E.Coli , Klebsiella

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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

Groups	Lactose fermentation	Colonies on MacConkey agar	Examples
Lactose fermenters (LF)- all are coliform bacilli	Ferment lactose producing acid	Produce pink colored colonies, (acid changes the color of neutral red indicator to pink)	Escherichia, Klebsiella Citrobacter
Non lactose fermenters (NLF)	Do not ferment lactose	Produce pale or colorless colonies	Salmonella, Shigella Proteus, Morganella, Providencia and Yersinia
Late lactose fermenters (LLF or previously called as paracolon bacilli	Ferment lactose after 2-8 days of incubation	At 24 hrs incubation- produce pale or colorless colonies, After 2 days- produce pink color colonies	Shigella sonnei

Classification: Common morphogical, 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

Tribe	Genus
Tribe I-Escherichieae	Escherichia, Shigella
Tribe II-Edwardsielleae	Edwardsiella
Tribe III-Salmonelleae	Salmonella
Tribe IV-Citrobactereae	Citrobacter
Tribe V- Klebsiellaae	Klebsiella, Enterobacter, Hafnia, Serratia, Pantoea
Tribe VI-Proteeae	Proteus, Morgenella, Providencia
Tribe VII- Yersinieae	Yersinia

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 → 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

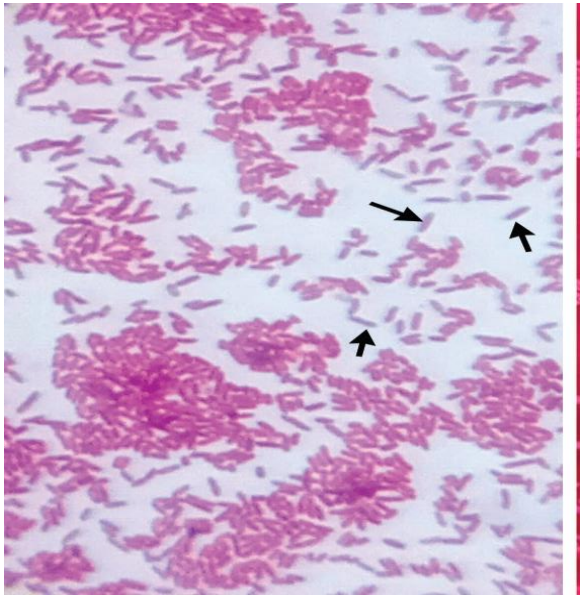
Specimens collected	Disease
Pus, exudates and wound swab	Cellulitis or pyogenic wound infection
Urine	UTI
Stool	Diarrhea
CSF	Meningitis
Peritoneal exudate	Peritonitis
Sputum	Pneumonia
Tracheal aspirate	Ventilator associated pneumonia
Blood	Bacteremia

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

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: C**ommonest 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- refrigerator 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 ≥**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 ≤ 10^4 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

Enteroaggregative E. coli (EAEC)

- 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*** **-** enteroaggregative 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 β-lactamases →resistant to most β-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. Peumoniae, ozaenae and rhinoscleromatis***

- **Lactose fermenters**

- **Non-motile and capsulated**

Morphology

- Short , coccobacilli, Gram negative, capsulated, non motile 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

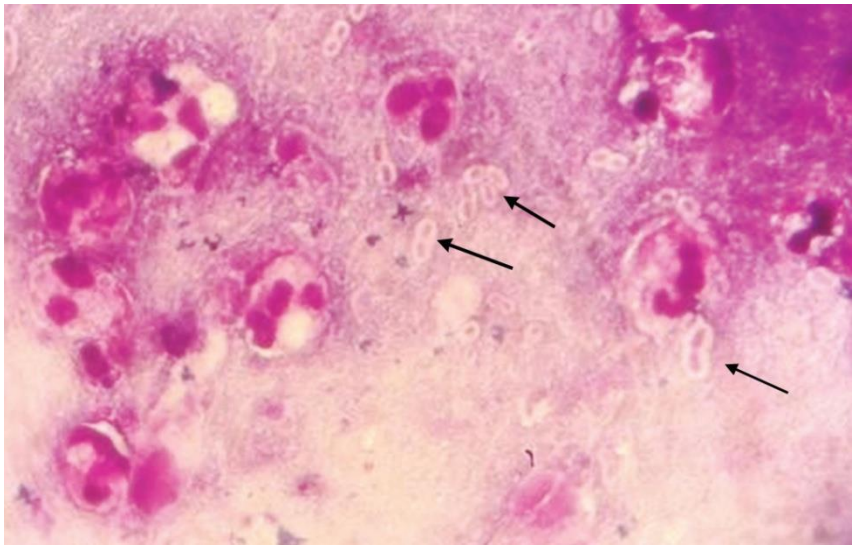
- Colonizes the oropharynx of hospitalized patients
- Common cause of nosocomial infections
- Most hospital strains - multidrug resistant

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

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*

HAVE A NICE DAY

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