GRAM NEGATIVE, AEROBIC, FACULTATIVE ANAEROBE, MICROAEROPHILIC WITH SIMPLE CULTURE REQUIREMENTS
ACCORDING TO RESPIRATORY ENZYME (OXIDASE ENZYME)

- Reaction group I
  - oxidase +ve

- Reaction group II
  - oxidase -ve

According to: glucose metabolism (OF test)

- Reaction group I:
  - la
    - Fermentation oxidatin ( -ve O2 )
  - lb
  - lc
    - inactive type
    - no breakdown

- Reaction group II:
  - llia
  - llb
  - llc
    - the same
The oxidase test is a test used in microbiology to determine if a bacterium produces certain cytochrome c oxidases. It uses disks impregnated with a reagent such as N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) or N,N-dimethyl-p-phenylenediamine (DMPD), which is also a redox indicator.
OF Test

Media used:

Hugh and Leifson’s medium, commonly called as OF medium which contain tryptone and bromothymol blue (an indicator). One of the sugars, such as glucose, xylose, mannitol, lactose, sucrose, and maltose is added to the medium which serves as the fermentable carbohydrate.

One tube is overlaid with mineral oil or melted paraffin producing an anaerobic environment. The other tube is left open to the air.
Positive: A positive carbohydrate utilization test is indicated by the development of a **yellow color** in the medium.

**Oxidative:** Development of a yellow colouration in the open tube only.

**Fermentative:** Development of a yellow colouration in both open and closed tubes.

Negative: A negative carbohydrate utilization test is indicated by the absence of a yellow color (media remains **green** or turns blue).

**Non-oxidizer/Non-fermenter**
group II A

Oxidase -ve

OF test fermentative
Family : Enterobacteriaceae

- Distribution
- General characters :
  - no production of oxidase
  - nitrate reduced to nitrite
  - fermentation of glucose with or without gas
  - growth on simple media & selective media
  - gram negative rods
  - no spore formation
  - motile ( peritrichous flagella ) or non motile
CLASSIFICATION OF THE ENTEROBACTERIACEAE

- Depends on lactose fermentation on macConkey’s medium,

- Lactose fermenters (LF)

  - E. coli
  - Klebsiella
  - Enterobacter
  - Citrobacter

- Non lactose fermenters (NLF)

  - Salmonella
  - Shigella
  - Proteus
  - Yersinia
  - Providence
THE COLIFORMS

- Escherichia, Enterobacter, Klebsiella & Citrobacter

- Not all coliforms are associated with the intestinal tract

- Fecal coliforms
  - Escherichia
  - Klebsiella & enterobacter

- non fecal coliforms
Eijkman test or Differential coliform test:
Christiaan Eijkman (1858-1930)

is a test used for the identification of coliform bacteria from warm-blooded animals

**based on:** the bacteria's ability to produce gas when grown in glucose media

at 46°C (114.8°F)

The test to determine whether coliform bacteria come from warm-blooded animals. By means of this test it can be readily established if water has been polluted by human and animal defecation containing colibacilli
**GENUS : ESCHERICIA**
**SPECIES : E.COLI**

- Incidence & veterinary significance

  - comensal
  - economic importance
    - septicaemia & diarrhea
    - enterotoxaemia (oedema disease)
    - dysentery of rabbits
    - mastitis cows & other species
    - septicaemia & granulomatosis

  in poultry
MORPHOLOGY

- Plump to coccoid
- Gram negative rod 1.1-1.5 X 2-6 µm
- Motile
- Single or in pairs
- Capsulated
- Non sporulated
CULTURAL CHARACTERS

- At 37°C onto nutrient agar, blood agar selective media

<table>
<thead>
<tr>
<th>S-form</th>
<th>R-form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round, small</td>
<td>Large, dry</td>
</tr>
<tr>
<td>Smooth outline</td>
<td>Irregular outline</td>
</tr>
<tr>
<td>Greyish white, shiny</td>
<td>Pathogenic or heamolytic</td>
</tr>
</tbody>
</table>
- In nutrient broth

- After 12 hours: regular turbidity
  - after 24 hours: heavy powdery sediment

**Bacterial Cultures in Broth Media**

- **a.** Sterile (uninoculated broth) - note how clear the media is
- **b.** Broth showing slight turbidity (some bacterial growth)
- **c.** Broth showing significant turbidity (a lot of bacterial growth)
- **d.** Broth that hasn’t been agitated (shaken)
VIRULENCE FACTORS
(ANTIGENIC STRUCTURES)

- Somatic antigen  O - antigen  >160
- Lipopolysaccharides, heat labile,
- Determined by tube, slide agglutination test
- Capsular antigen  K - antigen  (91)
- Polysaccharides, ,

- K-A antigen  K-B antigen
- Polysaccharide  acid polysaccharide
- Heat stable  heat labile
- Flagellar antigen  H - antigen  (49)
- Protein in nature, heat labile, determined by tube agglutination
- Fimbrial antigen  L - antigen
- Pili antigen, protein in nature, (adhesion antigen)
- Important for identification of the enteropathogenic strains,
determined by slide agglutination & ELISA technique
TOXINS OF E.COLI

1- Enterotoxins:

- Enterotoxic E.coli (ETEC)

Young animals

Heat labile

Heat stable

ST a

ST b
2- Neurotoxins

Haemolytic strain of E.coli

- Lipoprotein, thermolabile
- Can be neutralize with antitoxic sera
- Induced in mice central nervous system disturbances
3- Endotoxins:
Occur in all strains of E.coli

- Protein-phospholipid polysaccharide complexes
  - toxicity
  - serological specificity

- Signs of shock
4- Colicins

- Protein
- Bactericidal effect
- Differentiate of E.coli strains to:

  - Colicin +ve E.coli
  - Colicin -ve E.coli
BIOCHEMICAL REACTION

- Lactose fermenter: +ve

<table>
<thead>
<tr>
<th>I</th>
<th>M</th>
<th>V</th>
<th>C</th>
<th>U</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

[Image of four test tubes labeled A, B, C, D, with different colored liquids.]
DISEASES OF E. COLI

- 1- intestinal diseases
- 5 virotypes cause diarrheal disease are recognized:
  - A- ETEC, SI, without fever, non-invasive
  - heat labile & heat stable enterotoxin
  - B- EIEC, LI, highly invasive, no toxins
  - C- EHEC, LI, moderately invasive
  - D- EPEC, SI, diarrhea with fever, no toxins
  - E- EAggEC, SI, non-invasive, diarrhea without fever, produce hemolysin & heat stable enterotoxin
2- urinary tract infection: 
- UPEC 
- Due to enterotoxins 
  - kidney invasion 
  - renal failure 

3- endotoxic shock 
- fever, hypotension due to endotoxemia 

4- wound sepsis
LABORATORY DIAGNOSIS

**Speciemens**
Intestinal content, fecal samples, organs, milk samples

**Isolation by culture**
Direct culture on to lactose containing selective media
Incubation: 24h, 37°C
E. coli lactose +ve colonies

**Biochemical identification**
- Motility: +ve
- Urease: -ve
- Indole: +ve
- H2S: -ve
- MR: +ve
- VP: -ve
- citrate: -ve

**Serological typing with E. coli O-antisera, K antigen**
GENUS : KLEBSIELLA

Species : K. pneumoniae

- K. pneumoniae subsp. Pneumoniae
- K. pneumoniae subsp. Ozaenae
- K. pneumoniae subsp. rhinoscleromatis
MORPHOLOGY

- Gram negative thick rod 0.5 - 1X 1 - 3µm
- non motile
- Single or pairs or short chains
- Capsulated (mucoid colonies)
- Non sporulatd
CULTURAL CHARACTERS

- Grows on ordinary media 18 - 24 hrs

- **Nutrient broth** → turbidity + mucoid sediment

- **macConkey agar** → large, convex, circular, red, mucoid (lactose fermenter)
BIOCHEMICAL CHARACTERS

- Lactose fermenter: + ve
- Indole: - ve
- MR: - ve
- VP: + ve
- Citrate: + ve
- Urease: + ve
- H2s: - ve
DISEASES OF KLEBSIELLA

- In horses: inflammation of the genital mucosa & abortion, generalized infections of foals
- In cattle: mastitis, generalized infections & entritis of calves
- In pigs: piglet diarrhea, nasopharyngeal region & digestive tract without clinical signs
- In poultry
ANTIGENIC STRUCTURE

- **O - antigens**: of little importance in the differentiation
  
  why?????

- **K - antigen**: more than 70 strains can be identified using the K. antigen
LABORATORY DIAGNOSIS

**specimens**
- Fecal, organ or milk samples, swabs from genital mucosa of horses, food materials

**Isolation by culture**
- On ordinary media → very mucoid, lactose +ve colonies

**Biochemical identification**
- Motility -ve
- Urease +ve
- Indole -ve
- H2S -ve
- MR -ve
- VP +ve
- Citrate +ve

- Serological typing with O-antisera without significance
- Identification of K. antigen provides real information about the virulence of the strain
GENUS : CITROBACTER

- Species :
  - C. freundii
  - C. diversus
  - C. amalonticus
DISEASES CAUSED BY CITROBACTER

- **In cattle**: mastitis, abortion, diarrhea
- **In sheep & goats**: diarrhea
- Can be isolated from frogs, snakes & fishes
**BIOCHEMICAL PROPERTIES**

- Lactose fermenter: + ve
- Indole: - ve
- MR: + ve
- VP: - ve
- Citrate: + ve
- Urease: + ve
- H2s: + ve
NON LACTOSE FERMENTERS NLF

- Salmonella
- Shigella
- Proteus
- Pseudomonas
SALMONELLA

- Comprise a large group of serotypes (>1600)
- World wide & infect a wide variety of hosts As man, animals & poultry
- Salmonella are typical intestinal pathogens & contaminate the environment by the organism in the feces and transfer infection to other
- Salmonella occurs as a common inhabitant in reptiles
- Some types are host specific
<table>
<thead>
<tr>
<th>species</th>
<th>group</th>
<th>host</th>
<th>disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Paratyphi A</td>
<td>A</td>
<td>Man</td>
<td>Paratyphoid A fever</td>
</tr>
<tr>
<td>S. Paratyphi B</td>
<td>B</td>
<td>Man</td>
<td>Paratyphoid B fever</td>
</tr>
<tr>
<td>S. Abortus equi</td>
<td>B</td>
<td>Equine</td>
<td>Equine abortion &amp; infertility</td>
</tr>
<tr>
<td>S. Abortus ovis</td>
<td></td>
<td>Ovines</td>
<td>Ovine abortion</td>
</tr>
<tr>
<td>S. cholera suis</td>
<td>C</td>
<td>swine</td>
<td>Piglet typhus Infectious enteritis</td>
</tr>
<tr>
<td>S. typhi</td>
<td>D</td>
<td>man</td>
<td>Human typhoid fever</td>
</tr>
<tr>
<td>S. gallinarum</td>
<td></td>
<td>poultry</td>
<td>Pullorum disease</td>
</tr>
</tbody>
</table>
Genus: Salmonella
Species: S. typhi
S. typhimurium
S. enteriditis
S. gallinarum-pullorum
S. dublin
S. infantis
S. derby
S. agona
S. panama
S. heidlberg
MORPHOLOGY

- Cocco-bacilli
- Gram -ve
- Medium sized rods
- Actively motile expect S. gallinarum and pullorum
- Many species developed fimbria
CULTURAL CHARACTERS

- In nutrient broth: Turbidity, without pellicle formation.
- Nutrient agar: Round colonies, smooth, convex with grayish colour.

*S. gallinarum* & *S. abortus ovis*

Small, dew drop like colonies after 48 hours incubation.
- Onto blood agar → the colonies relatively large, non-haemolytic
- Onto MacConkey agar → pale in colour (non-lactose fermenter)
ENRICHED MEDIA

There is a more complex problem for salmonella isolation due to their presence in the intestinal tract.

Examples:
- Tetrathionate brilliant green broth
- Selenite F broth

by growing the fecal samples or intestinal contents on the enriched media for 18 - 24 hours to inhibit the growth of the other contaminants as E.coli, proteus
BIOCHEMICAL REACTIONS

- Lactose: -ve
- Indole: -ve
- MR: +ve
- VP: -ve
- Citrate: +ve
- Urease: -ve
- H2S: +ve
VIABILITY & RESISTANCE

- Salmonellae easily killed at 60°C for 20 min.
- Quickly destroyed by the common chemical disinfectant
Identification of salmonella species depends mainly on the serotyping according to Kaufman & White scheme of the antigenic structure of salmonella.
ANTIGENIC STRUCTURES

- **Somatic antigens**  O-antigen
  - Present in the cell wall
  - Lipopolysaccharide-protein complex
  - Designated by arabic numbers from 1 - 65
  - The majority of salmonallae possess more than one of somatic antigens (3-4) on their surface
Flagellar & fimbrial antigens (H-antigens)
- Present in motile strains except S.gallinarum
- Protein in nature
- Salmonella have 2 phases of flagellar antigens
  - Phase 1 H
  - Phase 2 H
- Identified by alpha-betical a,b,c,d ....
- 20 H antigens are Signified by 2 numbers
- 1,2 - 1,3 etc
- or 2-3 letters as I,W, enx
- enz .......
- Virulent antigen (Vi - antigen)
  - Present in freshly isolates of S. typhi
  - Vi - antigen lost by subculturing
# Examples of Salmonella Serotypes

<table>
<thead>
<tr>
<th></th>
<th>S. paratyphi A</th>
<th>S. paratyphi B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>group</strong></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td><strong>O-antigen</strong></td>
<td>1,2,12</td>
<td>1,4,5,12</td>
</tr>
<tr>
<td><strong>H-antigen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phase 1</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>phase 2</td>
<td>-</td>
<td>1,2</td>
</tr>
<tr>
<td><strong>Monophase</strong></td>
<td>Monophasic</td>
<td>Diphasic</td>
</tr>
</tbody>
</table>
DISEASES CAUSED BY SALMONELLA

- specific enteric diseases
  mainly in man as human typhoid
  3 types of human paratyphi
- abortions in mares & ewes
  host specific S. abortus equi
  S. abortus ovis
- septicaemic diseases in newly born animals
- enteric disorders in adults
  non host specific
- salmonella in fowls
SEROLOGICAL EXAMINATION
AGGLUTINATION TESTS

- Antigens used stained or unstained
- There are 2 methods for application:

  - Rapid slide or plate test for pullorum or fowl typhoid
  - Tube agglutination test or slow method

  as Widal test human typhoid 
  abortion of mares 
  & ewes
BACTERIOPHAGE TYPING

- More accurate
- Indicates the origin & non host specific salmonella serotypes
- Studying the epidemiology as in *S. typhimurium*