

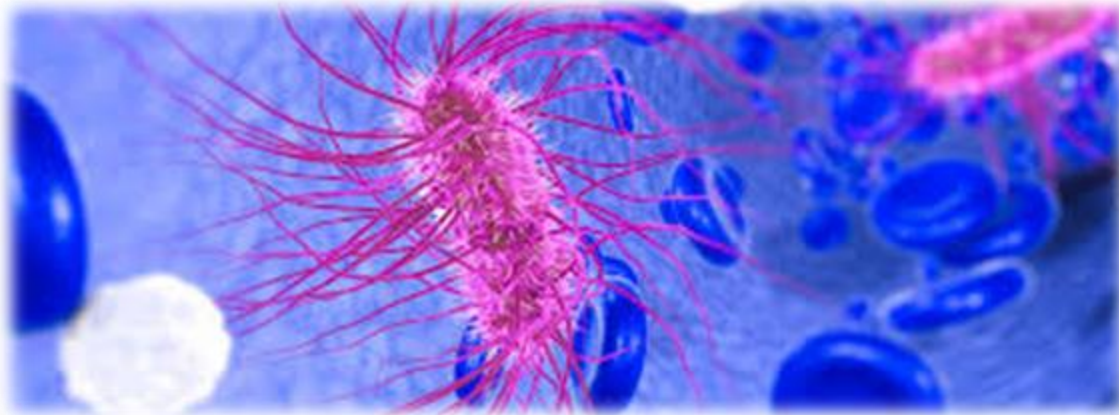


## **FAMILY : ENTEROBACTERIACEAE II**

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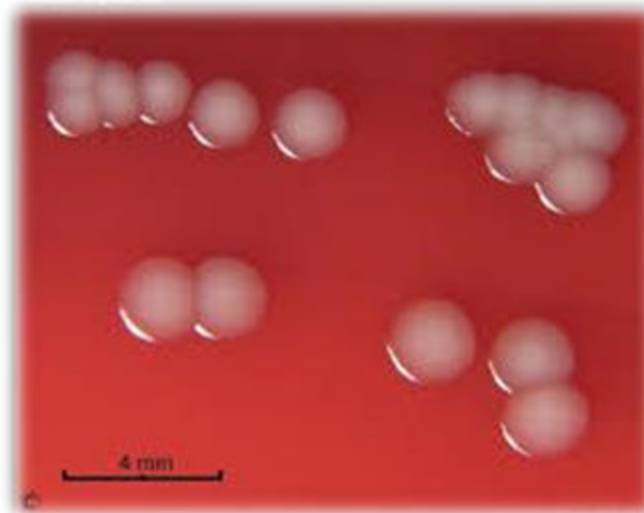
# GENUS: ESCHERICHIA

- Type species: *Escherichia coli*
- Isolated by **Theobord Escherich** from feces of new borne baby
- Gram negative bacillus of 2-3  $\mu\text{m}$  length
- Non spore forming organism
- Most of them are motile with **eritrichous flagella**
- Extra intestinal isolates are capsulated



# GROWTH REQUIREMENT

- Aerobic or facultative anaerobic
- Temperature for growth is 15-45°C.
- Most of the virulent organism grow at temperature as high as 45°C.
- Organism grow on ordinary medium and colonies are large circular and low convex



# GROWTH ON SELECTIVE MEDIA

- Most important is **MaConkey's medium** which contain the inhibitor substance bile, substrate lactose, and indicator is neutral red. Since the organism is lactose fermenting produce rosy pink colonies.



# ENDO AGAR

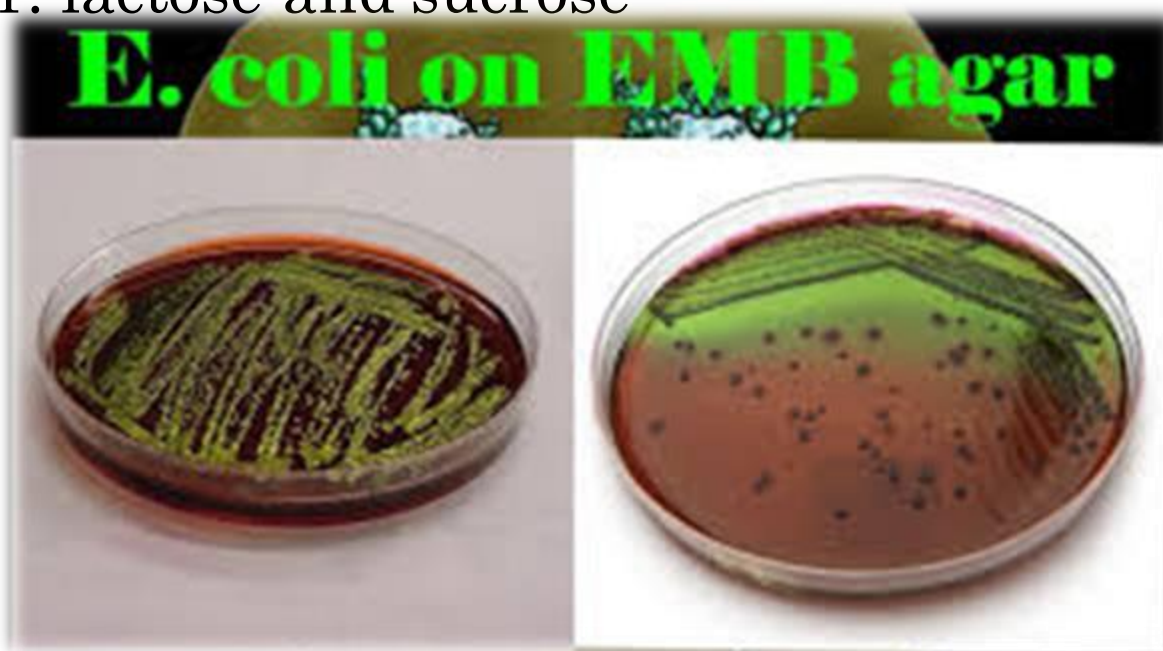
- Endo agar : colonies are red with metallic sheen





# EMB AGAR

- Eosine methylene blue agar. It will produce brownish black colonies with greenish metallic sheen. It is characteristic appearance of *Escherichia coli*
- Sugar: lactose and sucrose



# BIOCHEMICAL CHARACTERISTICS

- Catalase positive and oxidase negative
- Nitrate reduction test is positive
- **Imvic test**: Indole, Methyl Red, Voges Proskauer test, Citrate utilization
- *E. coli* ++--

## Nitrate Reduction Test



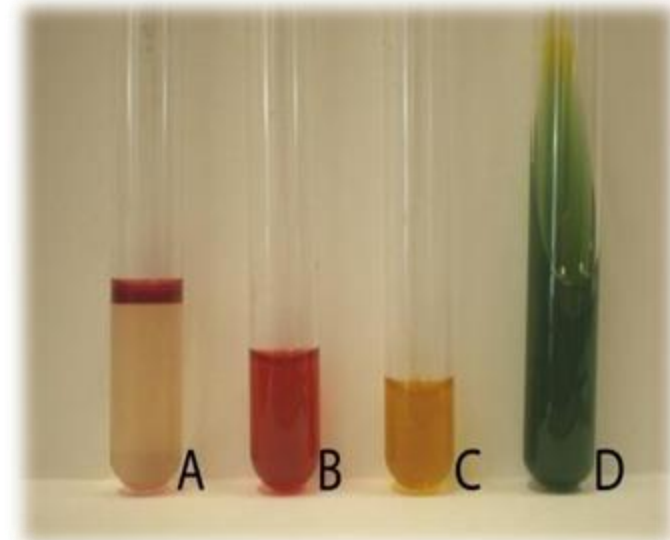
Nitrate negative



Nitrate positive

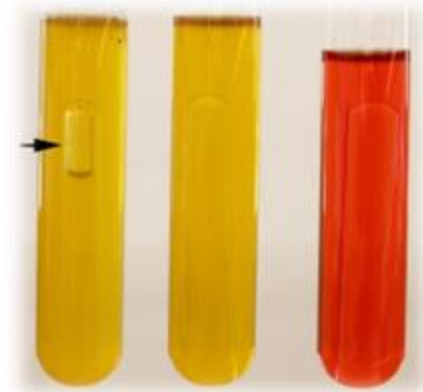


Dr. Binay Joseph



# EIJKMAN'S TEST

- It detect the ability of *E. coli* to produce acid and gas from lactose at 44°C. Other organism produce acid and gas at 37°C but not at 44°C
- Eijkman's test is a presumptive test for *E.coli*.
- So *E.coli* will give Emvic test (++-+-) which stands for Eijkman test, methyl red, vogus Prauskuer test, indole test, and citrate utilization test





# GROWTH ON TSI AGAR SLANT

- In TSI agar sugars like sucrose, lactose and glucose present. E. coli can utilise all sugars
- Indicator in the medium is phenyl red.
- It will produce **acid butt acid slant (yellow butt and yellow slant)** and **shows gas production**. So there will be breakage of the medium
- Organism is negative for **urease** production



# ANTIGENS

- Important antigens are somatic antigen, flagellar antigen, capsular antigen and fimbrial antigen
- Over 165 somatic antigens present and based on somatic antigens over 165 serotypes are present
- Over 100 flagellar antigens present
- 3 types of capsular antigens present **L, A, B**.
- Fimbrial antigen is associated with enterotoxin production
- The most significant adhesins in strains of *E. coli* producing disease in domestic animals are K88 (F4), K99 (F5), 987P (F6), F18 and F41
- There is an enterobacterial common antigen **ECA** in all organism of the family, a thermostable somatic antigen



# TOXINS

- Important factors associated with virulence of *E. coli* are
- lipid A
- haemolysin
- heat labile and heat stable enterotoxin
- vero toxin ( toxin for vero cells)
- CNTF ( cytotoxic necrotising factor)



# VIRULENCE FACTORS OF *E. COLI*

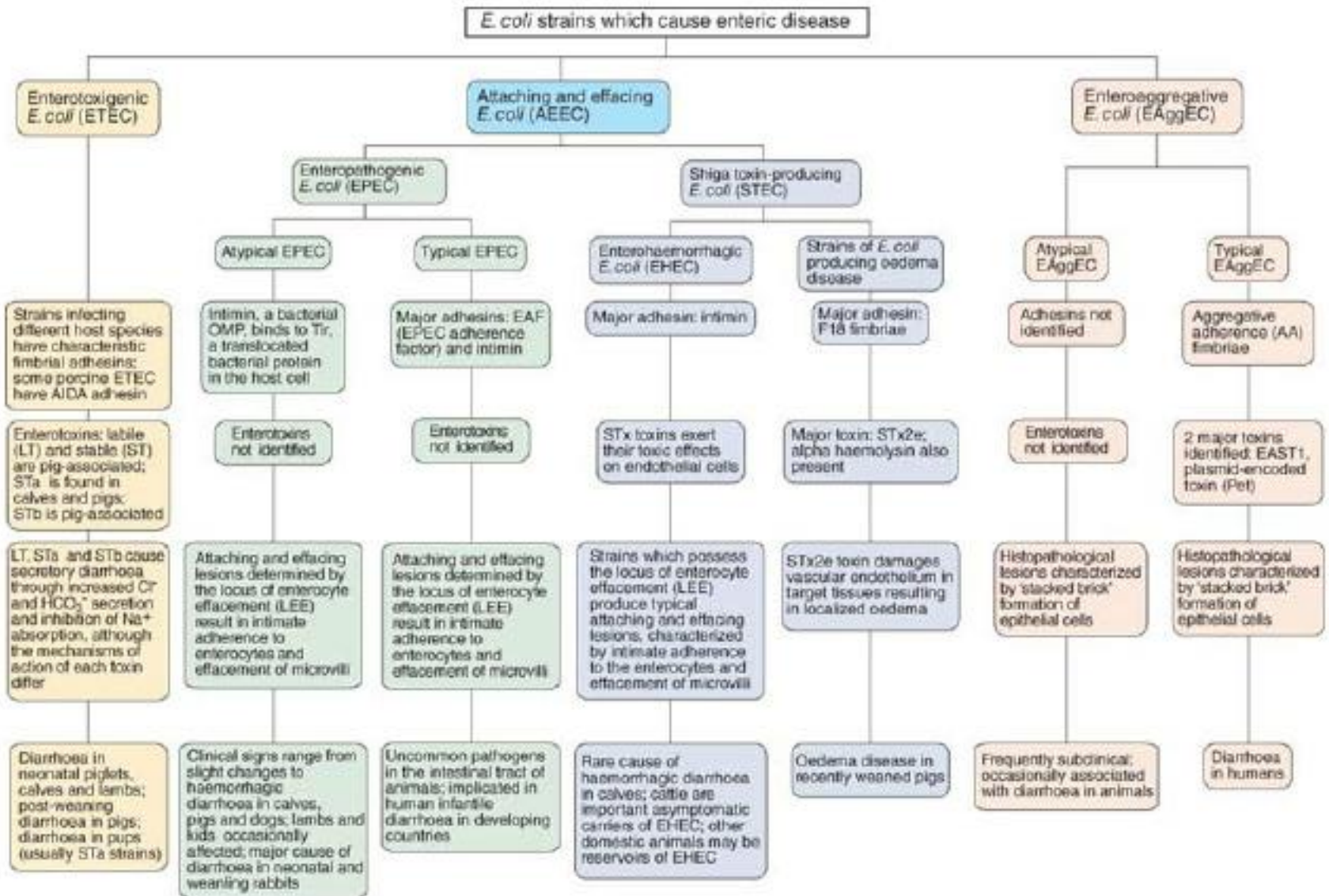
- Capsule: Capsular polysaccharides, which are produced by some *E. coli* strains, interfere with the phagocytic uptake of these organisms.
- Endotoxin, a lipopolysaccharide (LPS) component of the cell wall of Gram-negative organisms is released on death of the bacteria.
- It is composed of a lipid A moiety, core polysaccharide and specific side chains.
- The role of LPS in disease production includes pyrogenic activity, endothelial damage leading to disseminated intravascular coagulation, and endotoxic shock.
- These effects are of greatest significance in septicaemic disease.
- Fimbrial adhesins which are present on many strains of *E. coli* allow attachment to mucosal surfaces in the small intestine and in the lower urinary tract.



# VIRULOTYPES/PATHOTYPES OF *E. COLI*

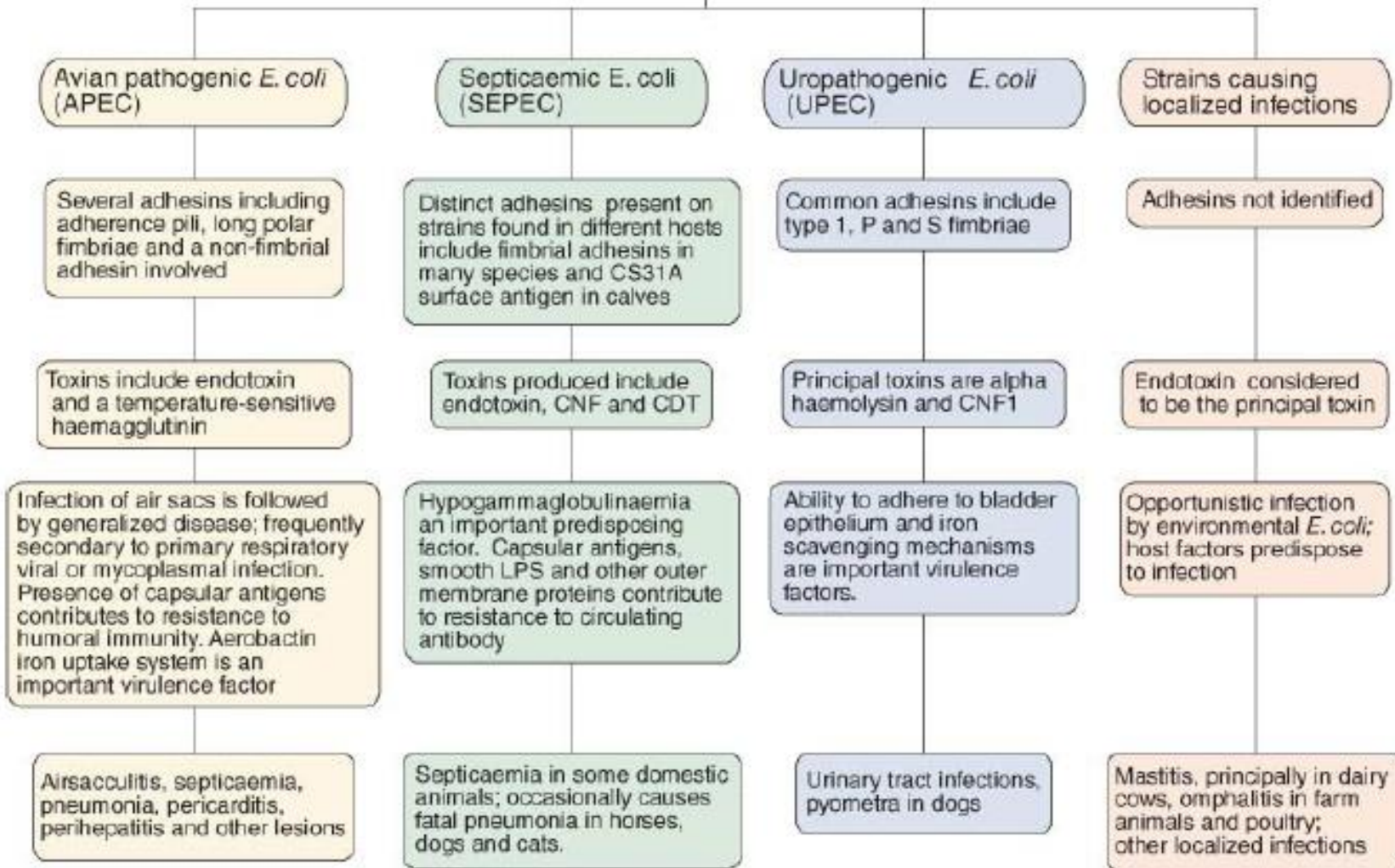
- Based on virulence *E. coli* can be classified as
  - Enteropathogenic *E. coli*
  - Enterotoxigenic *E. coli*
  - Enterohaemorrhagic *E. coli*
  - Enteroinvasive *E. coli*
  - Entero aggregative *E. coli*
  - Attaching and effacing *E. coli*
  - CTNF- PEC –cytotoxin necrotising factor positive *E. coli*







## *E. coli* strains associated with extraintestinal disease



## DISEASES

- In horse: joint ill, naval ill (i.e. infection affecting umbilical cord) and the condition called **sleepy foal disease**
- In cattle: colisepticaemia, colibacillosis and mastitis
- In calf: **calf scour or white scour**. The organism also produce joint ill or naval ill
- In pig, esp the unweaned pigs: piglet scour
- In weaned piglets: **oedema disease**
- In adult pigs: MMA: **Mastitis metritis agalactia syndrome**



- In sheep and goat: mastitis in adult animals and colibacillosis and colisepticaemia in young
- In poultry: colibacillosis, colisepticaemia, and coligranuloma (**hjarre's disease**) characterised by granulomatous lesions in visceral organs
- In chicks causes omphalitis; inflammation of yolk sac also known as **mushy chick disease**
- in dogs causes septicaemia, and mortality
- the septicaemic condition known as **fading puppy disease** which causes severe mortality
- In laboratory animals mucoid enteritis
- In human being traveller's diarrhoea and meningitis



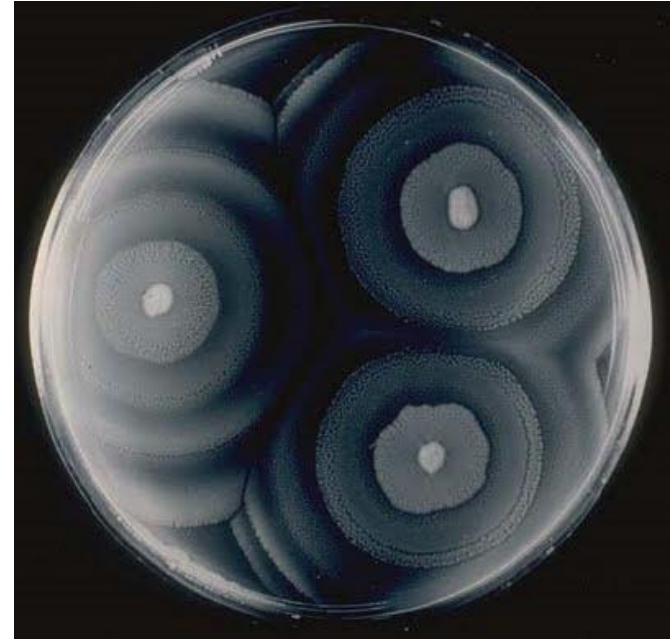
# DIAGNOSIS AND CONTROL

- Isolation and identification of organism
- Treatment :Various antibiotics
- Proper hygienic conditions



# GENUS: PROTEUS

- Type species: *Proteus vulgaris*
- Produce swarming type of motility
- Culture has fishy smell
- A non lactose fermenting organism
- Imvic test -+ -+ based on these the organism simulate Salmonella ( in cultural and biochemical characters)
- To differentiate Proteus from salmonella urease test is used, urease is negative for salmonella and positive for proteus
- Another test is phenyl alanine deaminase test. Proteus is positive for this test( only organism positive for this test in this family) and Salmonella negative for this test



# GENUS: KLEBSIELLA

- *Klebsiella pneumoniae*: Friedlander's bacillus
- Lactose fermenting organism
- Imvic test --++
- Positive for urease test





# GENUS: ENTEROBACTER

- Type sp. *Enterobacter cloaca*
- Imvic test --++ ( similar to Klebsiella)
- but Klebsiella is capsulated non motile organism

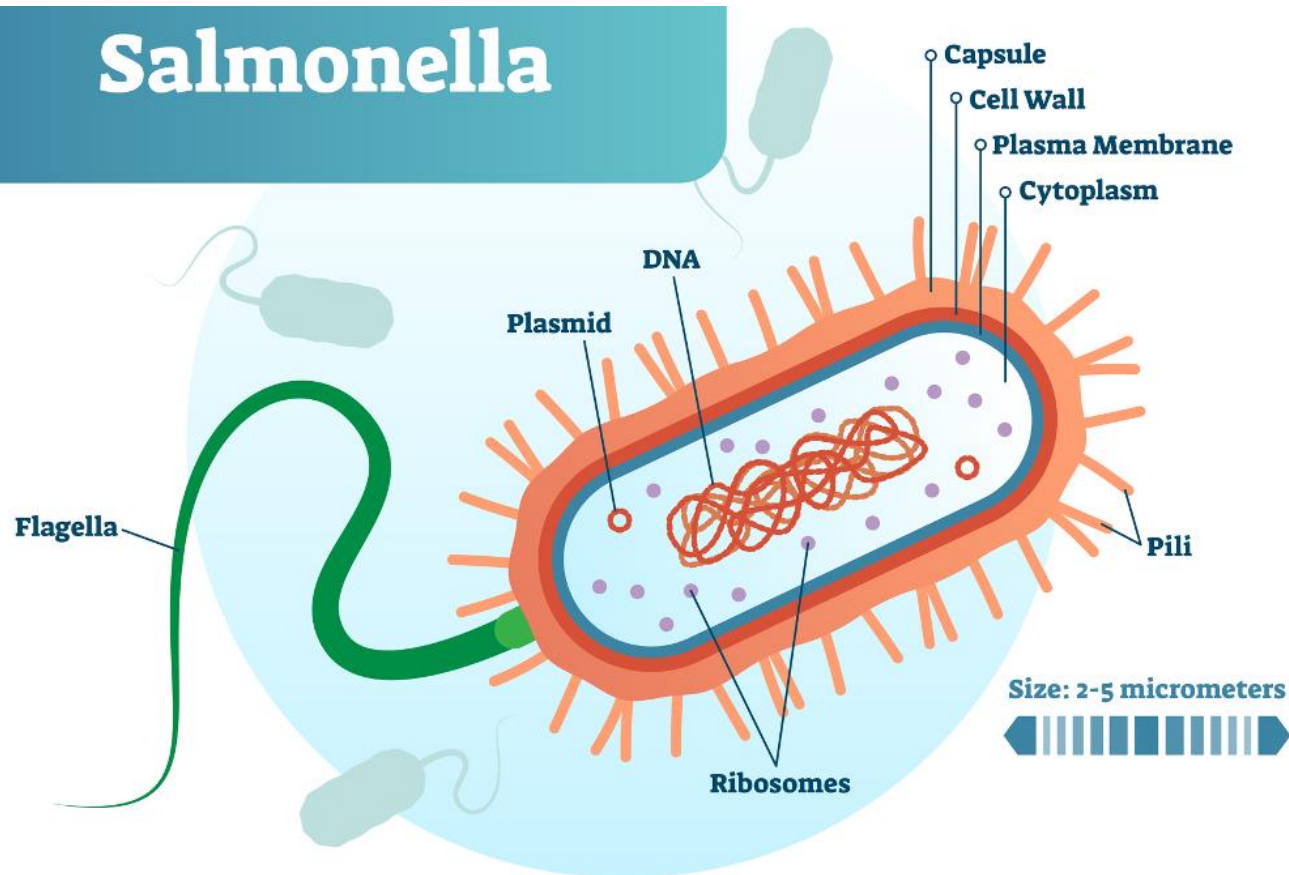
Enterobacter is motile

- *E.coli*, Klebsiella and Enterobacter are lactose fermenting organism together known as coliforms



# GENUS:SALMONELLA

- Named after E. Salmon who worked on human typhoid



## CONTD..

- According to latest classification there are only two species of salmonella
- *Salmonella bongori*
- *Salmonella enterica*
- *Salmonella enterica* is most important and has 6 sub species of these most important is *Salmonella enterica subspecies enterica*
- All other organism other than these two are serovars.
- About more than 2500 serovars are present



## CONTD..

- The organism of this genus is highly host specific
- *Salmonella* Dublin: cattle
- *Salmonella* Pullorum, *Salmonella* Gallinarum: poultry
- *Salmonella* Choleraesuis: for pig
- *Salmonella* Abortusovis : goat and ram
- *Salmonella* Abortusequi : for horse
- *Salmonella* Typhi and *Salmonella* Paratyphi: human
- *Salmonella* Enteritidis and *Salmonella* Typhimurium are non host specific affect variety of animals and human being



# MORPHOLOGY

- Gram negative bacilli, non capsulated, non spore forming
- All are motile except *Salmonella Pullorum* and *Salmonella Gallinarum*
- Most of them possess type I fimbriae which is mannose sensitive
- Organism also have mannose resistant fimbriae







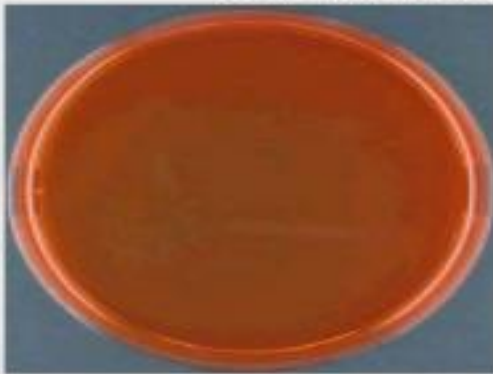
# CULTURAL CHARACTERISTICS

- MaConkey agar : colourless colony
- BGA: Pink colony
- XLD agar: Red colonies with black centre because of H<sub>2</sub>S production
- Other media are Salmonella Shigella agar ( SS agar) DCA (Deoxycholate Citrate agar and Bismuth Sulphite agar
- In broth it will produce uniform turbidity

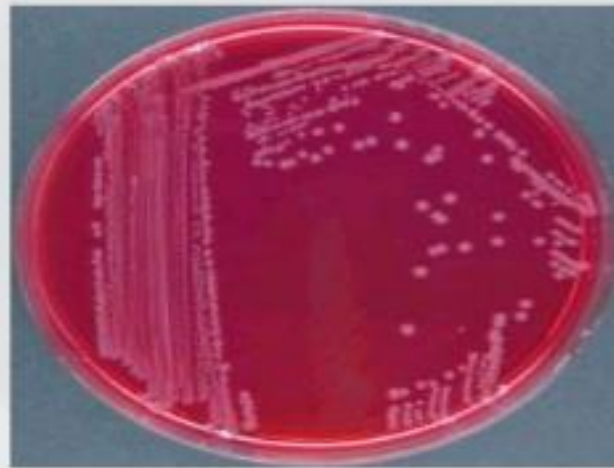


# SALMONELLA ON BGA

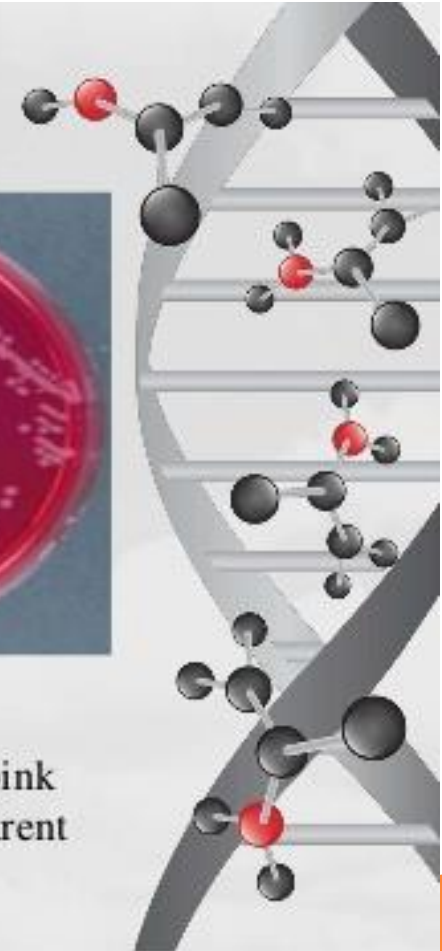
## Salmonella on BGA



an uninoculated plate



Salmonella on BGA . The colonies are small, opaque, pink or white. Can also be transparent and colorless.



# XLD AGAR

XLD agar:  
Red  
colonies  
with black  
centre  
because of  
H<sub>2</sub>S  
production



# BIOCHEMICAL CHARACTERISTICS

- Organism positive for catalase and negative for oxidase
- Nitrate reduction positive
- Can not ferment lactose
- *Salmonella* Gallinarum is differentiated from *Salmonella* Pullorum by ability to conduct maltose and dlacitol fermetation
- Imvic test is -+ -+
- On TSI acid but, alkaline slant and organism produce H<sub>2</sub>S.
- The organism is negative for urease test



# KAUFFMAN WHITE SCHEME OF SEROTYPING OF SALMONELLA

- Antigens two types : somatic and flagellar antigen
- Somatic antigen designated by arabic numerals from 1 to 67. single organism may possess more than one somatic antigen
- Organism possessing some somatic antigen are included in the same somatic group
- For example *Salmonella* Pullorum important somatic antigen are 9 and 12. for *Salmonella* Gallinarum somatic antigen are 1,9,12. so both in same somatic serogroup



- Then classification is based on flagellar antigen
- Two flagellar antigen as phase I and phase II antigen  
phase I flagellar antigen designated by a to z ( small letter)
- if number is more than 26 then designated as z1, z2, z3 etc.
- Phase II flagellar antigens designated by arabic numerals. If an organism possess both phase I and phase II antigen , they are known as biphasic of diphasic organism.
- Apart form this antigen *Salmonella* Typhi possess Vi antigen ( V indicate virulence) Vi seen only in newly isolated strain and may be los as a result of subculturing
- Vi antigen may be capsular antigen





- Under Kauffmann white scheme antigenic notation for *Salmonella Pullorum* is 9,12:-----:-----
- Antigenic notation has three parts, first part represents somatic antigen, 2<sup>nd</sup> part phase I flagellar antigen and third part represents phase II flagellar antigen
- Each part is separated by a colon: each component in same part separated by comma
- for *Salmonella Paratyphi* the antigenic notation is 1,4,[5],12:b:12
- underline for '1' indicates this particular antigen is having phage mediated transmission
- [ ] indicates this antigen seen only in particular strain



## PATHOGENICITY

- In human being *S. yphi* : Typhoid
- Most common diagnostic serological test for typhoid is Widal test
- *S. Paratyphi*: paratyphoid in human being
- *Salmonella* Enteritidis and *Salmonella* Typhimurium causes food poisoning in human being
- *Salmonella* Abortusovis : abortion in sheep
- *Salmonella* Abortusequi: abortion in equine
- *Salmonella* Choleraesuis: secondary invaders of hog cholerae or swine fever
- *Salmonella* Dublin: Enteritis, septicaemia and meningitis in calf and abortion in adult animal



# PULLORUM DISEASE

- Etiology: *Salmonella* Pullorum
- Bacillary white diarrhoea
- characterized by chalky white pasty feces in birds **below 2 weeks** of age
- Transmission by faeco oral route
- Egg transmission ( bacteria from feces enter through the holes of egg) and also transovarian transmission
- In adult birds it mainly affect ovary and adult birds act as carrier
- Most important lesion is pedunculate ovary



# PEDUNCULATED OVARY IN PULLORAM DISEASE



# DIAGNOSIS OF SALMONELLA PULLORUM INFECTION

- Tentative diagnosis based on history, symptoms and lesions
- **Confirmatory diagnosis**
- **Isolation and identification of organism** from faeces, liver and yolk. This material is first inoculated on enrichment medium as selenite broth, tetrathionate broth and incubate at 45°C for 18-24 hours. Then subculture on selective media such as McConkey agar, Salmonella Shigella agar, DCA and BGA
- **Whole blood agglutination test** : for flock diagnosis of salmonellosis in field condition. Take one drop of whole blood and 1 drop of crystal violet coloured antigen. In positive cases agglutination in 1-2 minutes



# FOWL TYPHOID

- Etiology: *Salmonella Gallinarum*
- Affect mainly adult birds only
- Transmission by feed and water
- There is egg transmission and transovarian transmission
- Two more organism affecting birds are *Salmonella Enteritidis* and *Salmonella Typhimurium* causing condition known as paratyphoid
- Reservoir of salmonellosis in birds is rodents



# DIAGNOSIS OF SALMONELLA GALLINARUM

- Tentative diagnosis ( history, only adult birds are affected)
- Lesions: brown coloured liver, splenomegaly and enteritis
- Isolation of organism from liver, intestine and bone marrow
- whole blood test and rapid agglutination
- Crystal violet antigen of *Salmonella pullorum* is used for this also because common 9, 12 antigen

Bronzed Greenish liver





# CONTROL

- Periodical conduct of whole blood test
- Slaughter of reactors
- burial of litter properly
- disinfect equipment with 1% phenol or 0.5% cresol
- fumigation of incubators
- Egg dipping
- Egg should not be used for hatching from affected birds
- New stock is introduced after slaughter only after 4-6 months
- feed storage area and poultry shed should be made rat proof





**THANK YOU!**