable toxin)

**Produced by:** Enterotoxigenic *E. Coli*

- Plasmid-coded

- ST is of two types: ST-I and ST-II

**Mechanism of action:**

- **ST-I:** Binds to the guanylate cyclase C → increased production of cGMP → fluid accumulation in gut lumen → diarrhea

- **ST-II:** causes fluid accumulation by an unknown mechanism

**Detection of ST:** Same as for LT
Verocytotoxin or Shiga-like toxin

- Produced by:
  - Enterohemorrhagic *E. Coli*
  - Bacteriophage-coded
- Cytotoxic to Vero cell lines,
- Also called Shiga-like toxin as it resembles Shiga toxin in its structure and function
Verocytotoxin or Shiga-like toxin

**Mechanism of action:**
- **Fragment B** binds to a globotriosyl ceramide (Gb3) receptor on intestinal epithelium
- **Fragment A** - Active fragment. Inhibits protein synthesis

**Detection of VT:**
- Serologically—Latex agglutination, ELISA
- Molecular methods—using specific DNA probe
- Cytotoxicity on Vero and HeLa cell lines
Clinical Manifestations

- one of the most common pathogen encountered clinically
- **Urinary tract infection (UTI):** uropathogenic *E. coli* (*UPEC*)
- **Diarrhea:** Six types of diarrheagenic *E. Coli*
  1. Enteropathogenic *E. coli* (*EPEC*)
  2. Enterotoxigenic *E. coli* (*ETEC*)
  3. Enteroinvasive *E. coli* (*EIEC*)
  4. Enterohemorrhagic *E. coli* (*EHEC*)
  5. Enteroaggregative *E. coli* (*EAEC*)
  6. Diffusely adherent *E. coli* (*DAEC*)
Clinical Manifestations

- **Abdominal infections**: Commonest cause of primary and secondary bacterial peritonitis
- **Visceral abscesses** - hepatic abscess
- **Pneumonia in hospitalized patients**—VAP
- **Meningitis** (especially neonatal)
- **Wound and soft tissue infection** - cellulitis and infection of wounds
- **Osteomyelitis, Endovascular infection and bacteremia**
## Laboratory Diagnosis – Specimen collection

<table>
<thead>
<tr>
<th>Specimens collected</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus, exudates and wound swab</td>
<td>Cellulitis or pyogenic wound infection</td>
</tr>
<tr>
<td>Urine</td>
<td>UTI</td>
</tr>
<tr>
<td>Stool</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>CSF</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Peritoneal exudate</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>Sputum</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>Ventilator associated pneumonia</td>
</tr>
<tr>
<td>Blood</td>
<td>Bacteremia</td>
</tr>
</tbody>
</table>
Laboratory Diagnosis

- **Direct Microscopy** – Gram negative Bacilli
- **Culture**: Aerobe and facultative anaerobe, nonfastidious
  - **Blood agar**: Colonies are big, circular, gray, moist and occasionally β hemolytic
  - **MacConkey agar**: pink due to lactose fermentation

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Laboratory Diagnosis

- **Liquid medium** - uniform turbidity
- **Culture smear and motility testing**: Scattered gram-negative bacilli
- **Hanging drop** – Motile bacilli

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Catalase positive and oxidase negative

Nitrate is reduced to nitrite

ICUT tests:
- Indole test: Positive
- Citrate & Urease test: Negative
- Triple sugar iron agar) test: Shows acid/acid, gas present, H2S absent

Biochemical Tests

Sugar fermentation test: ferments most of the sugars, such as glucose, lactose, mannitol, maltose (but not sucrose), with production of acid and gas.

MR (methyl red) test: Positive

VP (Voges-Proskauer) test: Negative
Laboratory Diagnosis of UPEC

- **Specimen Collection**
  - **Clean voided midstream urine:** Commonest specimen - collected after properly cleaning urethral meatus or glans

- **Suprapubic aspiration of urine from the bladder:** most ideal specimen - for patients in coma or infants

- **Catheterized patients** - from the catheter tube (after clamping and disinfecting); but not from the uro bag

- **Transport**
  - Processed immediately. Expected delay- refrigerate or adding boric acid, glycerol or formate
• **Direct Examination & Screening Tests**
  
  • **Wet mount examination:** Pyuria of more than 8 pus cells/mm³ or 4 lakh pus cells excreted in urine/hour is significant
  
  • **Leukocyte esterase test:** Rapid and cheaper method
  
  • **Nitrate reduction test (Griess test)**
  
  • **Gram staining of urine is not a reliable indicator as—**
    - Bacterial count in urine is usually low
    - Pus cells rapidly deteriorate in urine
    - Limited to pyelonephritis and invasive UTI - count of ≥1 bacteria/oil immersion field is significant
Culture media: MacConkey agar and blood agar or CLED agar

Kass concept of significant bacteriuria:
- A count of $\geq 10^5$ colony forming units (CFU)/mL of urine is considered as significant → Indicates infection
- Count between $10^4$ to $10^5$ CFU/mL indicates doubtful significance - clinically correlated
- **Low count of \( \leq 10^4 \text{ CFU/mL} \)** - Commensal bacteria due to contamination during voiding. Low counts may be significant:
  - Patient on antibiotic or on diuretic treatment
  - Infection with some gram-positive organisms such as *S. aureus* and *Candida*
  - Pyelonephritis and acute urethral syndrome
  - Sample taken by suprapubic aspiration
- **Quantitative culture:** Semi-quantitative method - standardized loop technique
- Quantitative method such as pour plate method.
**Diarrhea (Diarrheagenic E. coli)**

- Diarrheagenic *E. coli* are **antigenically distinct** from the commensal *E. coli* which colonize the intestine.
- Only few **serotypes of *E. coli*** which express the enterotoxin or other virulence mechanisms can cause diarrhea.
- **Six types** of diarrheagenic *E. coli*. 
Enteropathogenic E. coli (EPEC)

- Causes infantile diarrhea (outbreaks) and occasionally sporadic diarrhea in adults
- Nontoxigenic and noninvasive
- **Mechanism of diarrhea:**
  - Adhesion to intestinal mucosa mediated by plasmid coded bundle-forming pili
- **Attaching and effacing lesions:** leads to disruption of brush border epithelium causing increased secretion and watery diarrhea
Enterotoxigenic E. coli (ETEC)

- Most common cause of **traveler’s diarrhea** (25–75%)
- Acute watery diarrhea in infants and adults
- Common serotypes—O6, O8, O15, O25, O27, O153, O159, etc.
- Toxigenic, but not invasive
- **Pathogenesis of ETEC is by:**
  - Attachment to intestinal mucosa mediated by fimbrial protein colonization factor antigen (CFA)
  - **Toxin production**—(1) heat-labile toxin or LT (acts by ↑cAMP), (2) heat-stable toxin or ST (acts by ↑cGMP)
Enteroinvasive E. coli (EIEC)

- Common serotypes - O28, O112, O114, O124, O136, O143, O144, O152, O164

- **Pathogenesis:** Invasive
  - Mediated by a plasmid-coded antigen called virulence marker antigen (VMA)
  - Biochemically, genetically & pathogenically related to *Shigella*

- **Manifestations:** Ulceration of bowel, dysentery

- **Diagnosis:** Detection of VMA by ELISA
  - HeLa cell invasion assay, DNA probes to screen faeces
  - **Sereny test:** On instillation into the eyes of guinea pigs, EIEC cause keratoconjunctivitis, no longer used.
Enterohemorrhagic E. coli (EHEC)

- **Serotypes associated with EHEC are:** O157:H7 (most common serotype)
- **Other serotypes** - O26:H11, O6, O55, O91, O103, O111 & O113
- **Transmitted by contaminated food,** i.e. consumption of lettuce, spinach, sprouts and undercooked ground beef
- **Prevalent mainly in industrialized countries**
- **Low infective dose:** Few organisms (<$10^2$ bacilli) are required to initiate the infection
- **Pathogenesis:** secretes verocytotoxin or Shiga-like toxin
Shiga-like Toxin

- **Mechanism of action:** inhibits protein synthesis by inhibiting the 28S subunit of 60S ribosome.
- Stx2 is more commonly associated with HUS than Stx1
- **Manifestations:** predilection for endothelium → capillary microangiopathy
- **Hemorrhagic colitis:** gross bloody diarrhea, abdominal pain and fecal leukocytosis but no fever
- **Hemorrhagic uremic syndrome (HUS):** injury to small vessels of the kidney and brain → bloody diarrhea, thrombocytopenia, renal failure and encephalopathy but without fever
Diagnosis:

- **Sorbitol MacConkey agar:** Unlike other *E. coli*, does not ferment sorbitol and produces pale colonies

- **Toxin detection:**
  - Demonstration of cytotoxicity in Vero cell lines (gold standard method)
  - Fecal toxin detection by ELISA or rapid tests
  - **PCR** - to differentiate genes coding for Stx1 and Stx2
**Enteropathogenic E. coli (EPEC)**

- Adheres to HEp-2 cells in a stacked-brick fashion
- Most strains are “O” untypeable but “H” typeable

**Pathogenesis:**
- Intestinal colonization mediated by aggregative adhesion fimbriae I
- EAST 1 toxin

**Manifestations:** Persistent and acute diarrhea

**E. coli O104: H4** - enteropathogenic strain that has caused major outbreaks in Germany in 2011. Also produces Shiga-like toxin and can cause HUS
Treatment *E. coli*

- **Extra-intestinal *E. coli***
- Based upon antimicrobial susceptibility test report
- Hospital strains mostly MDR. Often produce ESBLs or AmpC $\beta$-lactamases $\rightarrow$ resistant to most $\beta$-lactams except carbapenems
- Carbapenems, amikacin or BL/BLIs - agents of choice for hospital acquired MDR *E. coli* isolates
• Extra-intestinal *E. Coli*

• Carbapenem resistant isolates - Polymyxins, fosfomycin or tigecycline

• Diarrheagenic *E. Coli* - fluid replacement
  - Antimicrobials to be avoided
KLEBSIELLEAE
Genera *Klebsiella*, *Enterobacter*, *Hafnia* and *Serratia* differ from all other tribes being VP positive but MR negative.

*Klebsiella* - found as commensals in human intestines and as saprophytes in soil.

Genus *Klebsiella* has three species—*K. Pneumoniae*, *K. Oxytoca* and *K. granulomatis*.

*K. pneumoniae*: 3 Subspp. *Pneumoniae*, *ozaenae* and *rhinoscleromatis*.

Lactose fermenters

Non-motile and capsulated
Morphology

- Short, coccobacilli, Gram negative, capsulated, nonmotile bacilli
- Size 1-2 µm x 0.5-0.8 µm
- **Culture**: MA - Colonies are large, mucoid, LF,

**Biochemicals;**
Ferments sugar (G,L,S,M,) with production of acid and gas
Urease positive, indole-ve, VP positive,, citrate utilizing (IMViC --++)
Antigenic structure

1. Capsular (K) antigen: on the basis of capsular antigens, Klebsiella classified into 80 (1-80) serotypes.
   • Identification of capsular antigens usually done by capsular swelling reaction with capsular antiserum

2. Somatic (O) antigen: Klebsiella contains five (01-05) different somatic or O antigens in various combinations
Methods of typing

- Phage typing, biotyping, bacteriocin (klebocin or pneumocin) typing and resistotyping

- Many Klebsiella strains produce bacteriocins k/a Klebocins or pneumocins which show a narrow range of activity on other Klebsiella strains

- Klebocin typing and capsular serotyping together may be very useful for epidemiological studies
Pathogenesis

❖ *Klebsiella pneumoniae subspecies pneumoniae*:
- Most pathogenic
- Severe lobar pneumonia - destructive with production of thick, mucoid, brick red sputum
- Urinary tract infections, meningitis (neonates), septicemia and pyogenic infections such as abscesses and wound infections
- Colonizes the oropharynx of hospitalized patients
- Common cause of nosocomial infections
- Most hospital strains - multidrug resistant
Pathogenesis

- **K. pneumoniae subspecies ozaenae**
  - Atrophic rhinitis (or ozena) - foul smelling nasal discharge
  - Biochemically inactive
- **K. pneumoniae subspecies rhinoscleromatis**
  - Rhinoscleroma - chronic granulomatous hypertrophy of the nose
  - South eastern Europe, India and in Central America
  - Biochemically inactive
Laboratory Diagnosis

- **Gram staining:** short, plump, straight capsulated gram-negative rods

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Klebsiella

- **Culture:**
- **MacConkey agar** - large dome shaped mucoid (due to capsule) sticky, pink colour, lactose fermenting colonies

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Biochemical identification:

- **ICUT test:**
  - **Indole test:** Negative
  - **Citrate test & Urease test:** Positive
  - **Triple sugar iron agar test:** Acid/acid, gas present, H2S absent

- **Sugar fermentation test:** Ferments most of the sugars glucose, lactose, mannitol, maltose (but not sucrose), with production of acid and gas
• **VP (Voges-Proskauer) test:** Positive

• **MR (methyl red) test:** Negative

• *K. oxytoca* is biochemically similar to *K. pneumoniae*, but differs from the latter by being indole positive

• **Treatment:** Most clinical isolates are MDR

  - Guideline for treatment is same as that for *E. coli*