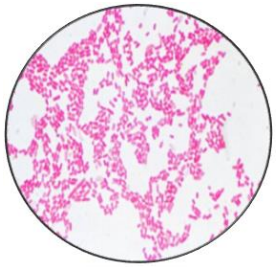
Enterobacteriaceae



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Department of Environmental Health

27.2.2025

Enterobacteriaceae

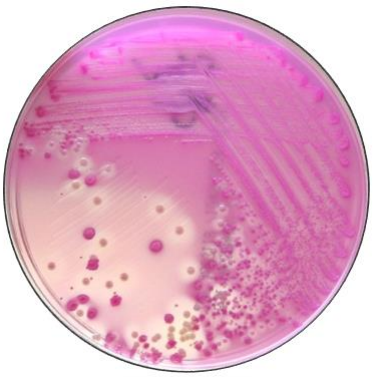
The most important species:

1- ***Escherichia coli***

2- ***Klebsiella* spp.**

3- ***Salmonella* spp.**

4- ***Proteus spp.***



Enterobacteriaceae

Enterobacteriaceae general characteristics:

1- Gram-negative non-spore forming bacteria.

2- Medium-sized(0.4-0.6×2-3µm).

3- The motile species have peritrichous arrangement of flagella.

4- Facultative anaerobic.

5- Catalase - positive and oxidase - negative.

6- Reduce nitrate to nitrite.

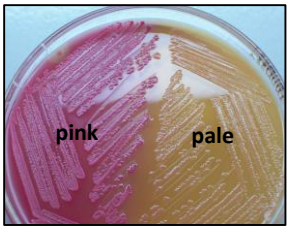
7- Able to grow on non-enriched media such as nutrient agar.

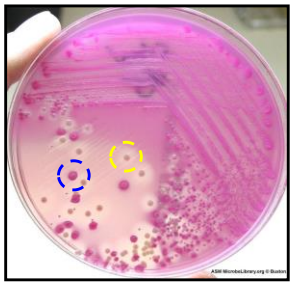
Enterobacteriaceae on MacConkey agar:

Lactose fermentation in MacConkey agar:

1- The colonies of lactose fermenters are **pink** due to acid production from lactose (***E. coli, Klebsiella***).

2- The colonies of non-lactose fermenters have **pale** and are alkaline due to utilization of peptones in the medium (***Salmonella, Shigella, Proteus***).





Enterobacteriaceae classification according to biochemical reaction tests (Table 1):

I	Indole production
M	Methyl red
V	Voges – Proskauer
C	Citrate utilization

IMVC

H	H_2S production
U	Urease
M	Motility

HUM

Table 1: Biochemical reactions for main members of the Enterobacteriaceae

Genus	I	M	V	C	H	U	M
E. coli	+	+	-	-	-	-	+
Klebsiella	-	-	+	+	-	+	-
Salmonella	-	+	-	+	+	-	+
Proteus	v	+	-	-	+	+	+

v = variable

Diagnosis of Enterobacteriaceae

Specimens for bacterial examination should be cultured aerobically on MacConkey agar at 37˚C for 24 hours.

General identification criteria for isolates include:

1- Gram-negative rods.

2- Catalase-positive, oxidase-negative.

3- Growth and appearance on MacConkey agar (lactose fermenter or non-lactose fermenter).

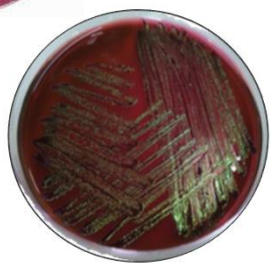
4- Biochemical profile including IMVC and HUM)for definitive identification.

Diseases produced by Enterobacteriaceae

ORGANISM	DISEASES
Lactose Positive	
Escherichia coli	Diarrhea, sepsis, urinary tract infection, neonatal meningitis
Klebsiella, Serratia, Citrobacter, Enterobacter species	Opportunistic infections (e.g., pneumonia, sepsis, neonatal meningitis)
Lactose Negative	
Salmonella species	Diarrhea, typhoid fever, bacteremia; localized infections in bone, meninges, liver
Shigella species	Dysentery
Proteus species	Urinary tract infection
Yersinia species	Plague, diarrhea, mesenteric lymphadenitis

1- *Escherichia coli*

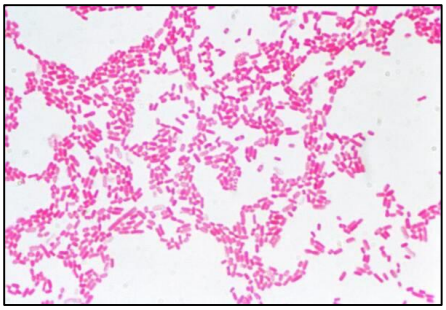
- Morphology and Staining
- Cultural Characteristics
- Biochemical Tests
- Diagnosis



Morphology and Staining

Escherichia coli

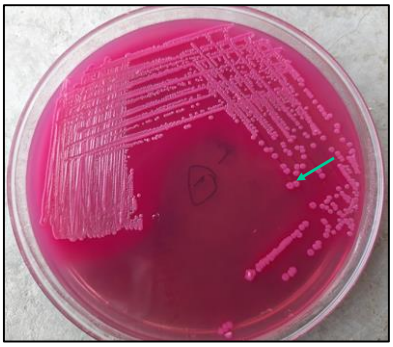
Theodor Escherich, 1885.



Gram-negative, medium-sized rod (3-1 × 0.6 µm).

Cultural Characteristics

Escherichia coli on MacConkey agar **ferment lactose** and produce **pink** colonies.



E. coli on Eosin Methylene Blue agar (EMB) produce the characteristic phenomena greenish color called metallic sheen.

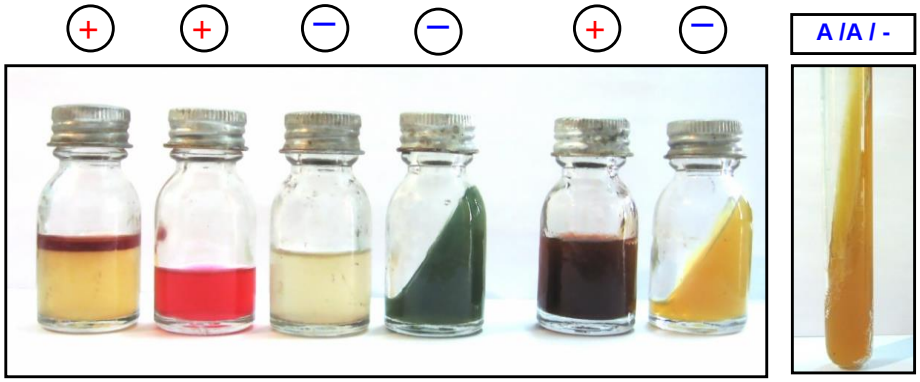
Metallic sheen

Biochemical tests

Species	Indole production	Methyl red	Voges – Proskauer	Citrate	Hydrogen sulphide	Urease	Motility (36 °C)	Gelatin liquefaction	Lactose	Maltose	Mannitol	Sucrose
E. coli	+	+	-	-	-	-	(+)	-	+	+	+	d

+ = 90 – 100% strain positive
(+) = 76 – 89% Positive
- = 0 – 10% Positive
d = 26 – 75% Positive

Escherichia coli



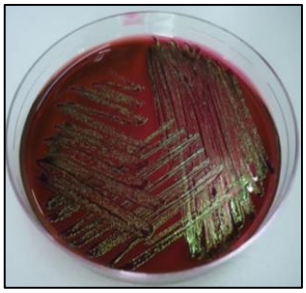
I M V C N U H

I = Indole, **M** = Methyl red reaction, **V** =Voges – Proskauer reaction, **C** = Citrate utilization, **N** = Nitrate reduction, **U** = Urease, **H** = Hydrogen sulfide production (H_2S) test, **A** = Acid,

Diagnosis

Identification criteria for *E. coli.*

- On MacConkey agar, colonies are bright pink.
- Using IMVIC tests for confirmation (Table.1).
- On EMB agar, the colonies of *E. coli* have a metallic sheen.



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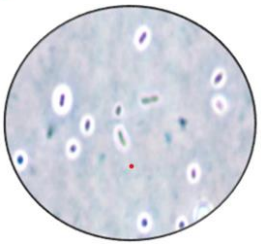
2- *Klebsiella* spp.

- Morphology and Staining
- Cultural Characteristics
- Biochemical Tests
- Diagnosis



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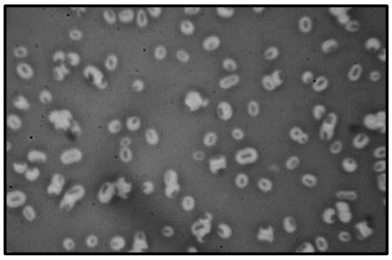
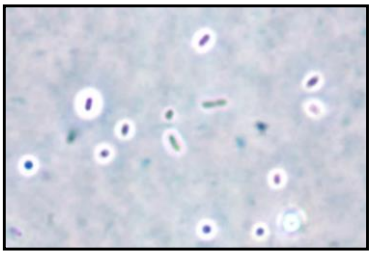
Morphology and Staining

Klebsiella pneumoniae

1-Gram -negative, medium-sized rods 2-1 × 0.5-0.8 µm.

2-Non-motile (because of capsule), non-spore forming bacteria.

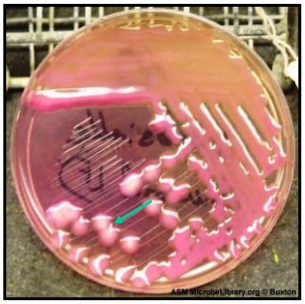


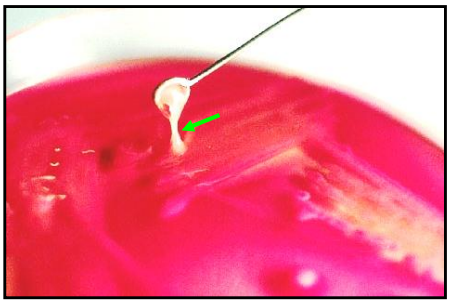


Capsule stain

Cultural Characteristics

Klebsiella pneumoniae on MacConkey agar is a lactose-fermenter with characteristic large mucoid pink colonies.



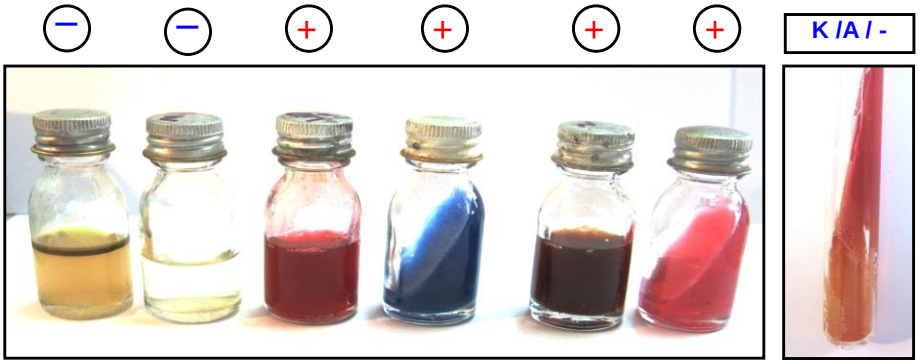


Biochemical tests

Species	Indole production	Methyl red	Voges – Proskauer	Citrate	Hydrogen sulphide	Urease	Motility (36 °C)	Gelatin liquefaction	Lactose	Maltose	Mannitol	Sucrose
K. pneumoniae	-	(-)	+	+	-	+	-	-	+	+	+	+

+ = 90 – 100 % strain positive
(-) = 11 –25 % Positive
- = 0 – 10 % Positive

Klebsiella pneumoniae

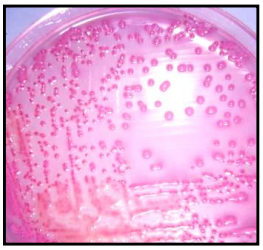


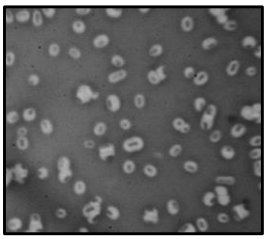
I = Indole, **M** = Methyl red reaction, **V** =Voges – Proskauer reaction, **C** = Citrate utilization, **N** = Nitrate reduction, **U** = Urease, **H** = Hydrogen sulfide production (H_2S) test, **K**= Alkaline, **A** = Acid,

Diagnosis

Identification criteria for *Klebsiella pneumoniae*.

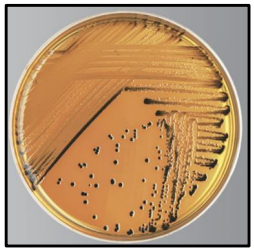
- On MacConkey agar, colonies are mucoid pink.
- Using IMVIC tests for confirmation (urease +ve) (Table.1)
- Using capsule stain (negative stain), Klebsiella pneumoniae have clear capsule.





3- *Salmonella* spp.

- Species
- Morphology and Staining
- Cultural Characteristics
- Biochemical Tests
- Diagnosis



Species

All Salmonella species are divided into 2 main species only:

1. ***Salmonella enterica***

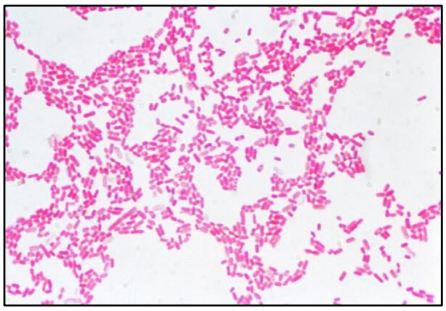
- *Salmonella entericaa subsp. enterica ser. Typhi* (***Salmonella typhi***)
- *Salmonella entericaa subsp. enterica ser. Typhimurium* (***Salmonella typhimurium***)

2. ***Salmonella bongori***

Morphology and Staining

***Salmonella* spp.**

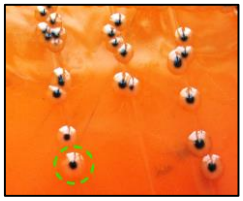
Daniel Elmer Salmon, an American veterinary surgeon, 1900.



Gram-negative, medium-sized rod (3-1 × 0.6 µm).

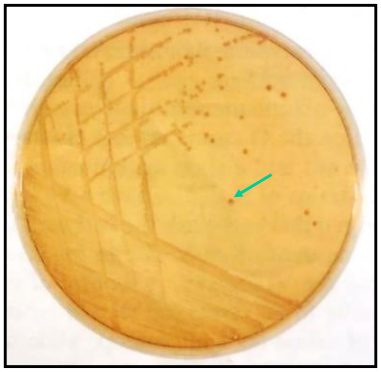
Cultural Characteristics

1. On MacConkey agar *Salmonella* spp. produce pail non-lactose fermenter colonies.

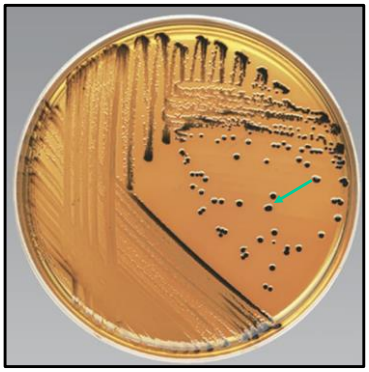


2. On Salmonella Shigella agar is a *Salmonella* spp. produce characteristic black center colonies.

3. Tetrathionate broth and selenite broth enrichment broth for salmonella growth.



MacConkey Agar
Pale colonies



Salmonella Shigella Agar
Black center colonies

Biochemical tests

Species	Indole production	Methyl red	Voges – Proskauer	Citrate	Hydrogen sulphide	Urease	Motility (36 °C)	Gelatin liquefaction	Lactose	Maltose	Mannitol	Sucrose
Salmonella	-	+	-	+	+	-	+	-	-	+	+	-

+ = 90 – 100 % strain positive - = 0 – 10 % Positive

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Salmonella

− + − + + − K /A / H_2S

I M V C N U H

I = Indole, **M** = Methyl red reaction, **V** =Voges – Proskauer reaction, **C** = Citrate utilization, **N** = Nitrate reduction, **U** = Urease, **H** = Hydrogen sulfide production (H_2S) test, **K**= Alkaline, **A** = Acid,

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Diagnosis

- **Identification criteria for *Salmonella*.**
- Specimens should be cultured in tetrathionae broth for preliminary enrichment and subculture in selective plating media such as (Salmonella-Shigella agar, Brilliant green agar).
- On MacConkey agar, colonies are pale.
- On Salmonella Shigella agar produce black center colonies.
- Using IMVIC tests for confirmation especially, (TSI +ve) (Table.1).

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4- *Proteus* spp.

- Morphology and Staining
- Cultural Characteristics
- Biochemical Tests
- Diagnosis

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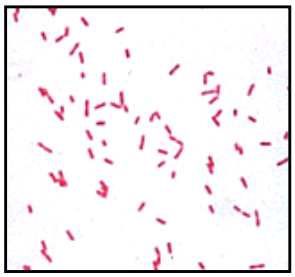
Morphology and Staining

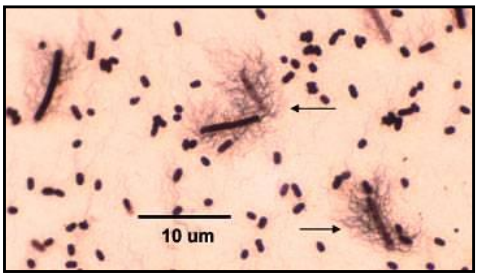
Proteus mirabilis

Proteus vulgaris

1-Gram - negative, small rods 1-3 × 0.5 µm

2-Actively motile peritrichous flagella, non capsulate.



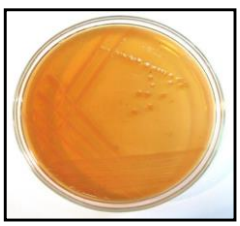


Cultural Characteristics

1- On MacConkey agar, *Proteus* do not ferment lactose and form pale colonies.

2- On blood agar, *Proteus* spp. produce swarming phenomena due to vigorous motility, it can be inhibited by increasing the concentration of agar in the medium.

3- On brain heart infusion agar, *Proteus* spp. growth (swarming phenomenon).



MacConkey agar

Blood agar

BHI agar

Swarming phenomena for *Proteus*

Blood agar

BHI agar

Biochemical tests

Species	Indole production	Methyl red	Voges - proskauer	Citrate	Hydrogen sulphide	Urease	Motility (36 °C)	Gelatin liquefaction	Lactose	Maltose	Mannitol	Sucrose
Pr. mirabilis	-	+	(-)	d	+	+	+	+	-	-	-	(-)
Pr. vulgaris	+	+	-	(-)	+	+	+	+	-	+	-	+

+ = 90 – 100% strain positive
(-) = 11 –25 % Positive
- = 0 – 10% Positive
d = 26 – 75% Positive

Proteus

I M V C N U H

I = Indole, **M** = Methyl red reaction, **V** =Voges – Proskauer reaction, **C** = Citrate utilization, **N** = Nitrate reduction, **U** = Urease, **H** = Hydrogen sulfide production (H_2S) test, **K**= Alkaline, **A** = Acid,

Diagnosis

Identification criteria for *Proteus* spp.

- On MacConkey agar, colonies are pale.
- Using IMVIC tests for confirmation (Table.1). (urease +ve)
- On BHI agar and blood agar, *Proteus* produce swarming phenomena.


Thanks