

IDENTIFICATION OF PATHOGENIC BACTERIA IN CLINICAL MICROBIOLOGY LABORATORY

G.HARIPRASAD M.Sc.,M.Phil.,Ph.D

Department of Microbiology

Thoothukudi Govt. Medical College

Thoothukudi

Identification of Gram Positive Cocci(I)

Staphylococcus species

Gram stain – Gram positive cocci in clusters

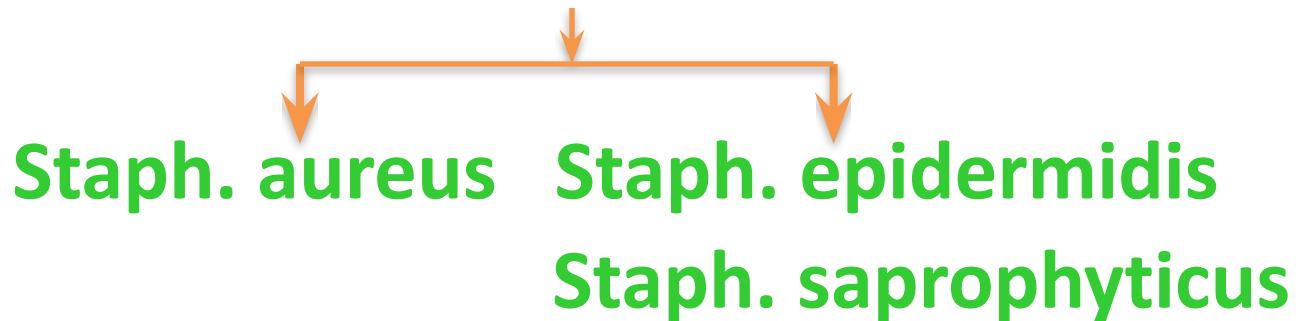
Motility – Non-motile

Catalase – Positive

Oxidase – Negative

So it is Staphylococcus

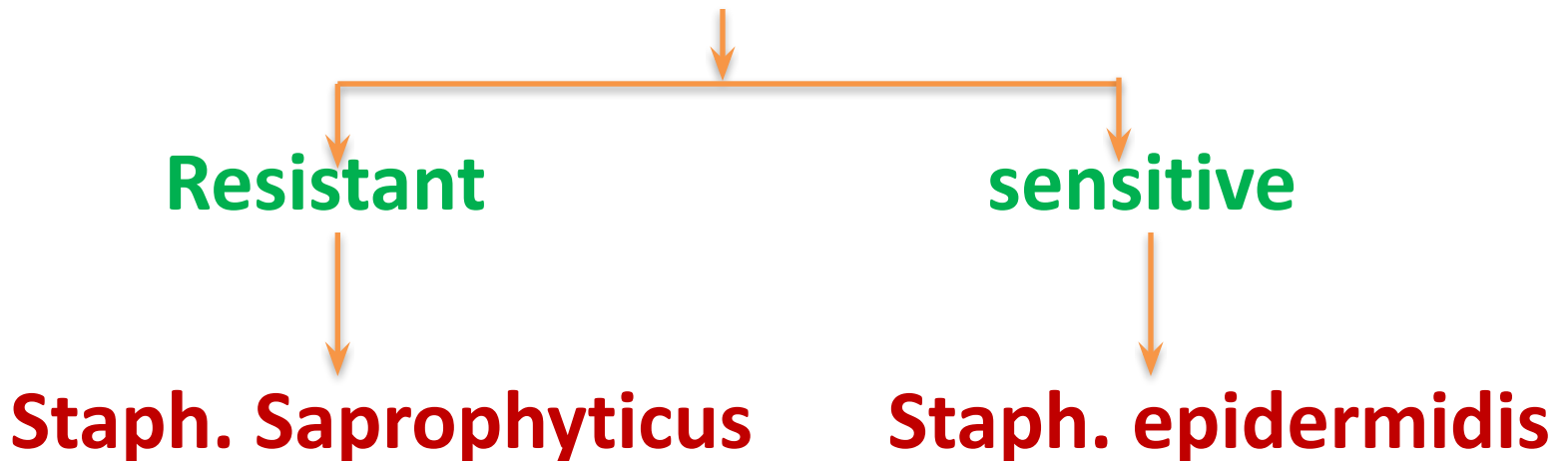
Coagulase Test



Identification of Gram Positive Cocci(I)

Staphylococcus species – cont.,

Novobiocin sensitivity



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)**

Optochin sensitivity test

(Sensitive)

(Resistant)

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.

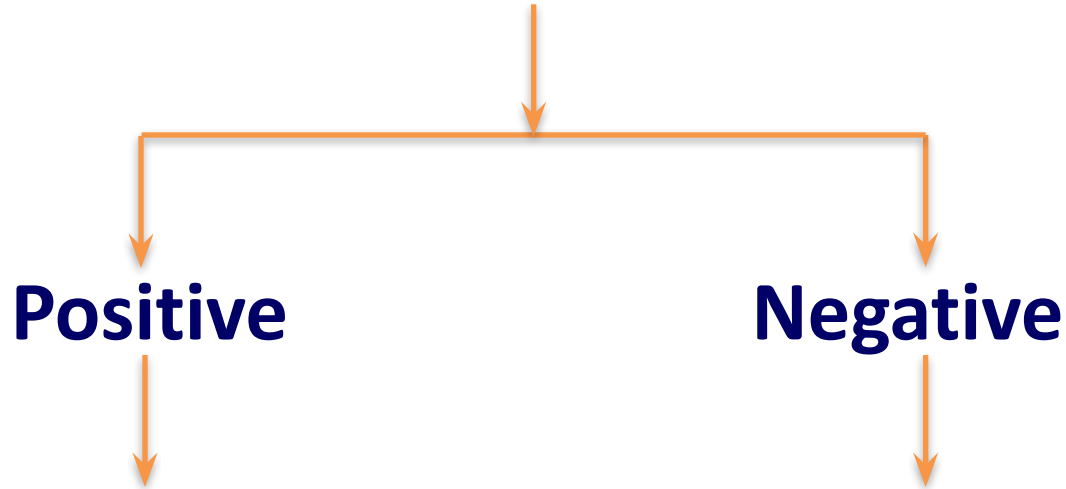
Viridans streptococcus and Streptococcus pneumoniae are always alpha-haemolytic.

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



Group D streptococcus

Enterococcus

(Grow in MacConkey's agar)

(Tiny deep pink (LF) colonies)

Negative

Pneumococcus

Viridans streptococci

(Not Grow in MacConkey's agar)

Growth in 6.5% salt (Salt tolerance test)

Positive (Growth)

Negative (No growth)

Enterococcus

Group D streptococcus

PYR

(+ve)

(-ve)

Sensitivity

(R)

(S)

to SXT

Ability to

grow at 45°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)

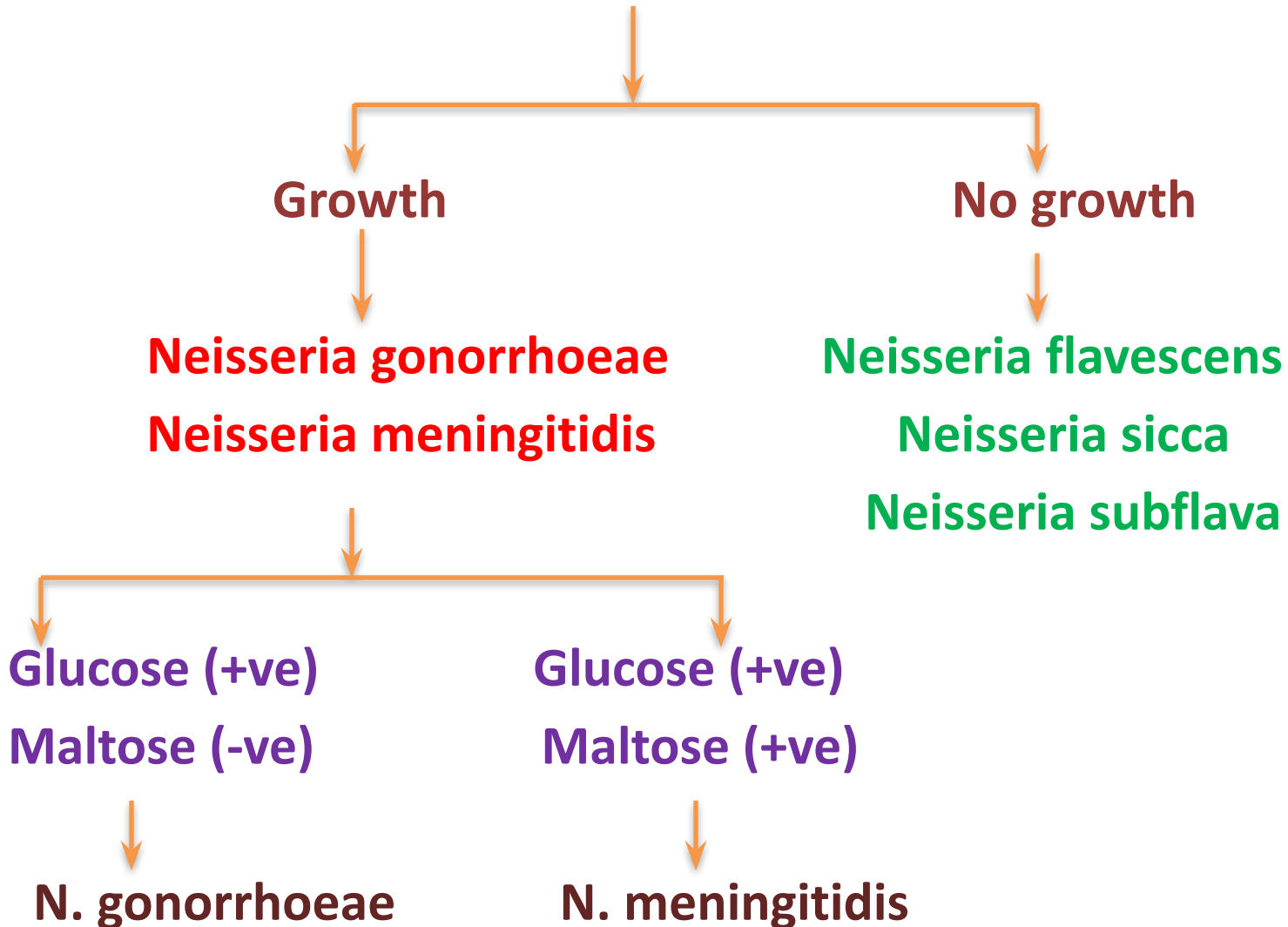
(Neisseria meningitidis)

(Neisseria flavescens)

(Neisseria sicca)

(Neisseria subflava)

Growth on THAYER-MARTIN MEDIUM (selective medium)



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.

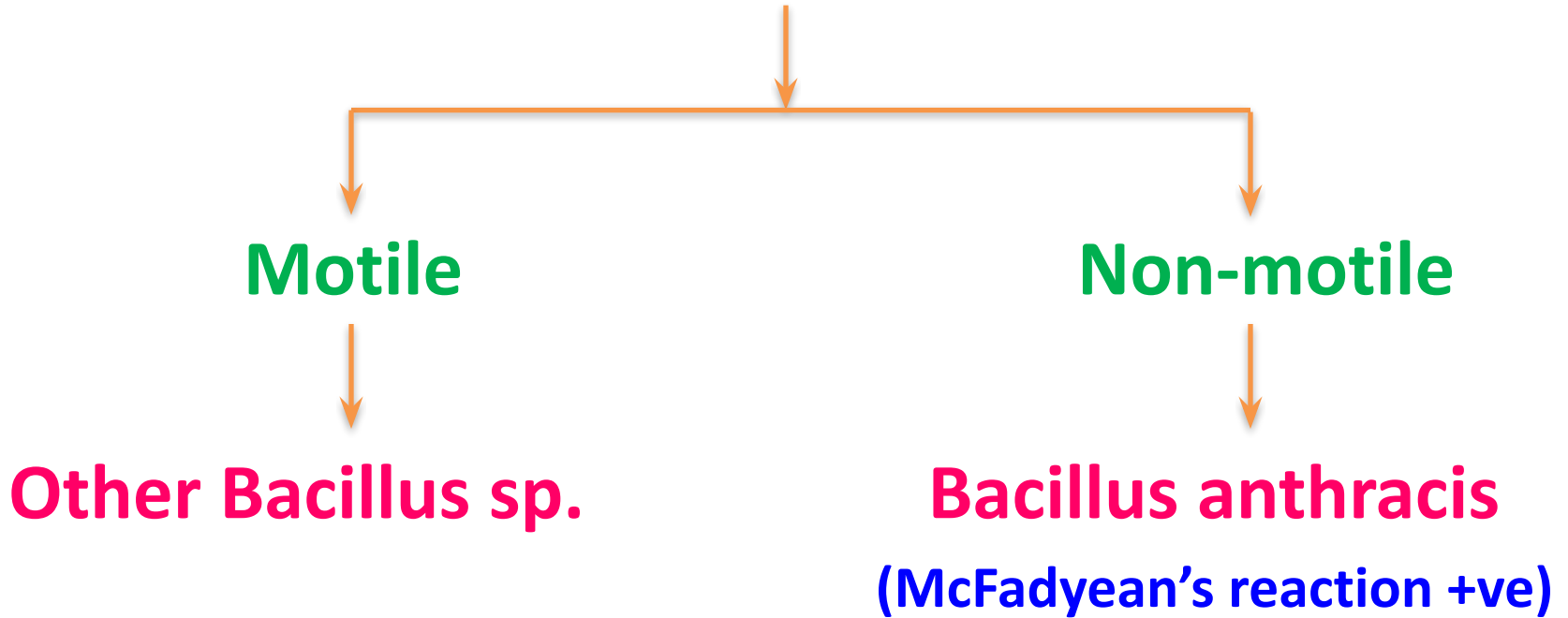
Bacillus

Clostridium

(Aerobic)

(Anaerobic)

Bacillus species



Catalase

(+)

(-)

Corynebacterium

Listeria

kurthia

Erysipelothrix

Lactobacillus

Actinomyces

Beta hemolytic on BA

(Yes)

(Listeria)

(Corynebacterium)

(No)

(Kurthia)

H₂S Production

(+)

Erysipelothrix

(-)

Lactobacillus

Actinomyces

Listeria

Corynebacterium



(Esculin Hydrolysis)



(+)

(-)



Listeria

Corynebacterium

(Motile at 25⁰C)

(Non-motile)

(Non-Motile at 37⁰C)

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

Diphtheroids, morphologically similar to C. diphtheriae can grow on ordinary media like Nutrient agar. But Corynebacterium diphtheriae 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

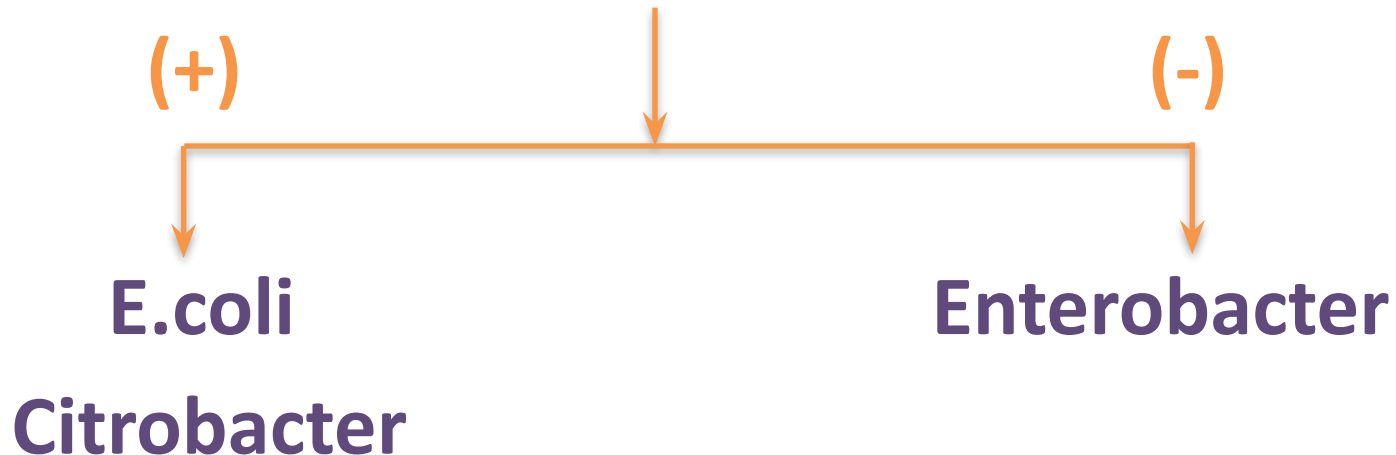
Motility – Motile

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

Klebsiella being non-motile, it is omitted

Indole test

Methyl-Red test



Citrate test

(+)

(-)

Citrobacter

E.coli

Serratia

Citrobacter

Serratia

(No red pigment)

(Red pigment)

IMVIC REACTIONS FOR E.COLI

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

Other biochemical reactions:

TSI – A/A, Gas (+), H₂S (-)

Urease – (-)

Remember:

E.Coli is the most common 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 – Muroid 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

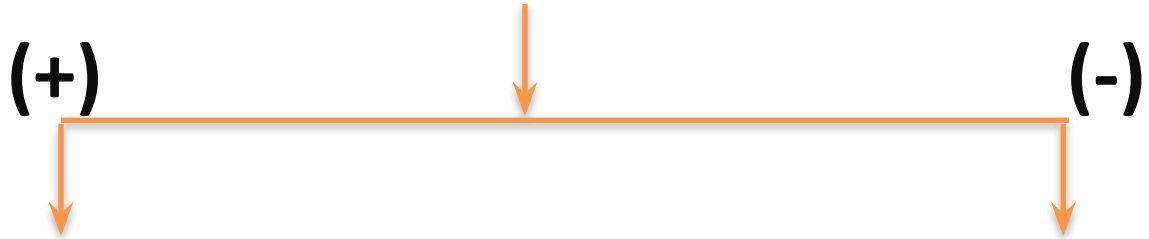
Klebsiella pneumoniae

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

Malonate

(+)

(-)

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 (+), H₂S (-)

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



(+)

(-)

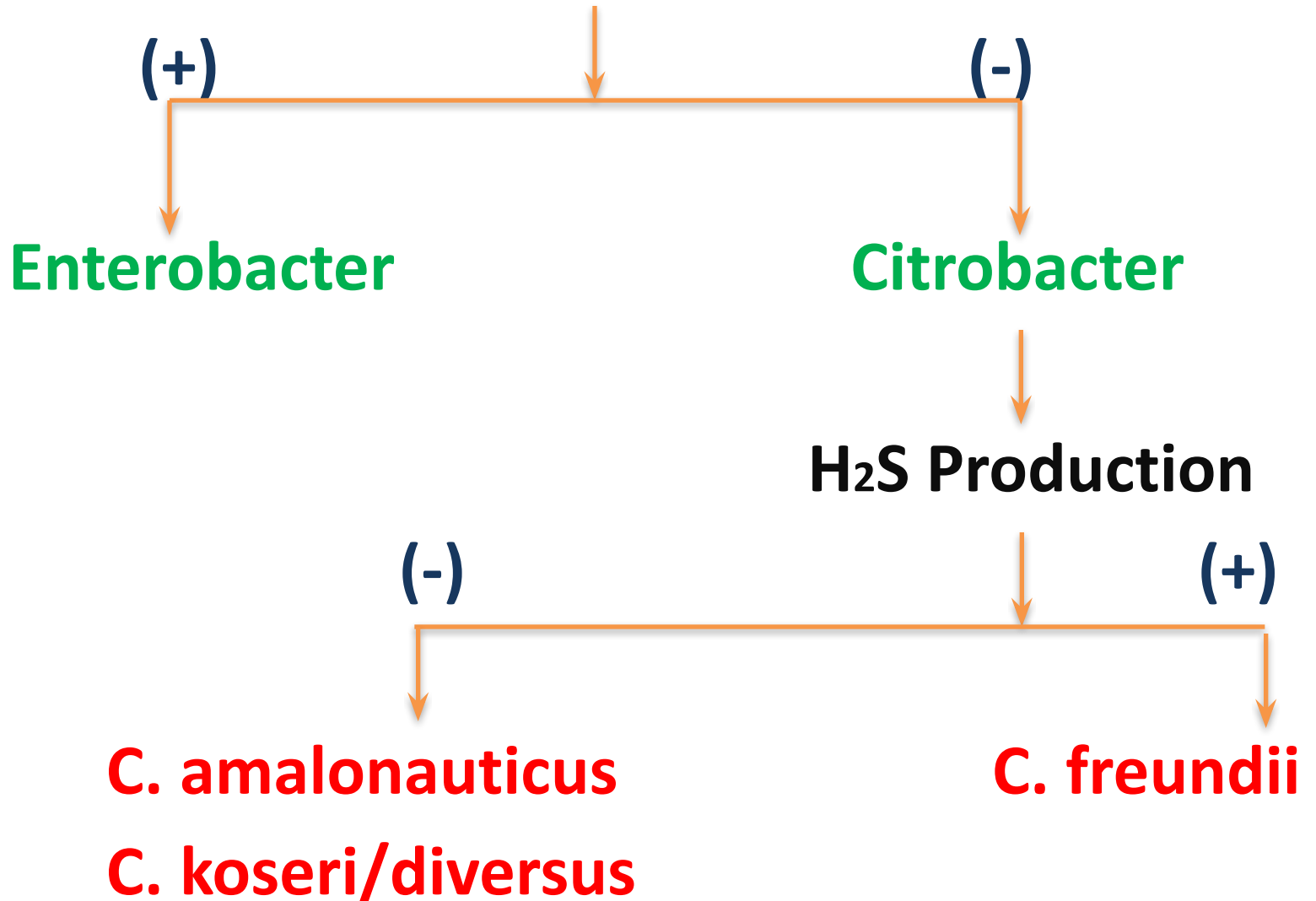


Citrobacter

E. coli

Enterobacter

VP TEST



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), H₂S (+/-)

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

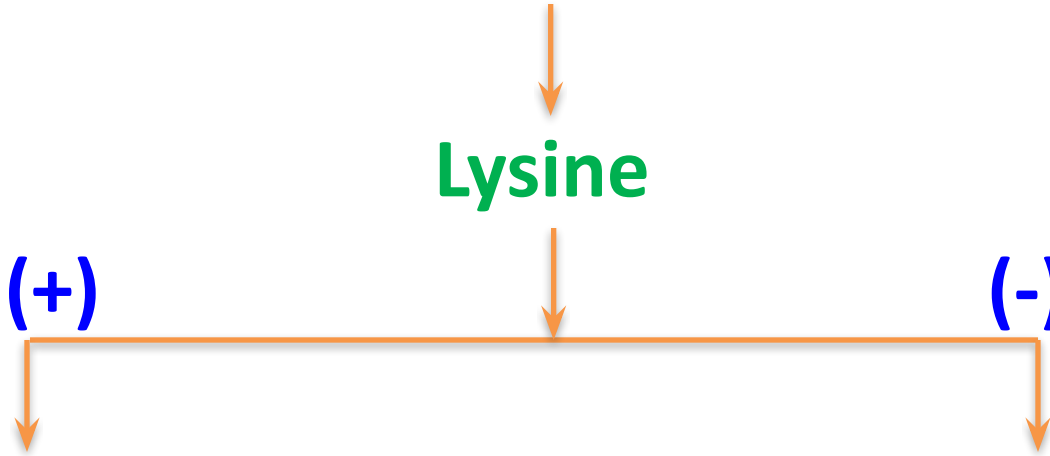
Lysine

(+)

(-)

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

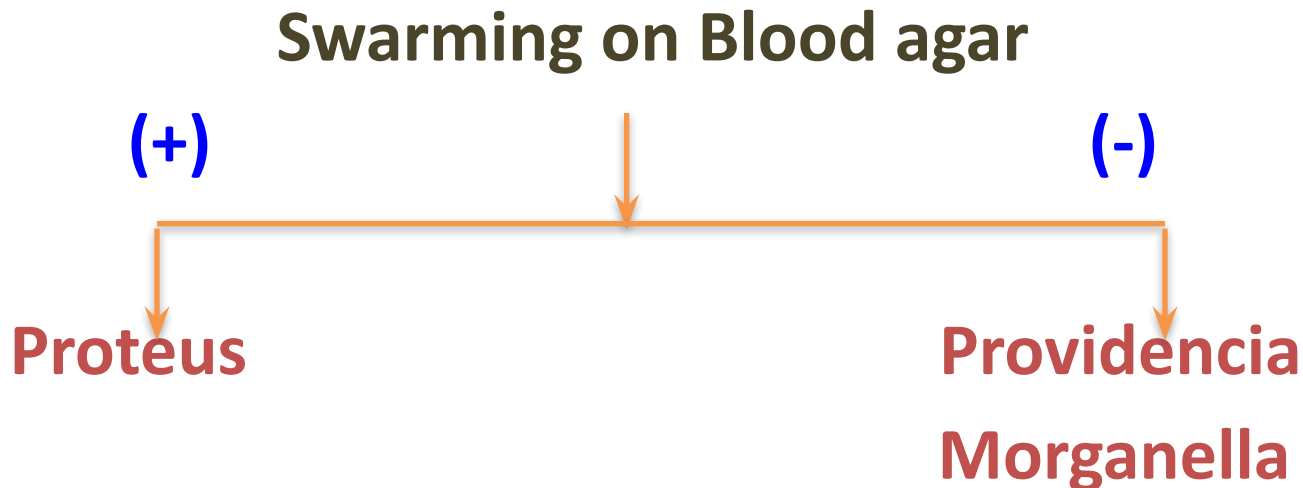
**On Mac – NLF – so E.coli, Citrobacter, Enterobacter are
omitted**

- 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 morganii

Prov. alcalifaciens

Prov. rettgeri

Prov. stuartii

Providencia alcalifaciens

Providencia rettgeri

Providencia stuartii



Gas from glucose



(-)

(+)



Prov. rettgeri

Prov. stuartii



Prov. alcalifaciens

Prov. rettgeri

Prov. Stuartii



Fermentation of trehalose



(+)

(-)



Prov. stuartii

Prov. rettgeri

Other biochemicals for Proteus species

MR – (+)

VP – (-)

Urease - (+)

Citrate – (-)

TSI – K/A, Gas(+), H₂S (+)

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 (-), H₂S (-)

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 Enterobacteriaceae

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

H₂S Production

(+)

(-)

S. typhi

S. paratyphi A

S. paratyphi B

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 H₂S (+)

S. paratyphi A

K/A, Gas (+), H₂S (-)

S. paratyphi B

K/A, Gas (+), abundant H₂S (+)

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-enterobacteriaeaceae 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 (-), H₂S (-)

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- Enterobacteriaceae

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

Aeromonas

Plesiomonas

Arginine

(+)

(-)

Vibrio species

Plesiomonas species

TCBS Medium

Yellow colonies

Green colonies

V. Cholerae

V. parahaemolyticus

V. alginolyticus

Vibrio cholerae
Vibrio alginolyticus

Swarming on Blood agar

(+)

(-)

V. alginolyticus

V. cholerae

Two biotypes

Classical

ElTor

Vibrio Cholerae

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graph TD; A[Vibrio Cholerae] --> B[Classical biotype]; A --> C[ElTor biotype]
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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 (-), H₂S (-)

Serotyping of Vibrio cholerae

Vibrio cholerae O1 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.**

Mail ID:

hprasad411@gmail.com