Diagnosis of puerperal sepsis and diagnosis of organisms causing spontaneous and recurrent abortion.

- Module (Reproductive systems)

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Learning outcomes:

By the end of this practice the students will be able to :

1. Define puerperal sepsis and list the causative organisms.
2. Recognize how to identify aerobic and anaerobic causative organisms of puerperal sepsis in the lab.
3. Describe the proper sample for diagnosis of puerperal sepsis.
4. Recognize the approach to diagnose bacterial and viral causes of spontaneous and recurrent abortion.
5. Identify samples, processing of and selection of proper culture media

Laboratory diagnosis of organisms causing puerperal sepsis :

- S. Pyogens (the major cause).
- Coliform bacilli.
- S. aureus.
- Mycoplasma hominis.
- Anaerobic infections

- Presenting Complaint: Emily Johnson, a 28-year-old woman who recently gave birth to her first child, presents to the emergency department with complaints of fever, abdominal pain, and foul-smelling vaginal discharge. She delivered a healthy baby girl vaginally 5 days ago. Emily noticed the symptoms starting 2 days postpartum but initially dismissed them as normal postpartum changes.

- Diagnostic Workup:

1. Blood tests reveal an elevated white blood cell count (WBC).

2. Cultures of blood and vaginal discharge are obtained for microbiological analysis.

3. Imaging studies, such as ultrasound, may be performed to assess the condition of the uterus and surrounding structures.

- **What is your probaple diagnosis?**

Pureperal sepsis

Definition:

It is uterine infection during peurperium (28 days after labour).

Cansative organisms:

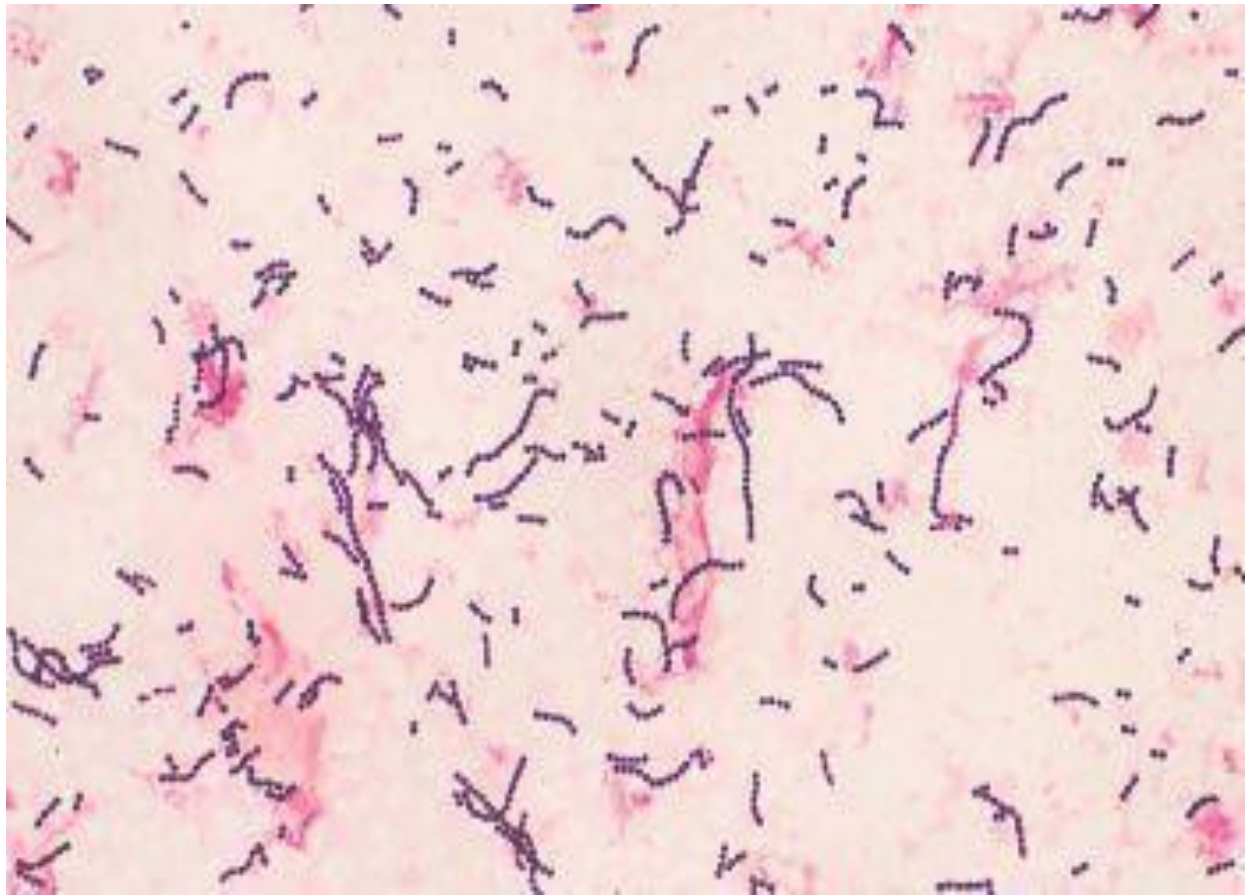
a) S. Pyogens (the major cause).
b) Coliform bacilli
c) S. aureus.
d) Mycoplasma hominis.
e) Anaerobic infections.

Diagnosis of puerperal sepsis

- Sample: cervical or vaginal swab

A- Group A Beta-hemolytic Streptococci (S. pyogenes)

1- Direct film stained with Gram stain for characteristic morphology



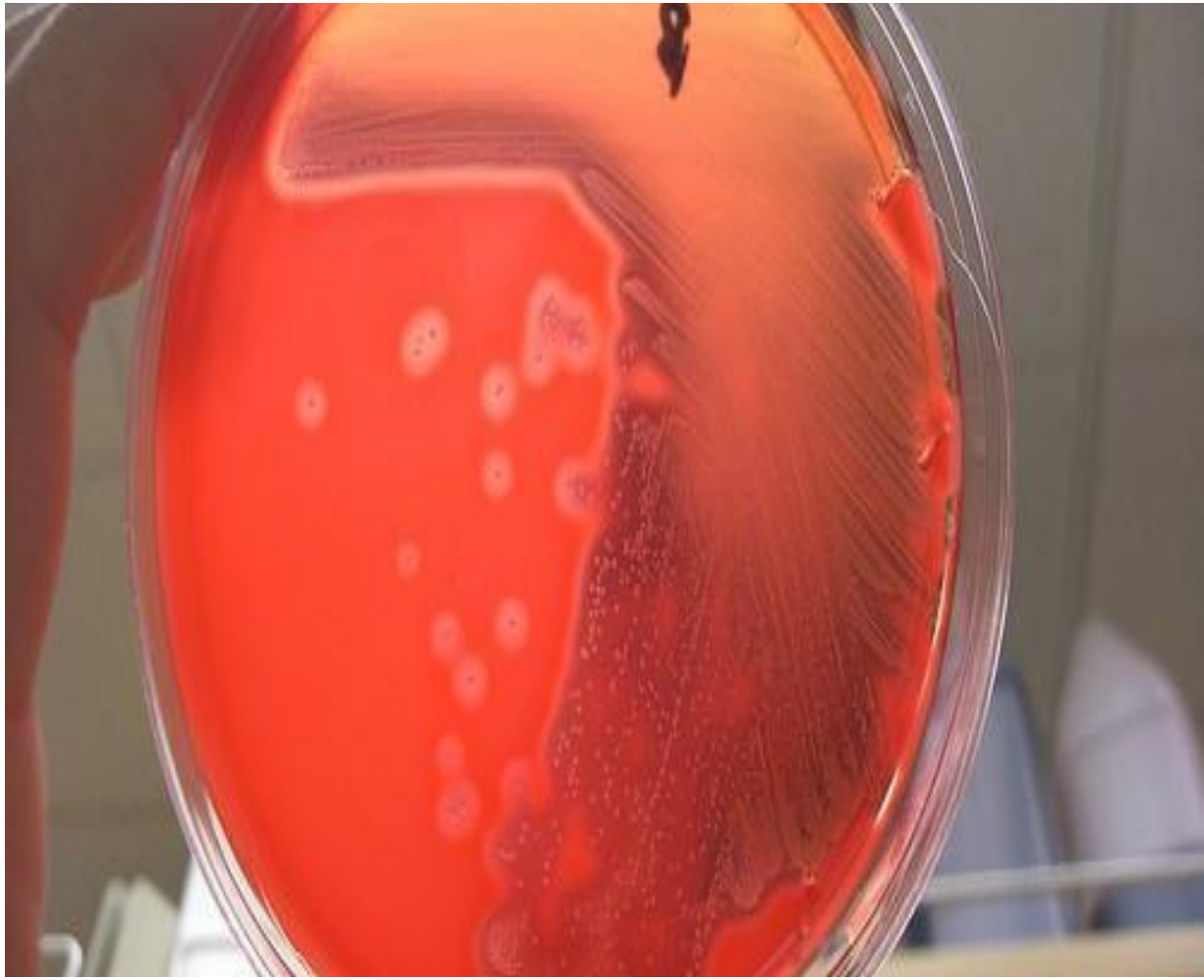
Morphology:

- Gram positive cocci
- Arranged in long chains
- Non spore forming, non motile
- A capsule of hyaluronic acid.

2- Culture

- **Ch.**
  1. Facultative anaerobes.
  2. Can grow in normal atmospheric Co2 concentration,but 10% Co2 enhances growth .
  3. Optimum temperature→ 37 ° C.
- **Media:**
  1. Cannot grow on ordinary media.
  2. On blood agar →β haemolysis.
- Colonies are small (pin point) and translucent.

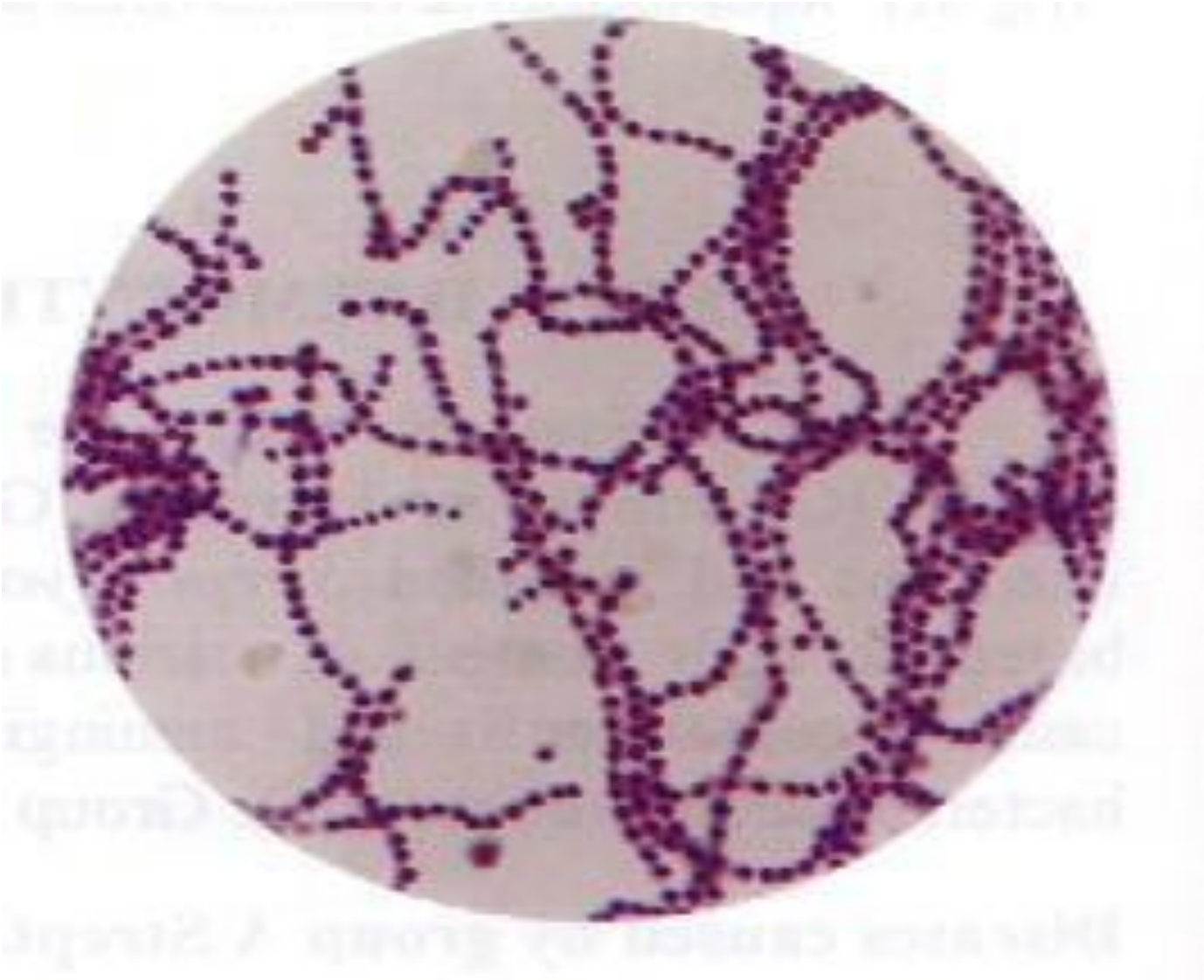
Streptococcus pyogenes on Blood agar



3- Growth can be identified systemically by:

- Film stained by gram: to see the morphology
- BR: Catalase test : negative
- Bacitracin (0.04 μg) sensitivity
- Specific identification of *S. pyogens* can be done by reaction with specific antibodies .

Gram positive cocci arranged in long chains: (S. pyogenes)



Catalase test

Use

Differentiate bacteria that produce catalase enzyme, Staphylococci, from non catalase producing bacteria such as Streptococci.

Principle

- the test is based on that some organisms produce catalase enzyme which breakdown the hydrogen peroxide to oxygen and water.

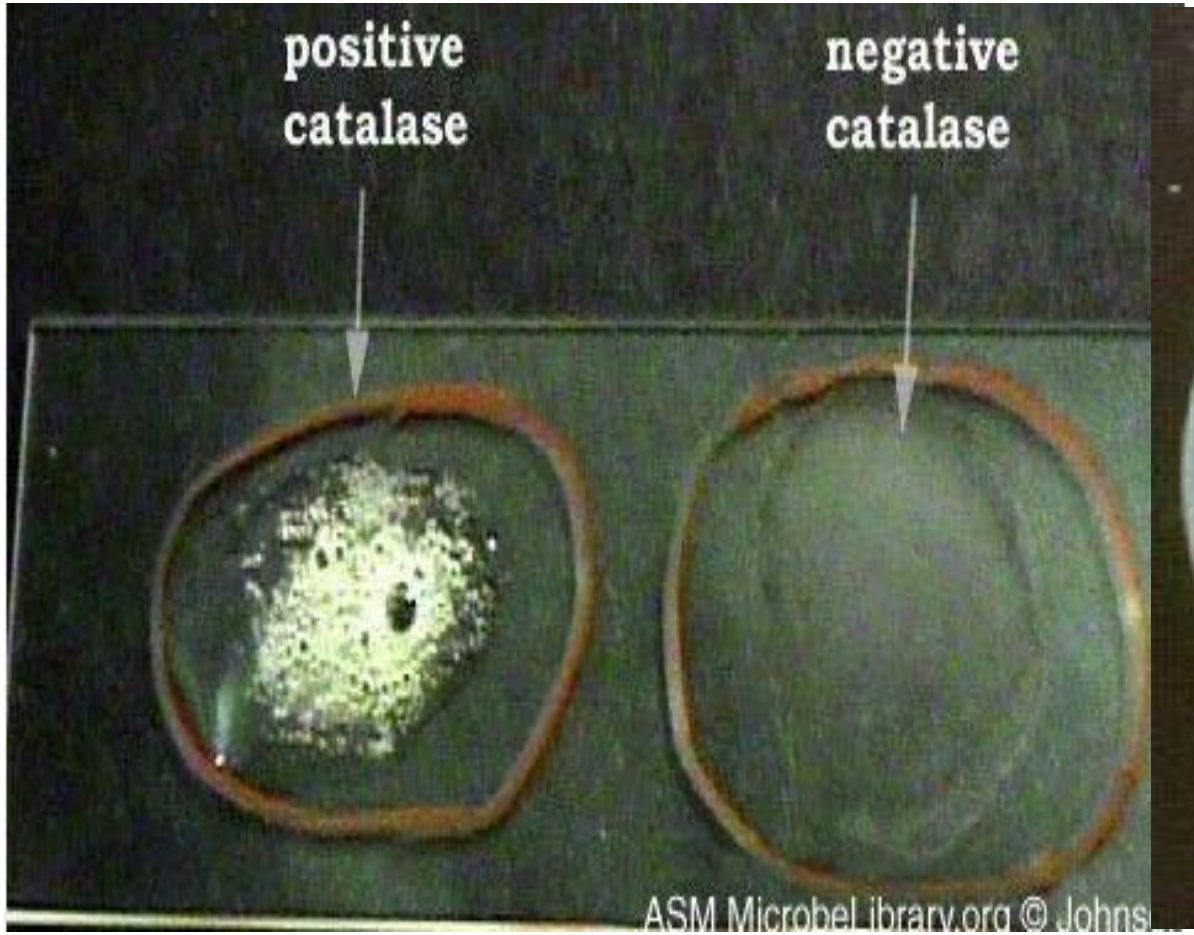
Method

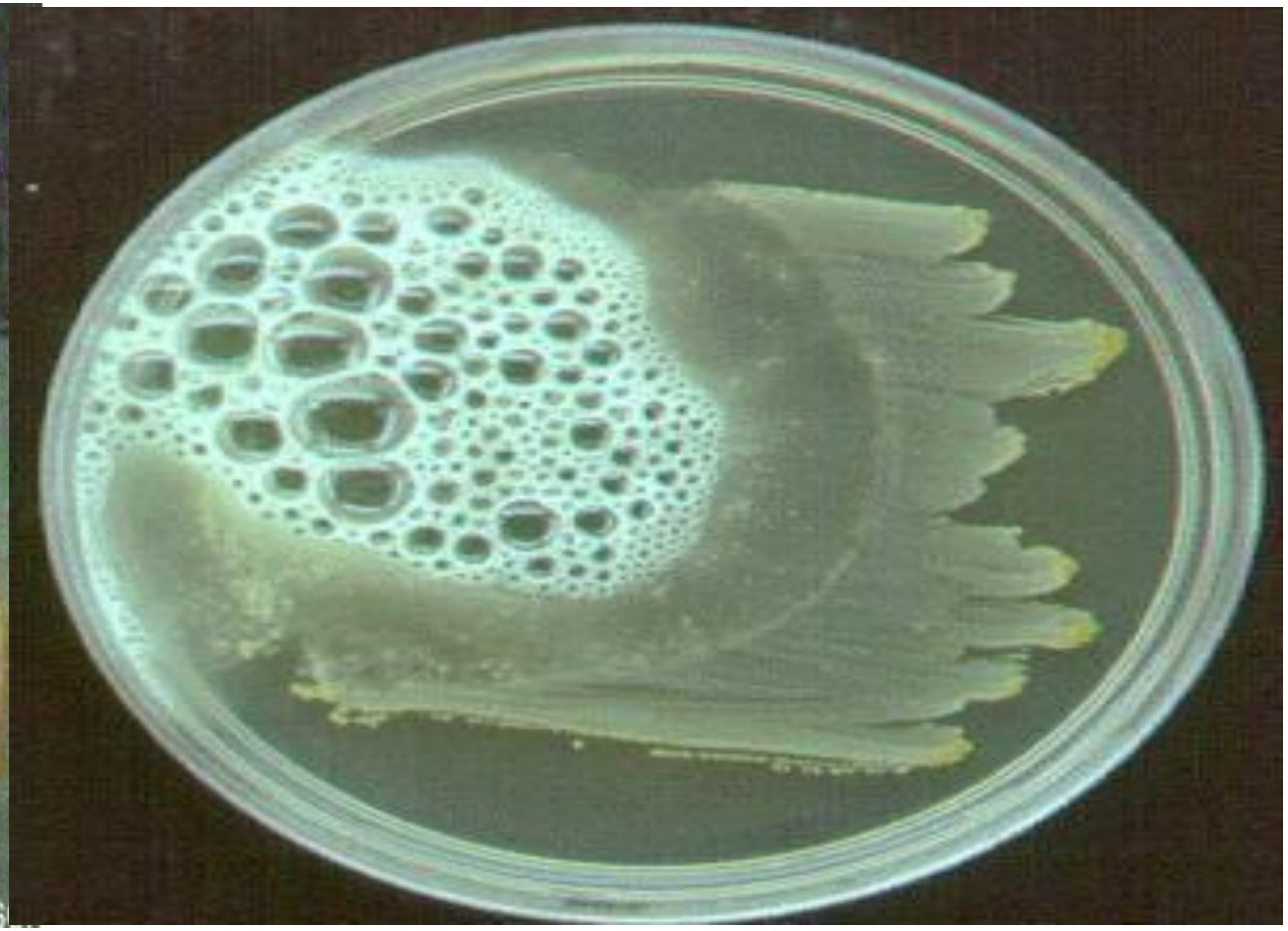
Few drops of 3% hydrogen peroxide solution, are placed on a clean glass slide; colony of the organism is removed and immersed in the hydrogen peroxide solution.

Interpretation

Immediate bubbling indicates positive results.

Catalase test:





Bacitracin sensitivity test

Method:

placing a filter paper disc impregnated with bacitracin on the surface of the culture plate after inoculating it with the organism .

Interpretation:

A zone of inhibition of growth occurs around the disc. differentiate them from other beta hemolytic streptococci which are bacitracin resistant.

Bacitracin sensitivity test

Bacitracin resistant
No zone of inhibition around the disc.
Non group A β-hemolytic Streptococci

Bacitracin Sensitive zone of inhibition around the disc.
S.Pyogenes

B- Staphylococci

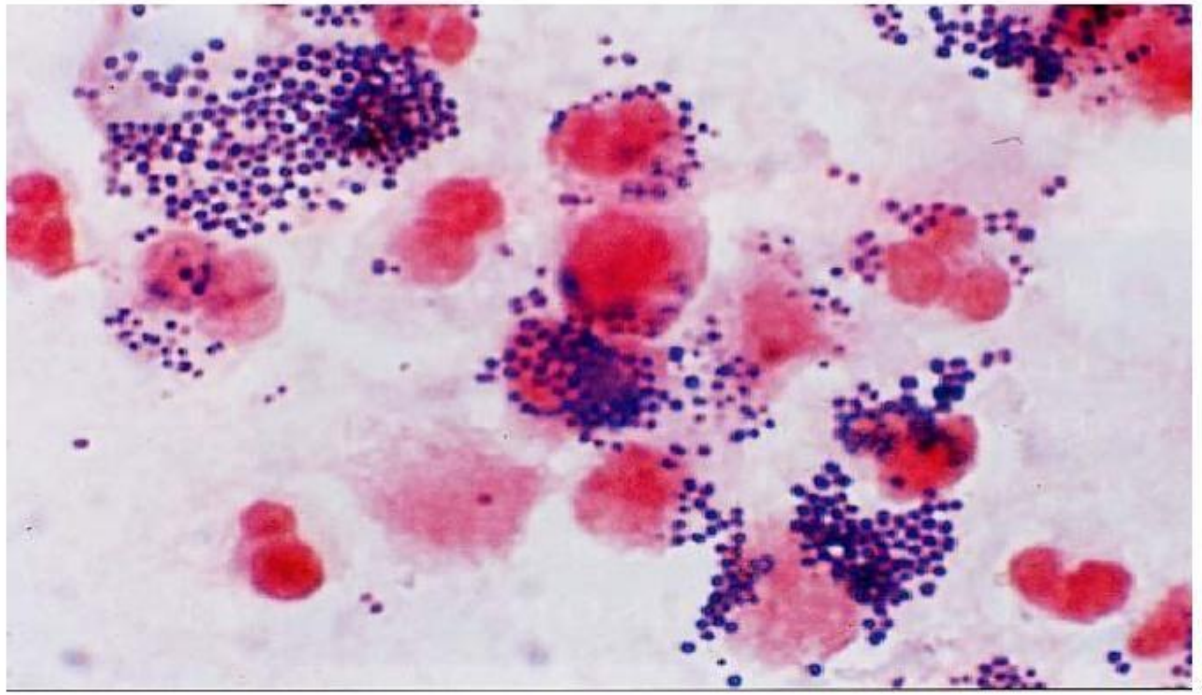
1- Direct film:

Stained with Gram stain for characteristic morphology:

- Gram positive cocci
- arranged in clusters
- non spore forming
- non motile

Gram staining showing Gram positive Cocci in clusters

– Probably Staphylococcus spp



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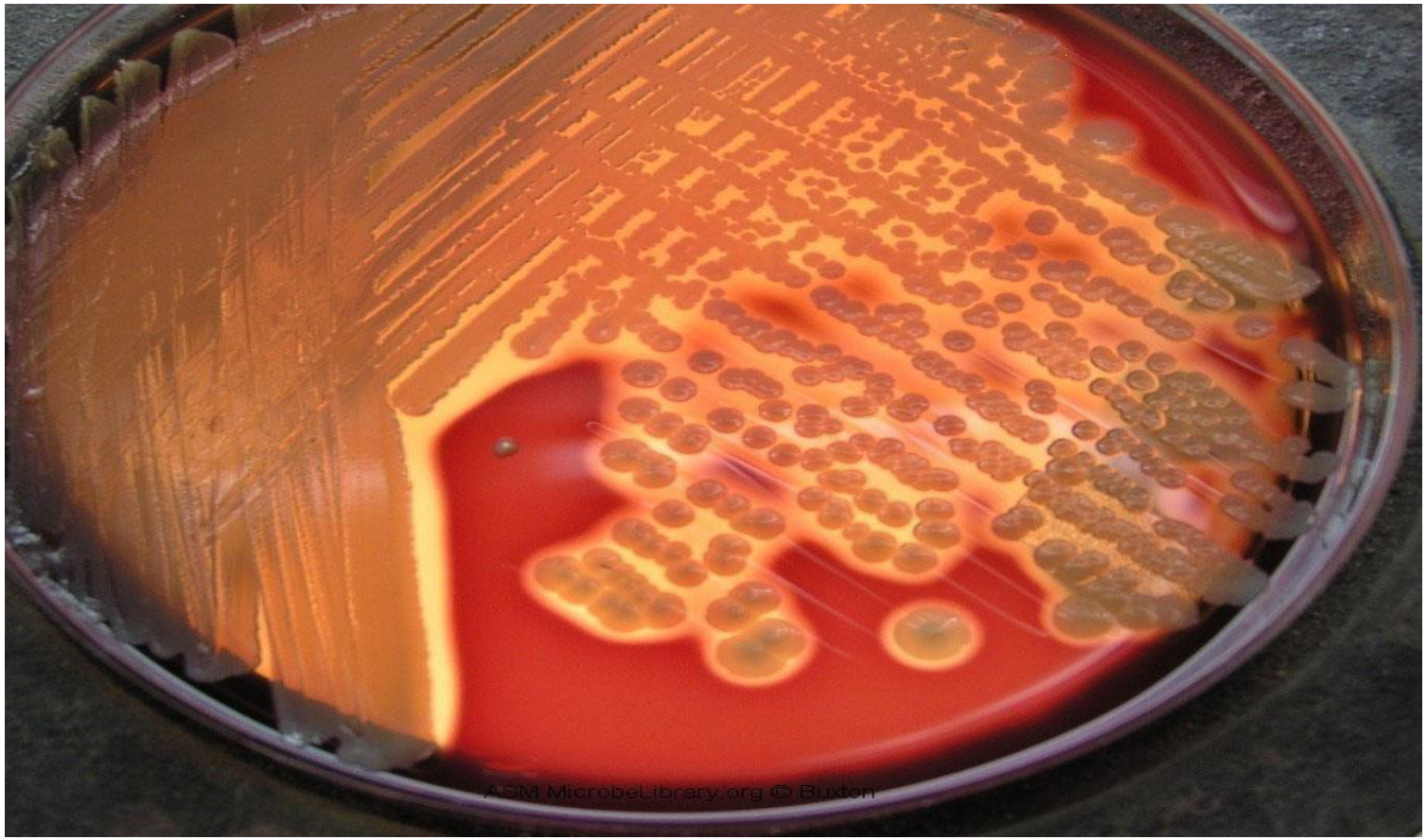
2-Culture:

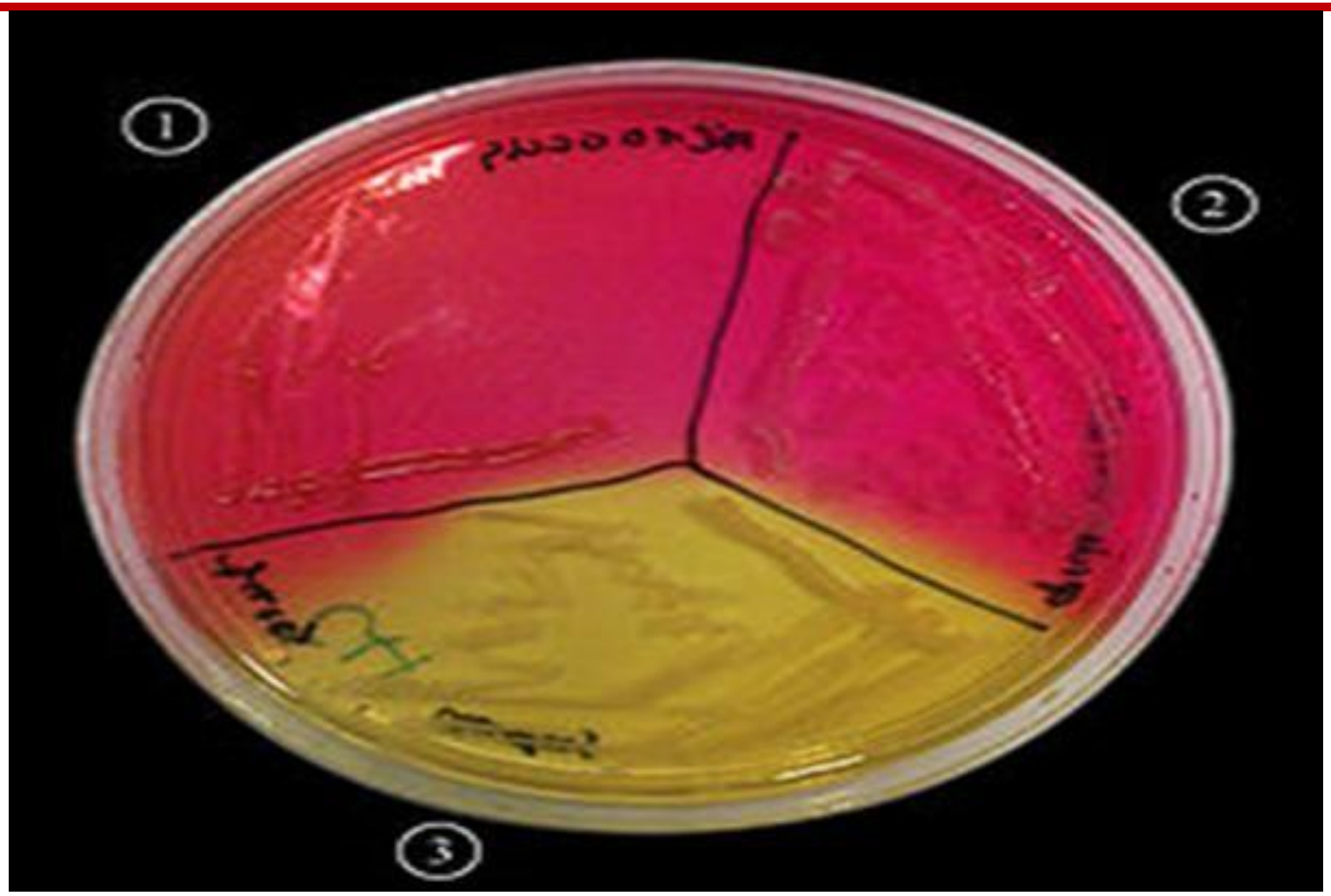
- **Ch.**
  1. Facultative anaerobes.
  2. normal atmospheric Co2 concentration
  3. Optimum temperature→ 37 ºC.
- **Media:**
  1. Can grow on ordinary media → golden yellow endopigments.
  2. On blood agar →beta haemolysis
  3. Mannitol salt agar→ yellow colonies (S. aureus grows in presence of 7.5% sodium chloride and ferment mannitol).

Golden yellow endopigments of *S. aureus*.

Staphylococcus aureus on Blood agar



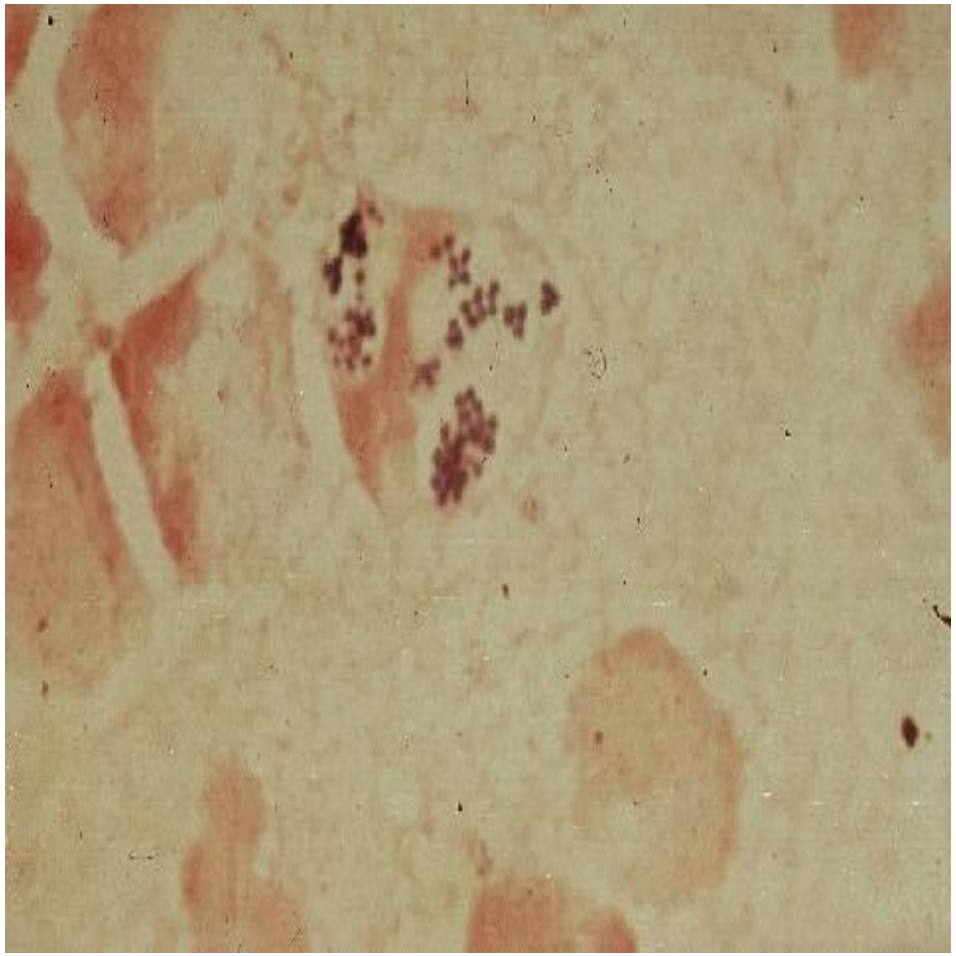
Selective media of S. aureus: Mannitol salt agar



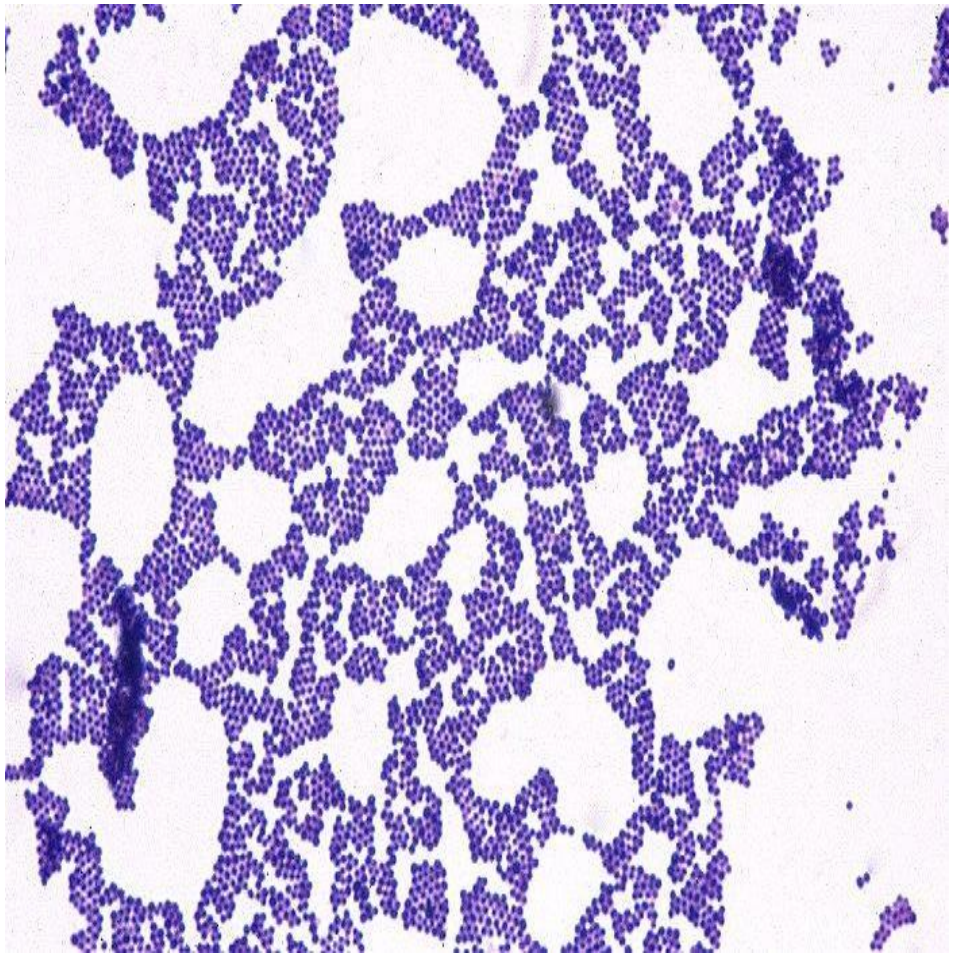
3- Growth can be identified systemically by

- Film stained by gram : to see the morphology
- BR:
  - Catalase: positive (differentiate it from streptococci
  - Coagulase: positive
  - DNase: positive
  - Mannitol fermentation.
- Antimicrobial susceptibility: to select the effective drug.

Staphylococci in pus



Staphylococci in culture



C- Coliform bacilli (lactose fermenter)

Laboratory diagnosis

1 Direct Gram stained film:

- **E.coli, Citrobacter** : Gram negative bacilli, non spore forming ,motile ,most are non capsulated
- **Klebsiella** : non spore forming, non motile and capsulated

2 Culture:

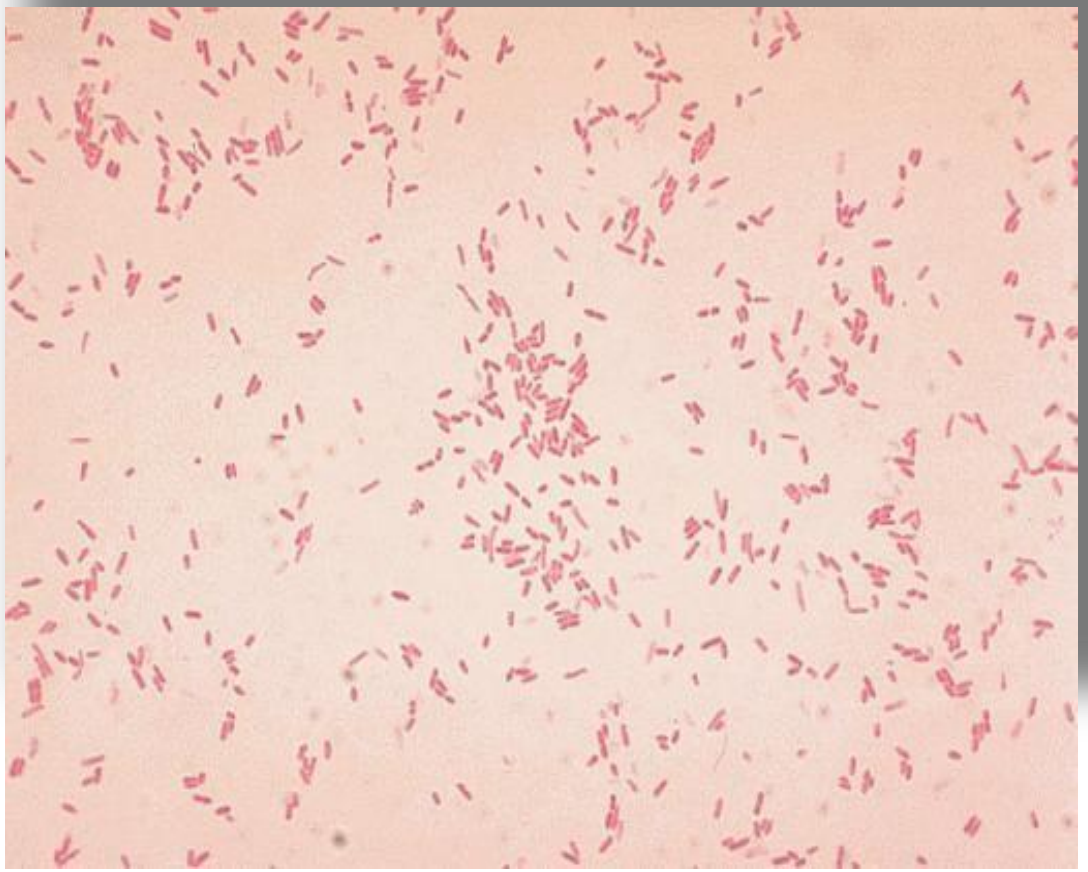
A- **Culture characters**:O2 – CO2 – Temp.

B- **Media**:

-Ordinary media: Grow

-Indicator media: Mac Conkey's →LF →Rose pink

Gram negative bacilli



LF colonies on MacConkey agar medium

Mucoid colonies = capsule

C- Colony identification:

-Colony characters

-Gram stained film from the culture (the previous morphology)

-B.Rs:-Ferment: (glucose, maltose, mannite, lactose and sucrose) with acid and gas production.

-IMVC.

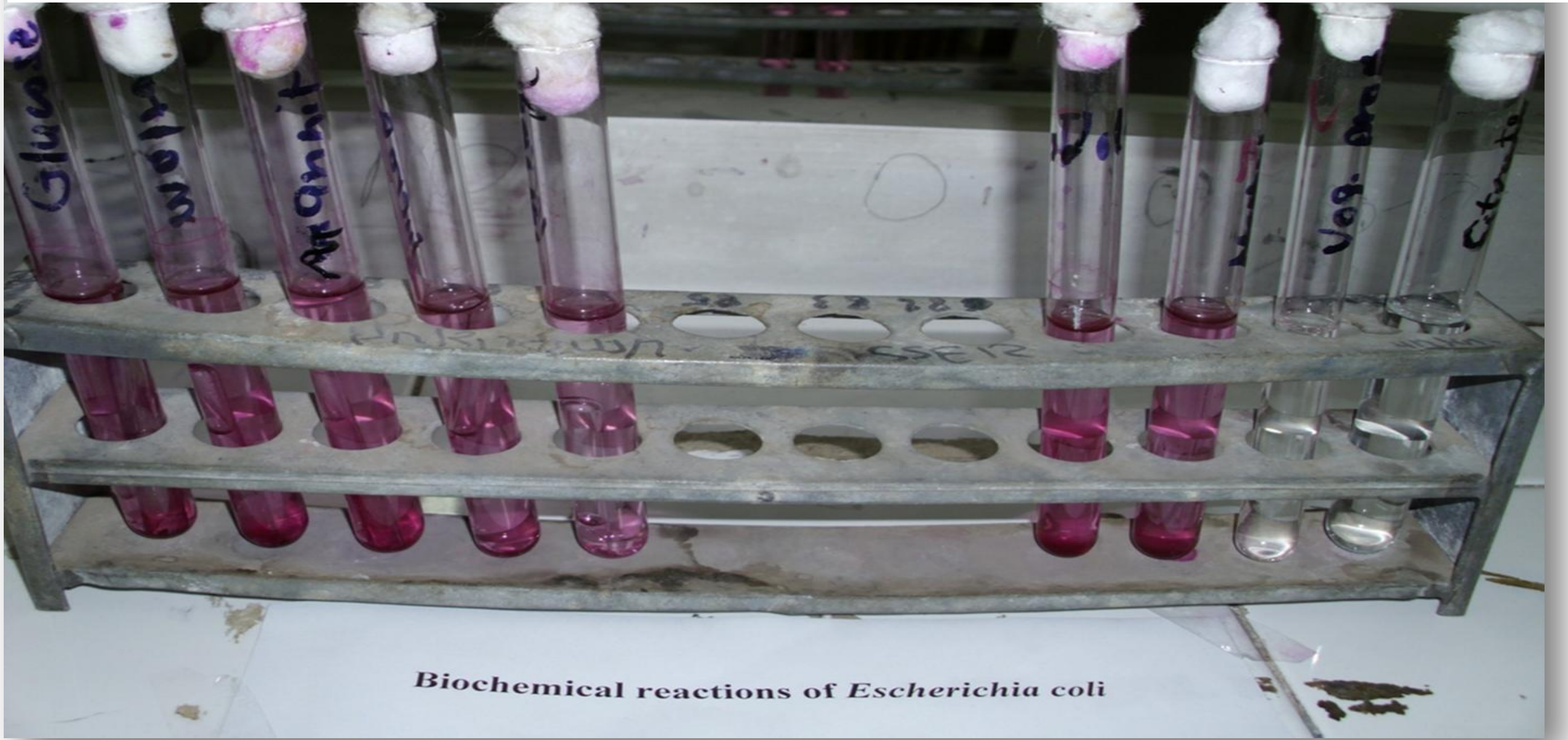
3 Typing

-Antibiotic sensitivity strain

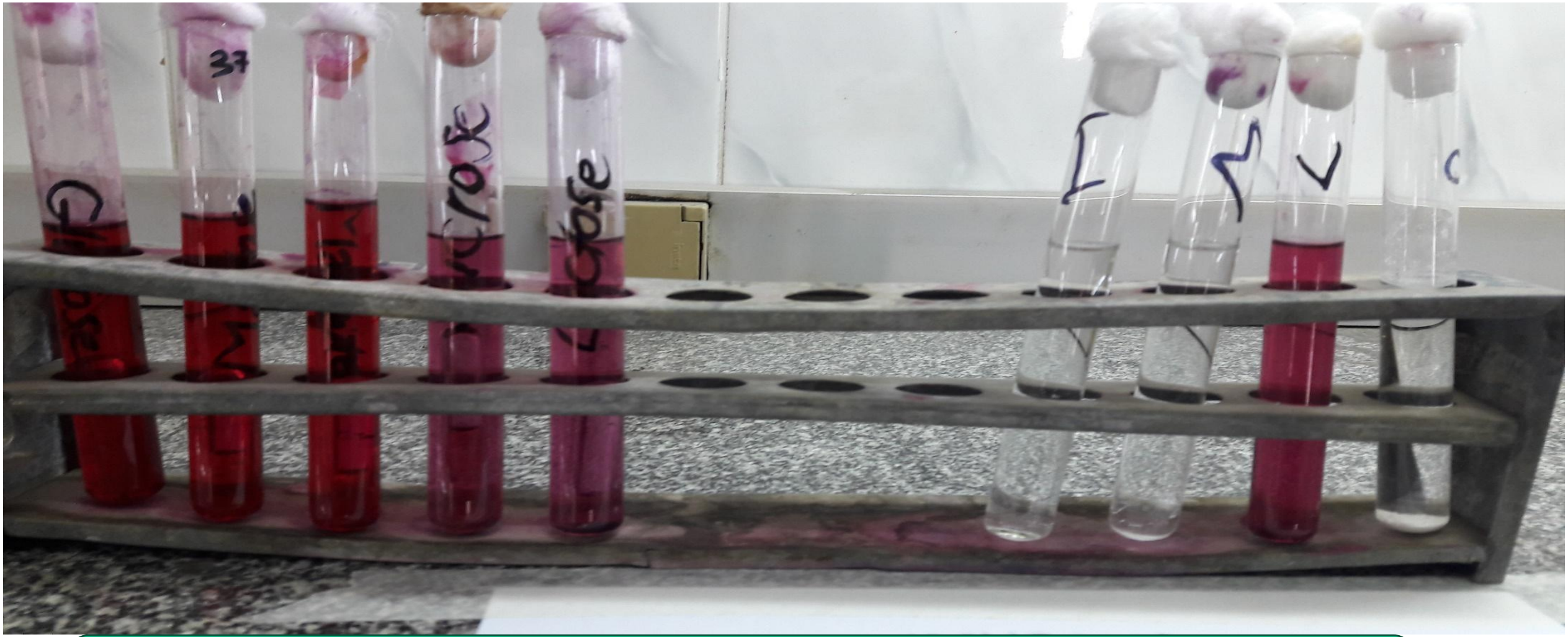
-Serotyping: to detect **O, H, K** antigens

IMVC test of Coliform group

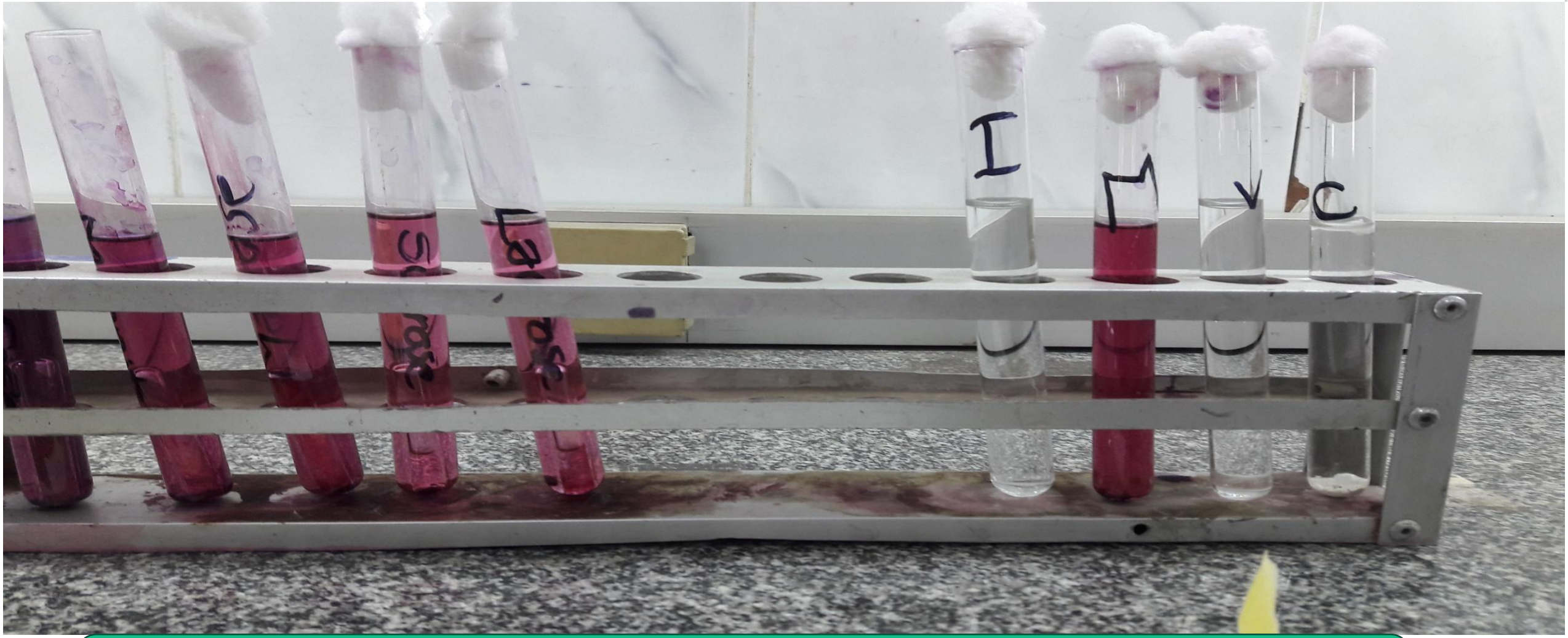
	Indole	MR	VP	Citrate
E.Coli	+	+	-	-
Klebsiella	-	-	+	+
Citrobacter	-	+	-	+



Biochemical reactions of *Escherichia coli*



Biochemical reactions of Klebsiella

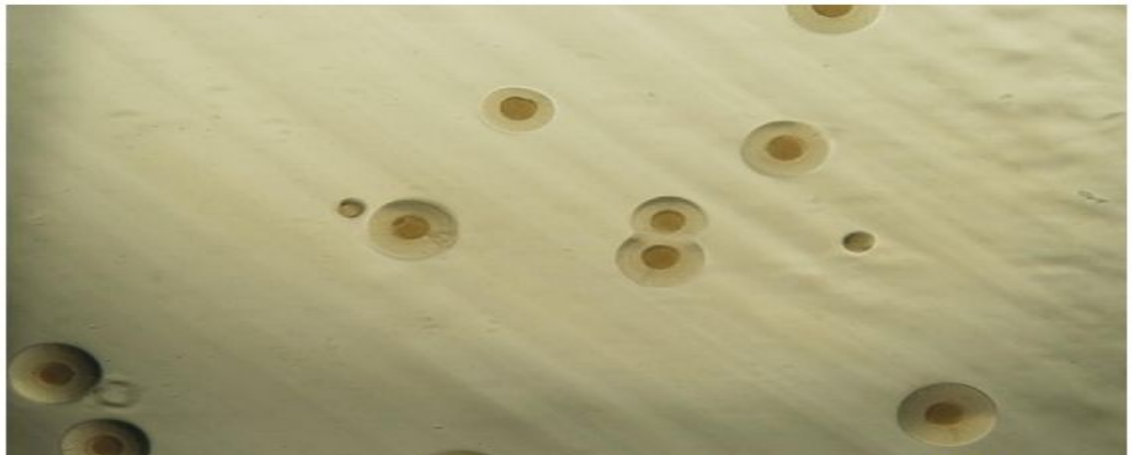


Biochemical reactions of Citrobacter

D- Mycoplasma hominis

- They are a group of very small organisms, lacking cell walls and highl pleomorphic.
- The cytoplasmic membrane contain sterols acquired from media or tissue.
- **Morphology:** Smallest **pleuromorphic bacteria** Size: 0.2µm in diameter Shape varies, they may be coccoid or filamentous depending upon species and growth conditions They lack cell wall so they are pleuromorphic and do not stain with conventional bacteriological stains

- **Culture**: on enriched media or special mycoplasma agar, give **fried egg colonies** after several days of incubation.
- Colonies can be identified by staining directly on agar with fluorescein-conjugated antibody or by demonstrating that a specific antiserum inhibits their growth.



- **Molecular technique:** DNA probes and PCR.
- **Serodiagnosis:**

  the main stay of diagnosis

  Specific antibodies detection by complement fixation, indirect hemagglutination and latex agglutination tests.

  Non specific antibodies detection by cold agglutination test: agglutination of group O RBCs.

E- Anaerobic infections

1-non sporing anaerobes:

a) Gram positive: peptococci, peptostreptococci, actinomyces

a) Gram negative: bacteroids, fusiform bacteria, villonella.

2- spore forming anaerobes: Clostredium group

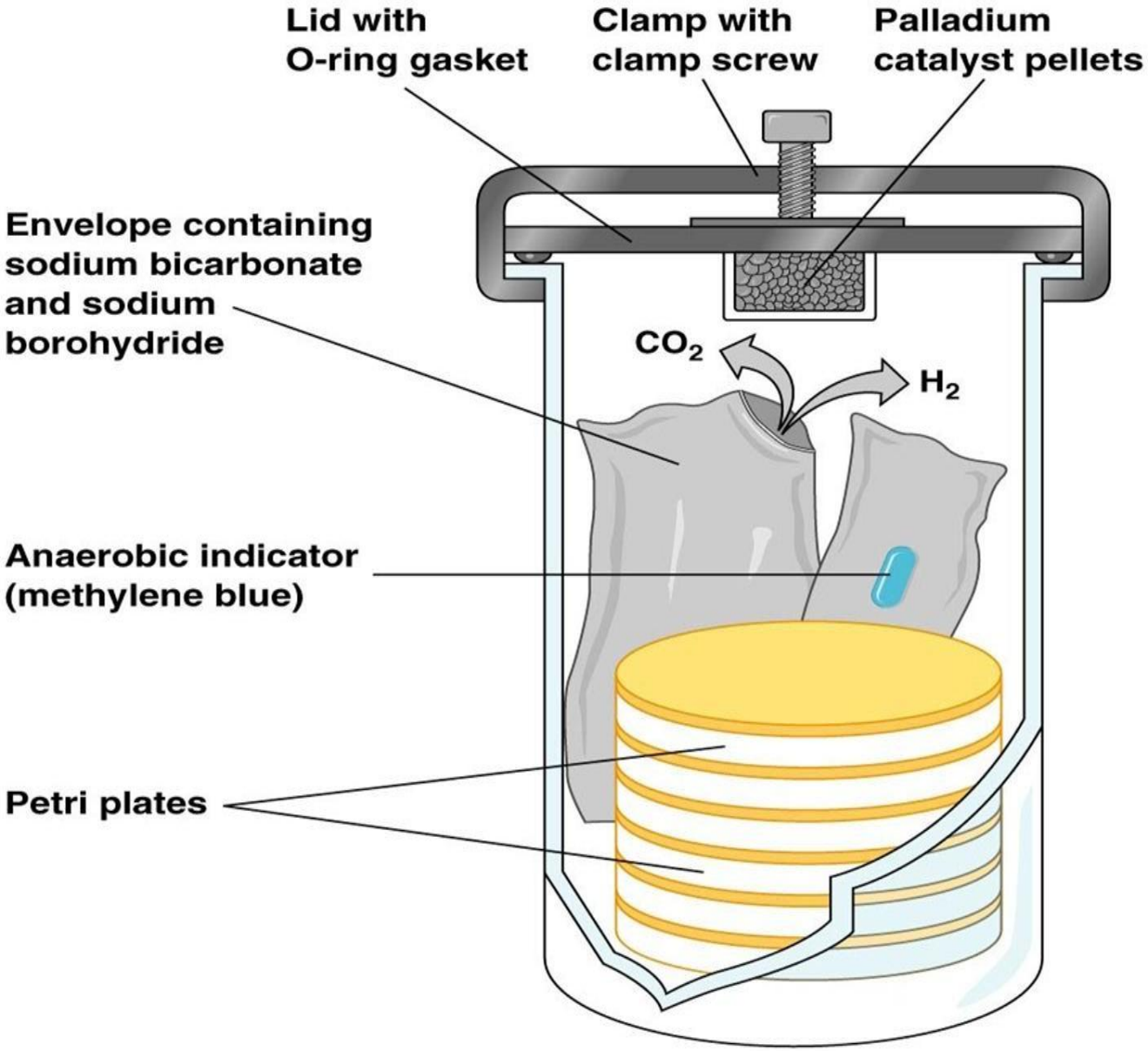
Methods of anaerobiosis

1)Deep agar

2)Media containing reducing compounds

3)Absorption of O2 by Na-pyrogallate

4) Replacement of Oxygen with hydrogen (Gas-bag jar)



Anaerobic jar

Bacterial and viral causes of spontaneous and recurrent abortion

VIRAL

- rubella virus
- Influenza virus
- Mumps virus
- Herpes simplex virus
- Varicella Zoster
- Measles virus
- Coxsackie A virus
- Cytomegalovirus
- HIV

BACTERIAL

- Brucella abortus
- Listeria monocytogenes.
- Treponema pallidum

References

- Practical Microbiology and Immunology 2018-2019: Textbook by staff members of medical Microbiology and Immunology Department; volume III; staphylococci and streptococci
- Baily and Scott diagnostic microbiology 13th edition (2014): chapter 14 Staphylococcus, and Similar Organisms, 232, CHAPTER 15 Streptococcus, and Similar Organisms, 247

Presenting Complaint: Emily Johnson, a 28-year-old woman who recently gave birth to her first child, presents to the emergency department with complaints of fever, abdominal pain, and foul-smelling vaginal discharge. She delivered a healthy baby girl vaginally 5 days ago. Emily noticed the symptoms starting 2 days postpartum but initially dismissed them as normal postpartum changes.

Diagnostic Workup:

1. Blood tests reveal an elevated white blood cell count (WBC).
2. Cultures of blood and vaginal discharge are obtained for microbiological analysis.
3. Imaging studies, such as ultrasound, may be performed to assess the condition of the uterus and surrounding structures.

What is your probable diagnosis?


THANK YOU