

**GRAM NEGATIVE, AEROBIC,
FACULTATIVE ANAEROBE,
MICROAEROPHILIC WITH
SIMPLE CULTURE
REQUIREMENTS**

ACCORDING TO RESPIRATORY ENZYME (OXIDASE ENZYME)



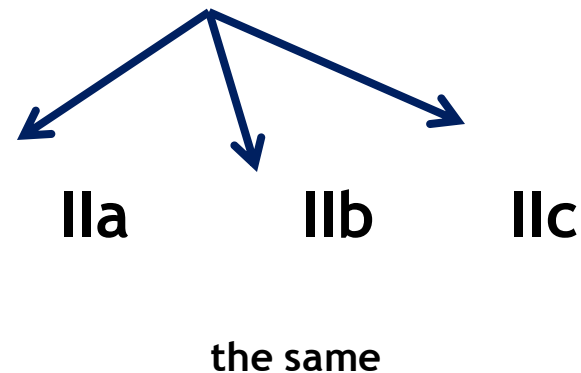
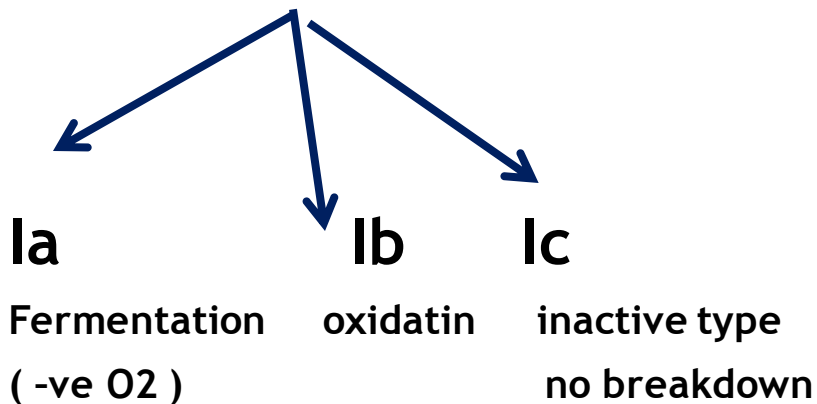
⊙ Reaction group I

oxidase +ve

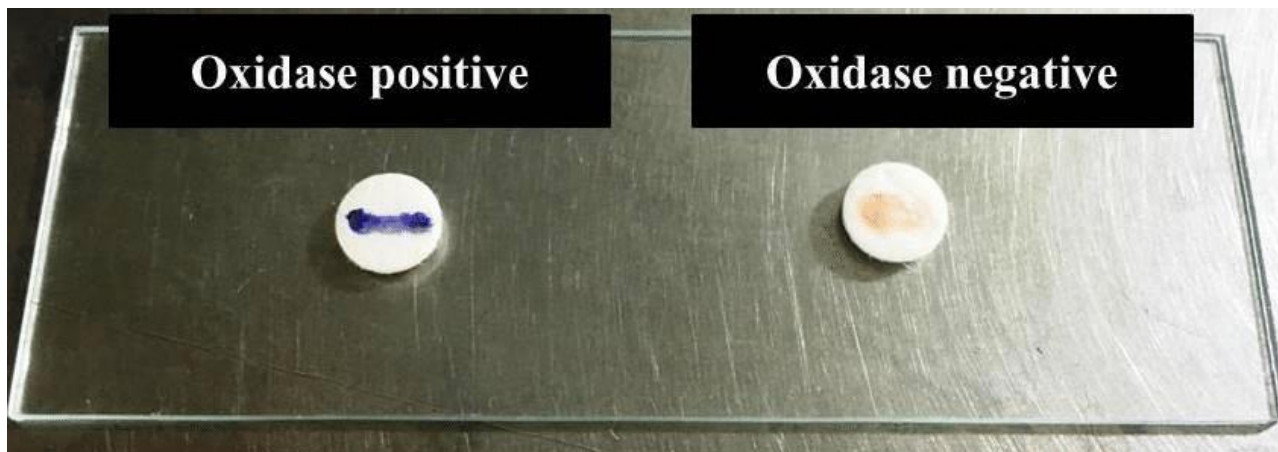
Reaction group II

oxidase -ve

according to: **glucose metabolism**
(OF test)



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

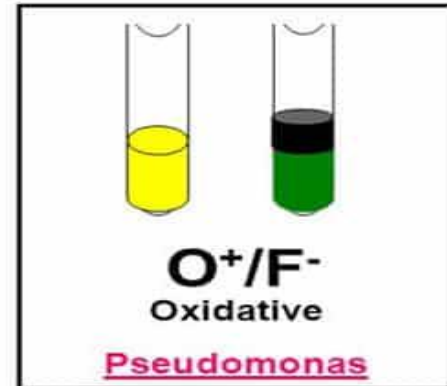
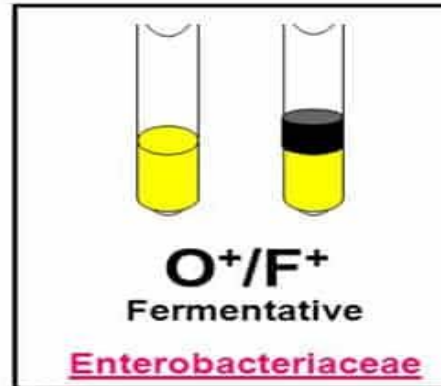
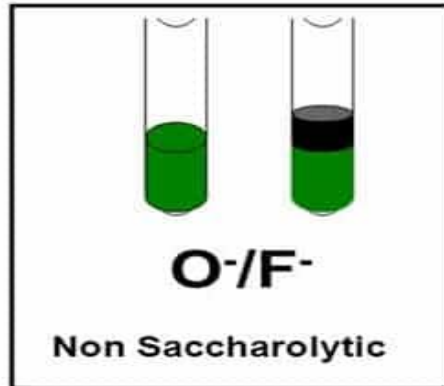
Media used:

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.

Results:**Positive Test:**

Microbiology Info.com



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

OXIDASE -VE , FACULTATIVE BACTERIA REACTION GP IIA

Family : Enterobacteriaceae

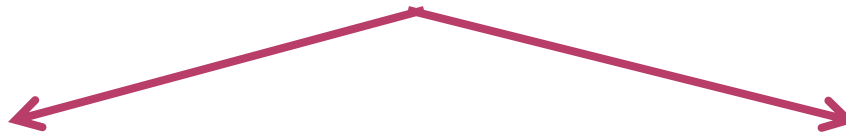
◉ Distribution

◉ 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)**

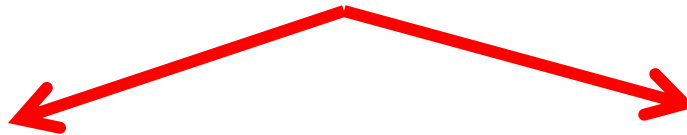
E. coli
Klebsiella
Enterobacter
Citrobacter

**non lactose
fermenters(NLF)**

Salmonella
Shigella
Proteus
Yersinia
Providencia

THE COLIFORMS

- ◉ Escherichia, Enterobacter, Klebsiella & Citrobacter
- ◉ Not all coliforms are associated with the intestinal tract



◉ Fecal coliforms

◉ **Escherichia**

non fecal coliforms

Klebsiella & enterobacter

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)

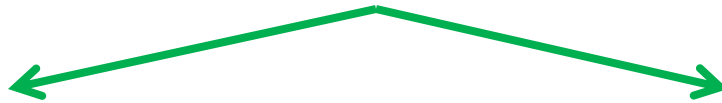
The test to determine whether coliform bacteria come from warm-blooded animals. By means of this test it can be readily established

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

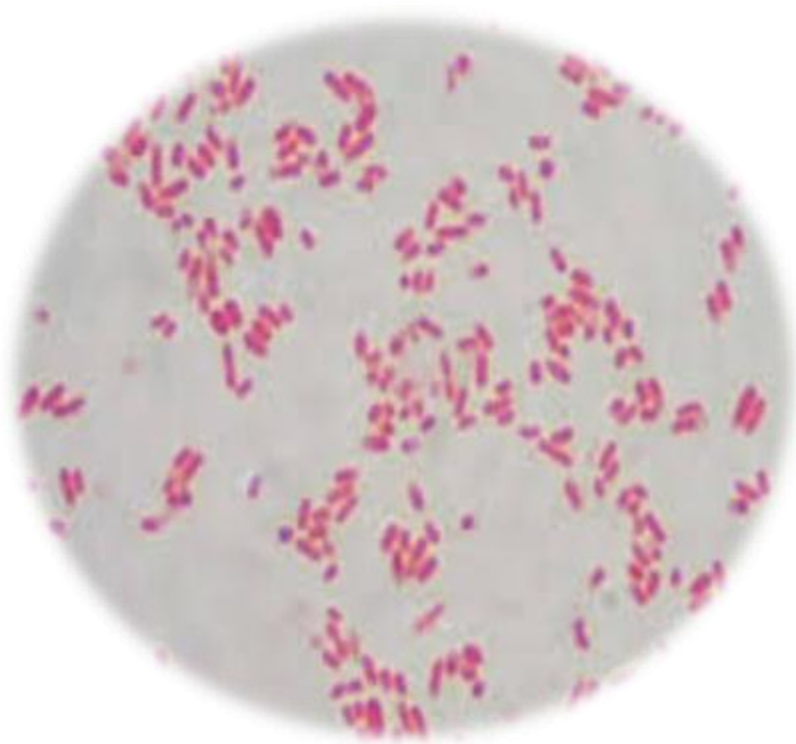
- mastitis cows & other species

- septicaemia & granulomatosis

in poultry

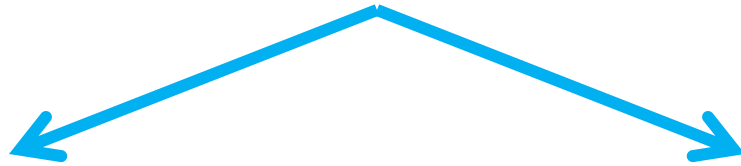
MORPHOLOGY

- ◉ 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



S- form

Round, small

Smooth outline

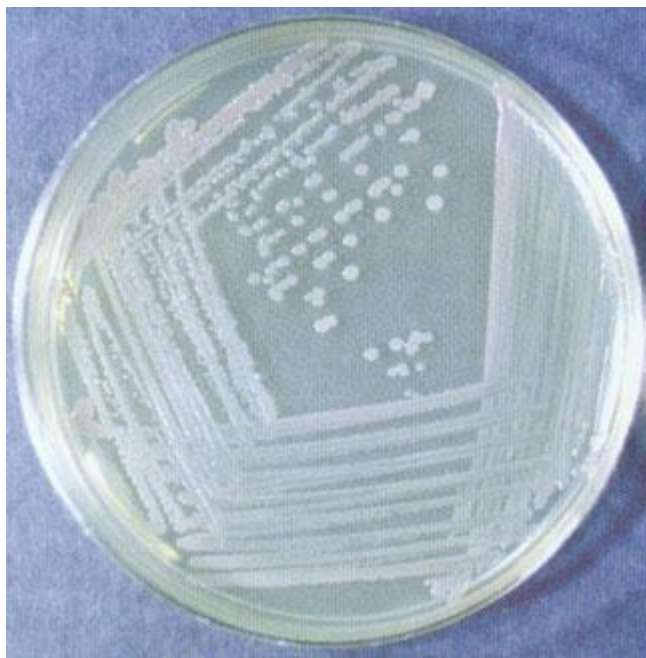
Greyish white, shiny

R- form

large ,dry

irregular outline

pathogenic or
heamolytic



Nutrient agar



Blood agar



MacConkey agar

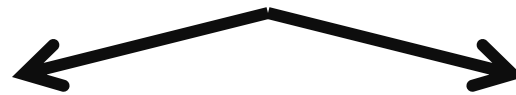


EMB

◉ In nutrient broth

◉ After 12 hours

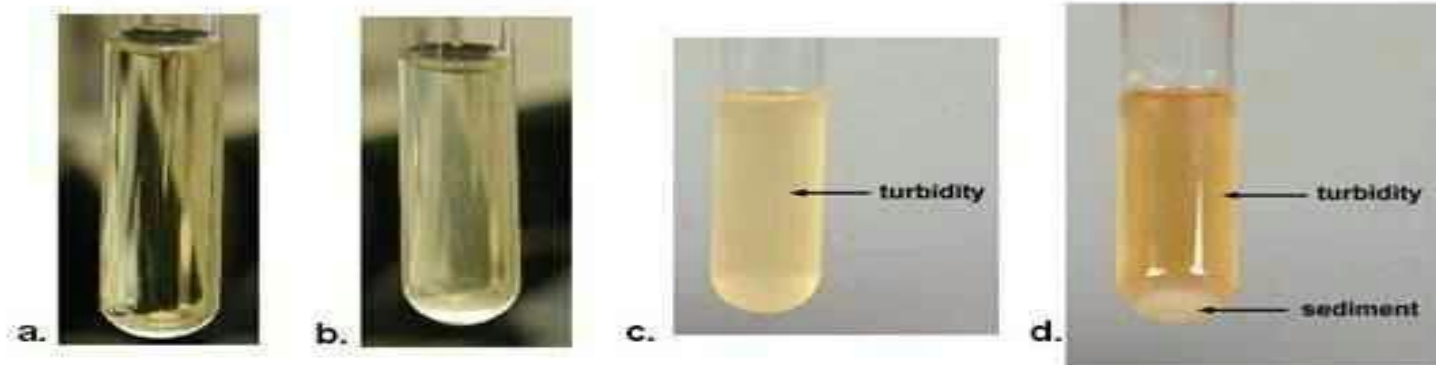
after 24 hours



regular turbidity


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)

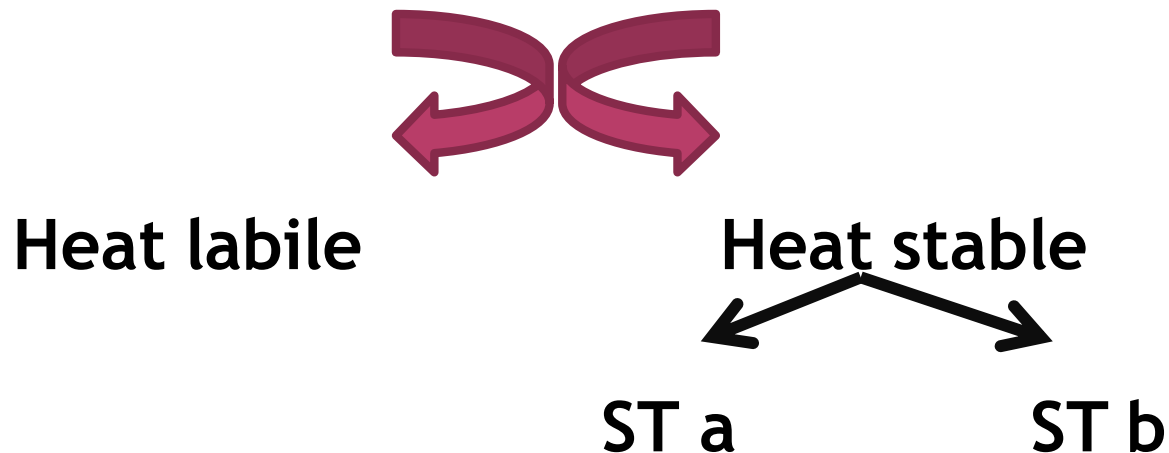
- **Somatic antigen** **O - antigen** >160
- Lipopolysaccharides, heat labile,
- Determined by tube, slide agglutination test
- **- Capsular antigen** **K - antigen** (91)
- Polysaccharides, ,

- **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:

- Entertoxic E.coli (ETEC)

Young animals



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

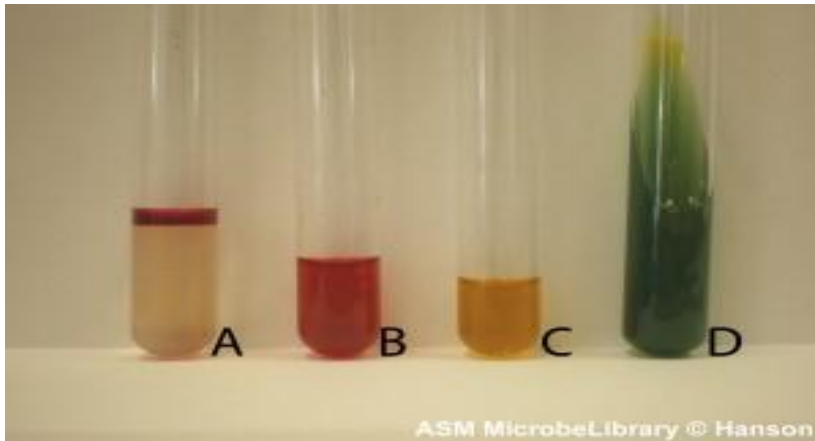
- ◉ Protein
- ◉ Bactericidal effect
- ◉ Differentiate of E.coli strains to :
 - ◉ { Colicin +ve E.coli
 - ◉ { Colicin -ve E.coli

BIOCHEMICAL REACTION

⊙ Lactose fermenter +ve

⊙ I M V C U H

+ + - - - -



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

specimens

Intestinal content, fecal samples, organs, milk samples

Isolation by culture

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

Biochemical identification

Motility	+ve	Indole	+ve	MR	+ve	VP	-ve	citrate	-ve
Urease	-ve	H ₂ S	-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	Indole	-ve	MR	-ve	VP	+ve	citrate	+ve
Urease	+ve	H ₂ S	-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 , diarrhoea
- ◉ In sheep & goats : diarrhoea
- ◉ Can be isolated from frogs,snaks & fishes

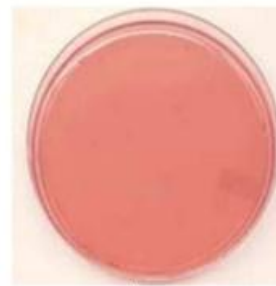
BIOCHEMICAL PROPERTIES

- ◉ Lactose fermenter + ve
- ◉ Indole - ve
- ◉ MR + ve
- ◉ VP - ve
- ◉ Citrate + ve
- ◉ Urease + ve
- ◉ H₂S + ve

NON LACTOSE FERMENTERS NLF

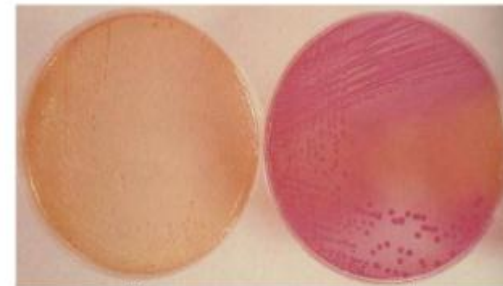
- ◉ Salmonella
- ◉ Shigella
- ◉ Proteus
- ◉ Pseudomonas

Growth of *Enterobacteriaceae* on MacConkey agar



↑
Uninoculated plate

Dr. Nabil El Aila
Diagnostic Microbiology



↓
Colorless colonies
Lactose non fermenters
Salmonella, Shigella, Proteus

↓
Pink colonies
Lactose fermenters
E. coli, Citrobacter, Klebsiella, Enterobacter

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 common inhabitant in reptiles
- ◉ some types are host specific

species	group	host	disease
S.Paratyphi A	A	Man	Paratyphoid A fever
S.Paratyphi B	B	Man	Paratyphoid B fever
S.Abortus equi		Equine	Equine abortion & infertility
S.Abortus ovis		Ovines	Ovine abortion
S.cholera suis	C	swine	Piglet typhus Infectious enteritis
S.typhi	D	man	Human typhoid fever
S.gallinarum		poultry	Pullorum disease

SALMONELLA

Genus : Salmonella

Species : S. typhi

S. typhimurium

S. enteriditis

S. gallinarum-pullorum

S. dublin

S. infantis

S. derby

S. agona

S. panama

S. heidelberg

MORPHOLOGY

- ◉ Cocco-bacilli
- ◉ Gram -ve
- ◉ Medium sized rods
- ◉ Actively motile except *S.gallinarum* and *pullorum*
- ◉ Many species developed fimbria

CULTURAL CHARACTERS

◉ In nutrient broth



Turbidity , without
Pellicle formation

nutrient agar

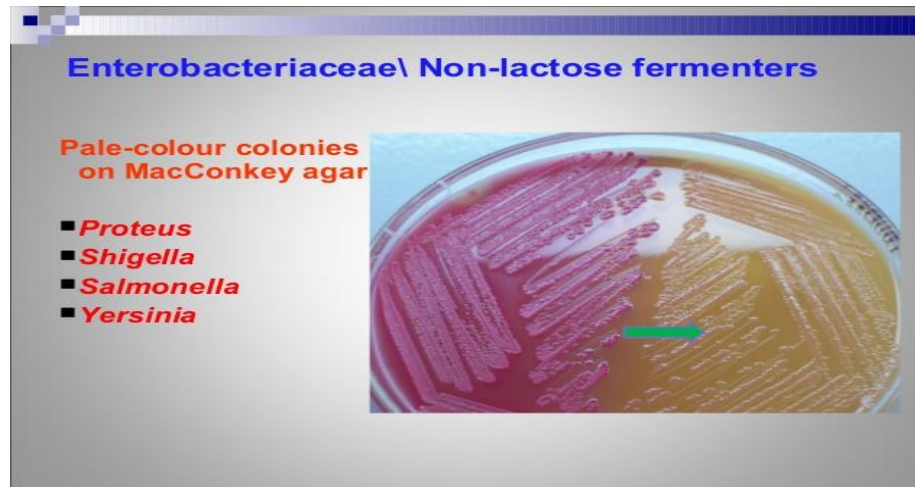


round colonies,
smooth, convex
with grayish colour

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 salmonellae 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

- Identified by alphabetical a,b,c,d

phase 2 H

- 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

S. paratyphi A

S. paratyphi B

group : A

B

O-antigen : 1,2,12

1,4,5,12

H-antigen

phase 1 : a

b

phase 2 : -

1,2

monophasic

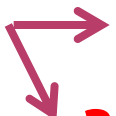
Diphasic

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 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