

# Enterobacteriaceae - I

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## About Enterobacteriaceae

- *A large Family of aerobic bacterial flora of intestine of humans and other animals.*
- Its members are nonsporting, non acid-fast, gram negative bacilli.
  
- **General features –**
  - *aerobic and facultatively anaerobic,*
  - *grow readily on ordinary media,*
  - *ferment glucose,*
  - *reduce nitrates to nitrites and*
  - *form catalase but not oxidase.*

## Classification based on lactose fermentation

### 1. Lactose fermenters

- ▶ *Escherichia coli*
- ▶ *Klebsiella sp.*

### 2. Late lactose fermenters

- ▶ *Shigella sonnei*

### 3. Non lactose fermenters

- ▶ *Salmonella*
- ▶ *Shigella etc.*



# Taxonomic classification

## ▶ Tribe I: Escherichia

### ▶ Genus

- ▶ Escherichia
- ▶ Edwardsville
- ▶ Citrobacter
- ▶ Salmonella
- ▶ Shigella

## ▶ Tribe II: Klebsiella

### ▶ Genus

- ▶ Klebsiella
- ▶ Enterobacter
- ▶ Hafnia
- ▶ Serratia

## ▶ Tribe III: Proteae

### ▶ Genus

- ▶ Proteus
- ▶ Morganella
- ▶ Providencia

## ▶ Tribe IV: Erwinieae

### ▶ Genus

- ▶ Erwinia

*Escherichia coli*

## History

- Genus *Escherichia* named after ***Theodore von Escherich, a German bacteriologist/paediatrician*** who was the first to describe the colon bacillus under the name *Bacterium coli commune* (1885).



## Difference species of Escherichia

### ➤ Species

- *E. coli*,
- *E. fergusonii*,
- *E. hermanii*,
- *E. vulneris*,
- *E. blattae* etc

## Morphology

- Gram negative bacilli
- 1-3 x 0.4-0.7  $\mu\text{m}$
- Single, pairs
- Motile by peritrichate flagella
- Found in some – capsules, fimbriae, immobility
- Non spore forming





## Culture characteristics

- Aerobe and facultative anaerobe
- 10-40°C (37°C)
- **S** = smooth forms seen in fresh isolates, easily emulsifiable in saline.
- **R** = rough forms seen in older cultures, with irregular dull surface, often auto-agglutinable in saline.
- **S-R variation** occurs as a result of repeated subcultures and is associated with the loss of surface antigens and usually of virulence.
- *Many pathogenic isolates have polysaccharide capsules.*
- *Some strains may occur in the mucoid form.*

## Culture characteristics

- **Nutrient agar** – colonies are large, thick, greyish white, moist, smooth, opaque or partially translucent discs.
- **Blood agar** - Many strains esp. pathogenic ones are haemolytic on blood agar.
- **MacConkey medium** - colonies are bright pink due to lactose fermentation.
- **Broth** – general turbidity, heavy deposit.

## Biochemical reactions

- ▶ Sugar fermentation – glucose, lactose, mannitol, maltose and many other sugars fermented with **acid and gas** production.
- ▶ *Sucrose generally not fermented.*
- ▶ IMViC ++--
- ▶ Gelatin -, H<sub>2</sub>S -, urease -.



## Reaction in TSI agar



- Acid butt
- Acid slant
- Gas production
- No H<sub>2</sub>S production

## Antigenic structure

- **O = somatic antigen**
- **K = capsular antigen**
- **H = flagellar antigen**
  
- So far, >170 types of O, 100 types of H and 75 types of K have been identified.
- Antigenic pattern of an organism based on these antigens is written as eg. O111:K58:H2, O54:K27:H41 etc.
- ***K antigen** is the acidic polysaccharide antigen located in the envelope or microcapsule (K for kapsel, german for capsule).*
- *It encloses the O antigen and renders the strain non-agglutinable by the O antiserum.*
- *It may also contribute to virulence by inhibiting phagocytosis.*

## Virulence factor

### ➤ Surface antigens: O and K

- O antigen – somatic lipopolysaccharide surface O antigen has endotoxic activity, protects the bacteria from phagocytosis and bactericidal effects of complement.
- K antigen – affords protection against phagocytosis and antibacterial factors in normal serum.

## Virulence factor

➤ **Fimbriae** – plasmid coded, found in small numbers and mediate mannose resistant haemagglutinins, act as virulence factors.

### ▶ Examples:

- CFA = colonization factor antigens in enterotoxigenic *E.coli* causing human diarrhea.
- P fimbriae which bind to uroepithelial cells and P blood group substance on human erythrocytes, have a role in urinary tract infection.

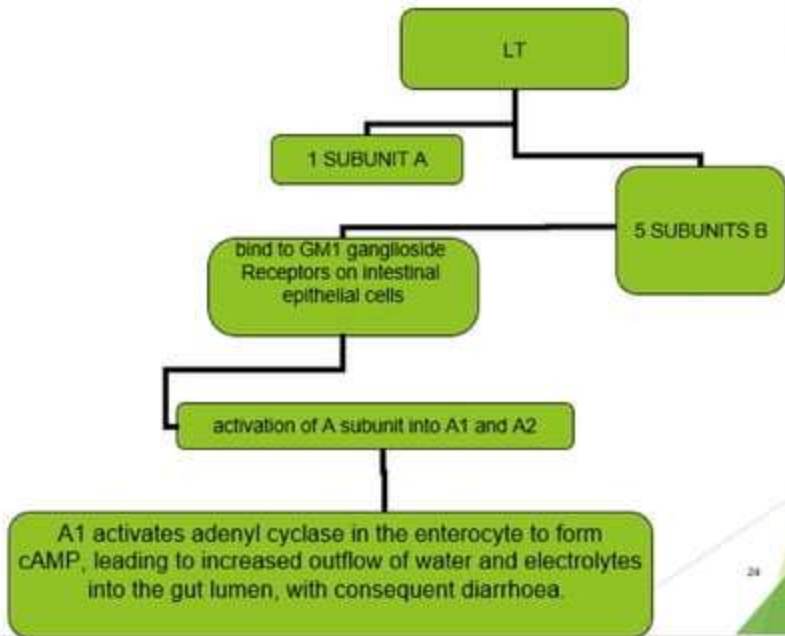
## Toxins

- *E. coli* produce two kinds of exotoxins – haemolysins and enterotoxins.
- Three distinct types of *E. coli* enterotoxins have been identified –
  - LT = heat labile toxin
  - ST = heat stable toxin
  - VT = Verotoxin (also known as SLT = shiga like toxin).



## LT = heat labile toxin

- Resembles cholera toxin in its structure - antigenic properties and mode of action.
- It is a complex of polypeptide subunits.
- Each unit of toxin has 1 subunit A and 5 subunits B.
- ***CT is about 100 times more potent than LT.***
- LT is a powerful antigen and can be detected by serological and biological tests.



## ST = heat stable toxin

- Low MW polypeptide, poorly antigenic.
- Two types known – STA/ST1 and STB/ST2.
- ST genes are carried on plasmids which may also carry other genes, such as for LT and drug resistance.

## VT = Verotoxin

- Named so because it was first detected by its **cytotoxic effect on Vero cells**, a cell line derived from African green monkey kidney cells.
- It is also known as **SLT = shiga like toxin** because it is similar to the shigella dysenteriae type 1 toxin in its physical, antigenic and biological properties.
- Has A and B subunits.
- VT genes appear to be phage encoded.
- VT1, VT2 identified.

## Clinical infections

- Urinary tract infections
- Diarrhoea
- Pyogenic infections
- Septicaemia

## Urinary tract infections

- ▶ **Community acquired:** E.coli and other coliforms account for the large majority of naturally acquired UTIs.
- ▶ **Hospital acquired:** Those acquired in the hospital, following instrumentation, are more often caused by other bacteria such as Pseudomonas and Proteus.
- ▶ The E.coli serotypes commonly responsible for UTI are those normally found in the faeces, **O groups 1,2,4,6,7,etc.**

## Kass' concept of significant bacteriuria

- Normal urine is sterile, but during voiding may get contaminated by genital commensals.
- Hence presence of bacteria in microscopy and culture of a urine sample need not necessarily mean UTI by that organism.
- To differentiate between actual pathogen and contaminant, Kass *et al.* devised the following formula.
  
- **Colony count in urine:**
  - $<10000/\text{ml}$  = contaminant
  - $10000$  to  $100000/\text{ml}$  = indecisive, repeat test.
  - $>100000/\text{ml}$  = significant bacteriuria.
  
- **Exceptions:** in patients on antibacterial or diuretic drugs and with some bacteria like Staph. aureus, even low counts may be significant.

## Urine sample collection

- ▶ Inform the patient about the procedure.
- ▶ Proper cleaning of area.
- ▶ **Clean voided midstream urine sample** is collected in a sterile leak-proof container.
- ▶ Sample container is labeled, request form filled and send along with the sample to the laboratory within an hour or two.
- ▶ If delay of more than 1-2 hours is there, the sample should be **refrigerated**.
- ▶ **Boric acid** can be used as a preservative to prevent the change in count of bacteria in the sample.



# Bacterial count

## ▶ Quantitative culture:

- Serial ten fold dilutions of urine are tested by the pour plate or surface culture methods. Complicated method.

## ▶ Semiquantitative culture:

- Fixed volume of urine placed on a non-inhibitory medium like blood agar with a standard loop for quantitative measurement.
  - Another loopful of urine placed on a indicator medium like MacConkey agar for presumptive diagnosis of causative organism.
- ▶ Culture is followed by biochemical tests etc for identification of the pathogen.

## Diarrhoea

- EPEC = ENTEROPATHOGENIC E.COLI
- ETEC = ENTEROTOXIGENIC E.COLI
- EIEC = ENTEROINVASIVE E.COLI
- EHEC = ENTEROHAEMORRHAGIC E.COLI
- EAEC = ENTEROAGGREGATIVE E.COLI

## EPEC = ENTEROPATHOGENIC E.COLI

- Associated with diarrhoea in infants and children.
- Institutional outbreaks, sporadic diarrhoea.
- Do not produce enterotoxins.
- Not invasive.
- Mode of action: Attach to mucosa of upper small intestine, cause **disruption of brush border microvilli.**
- Entero adherent E.coli is another name given to them.

## ETEC = ENTEROTOXIGENIC E.COLI

- Endemic in developing countries in tropics, all age groups.
- Mild watery diarrhea to fatal disease indistinguishable from cholera.
- Persons from developed countries visiting endemic areas often suffer from ETEC diarrhea – a condition known as **TRAVELER'S DIARRHEA**.
- Adhere to intestinal epithelium via fimbrial or colonization factor antigens (CFA I,II,III,IV, etc.).
- **Produce LT or ST or both.**
- Diagnosis done by demonstration of the toxin.

## EIEC = ENTEROINVASIVE E.COLI

- Resemble Shigella
- *Many are nonmotile, do not ferment lactose or ferment it late with only acid production, and do not form lysine decarboxylase.*
- Many of these show O antigen cross reactivity with Shigella.
- Named EIEC because they have the capacity to invade interstitial epithelial cells in vivo and penetrate HeLa cells in tissue culture.

▶ Clinically EIEC infection resembles shigellosis, ranging from mild diarrhoea to frank **dysentery**.

➤ **Sereny test:**

▪ Instillation of a suspension of freshly isolated EIEC or Shigella into the eyes of guinea pigs leads to mucopurulent conjunctivitis and severe keratitis.

▪ Mice can also be used.

➤ **Cell Penetration** in HeLa or HEP-2 cells.

➤ **Plasmid detection:**

➤ **VMA ELISA:** The plasmid codes for outer membrane antigens called the **virulence marker antigens** (VMA) which can be detected by the ELISA (VMA ELISA) test.

# EHEC = ENTEROHAEMORRHAGIC E.COLI

## ▶ Produce VT

- ▶ Mild diarrhea to fatal **haemorrhagic colitis and haemorrhagic uremic syndrome (HUS)** particularly in young children and elderly.
- ▶ Primary target of VT = vascular endothelial cells.
- ▶ **O157:H7**, O26:H1 etc
- ▶ The disease may occur **sporadically or as outbreaks of food poisoning.**
- ▶ Changing lifestyle and eating habits.
- ▶ Salad vegetables such as **radish and sprouts**, in which bacteria were found beneath the skin and in the deeper tissues.
- ▶ Diagnosis: demonstration of VT.

## EAEC = ENTEROAGGREGATIVE E.COLI

- Appear aggregated in a **stacked brick formation** on Hep-2 cells or glass.
- They have been associated with **persistent diarrhea**, especially in developing countries.
- They form a Low MW heat stable enterotoxin called **EAST1** (entero aggregative heat stable enterotoxin-1).





### ➤ PYOGENIC INFECTIONS:

- E.coli form the most common cause of intra-abdominal infections, such as peritonitis and abscess resulting from spillage of bowel contents.
- Pyogenic infections in the perianal area.
- ***Neonatal meningitis***

### ➤ SEPTICAEMIA:

- Blood stream invasion by E.coli may lead to fatal conditions like ***septic shock and systemic inflammatory response syndrome (SIRS)***.

Klebsiella

# Introduction

- GENUS KLEBSIELLA:

- *K. pneumoniae*
- *K. ozaenae*
- *K. rhinoscleromatis*
- *K. oxytoca* etc.

## Characteristics

- Non-motile
- Capsulated
- Grow on OM forming **large dome shaped mucoid colonies.**
- Short plump straight rods.
- **Capsular halo** seen prominently in gram stain.
- Commensals, saprophytes.



## *Klebsiella pneumoniae*

- Sugar fermentation – acid + gas
- IMViC --++
- Urease +
  
- Second most populous member of aerobic bacterial flora of the intestine.
  
- Important cause of nosocomial infections.
  
- Pneumonia, UTI, pyogenic infections, septicemia, and rarely diarrhoea.

## *Klebsiella pneumoniae*

- ▶ Serious disease with high case fatality.
- ▶ Middle age or older persons.
- ▶ Alcoholism, chronic bronchopulmonary disease, diabetes.
- ▶ Massive mucoid inflammatory exudate of lobar or lobular distribution, involving one or more lobes of the lung.
- ▶ Necrosis and abscess formation.
- ▶ **Serotypes 1, 2, 3.**

## Virulence factors

- **CAPSULE:** Mucoid capsule is anti phagocytic and acts as a major virulence factor.
- **PLASMID EXCHANGE:** Klebsiella participates in exchange of plasmids with other Enterobacteriaceae. The exchange of plasmid is presumed to be the basis for two constant characteristics of Klebsiella species.
  - a. **Antibiotic resistance**

Many strains are highly resistant to most antibiotics.
  - b. **Toxins**

Some Klebsiella strains carry plasmids that code for toxins similar to heat labile and heat stable exotoxins of E.coli.

# Clinical syndrome

## 1. PNEUMONIA

- *K. pneumoniae* is found in 10% normal individuals as normal flora of respiratory tract.
- Pneumonia in diabetics, alcoholics and immunocompromised patients.
- Lung abscess
- Lobar pneumonia – necrotic destruction of the alveolar spaces, cavity formation and production of thick blood tinged viscous sputum. Prognosis is grave with 50% mortality.



## Other diseases

2. URINARY TRACT INFECTIONS
3. SEPTICAEMIA
4. WOUND INFECTION
5. MENINGITIS
6. EPIDEMIC DIARRHOEA – in newborns.

Proteus

## Genus *Proteus*

- The genus *Proteus* currently consists of five named species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. myxofaciens* and *P. hauseri*

- Lactose non fermenters.
- The name proteus refers to their **pleomorphism**, after the greek god proteus who could assume any shape.
  
- **Urease +**
- **PPA +**
- Non capsulated, pleomorphic, motile rods.
  
- Weil Felix observed that flagellated strains growing on agar formed a thin surface film resembling the mist produced by breathing on glass and named this variety the Haunch form (haunch=film of breath).
- Non flagellated strains do not form, so called Ohne Haunch.
- **Hence O = somatic antigen, H = flagellar antigen.**

## Culture characteristics

- Putrefactive (fishy or seminal) odour
- Swarming observed on NA, BA
- Pale colonies on Mac and DCA and no swarming
- Indole + (except *P. mirabilis*)
- H<sub>2</sub>S + (*P. mirabilis* and *P. vulgaris*)
- MR +, VP –
- Citrate +

- Habitat: widely prevalent in nature, commensals, saprophytes, opportunistic pathogens.
- **Swarming:** wavy growth, thin filmy layer in concentric circles shown by *P. vulgaris* and *P. mirabilis* in culture plates.

➤ **To inhibit swarming:**

- 6% agar,
- sodium azide(1:500),
- alcohol(5-6%),
- sulphonamide,
- chloral hydrate(1:500),
- surface active agents or
- boric acid(1:1000).

On nutrient agar



# Clinical syndromes

## ▶ URINARY TRACT INFECTIONS

- Urease producing organisms cause liberation of ammonia which raises the pH of the urine. Alkalinized urine favors solubility of calcium that creates environment favorable for deposition of calcium and magnesium salts and the formation of kidney stones.
- Proteus infections tend to occur in patients in obstructive lesions of urinary tract following diagnostic instrumentation or during prolonged catheterization (exogenous infection).

## Other infections

- WOUND INFECTIONS
- SEPTICAEMIA
- ACUTE OTITIS MEDIA



## Weil Felix reaction

- They observed that certain nonmotile strains of proteus were agglutinated with sera from typhus fever patients.
- This heterophile agglutination due to sharing of a carbohydrate hapten by certain strains of proteus and rickettsia forms basis of Weil Felix reaction for Diagnosis of certain rickettsial infections.
- Nonmotile proteus strains **OX2, OX19 (*P. vulgaris*)** and **OXK (*P. mirabilis*)** used in agglutination test.



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DAY

"YOUR DISEASE IS CURABLE, BUT ONE SIDE EFFECT  
OF THIS DRUG IS POVERTY."