IDENTIFICATION OF PATHOGENIC BACTERIA IN CLINICAL MICROBIOLOGY LABORATORY

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Identification of Gram Positive Cocci(I)

Staphylococcus species

Gram stain – Gram positive cocci in clusters

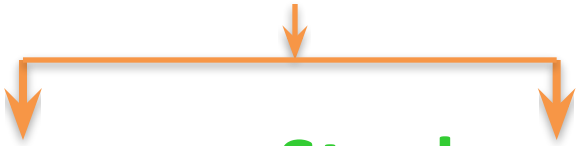
Motility – Non-motile

Catalase – Positive

Oxidase – Negative

So it is **Staphylococcus**

Coagulase Test



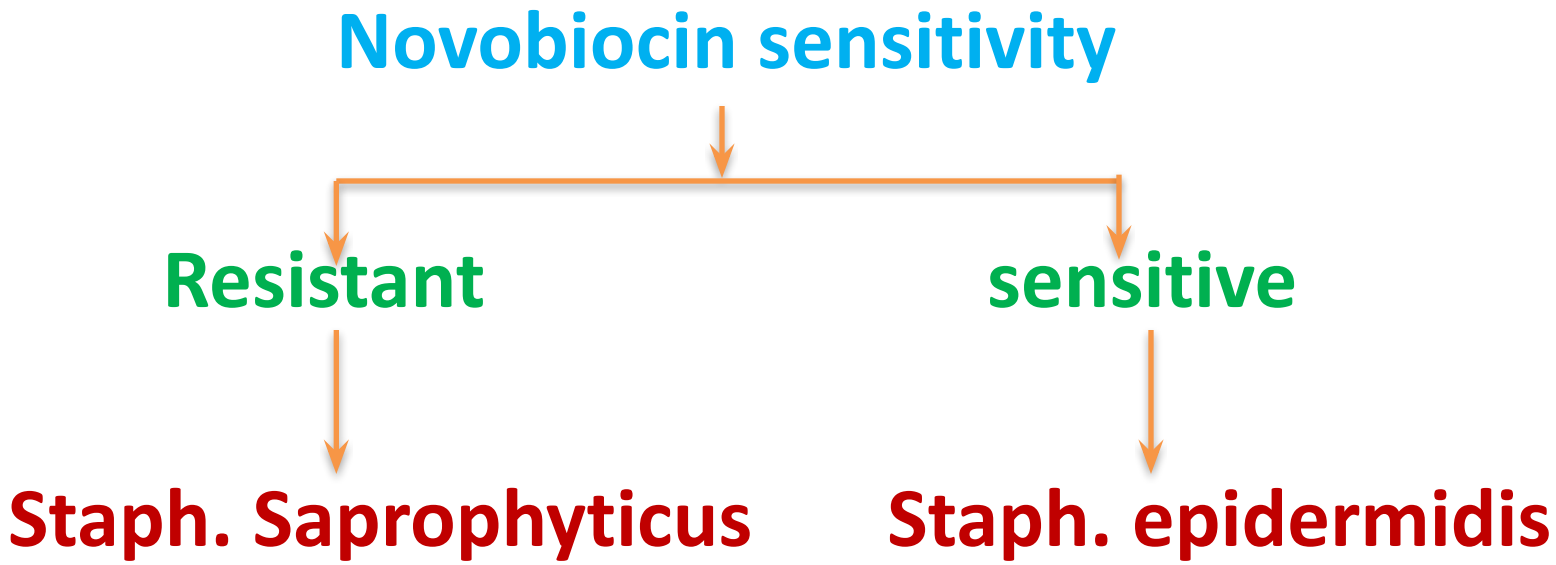
Staph. aureus

Staph. epidermidis

Staph. saprophyticus

Identification of Gram Positive Cocci(I)

Staphylococcus species – cont.,



Identification of Gram Positive Cocci(I)

Staphylococcus species- cont.,

- Differentiation between Staphylococci and Micrococci also may be important because both may be similar in microscopic morphology.

TEST	MICROCOCCI	STAPHYLOCOCCI
OF TEST	OXIDATIVE	FERMENTATIVE
MODIFIED OXIDASE TEST	POSITIVE	NEGATIVE
BACITRACIN SUSCEPTIBILITY	SENSITIVE	RESISTANT
FURAZOLIDONE SUSCEPTIBILITY	RESISTANT	SENSITIVE

(contd..)

Note:

Other characteristic features to identify *Staphylococcus aureus*

- **Mannitol fermentation – Positive**
- **DNAase test – Positive**
- **Beta-hemolytic on blood agar**
- **Golden yellow pigment**

Mannitol Salt Agar (Selective medium) –

Staph. aureus produce yellow color colonies due to mannitol fermentation.

IDENTIFICATION OF GRAM POSITIVE COCCI (II)
STREPTOCOCCUS

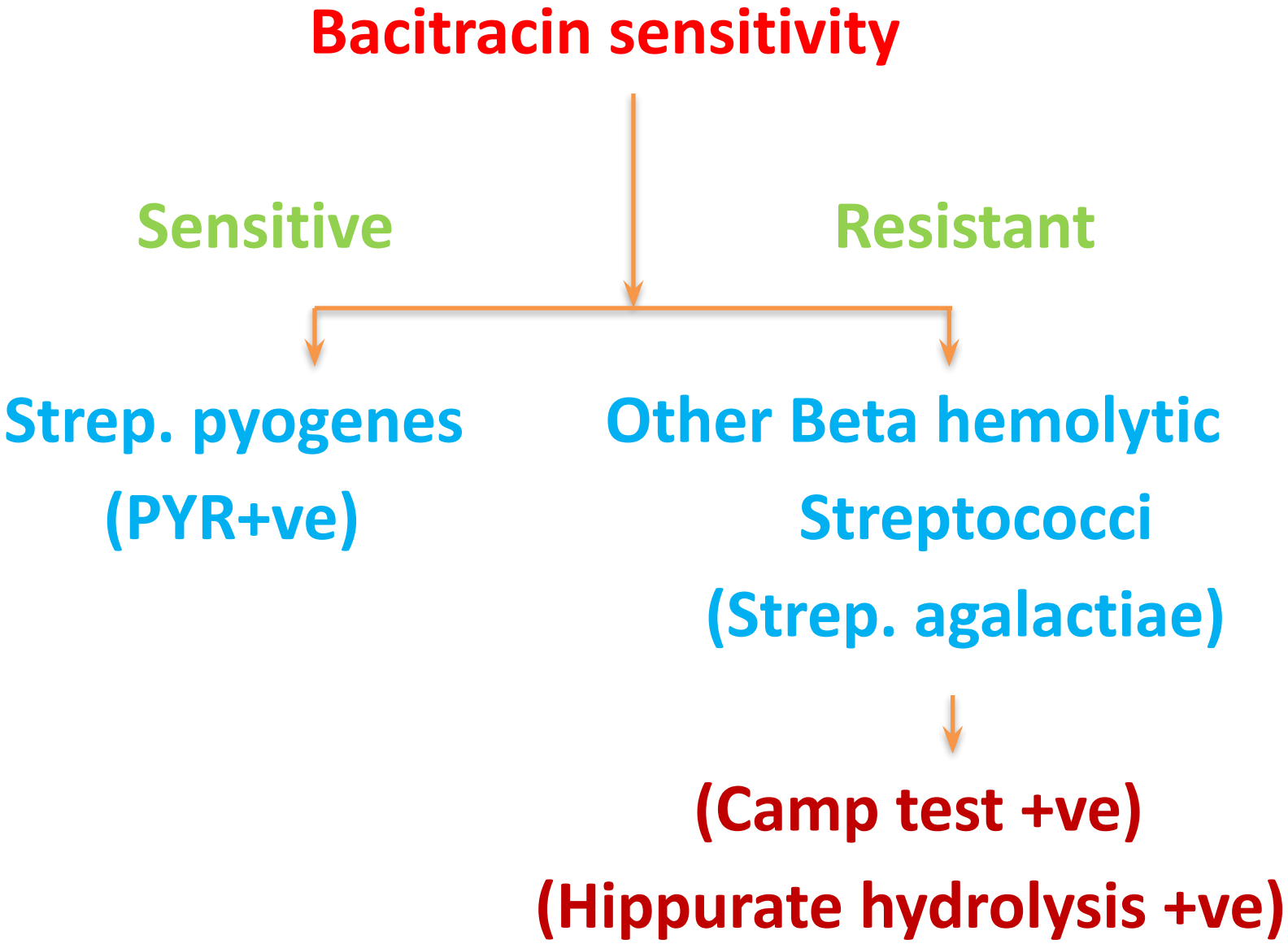
- **Gram stain – Gram positive cocci in chains**
- **Motility – Non-motile**
- **Catalase – Negative**
- **Oxidase – Negative**

So it is **Streptococcus**

Pin-point colonies with wide zone of Beta hemolysis on blood agar (constant property)

May be Beta-hemolytic streptococci like

- **Streptococcus pyogenes (Group A streptococci)**
- **Streptococcus agalactiae (Group B streptococci)**


Bacitracin sensitivity
Sensitive
Resistant
Strep. pyogenes
(PYR+ve)
Other Beta hemolytic
Streptococci
(Strep. agalactiae)
(Camp test +ve)
(Hippurate hydrolysis +ve)

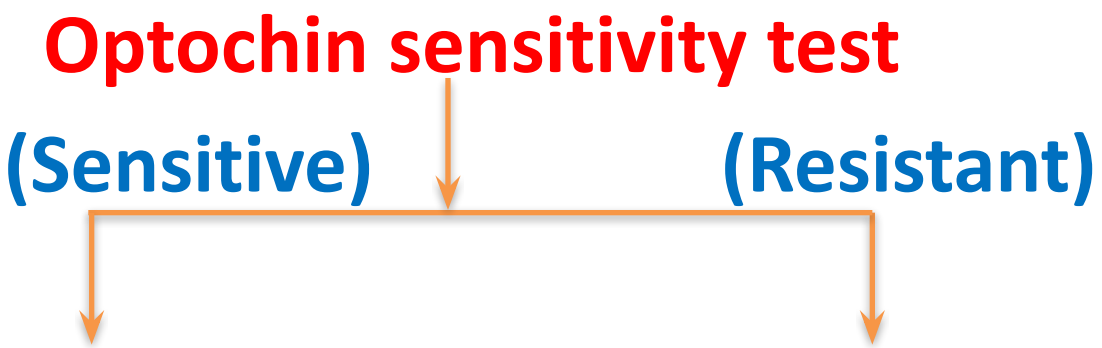
IDENTIFICATION OF GRAM POSITIVE COCCI (III)
PNEUMOCOCCUS

- **Gram stain** – **Gram positive cocci in pairs (lanceolate shape)**
- **Motility** – **Non-motile**
- **Catalase** – **Negative**
- **Oxidase** – **Negative**

On blood agar – ALPHA HEMOLYTIC

It may be Streptococcus pneumoniae

(or) Viridans streptococci (though it occur in chains predominantly sometimes may occur in pairs)



Pneumococcus

(Capsulated)

(Bile solubility test - Pos)

(Capsule swelling test – pos)

(Bile Esculin test – Neg)

Viridans streptococci

(Non-capsulated)

(Bile solubility test - Neg)

(Capsule swelling test – Neg)

(Bile Esculin Negative)

Note: Because the isolate is Gram positive cocci in pairs, we may also suspect Enterococcus sp., which may be alpha, beta or gamma hemolytic pattern on blood agar, hence Bile Esculin test, to which Enterococcus sp., is positive, can be used.

Note:

Pneumococcus – cause of Lobar pneumonia so it is most likely to be present in sputum of infected person.

Remember pneumococcus is also cause of meningitis (so also found in CSF)

Along with sputum, viridans streptococcus, which is a normal flora in oral cavity, may be present when sputum contaminated with saliva.

Viridans streptococcus, usually arranged in chains, may break into pairs looking like Streptococcus pneumoniae.

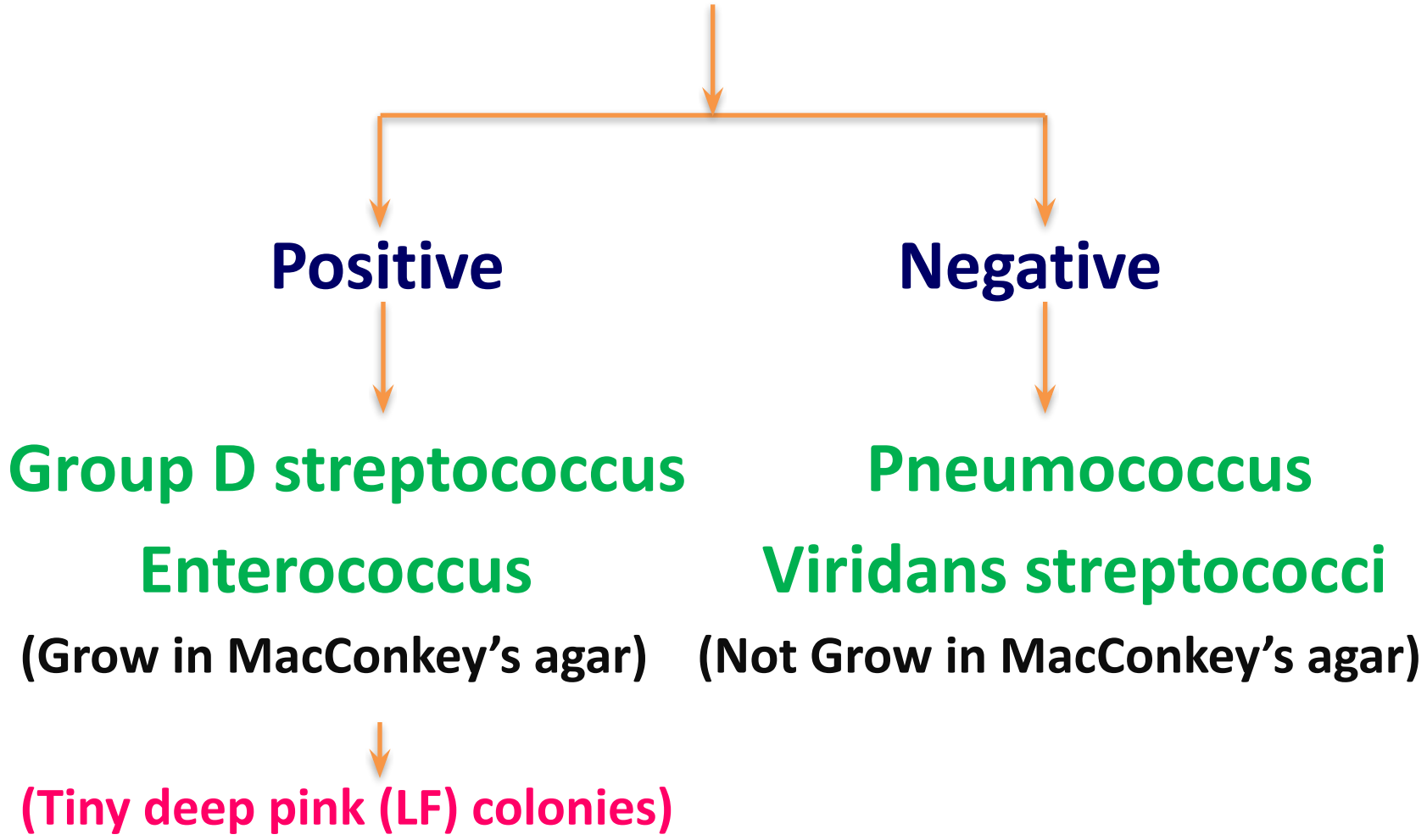
Viridans streptococcus and Streptococcus pneumoniae are always alpha-haemolytic.

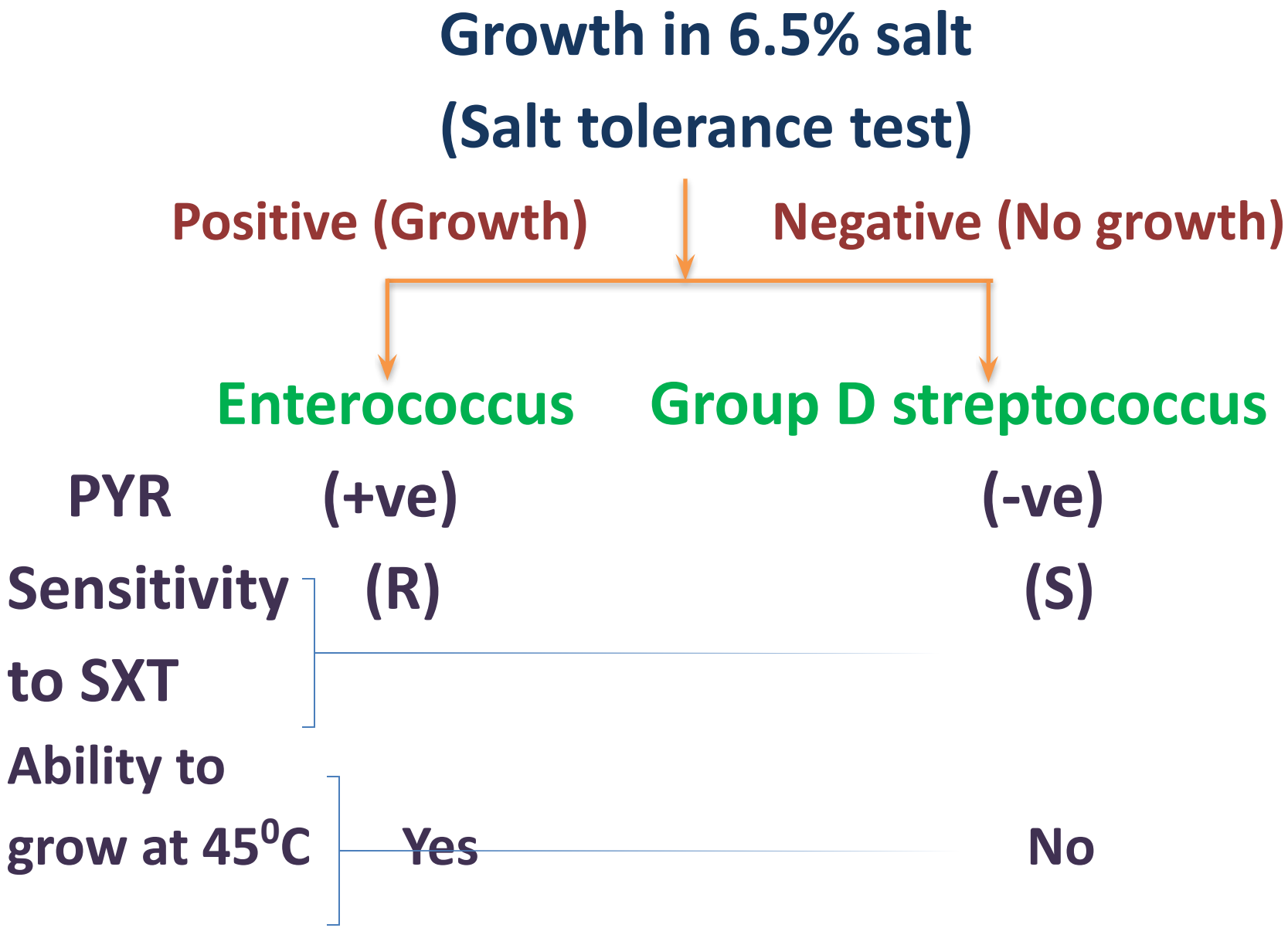
IDENTIFICATION OF GRAM POSITIVE COCCI- IV ENTEROCOCCUS

- **Gram stain – Gram positive cocci in pairs**
- **Motility – Non-motile**
- **Catalase – Negative**
- **Oxidase – Negative**

So it may be Enterococcus (or) Pneumococcus (or) viridans streptococci

BILE ESCULIN HYDROLYSIS TEST




Growth in 6.5% salt
(Salt tolerance test)
Positive (Growth)
Negative (No growth)
Enterococcus
Group D streptococcus
PYR
(+ve)
(-ve)
Sensitivity to SXT
(R)
(S)
Ability to grow at 45^{0}C
Yes
No

Note:

Enterococcus can be alpha or beta or gamma hemolytic on blood agar

Enterococcus faecalis and Enterococcus faecium are important pathogenic members in Genus Enterococcus

IDENTIFICATION OF GRAM NEGATIVE COCCI (I)
NEISSERIA SPECIES

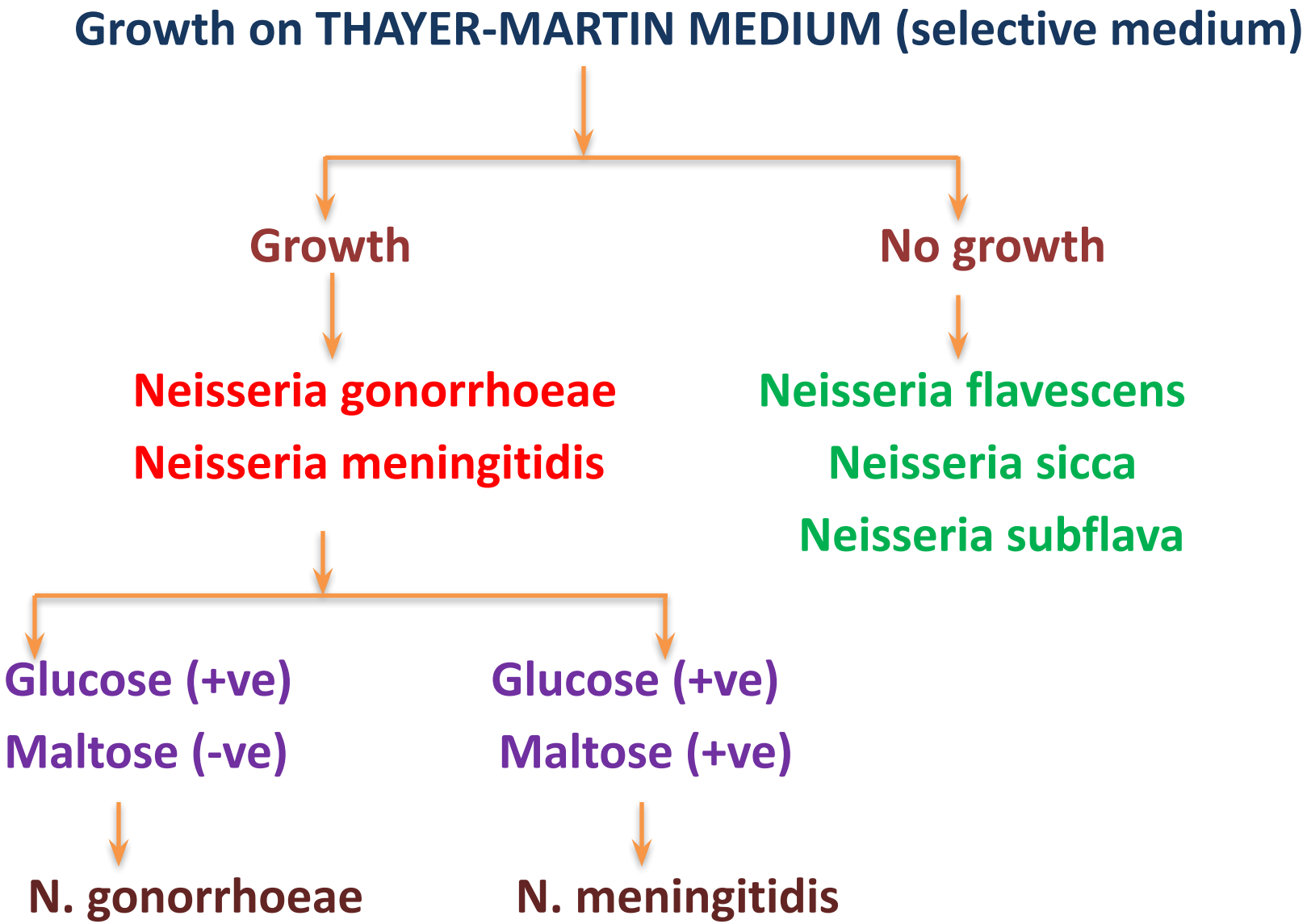
- **Gram Stain – Gram negative diplococci**
- **Motility – Non- motile**
- **Catalase – Positive**
- **Oxidase – Positive**

So it may be

Pathogenic Neisseria (or) **Non-pathogenic Neisseria**

(Neisseria gonorrhoeae)
(Neisseria meningitidis)

(Neisseria flavescens)
(Neisseria sicca)
(Neisseria subflava)


Growth on THAYER-MARTIN MEDIUM (selective medium)
Growth
No growth
Neisseria gonorrhoeae
Neisseria meningitidis
Neisseria flavescens
Neisseria sicca
Neisseria subflava
Glucose (+ve)
Maltose (-ve)
Glucose (+ve)
Maltose (+ve)
N. gonorrhoeae
N. meningitidis

Note:

- Non-pathogenic Neisseria like N. flavescens, N. sicca & N. subflava produce yellow pigment
- Non-pathogenic Neisseria can grow on Nutrient agar but pathogenic Neisseria don't.
- Catalase test for Neisseria can be done by superoxol test using 30% Hydrogen peroxide.
- N. gonorrhoeae – most likely to be present in urethral pus.
- N. meningitidis – most likely to be present in CSF.

IDENTIFICATION OF GRAM POSITIVE BACILLI

List of Gram positive Bacilli

Corynebacterium sp.

Listeria sp.

Erysipelothrix rhusiopathiae

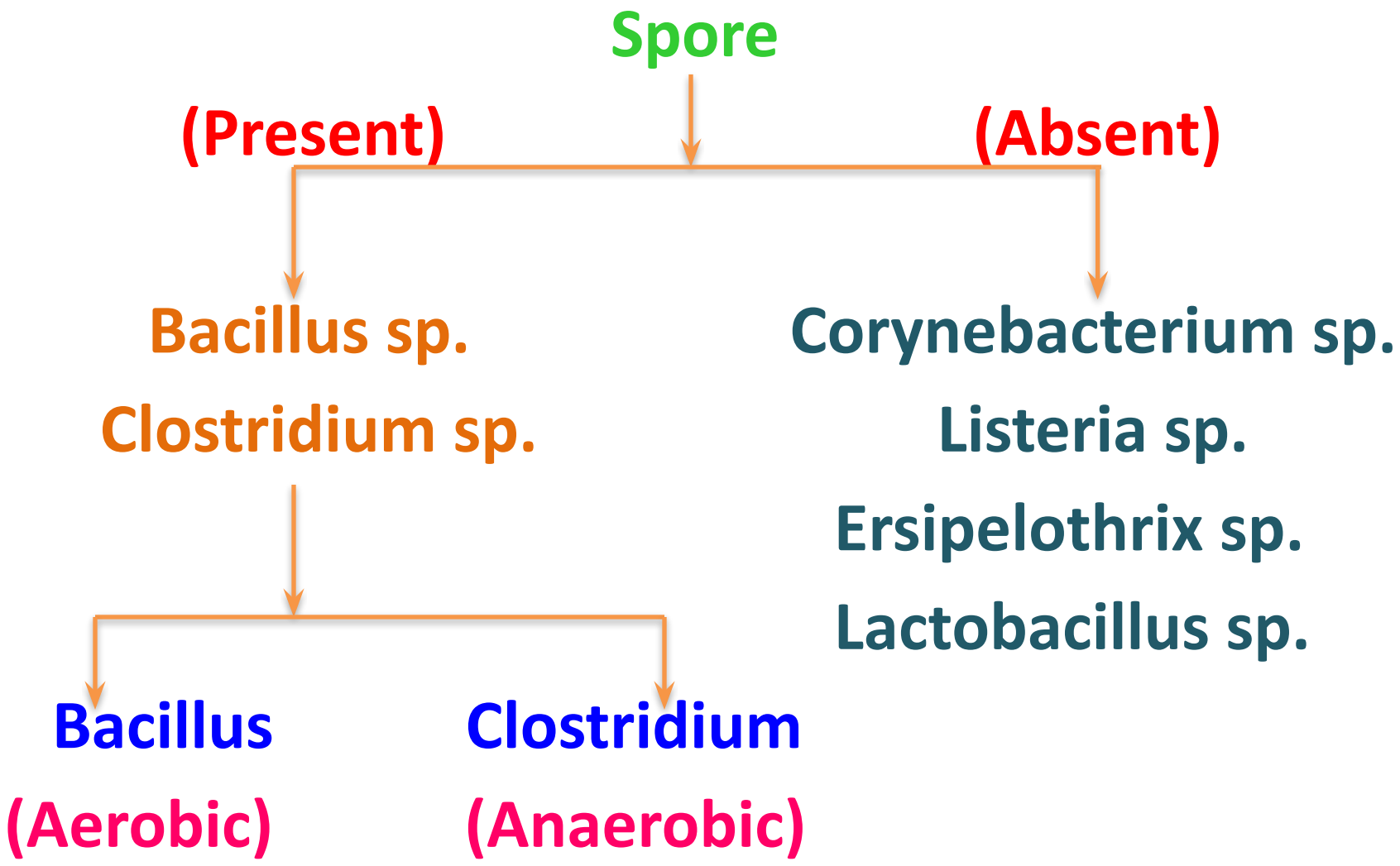
Lactobacillus sp.

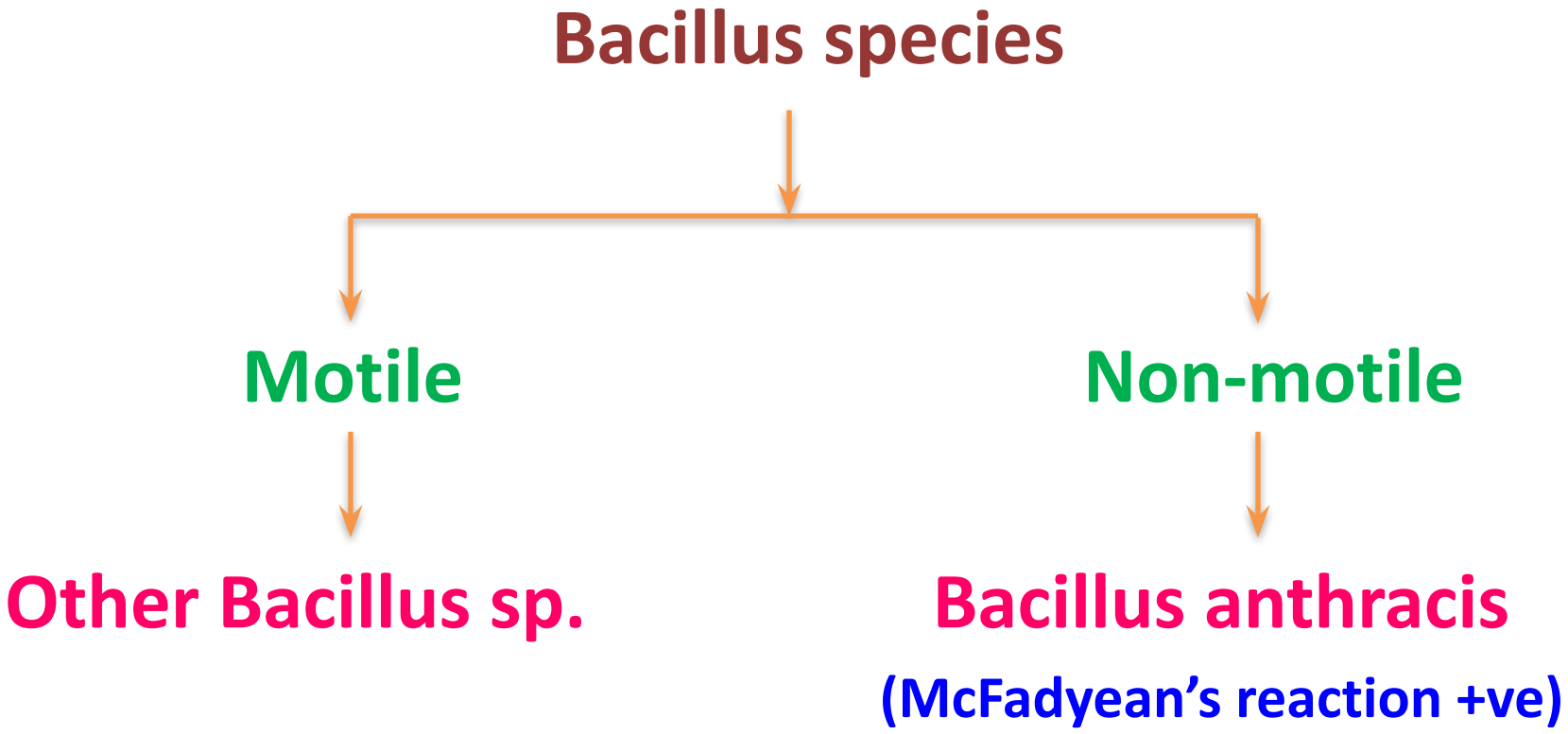
Kurthia sp.

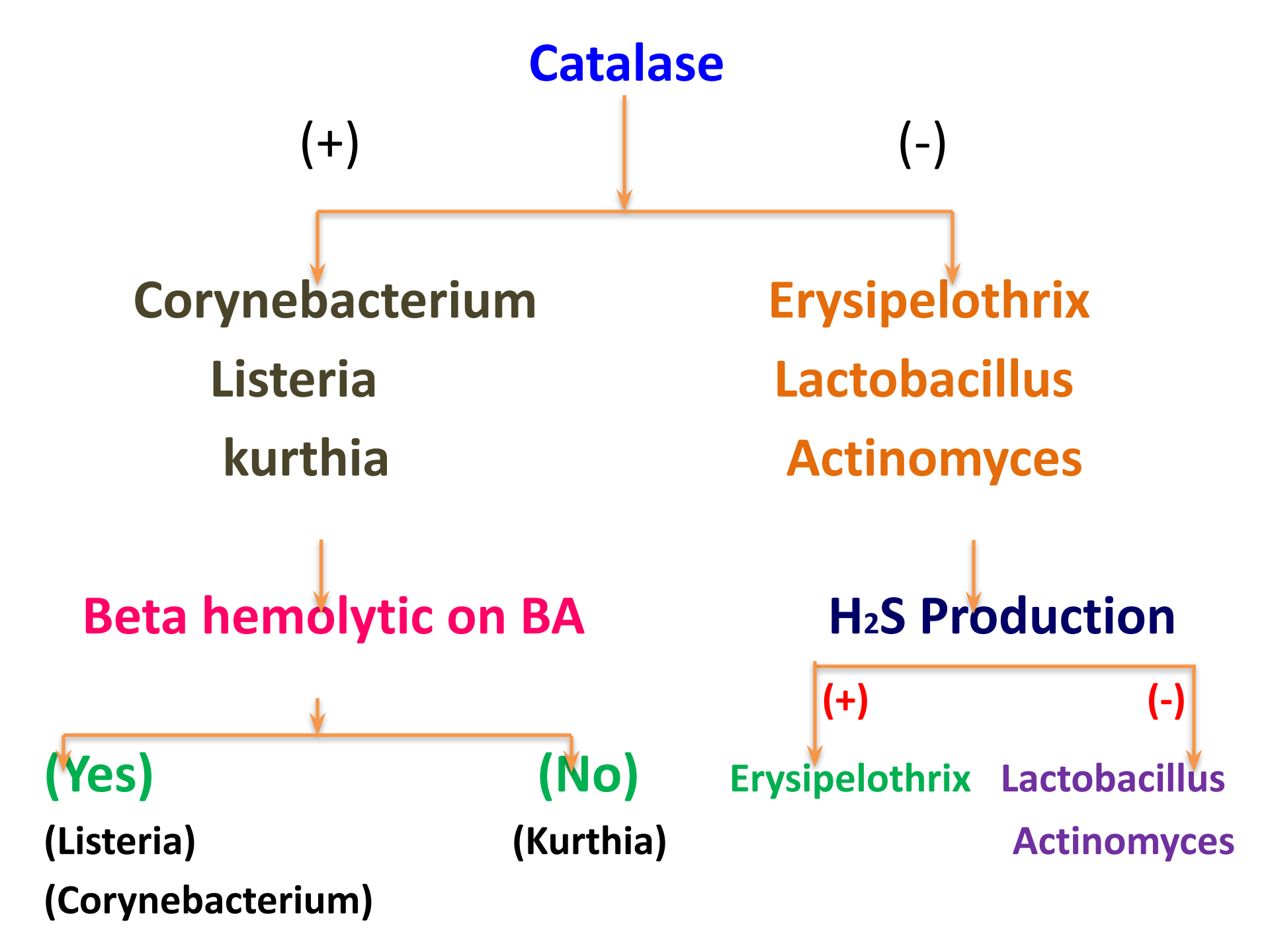
Actinomyces sp.

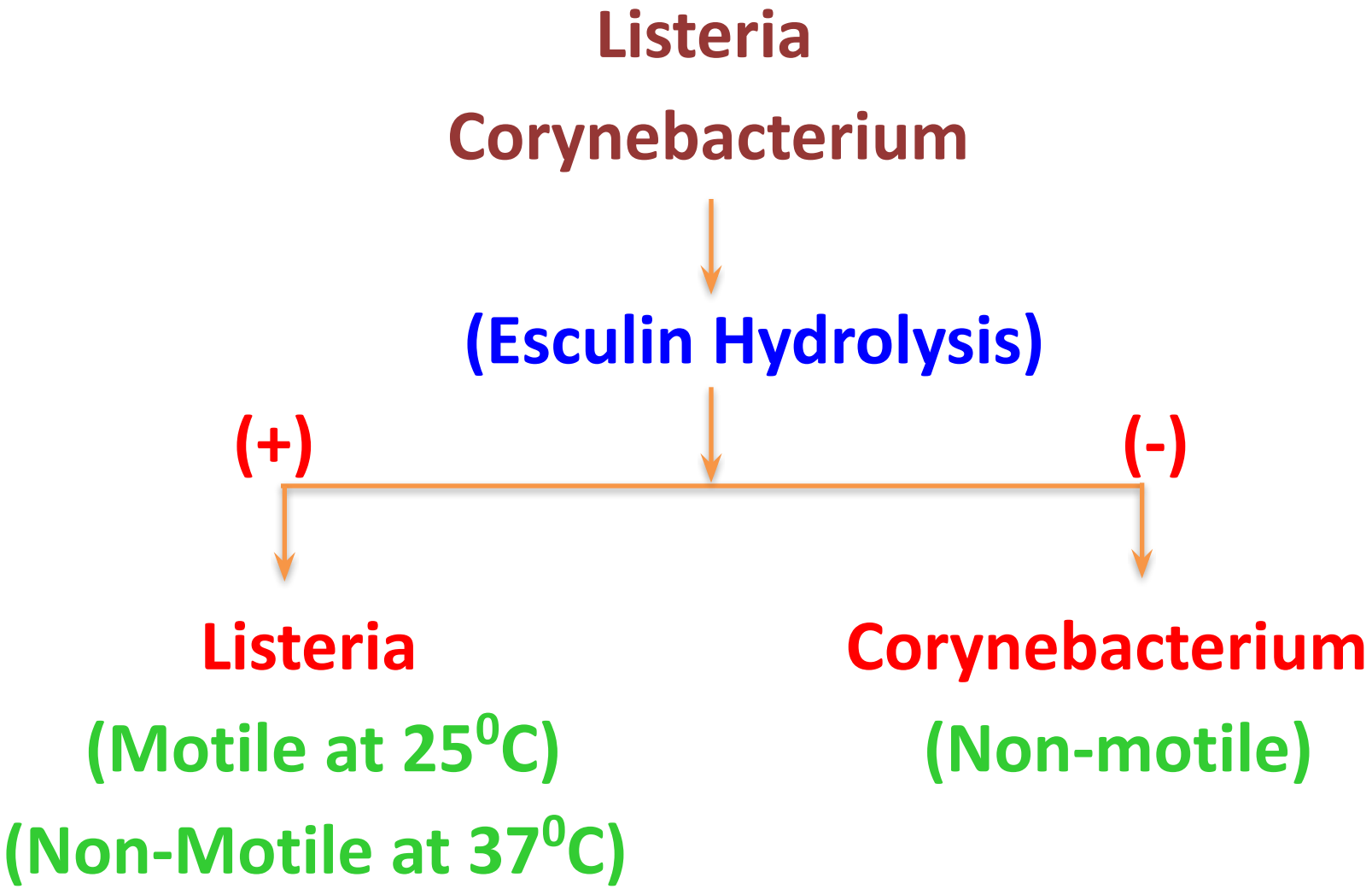
Bacillus sp.

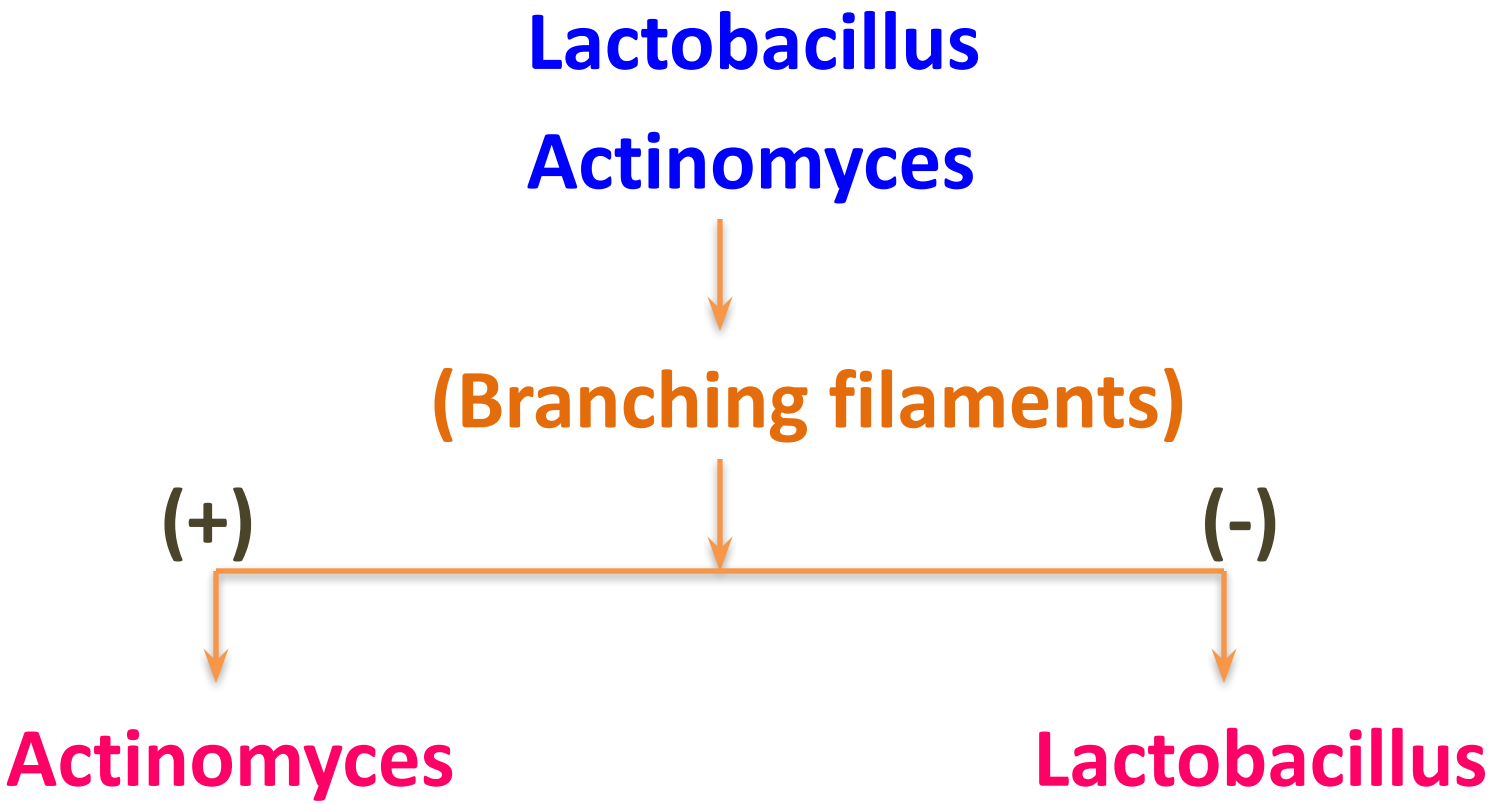
Clostridium sp.


Spore
(Present)
(Absent)
Bacillus sp.
Clostridium sp.
Corynebacterium sp.
Listeria sp.
Ersipelothrix sp.
Lactobacillus sp.
Bacillus
(Aerobic)
Clostridium
(Anaerobic)


Bacillus species
Motile
Non-motile
Other Bacillus sp.
Bacillus anthracis
(McFadyean's reaction +ve)


Catalase
(+)
(-)
Corynebacterium
Listeria
kurthia
Erysipelothrix
Lactobacillus
Actinomyces
Beta hemolytic on BA
H2S Production
(Yes)
(No)
(Listeria)
(Corynebacterium)
(Kurthia)
(+)
(-)
Erysipelothrix
Lactobacillus
Actinomyces


Listeria
Corynebacterium
(Esculin Hydrolysis)
(+)
(-)
Listeria
(Motile at 25^{0}C)
(Non-Motile at 37^{0}C)
Corynebacterium
(Non-motile)


Lactobacillus
Actinomyces
(Branching filaments)
(+)
(-)
Actinomyces
Lactobacillus

Note:

Other examples of anaerobic Gram positive bacilli – Eubacterium, Propionibacterium, Bifidobacterium, Mobilincus.

Remember, Actinomyces and Lactobacillus also contains anaerobic species and microaerophilic species.

Clostridium species (anaerobes) – another example of Gram positive bacilli

Diptheroids, morphologically similar to C. diptheriae can grow on ordinary media like Nutrient agar. But Corynebacterium diptheriae can only grow on enriched media like Blood agar and Loeffler serum slope.

IDENTIFICATION OF GRAM NEGATIVE BACILLI (I)
ESCHERICHIA COLI /E.COLI

- Gram stain – Gram negative bacilli
- Motility – Motile
- Catalase – Positive
- Oxidase – Negative

So it is one of the members of Enterobacteriaceae

Enterobacteriaceae includes E.coli, Klebsiella, Citrobacter, Enterobacter, Serratia, Salmonella, Shigella, Proteus

MacConkey's agar – Dry, flat LF colonies

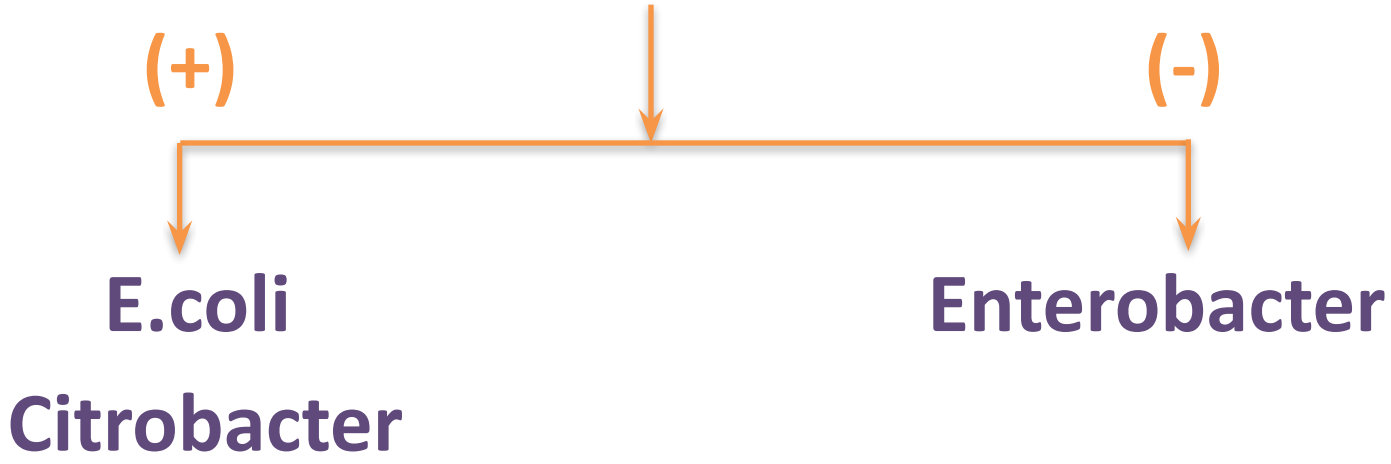
Motility – Motile

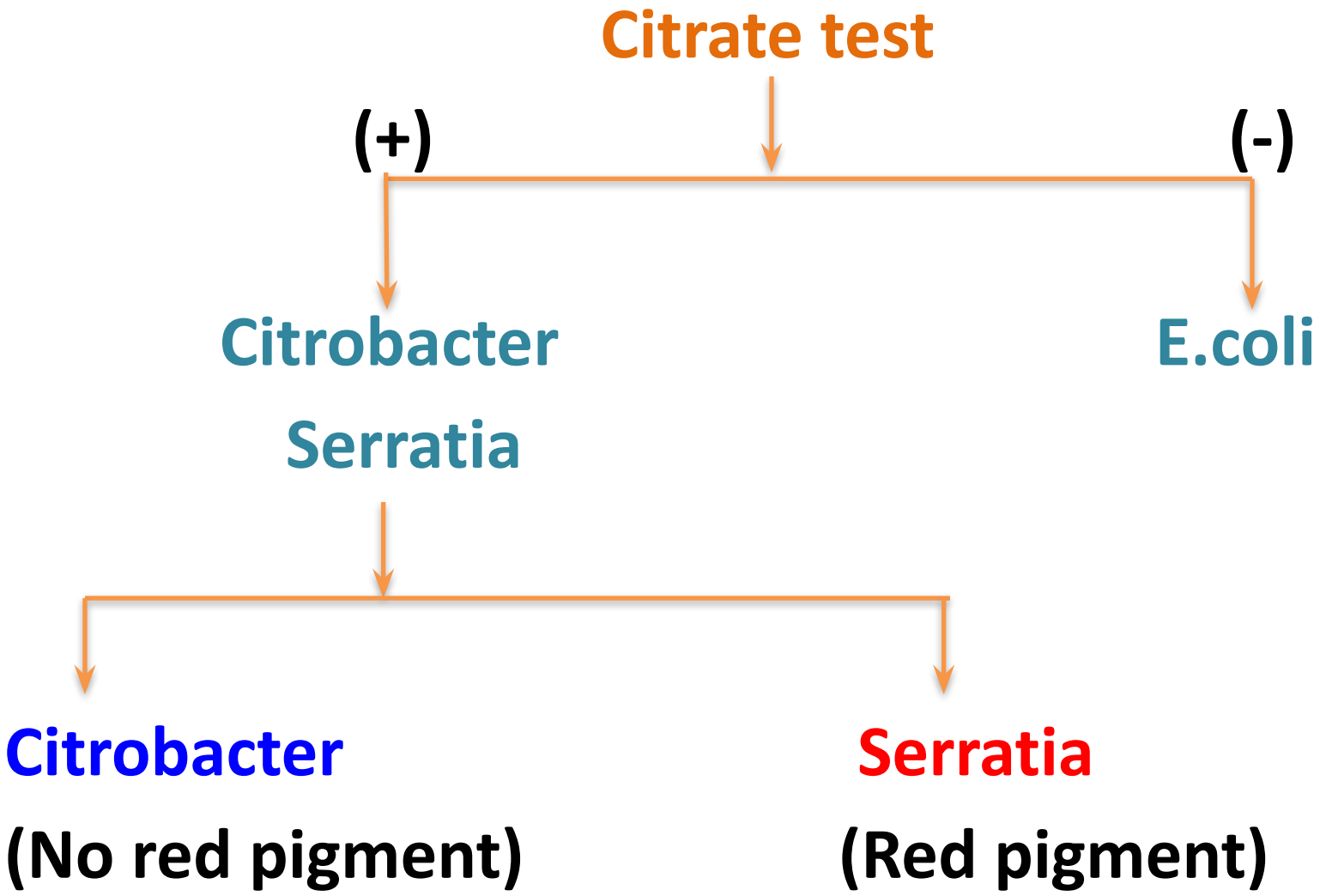
So it may be E.coli, Citrobacter, Enterobacter, Serratia

Klebsiella being non-motile, it is omitted

Indole test

Methyl-Red test




Citrate test
(+)
(-)
Citrobacter
Serratia
E.coli
Citrobacter
(No red pigment)
Serratia
(Red pigment)

IMVIC REACTIONS FOR E.COLI

- Indole – (+)
- Methyl red – (+)
- Voges-Proskauer – (-)
- Citrate - (-)

Other biochemical reactions:

TSI – A/A, Gas (+), H_2S (-)

Urease – (-)

Remember:

E.Coli is the most commones cause of lower urinary tract infection. In this case, E.coli is most likely to be present in urine sample of infected persons.

IDENTIFICATION OF GNB (II)
KLEBSIELLA SPECIES

- Gram stain – Gram Negative bacilli
- Motility – Non-motile
- Catalase – Positive
- Oxidase – Negative

So it is one of the members of Enterobacteriaceae

On MacConkey's agar – Mucoid LF Colonies

Motility – Non-motile

So it may be Klebsiella species

But it may not be E.coli, Citrobacter, Enterobacter or Serratia (because all are motile)

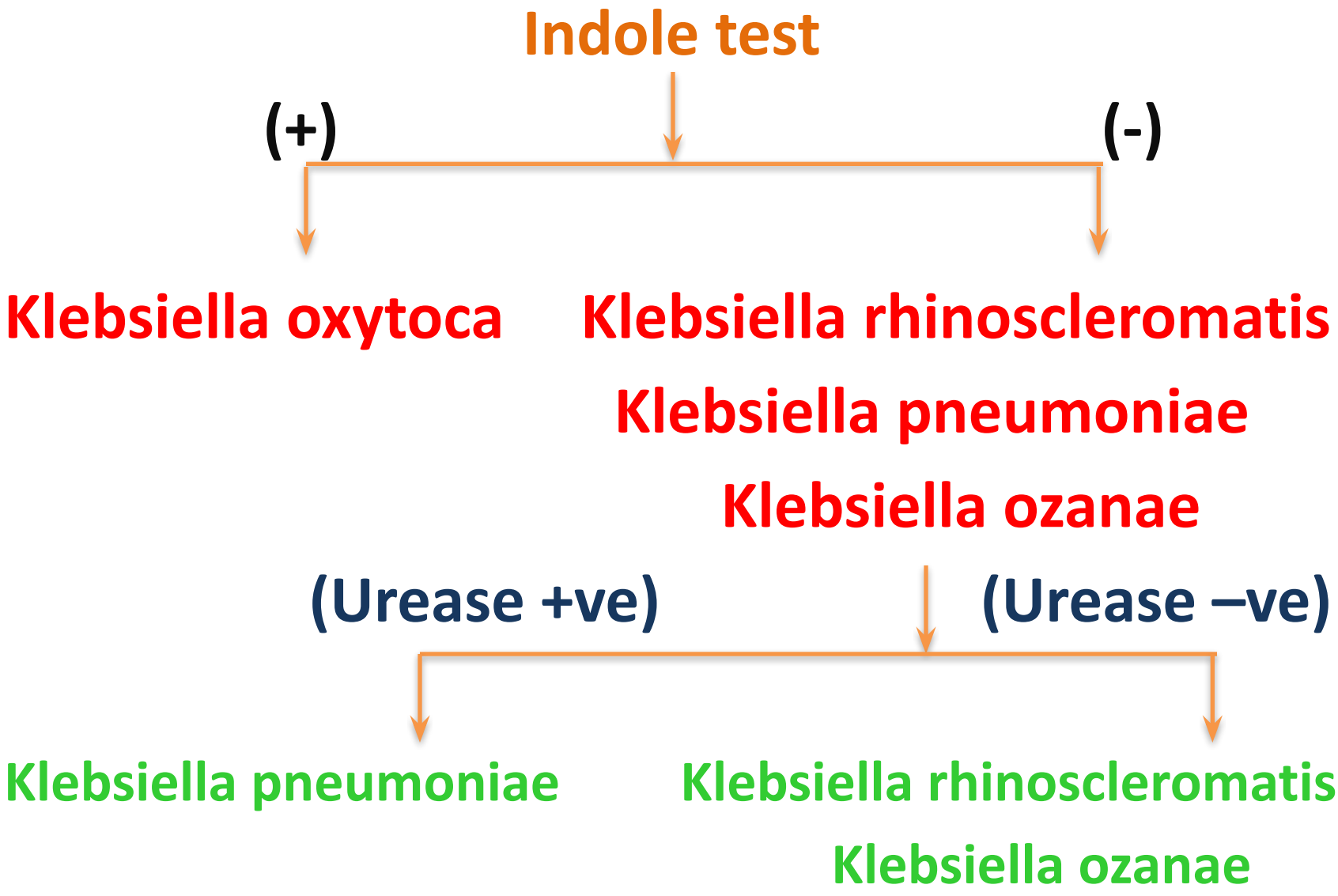
Klebsiella species

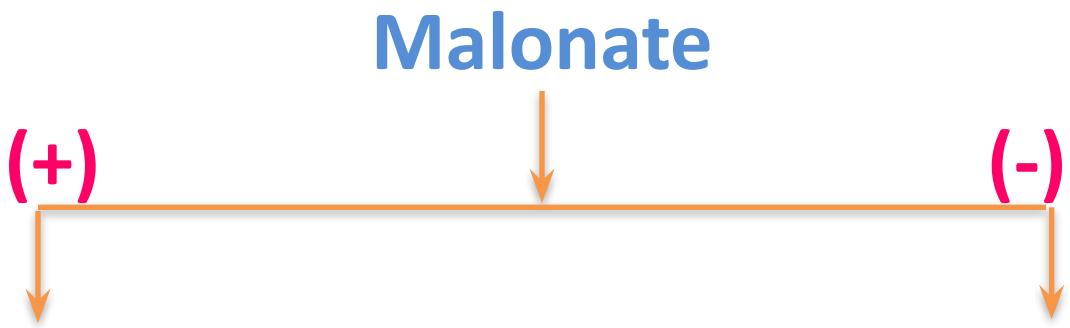
Klebsiella pneumoniae

Klebsiella oxytoca

Klebsiella rhinoscleromatis

Klebsiella ozanae


Indole test
(+)
(-)
Klebsiella oxytoca
Klebsiella rhinoscleromatis
Klebsiella pneumoniae
Klebsiella ozanae
(Urease +ve)
(Urease –ve)
Klebsiella pneumoniae
Klebsiella rhinoscleromatis
Klebsiella ozanae



Klebsiella rhinoscleromatis (VP POSITIVE)

Klebsiella ozanae (VP POSITIVE)

IMVIC REACTIONS FOR KLEBSIELLA PNEUMONIAE

- Indole – (-)
- Methyl red – (-)
- Voges-Proskauer – (+)
- Citrate - (+)

Other biochemical reactions:

TSI – A/A, Gas (+), H_2S (-)

Urease – (+)

Remember:

Klebsiella pneumoniae is also the most commonest cause of lower urinary tract infection. In this case, it is most likely to be present in urine sample of infected persons.

IDENTIFICATION OF GNB (III)
CITROBACTER

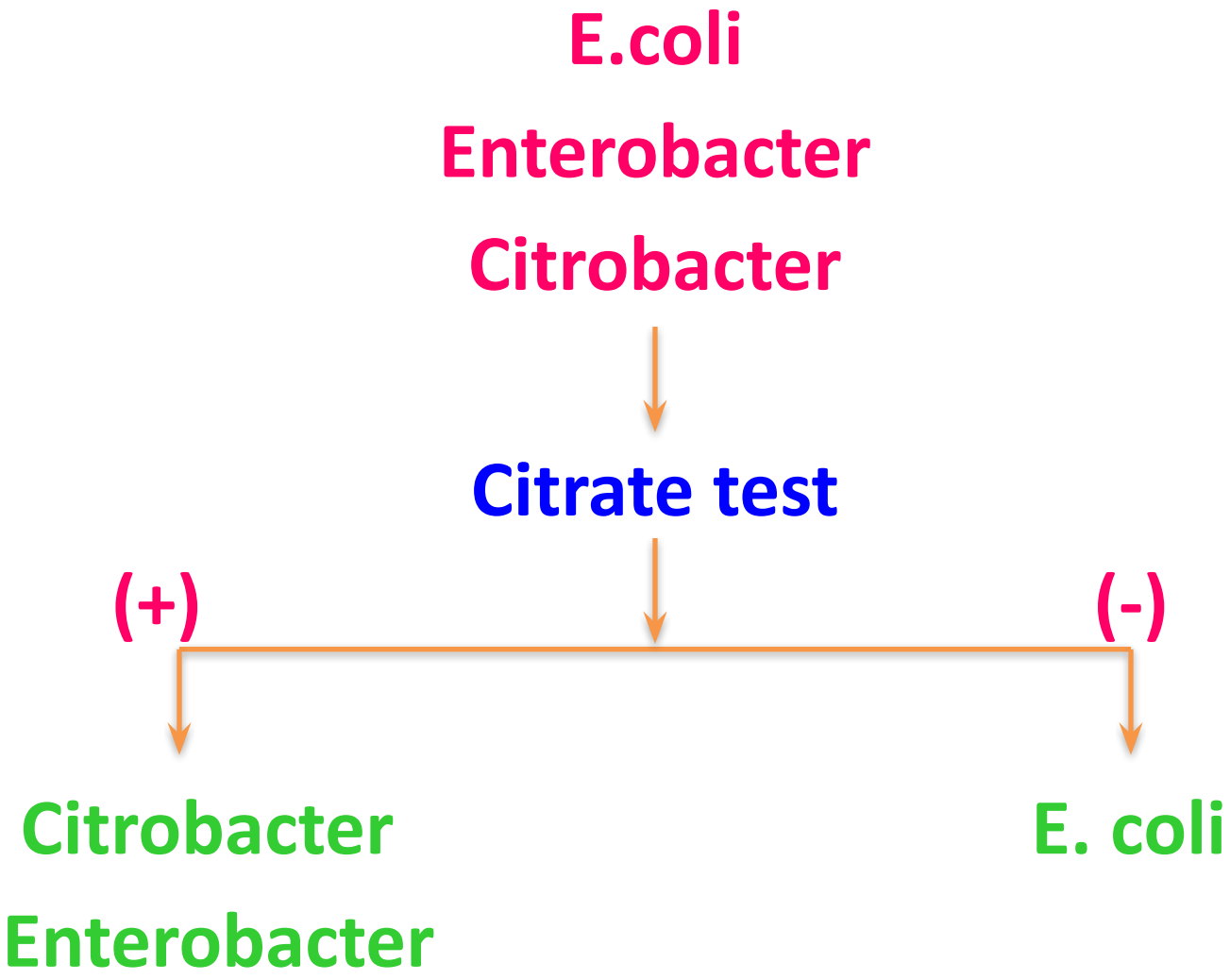
- Gram stain – Gram negative bacilli
- Motility – Motile
- Catalase – Positive
- Oxidase – Negative

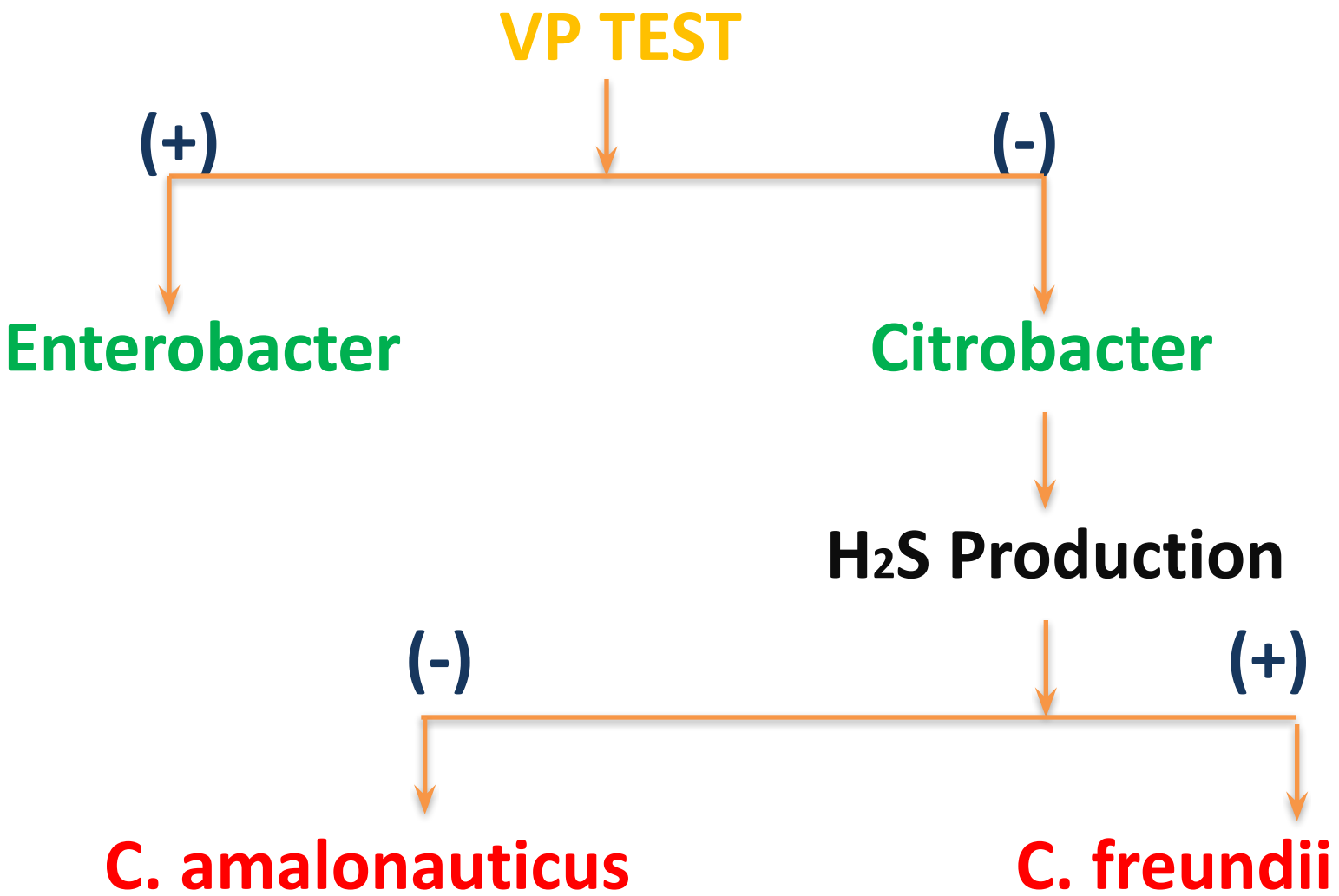
So it is one of the members of Enterobacteriaceae

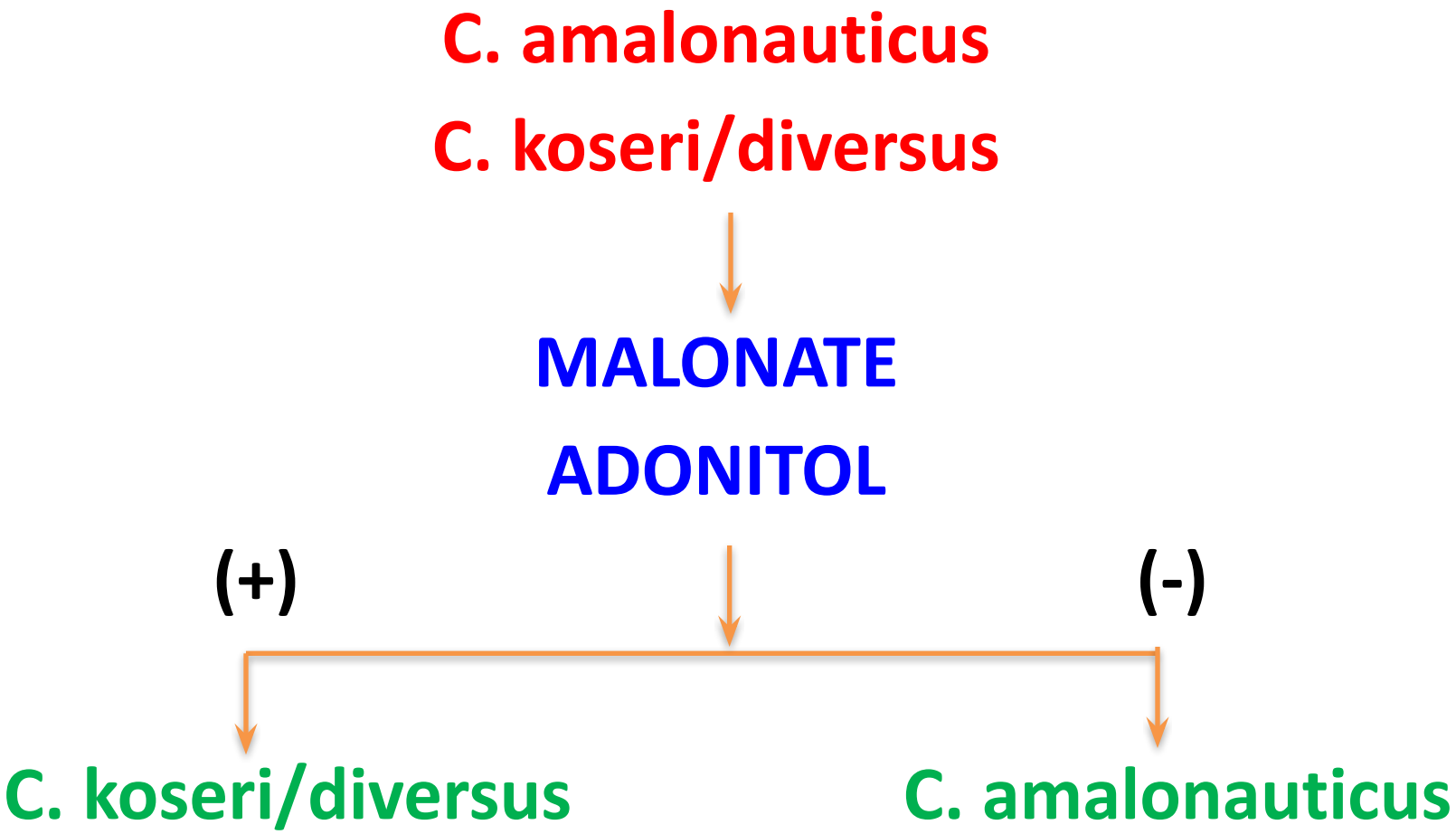
On Mac – LF colonies

Being motile

It may be E.coli or Enterobacter or Citrobacter


E.coli
Enterobacter
Citrobacter
Citrate test
(+)
(-)
Citrobacter
Enterobacter
E. coli


VP TEST
(+)
(-)
Enterobacter
Citrobacter
H2S Production
(-)
(+)
C. amalonauticus
C. koseri/diversus
C. freundii


C. amalonauticus
C. koseri/diversus
MALONATE
ADONITOL
(+)
(-)
C. koseri/diversus
C. amalonauticus

Other reactions of Citrobacter

- Indole – (+/-)
- MR – (+)
- VP – (-)
- Urease – (weakly positive)
- TSI – A/A, Gas (+ve), H_2S (+/-)

Remember citrobacter sometimes also may be Late lactose fermenter (ONPG – +VE)

Remember Citrobacter always Citrobacter (+)

IDENTIFICATION OF GNB (IV) ENTEROBACTER

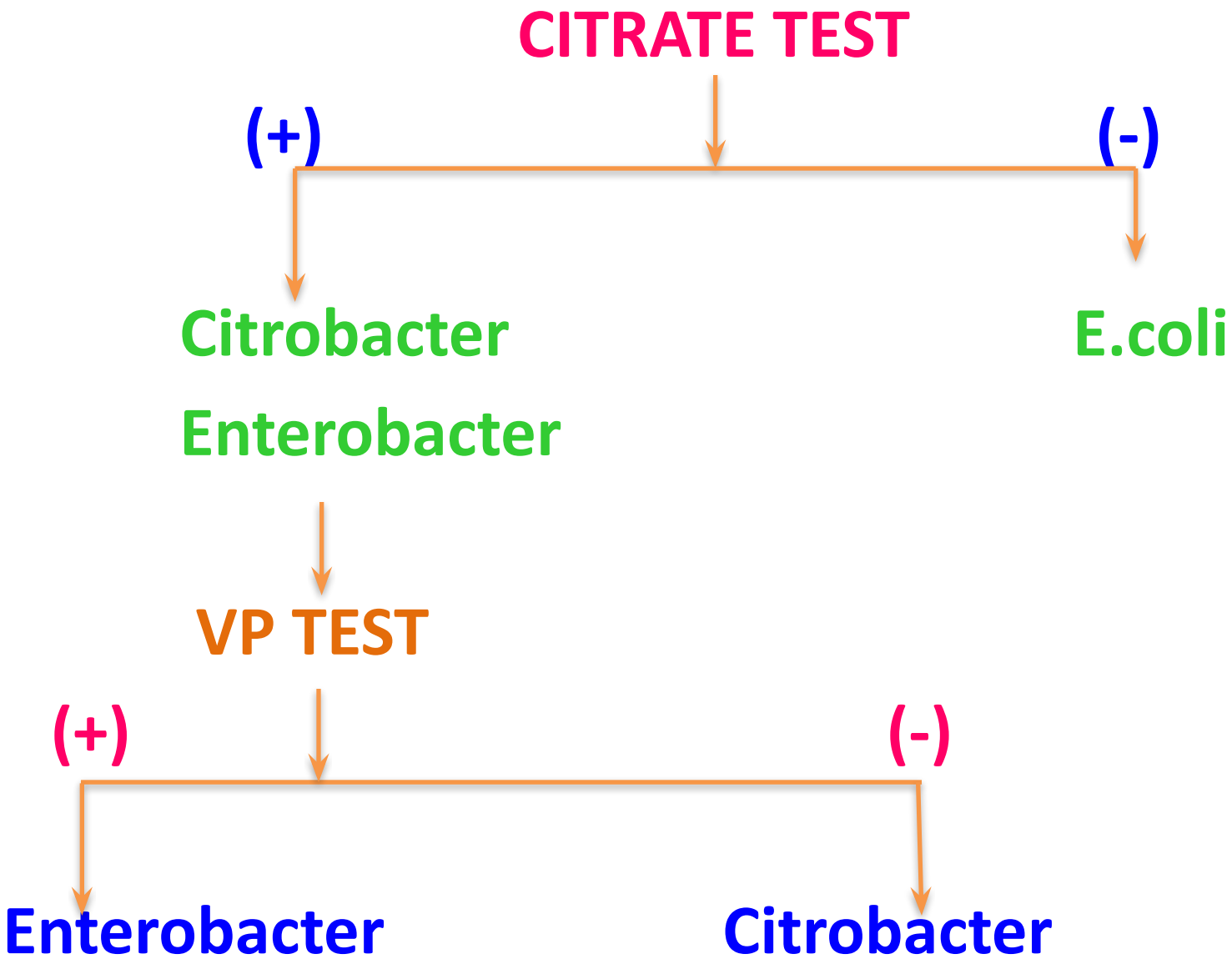
- Gram stain – Gram Negative Bacilli
- Motility – Motile
- Catalase – Positive
- Oxidase – Negative

So it is one of the members of Enterobacteriaceae

On Mac- LF colonies (Less mucoid)

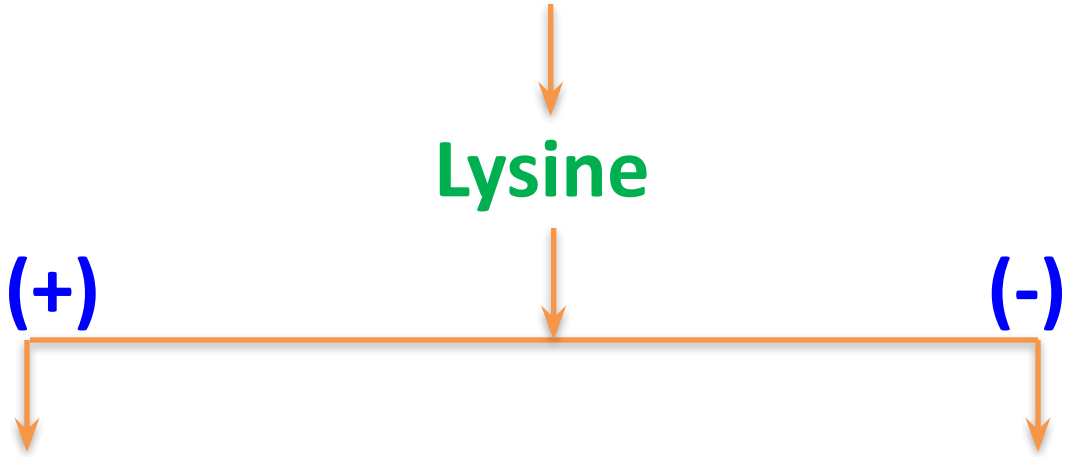
Being motile

It may be E.coli (or) Citrobacter (or) Enterobacter


CITRATE TEST
(+)
(-)
Citrobacter
Enterobacter
E.coli
VP TEST
(+)
(-)
Enterobacter
Citrobacter

Enterobacter cloacae

Enterobacter aerogenes



Ent. aerogenes

(Arginine- Pos)

Ent. Cloacae

(Arginine- Neg)

IDENTIFICATION OF GNB-V
PROTEUS, MORGANELLA, PROVIDENCIA

- Gram stain – Gram negative bacilli
- Motility –Motile
- Catalase – Positive
- Oxidase – Negative

So it is one of the members of Enterobacteriaceae

Being motile – Shigella is omitted

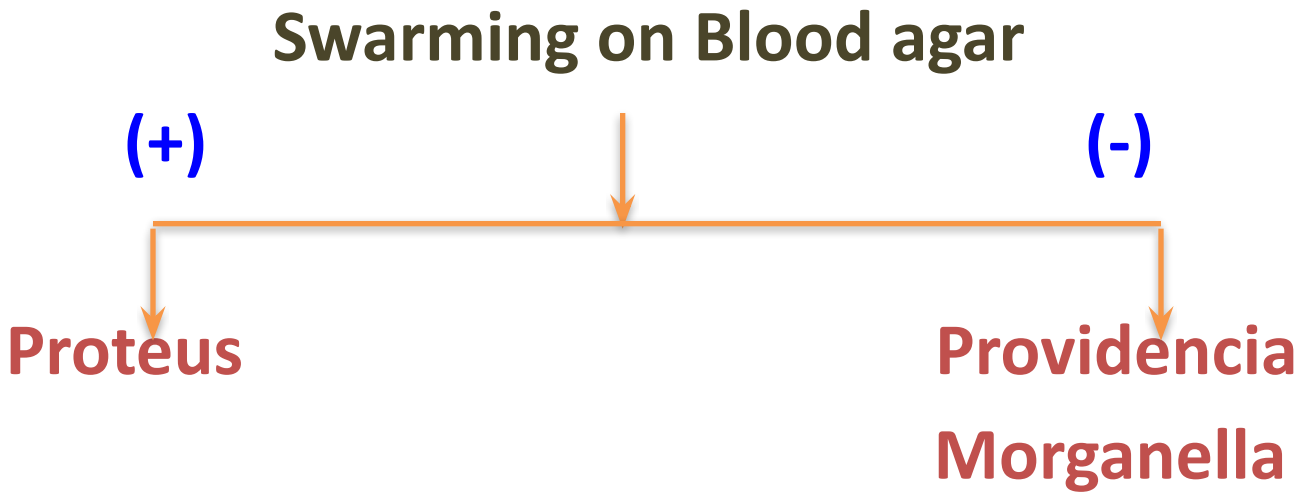
On Mac – NLF – so E.coli, Citrobacter, Enterobacter are omitted

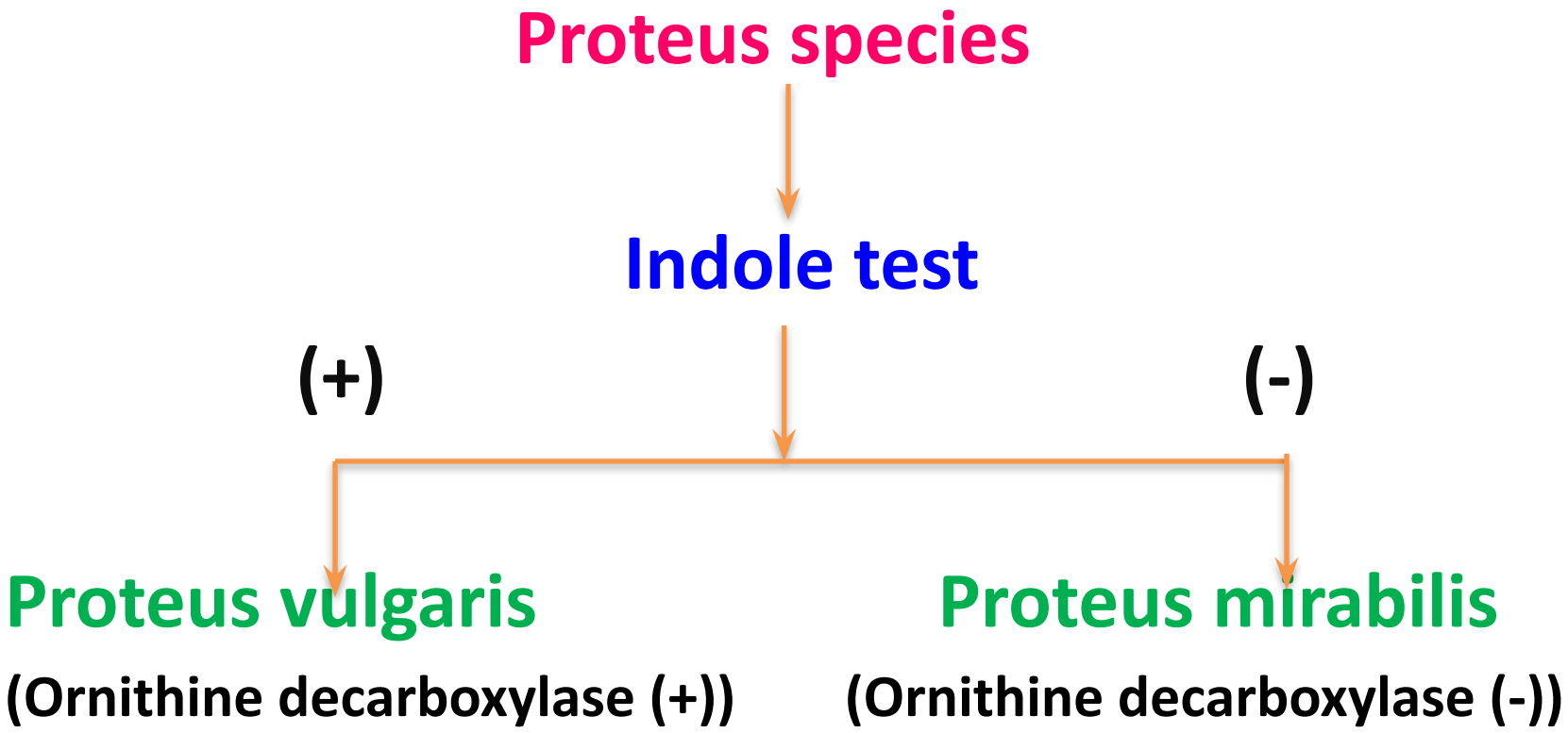
- **It may be Salmonella or Proteus or Morganella or Providencia**

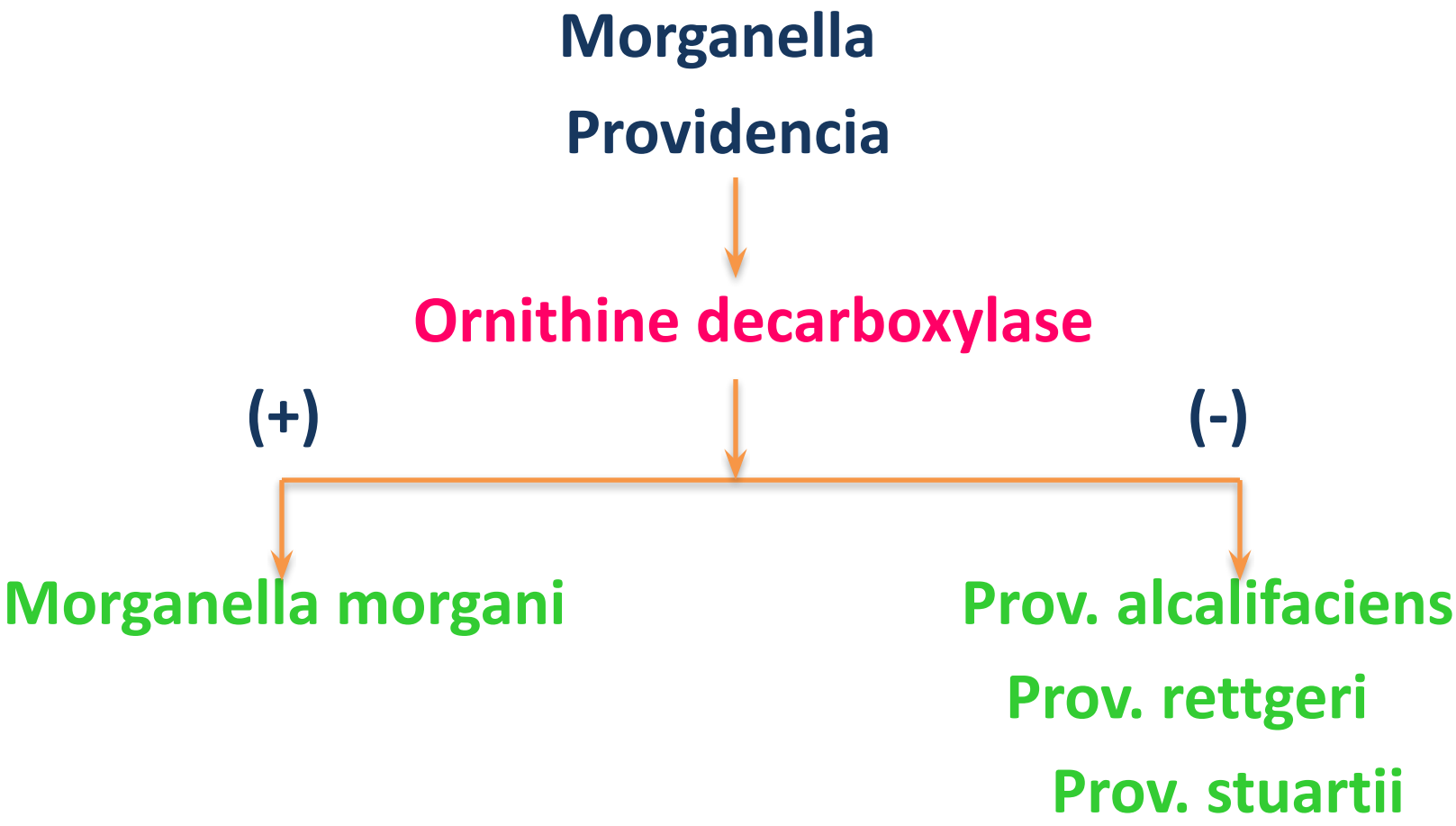
Being PPA/PAD test positive

It may not be Salmonella

It may be Proteus (or) Morganella (or) Providencia



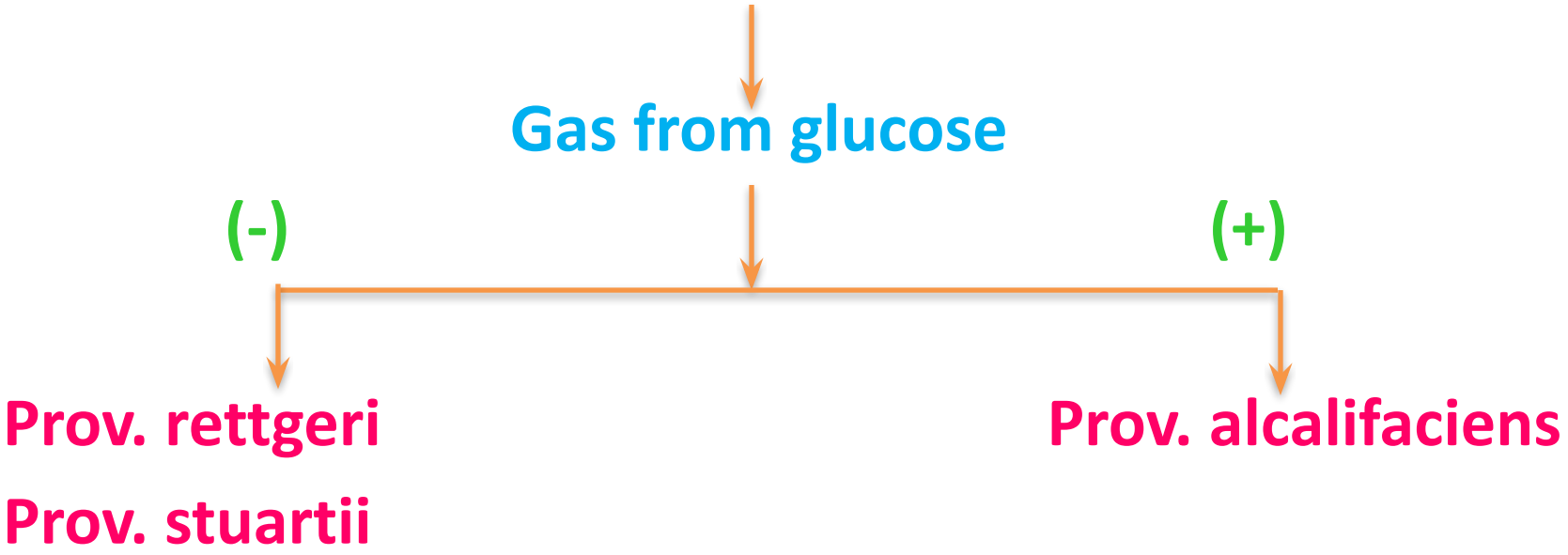

Proteus species
Indole test
(+)
(-)
Proteus vulgaris
(Ornithine decarboxylase (+))
Proteus mirabilis
(Ornithine decarboxylase (-))

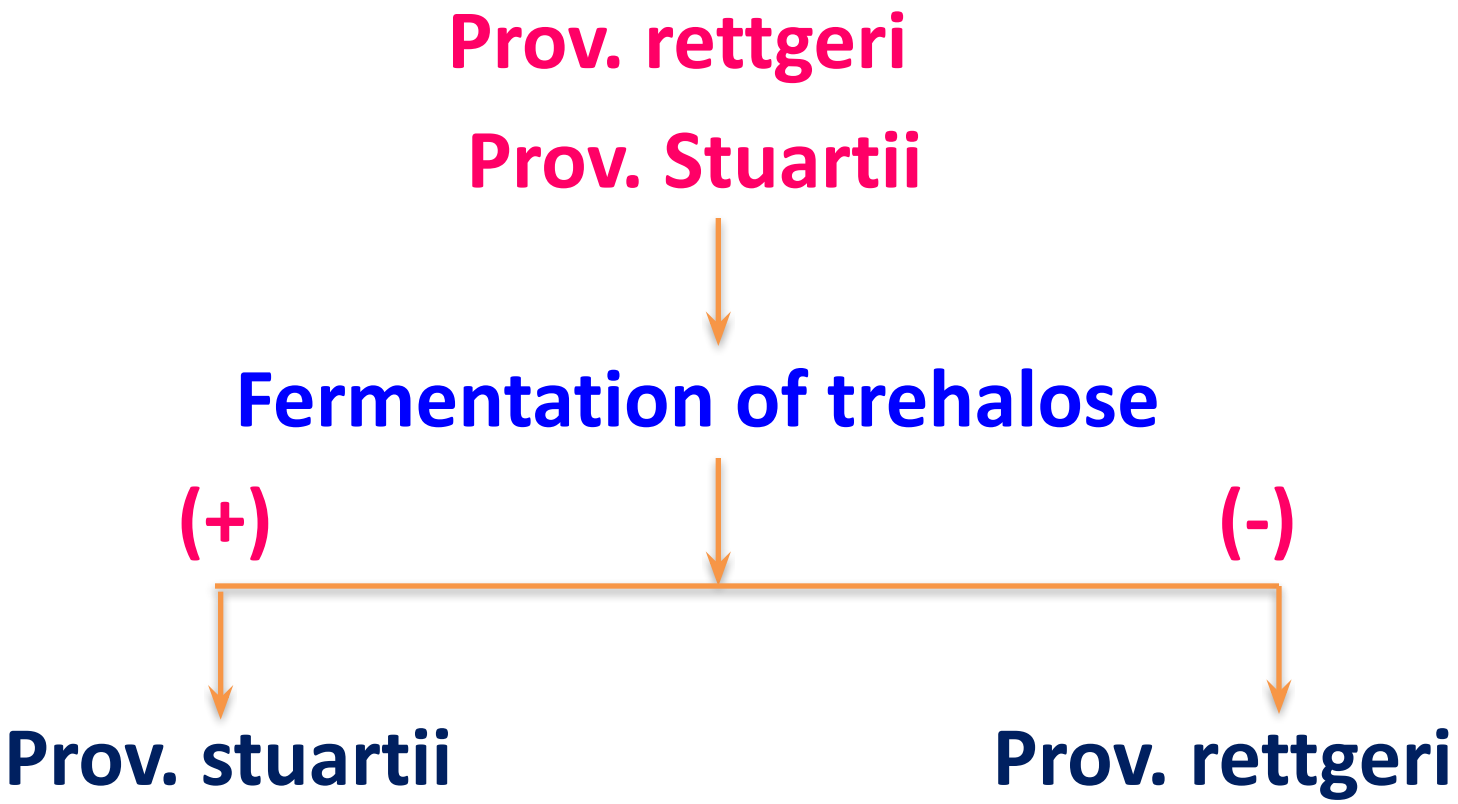

Morganella
Providencia
Ornithine decarboxylase
(+)
(-)
Morganella morgani
Prov. alcalifaciens
Prov. rettgeri
Prov. stuartii

Providencia alcalifaciens

Providencia rettgeri

Providencia stuartii




Prov. rettgeri
Prov. Stuartii
Fermentation of trehalose
(+)
(-)
Prov. stuartii
Prov. rettgeri

Other biochemicals for Proteus species

MR – (+)

VP – (-)

Urease - (+)

Citrate – (-)

TSI – K/A, Gas(+), H_2S (+)

IDENTIFICATION OF GNB – VI
SHIGELLA SPECIES

- Gram stain – Gram negative bacilli
- Motility – Non-motile
- Catalase – Positive
- Oxidase – Negative

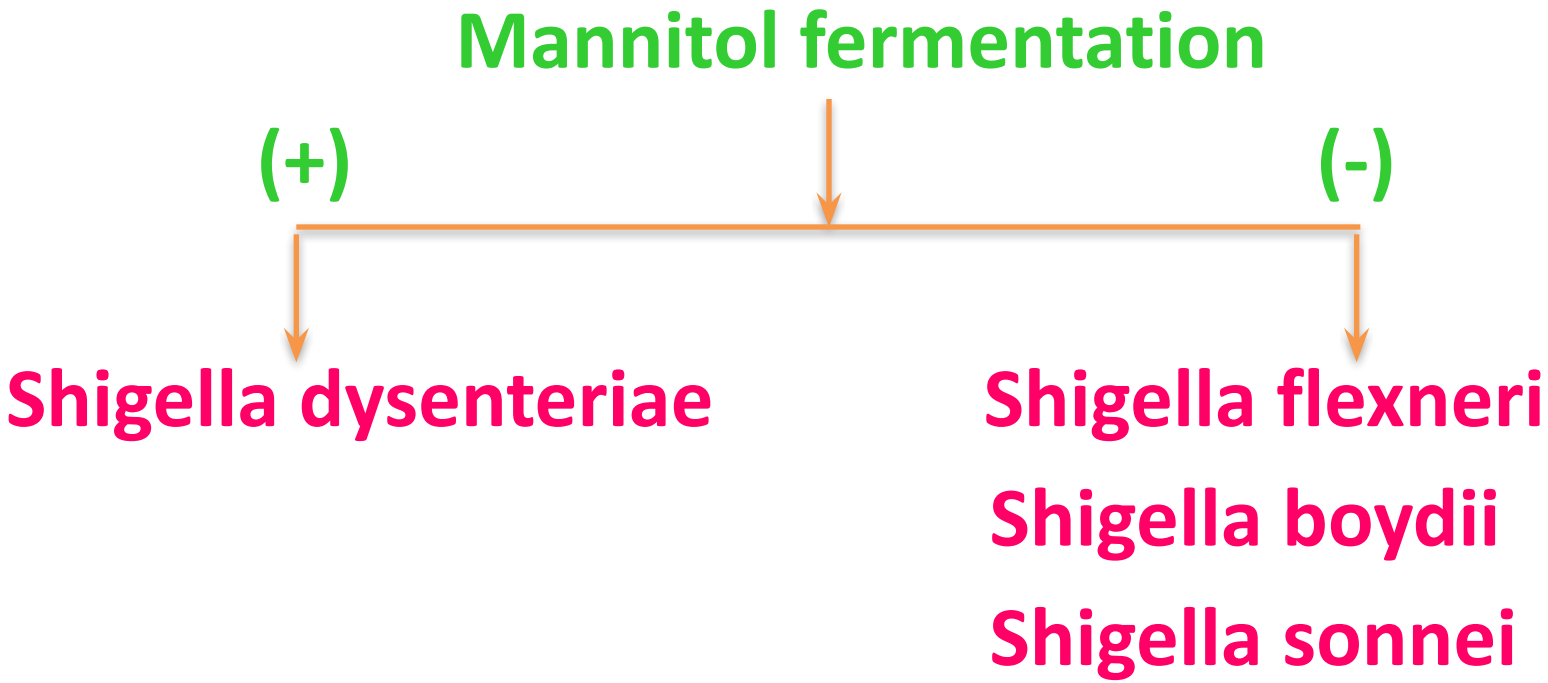
so it is one of the members of enterobacteriaceae

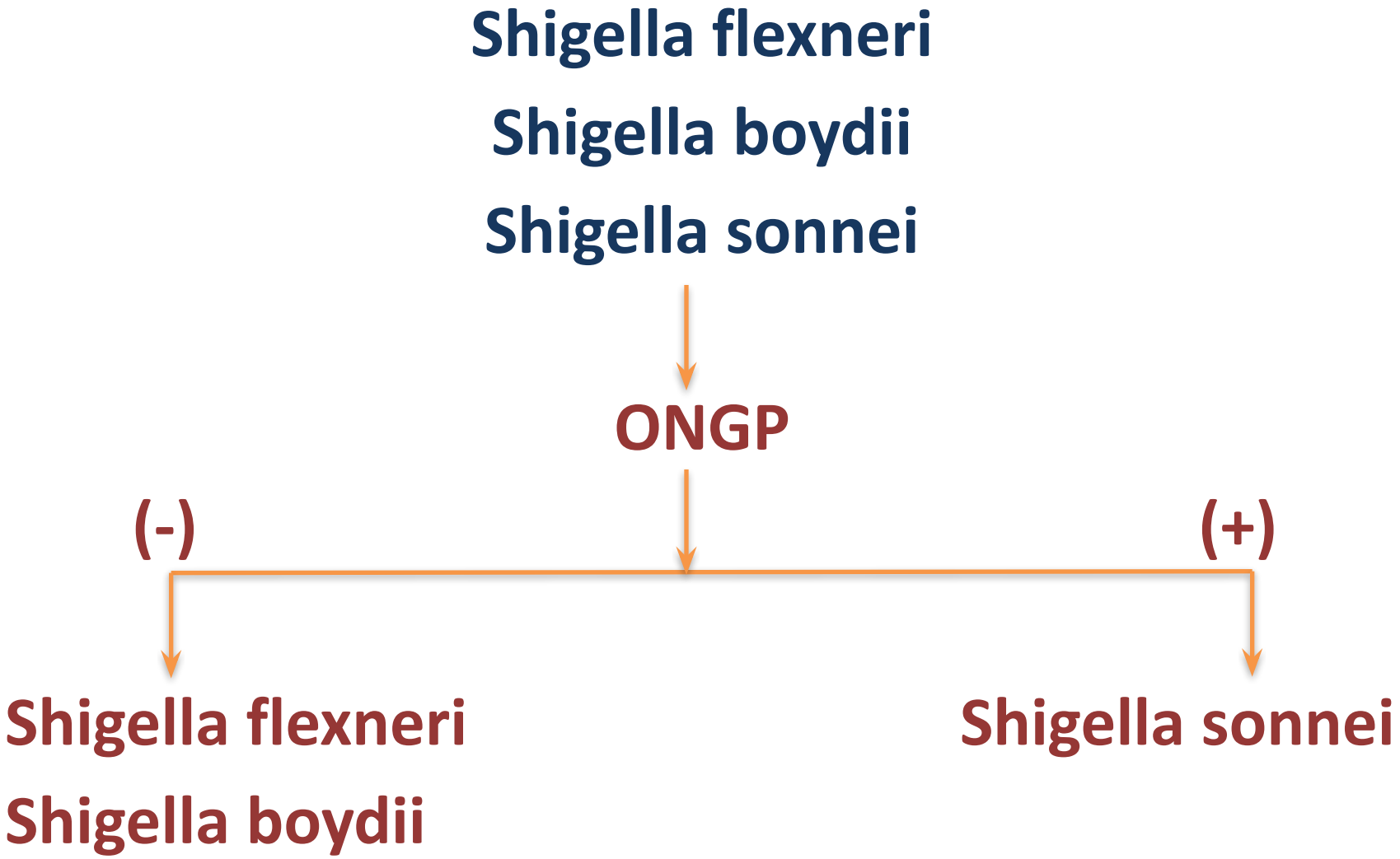
On mac – NLF colonies

Being non-motile

It is not Salmonella (or) Proteus (being motile)

It is Shigella species


Mannitol fermentation
(+)
(-)
Shigella dysenteriae
Shigella flexneri
Shigella boydii
Shigella sonnei


Shigella flexneri
Shigella boydii
Shigella sonnei
ONGP
(-)
(+)
Shigella flexneri
Shigella boydii
Shigella sonnei

Note:

Shigella dysenteriae type 1 is always catalase negative.

Differentiation between

Sh. Flexneri and Sh. Boydii can be done only by serological method using specific antisera because of non-availability of suitable biochemical reactions.

Other biochemicals for Shigella species

Indole – (+/-)

MR – (+)

VP – (-)

Citrate – (-)

Urease – (-)

TSI – K/A, Gas (-), H_2S (-)

IDENTIFICATION OF GNB – VII SALMONELLA SPECIES

- **Gram stain – Gram negative bacilli**
- **Motility – Motile**
- **Catalase – Positive**
- **Oxidase – Negative**

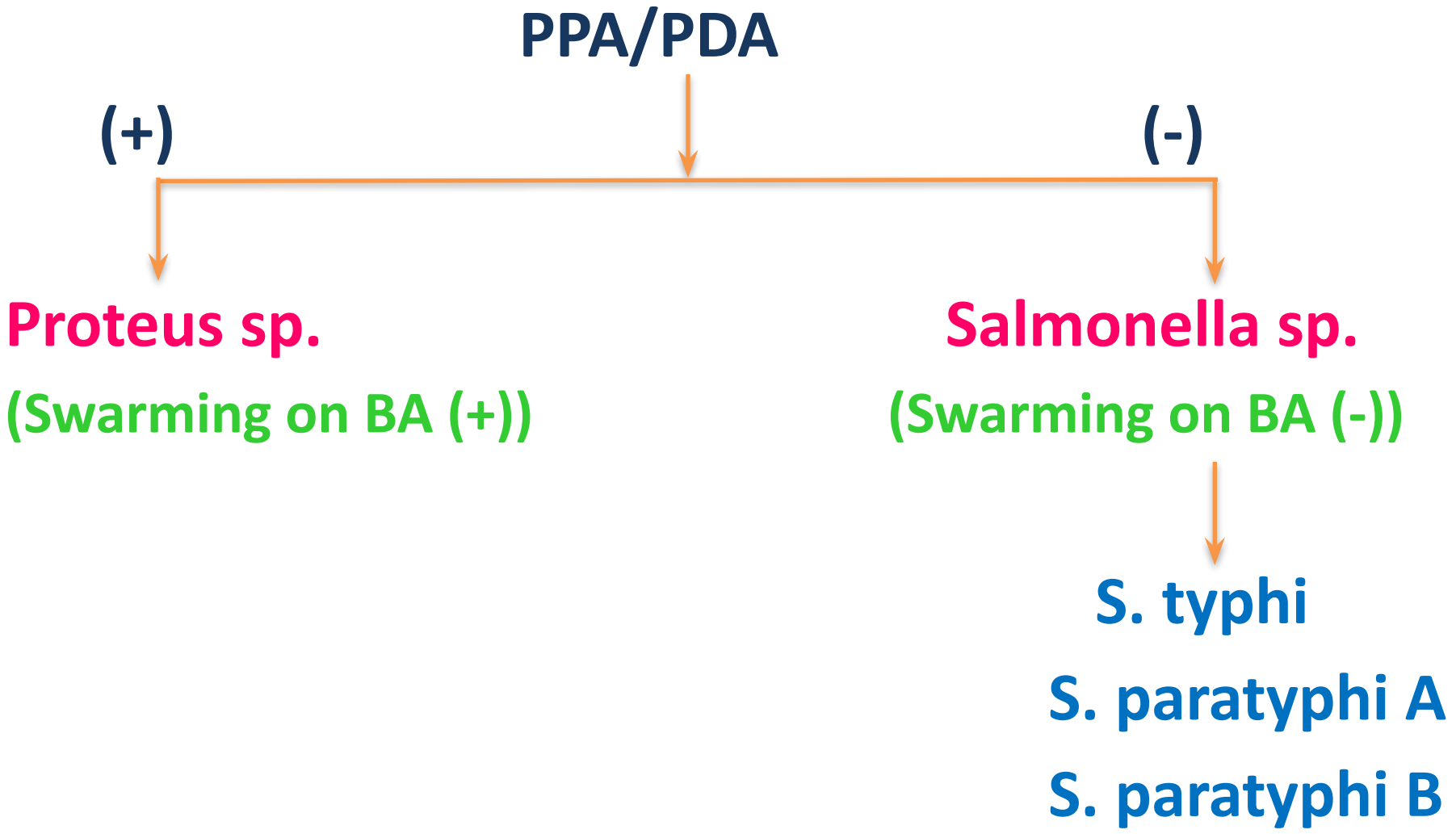
So it is one of the members of Enterobacteriaeceae

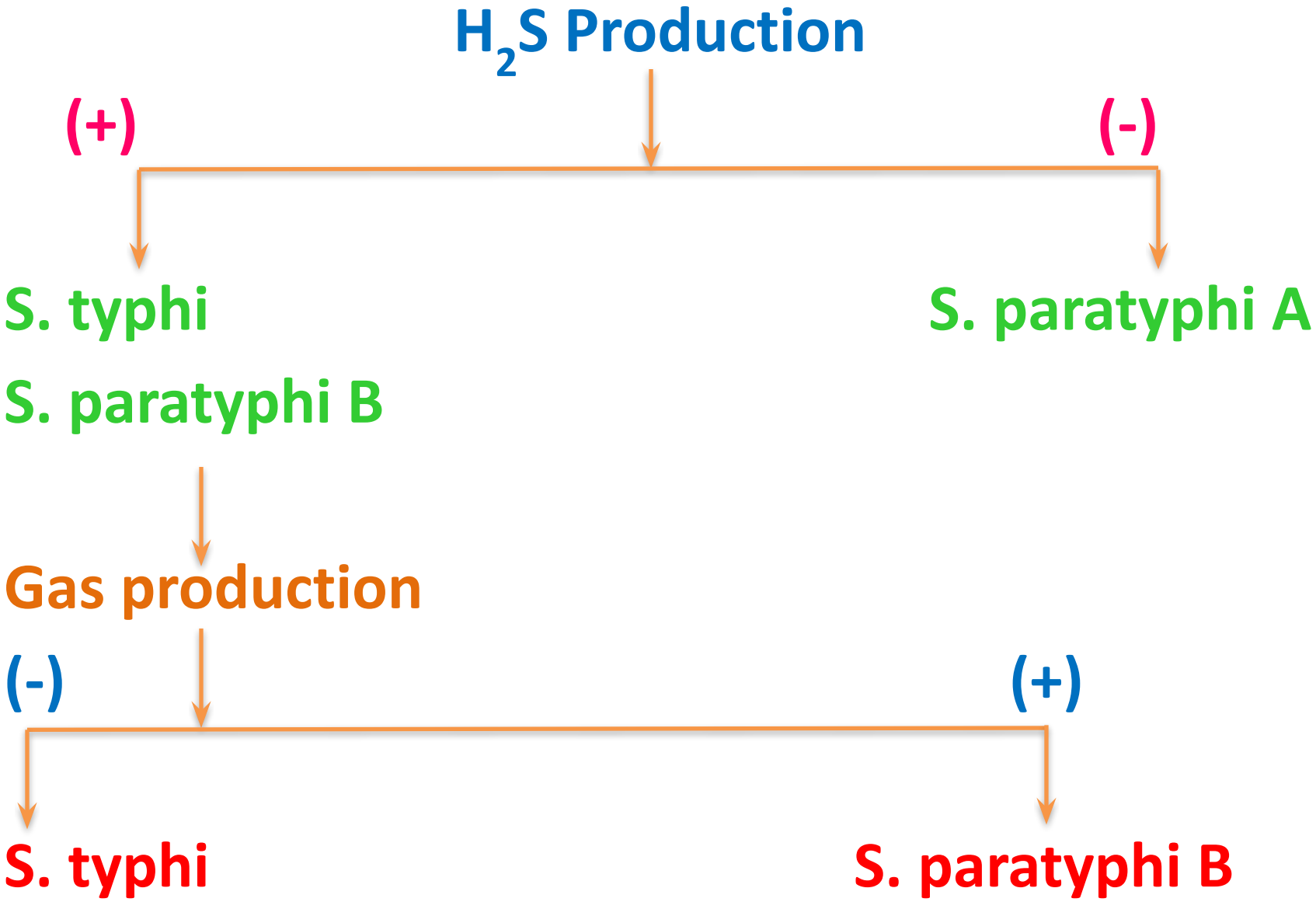
On Mac – NLF colonies

Being motile

It may not be Shigella (being non-motile)

It may be Salmonella or Proteus


PPA/PDA
(+)
(-)
Proteus sp.
(Swarming on BA (+))
Salmonella sp.
(Swarming on BA (-))
S. typhi
S. paratyphi A
S. paratyphi B


H_2S Production
(+)
(-)
S. typhi
S. paratyphi B
S. paratyphi A
Gas production
(-)
(+)
S. typhi
S. paratyphi B

Another way of differentiation of Salmonella species

- **Remember S. paratyphi B alone is citrate positive.**
- **Remember S. paratyphi A and S. paratyphi B both are xylose and arabinose positive**
- **Remember S. typhi is both xylose and arabinose negative.**

Differentiation of Salmonella species also may be possible by TSI reaction

S. typhi

K/A, Gas (-), speck of H_2S (+)

S. paratyphi A

K/A, Gas (+), H_2S (-)

S. paratyphi B

K/A, Gas (+), abundant H_2S (+)

Other biochemicals for Salmonella species

Indole – (-)

MR – (+)

VP – (-)

Citrate – (only S. paratyphi B positive)

Urease – (-)

IDENTIFICATION OF GNB – VIII

PSEUDOMONAS SPECIES

- Gram stain – Gram negative bacilli
- Motility – Motile
- Catalase – Positive
- Oxidase – Positive

So it is one of the members of

Non-enterobacteriaeceae like

Pseudomonas species

Vibrio species

On MAC – NLF colonies (irregular)

On NA – Bluish green (pyocyanin) pigmentation

OF –TEST – Oxidative reaction seen

It is more likely to be Pseudomonas aeruginosa

(because it is only pseudomonas species produce such type of bluish green pigment)

It may not be Vibrio species because it is fermentative in OF-TEST and not produce bluish green pigment

Other biochemicals for Pseudomonas aeruginosa

Indole – (-)

MR – (-)

VP – (-)

Citrate – (+)

Urease – (-)

TSI - K/K (or) K/no change, Gas (-), H_2S (-)

Nitrate reduction test – (+)

IDENTIFICATION OF GNB – IX
VIBRIO AND ASSOCIATED SPECIES

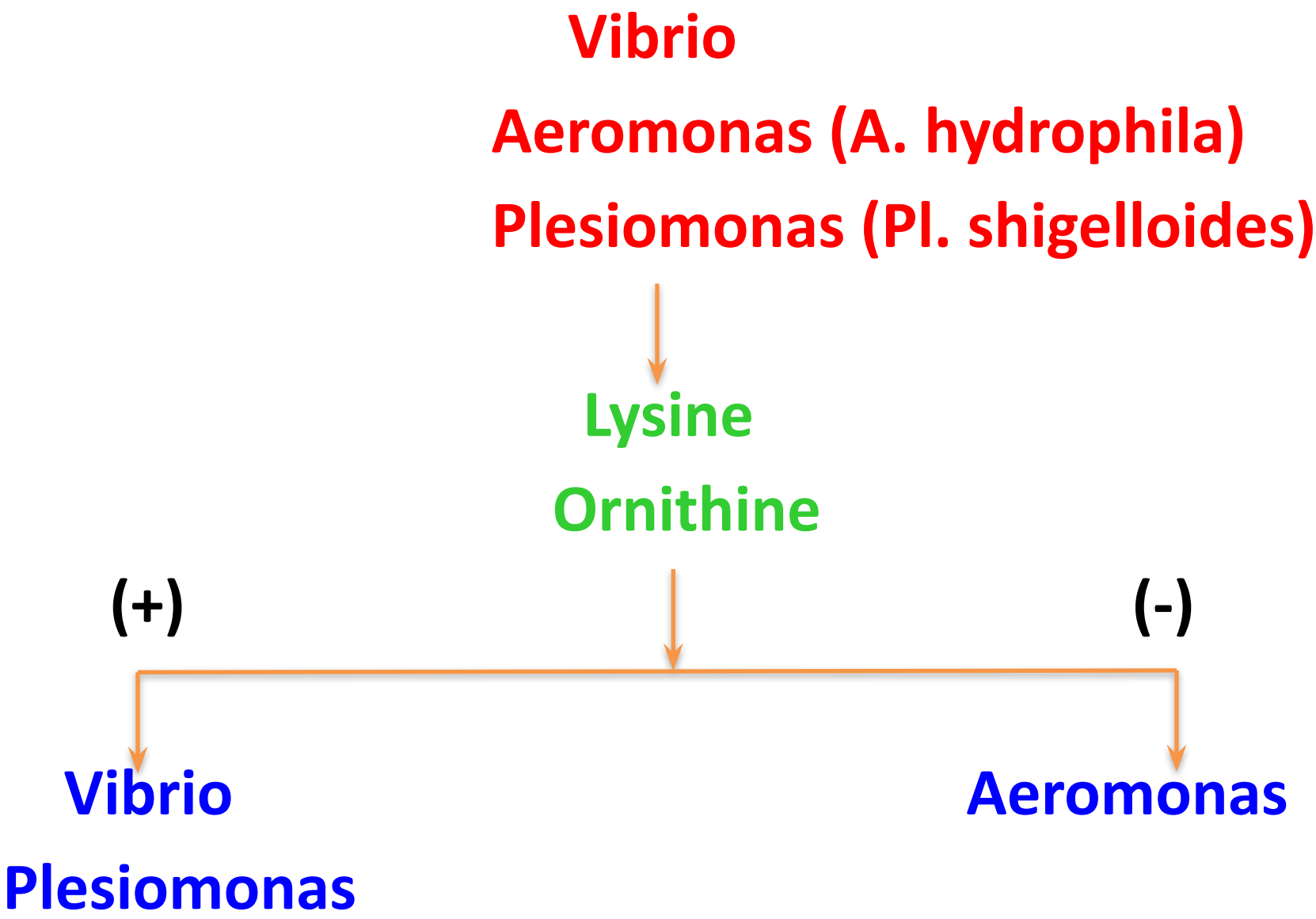
- Gram stain – Gram negative bacilli
- Motility – Motile (Darting motility)
- Catalase – Positive
- Oxidase – Positive

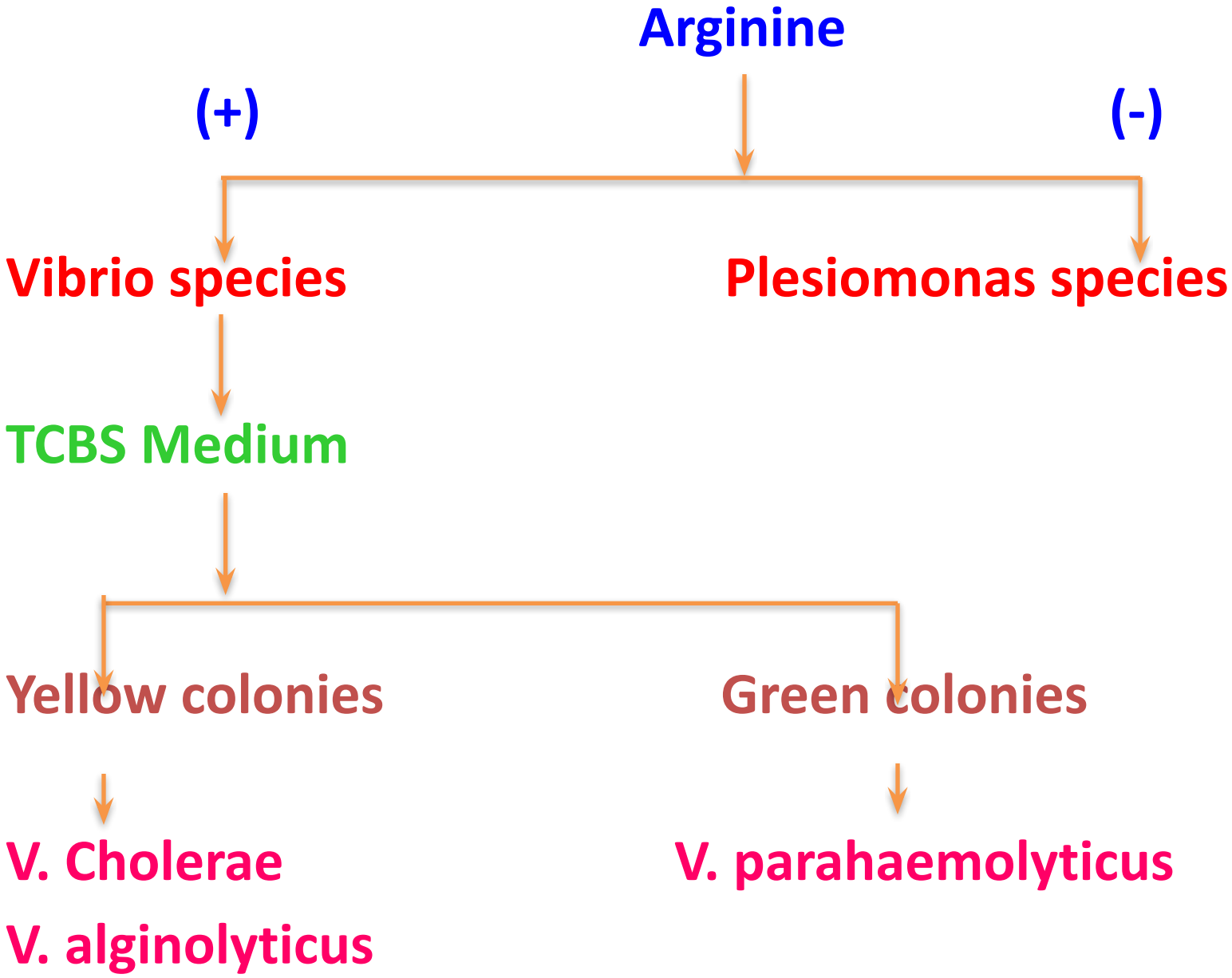
So it is one of the members of

Non- Enterobacteriaeceae

It may be Vibrio (or) Aeromonas (or) Plesiomonas

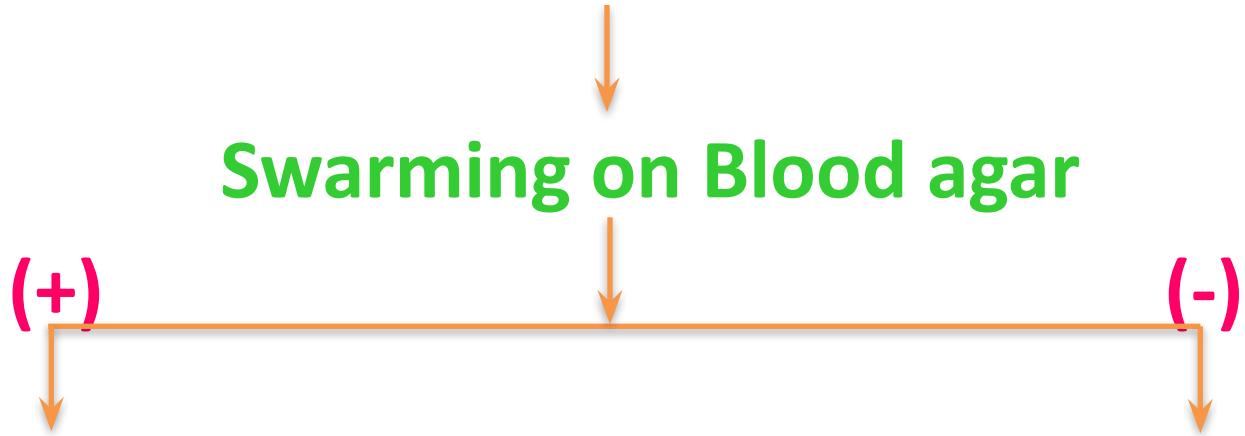
It may not Pseudomonas because no bluish green pigment seen


Vibrio
Aeromonas (A. hydrophila)
Plesiomonas (Pl. shigelloides)
Lysine
Ornithine
(+)
(-)
Vibrio
Plesiomonas
Aeromonas


Arginine
(+)
(-)
Vibrio species
Plesiomonas species
TCBS Medium
Yellow colonies
Green colonies
V. Cholerae
V. alginolyticus
V. parahaemolyticus

Vibrio cholerae

Vibrio alginolyticus



V. alginolyticus

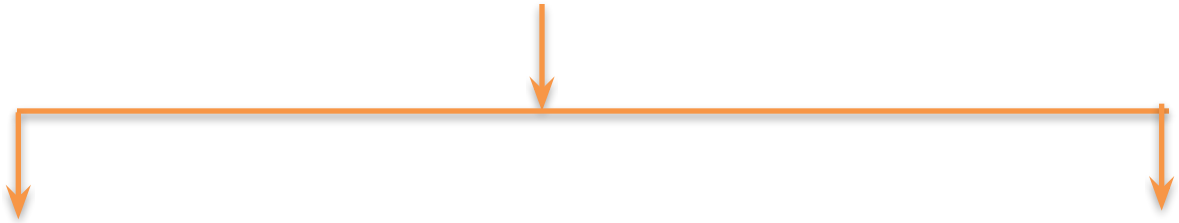
V. cholerae

Two biotypes

Classical

ElTor

Vibrio Cholerae



Classical biotype

VP – (-)

Non- haemolytic

Chick RBC agglutination (-)

ElTor biotype

VP – (+)

Haemolytic

Chick RBC agglutination (+)

Specific tests for Vibrio cholerae

- String test – (+)
- Cholera Red Reaction – (+)
- Gelatin liquefaction – (+)

Other reactions of Vibrio cholerae

MR – (-)

VP – (+/-)

Citrate (+)

Urease – (-)

TSI – A/A, Gas (-), H_2S (-)

Serotyping of Vibrio cholerae

Vibrio cholerae 01 antiserum helps to identify Vibrio cholerae O1 strain. This is followed by identification of specific subtypes like Inaba (or) Ogawa (or) Hikojima by using specific antisera.

- **This presentation was created for those who are working as a laboratory technician in clinical microbiology diagnostics.**
- **Also may find useful for UG, PG, DMLT, PGDMLT in Microbiology.**

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