AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 2.

Microbiology diagnosis of diseases, caused by Gram negative cocci (meningococci, gonococci) and opportunistic bacteria (klebsiella, proteus, acinetobacter, pseudomonas)

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. Classification of gram-negative cocci

2. Meningococci, morpho-biological characteristics, pathogenicity factors and diseases caused by it.

3. Methods of microbiological diagnosis of meningococcal infections.

4. Specific treatment and prevention of meningococcal infections.

5. Gonococci, morpho-biological characteristics, pathogenicity factors and diseases caused by them.

6. Microbiological diagnosis of acute and chronic gonorrhea.

7. Treatment and prevention of gonorrhea.

8. General characteristics of opportunistic bacteria, main representatives. Their role in the occurrence of purulent-inflammatory diseases and nosocomial infections.

9. Klebsiella genus, species, morpho-biological characteristics, pathogenicity factors, diseases caused by it and their microbiological diagnosis.

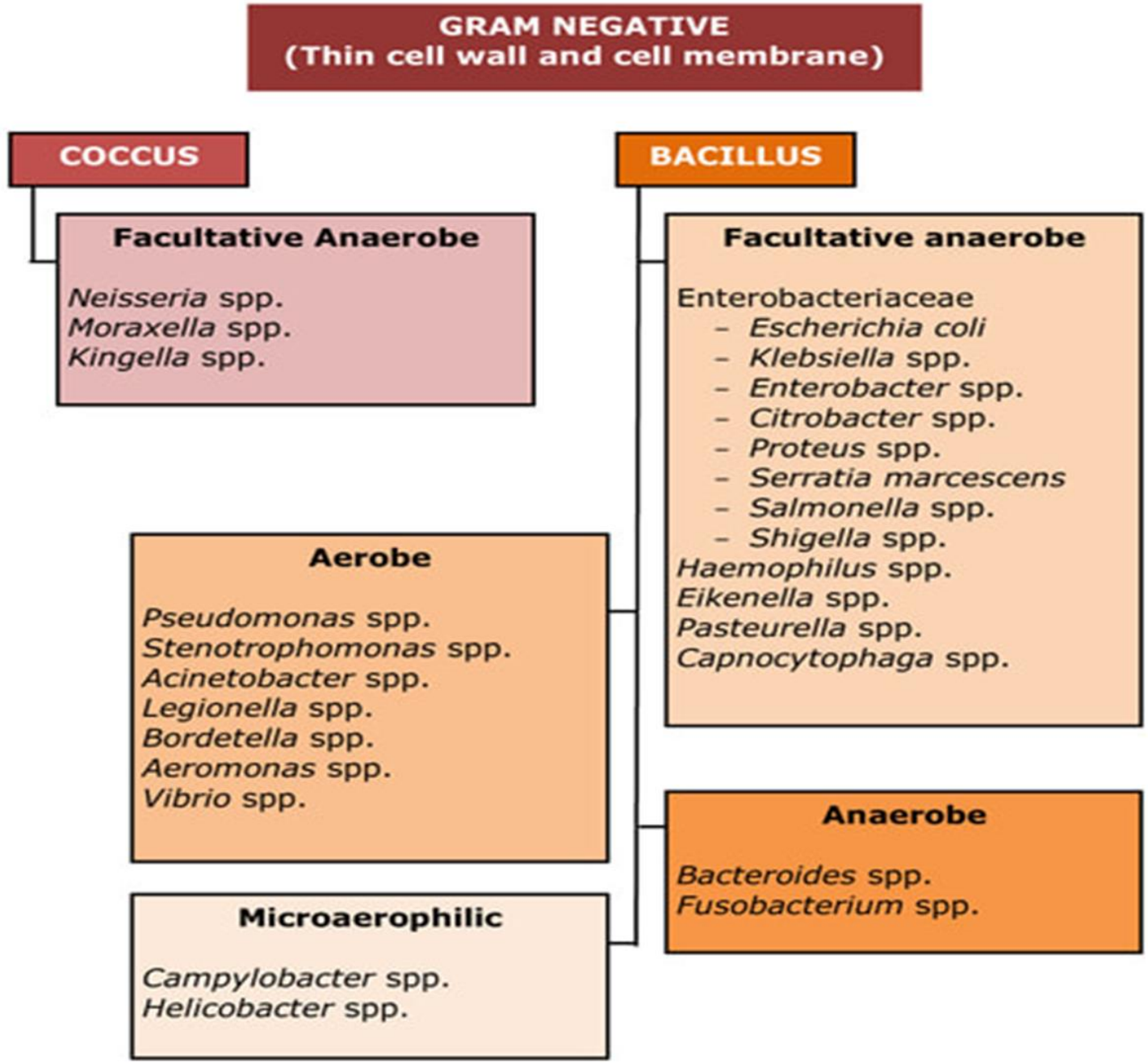
10. Proteus genus, species, morpho-biological characteristics, pathogenicity factors, diseases caused by it and their microbiological diagnosis

11. Acinetobacter genus, morpho-biological characteristics, pathogenicity factors, diseases caused by it and their microbiological diagnosis

12. Morpho-biological characteristics of Pseudomonas aeruginosa, pathogenicity factors, diseases caused by it and their microbiological diagnosis.

Purpose of the lesson:

- To inform students about the main characteristics of pathogenic neisseria (meningococci and gonococci), their role in the occurrence of diseases, to familiarize them with microbiological diagnostic methods, specific principles of prevention and treatment of these diseases. To acquaint students with the main characteristics of some opportunistic bacteria (klebsiella, proteus, acinetobacter, pseudomonas), microbiological diagnosis of diseases caused by them, specific treatment and prevention principles.


GRAM NEGATIVE
(Thin cell wall and cell membrane)
COCCUS
BACILLUS
Facultative Anaerobe
Neisseria spp.
Moraxella spp.
Kingella spp.
Facultative anaerobe
Enterobacteriaceae
– Escherichia coli
– Klebsiella spp.
– Enterobacter spp.
– Citrobacter spp.
– Proteus spp.
– Serratia marcescens
– Salmonella spp.
– Shigella spp.
Haemophilus spp.
Eikenella spp.
Pasteurella spp.
Capnocytophaga spp.
Aerobe
Pseudomonas spp.
Stenotrophomonas spp.
Acinetobacter spp.
Legionella spp.
Bordetella spp.
Aeromonas spp.
Vibrio spp.
Anaerobe
Bacteroides spp.
Fusobacterium spp.
Microaerophilic
Campylobacter spp.
Helicobacter spp.

Neisseria – Taxonomy

- (Domain): Bacteria
- (Kingdom): Pseudomonadota
- (Class): Betaproteobacteria
- (Order): Neisseriales
- (Family): Neisseriaceae
- (Genus): **Neisseria**

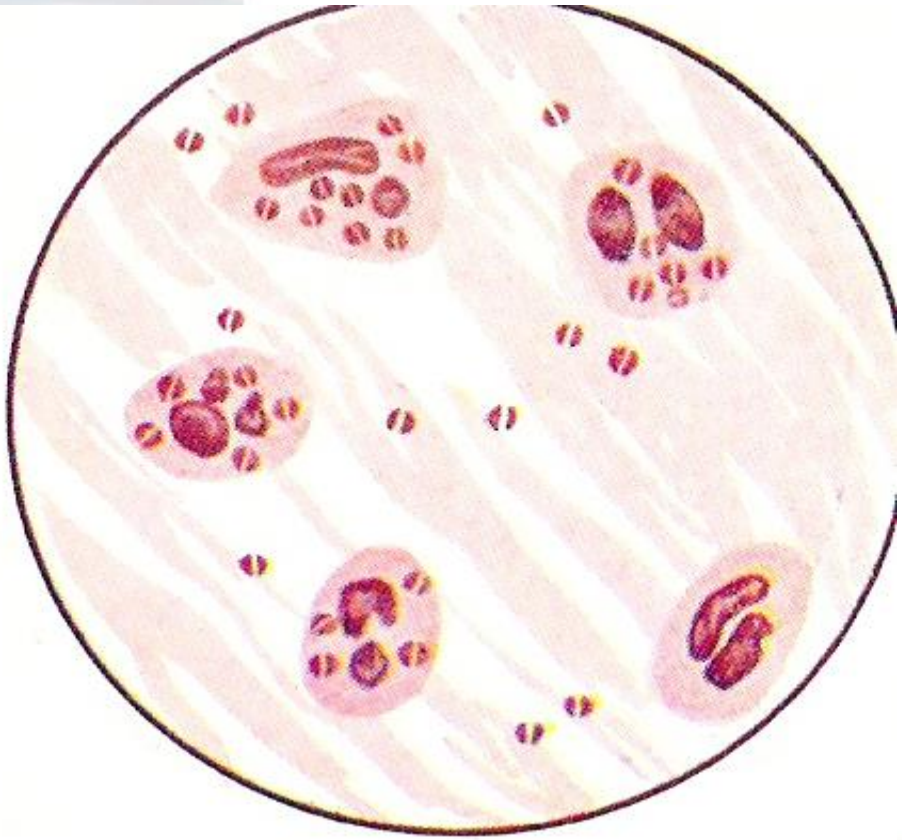
N. animalis
N. animaloris
N. bacilliformis
N. canis
N. cinerea
N. dentiae
N. elongata
N. flava
N. flavescens
N. gonorrhoeae
N. iguanae
N. lactamica
N. macacae
N. meningitidis
N. mucosa
N. oralis
N. perflava
N. pharyngis
N. polysaccharea
N. shayeganii
N. sicca
N. subflava
N. wadsworthii
N. weaveri
N. zoodegmatis

Neisseria and related spp. of human origin

- Neissseria meningitidis
- Neisseria gonorrhoeae
- N. lactamica, N. cinerea, N. polysacharea, N. subflava, N. sicca, N.mucosa, N.flavescens, N.elongata
- Moraxella (Branhamella) catarrhalis – coccoid form

SA PATHOLOGY

Morphology



- Capsulated Gram negative diplococci
- 0.5 – 1 µm
- Kidney shaped, flat sides adjacent
- Intracellular, usually
- Non-motile
- Non spore-forming

CULTURAL CHARACTERISTICS

Media used:

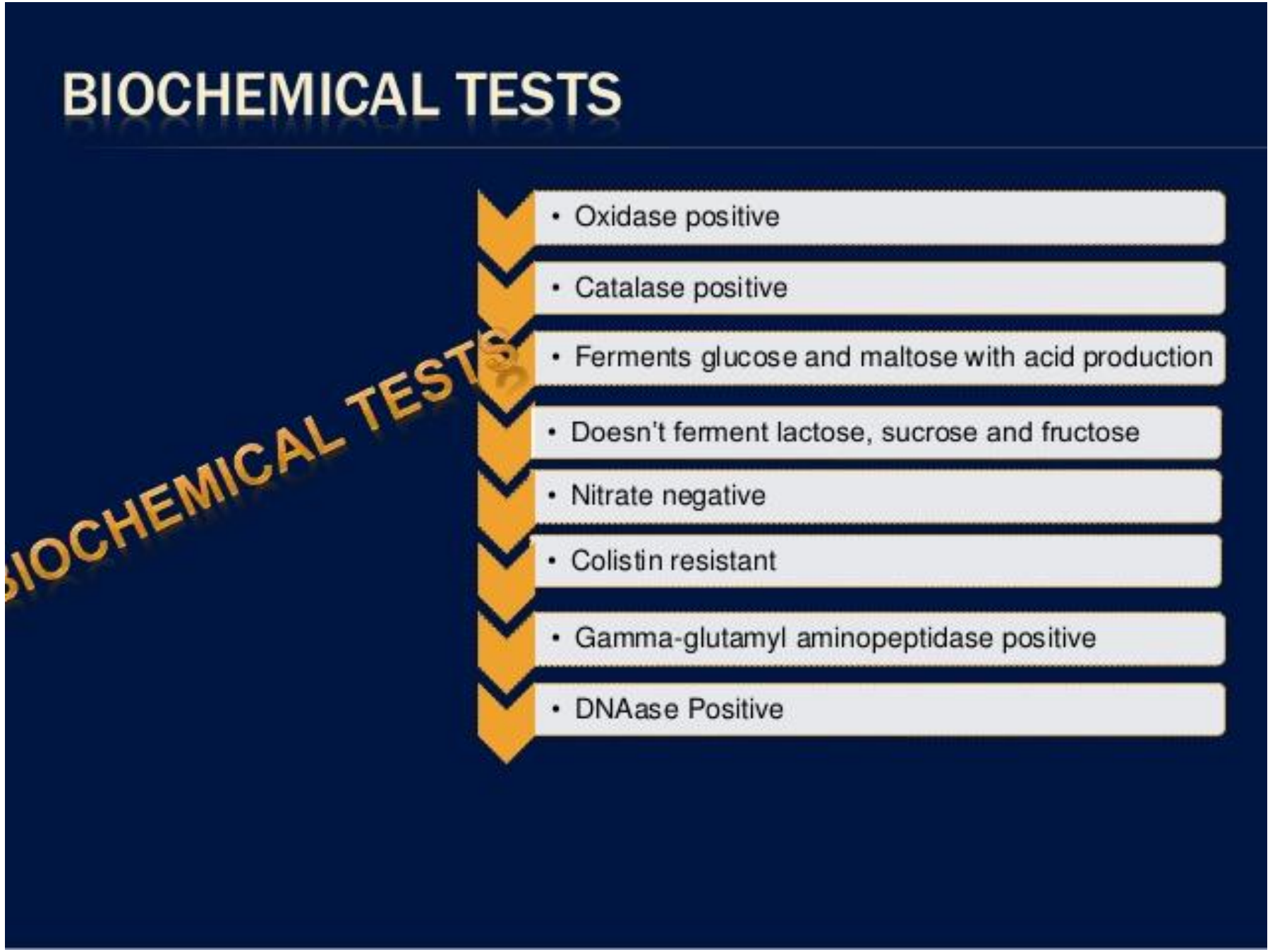
- non selective media:
  - Blood agar
  - Chocolate agar
  - Mueller-Hinton starch casein hydrolysate agar
- Selective media
  - Modified Thayer-Martin Agar

Colony characteristics

- Color: Bluish grey
- Shape: Round
- Size: About 1mm
- Surface: Smooth
- Elevation: Convex
- Opacity: Transluscent
- Consistency: Butyrous

N.meningitidis
Blood agar

BIOCHEMICAL TESTS



Virulence factors

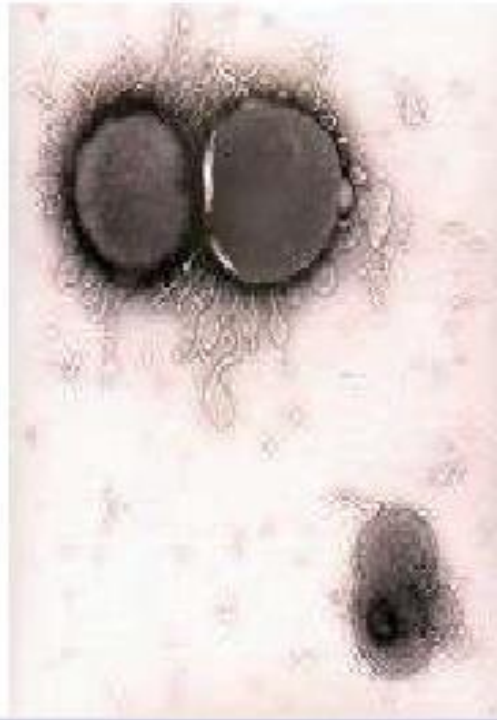
- **ANTIGENS**

  Capsular polysaccharide

  - 13 serogroups (**A**, B, **C**, D, **W 135**, X, **Y**, Z, H, K & L)
  - Used in **vaccine**
  - Serogroups A, B, C, Y, W135 account for about 90% of all infections

- **OMP**
  - 5 classes
  - Serogroups further subdivided into 20 serotypes

- **PILI**



- **TOXIN**

  ENDOTOXIN

  - Lipid A part of **lipopolysaccharide**
  - Causes fever and shock

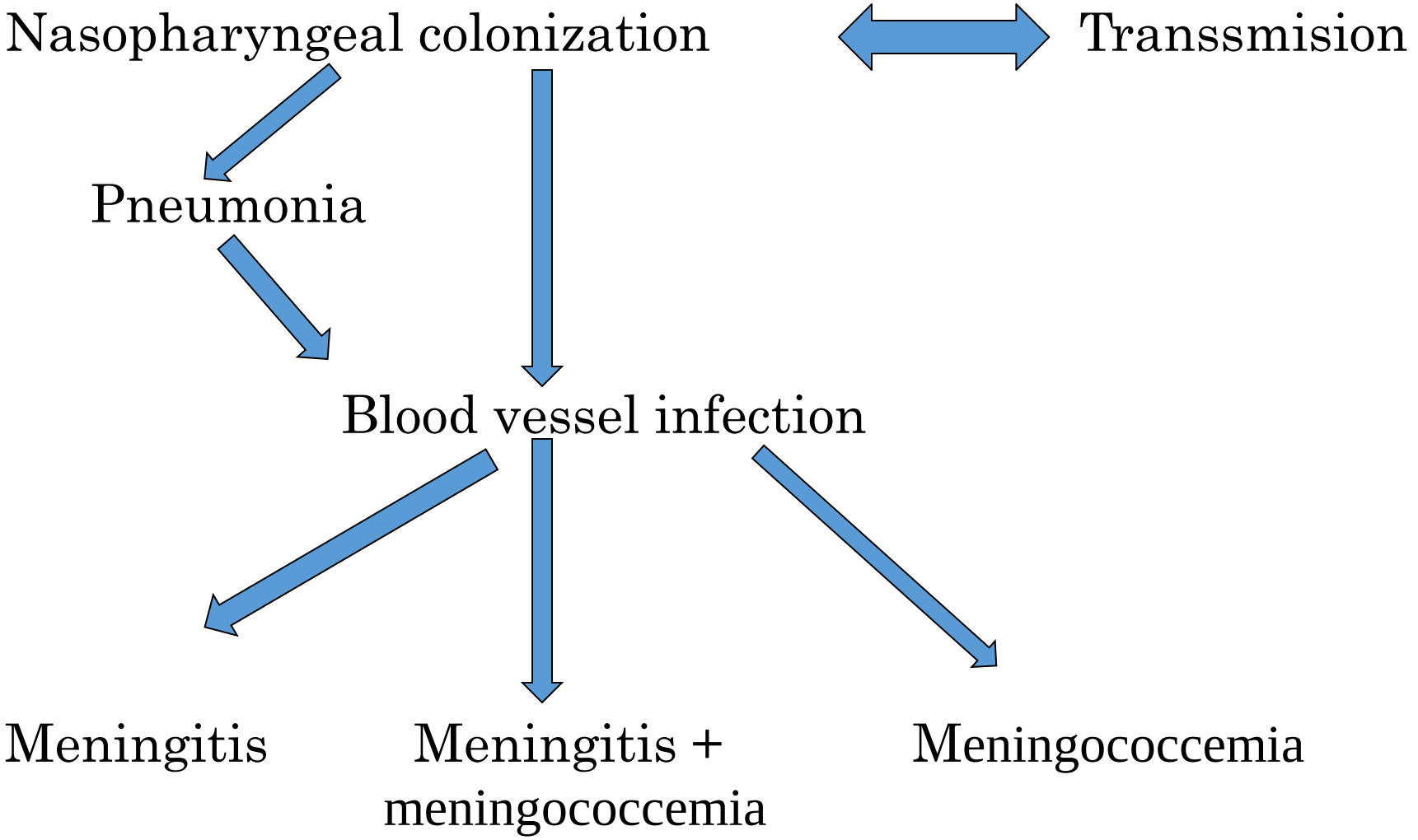
- **ENZYME**

  IgA Protease

TABLE 26-4 Virulence factors of *Neisseria meningitidis*

Virulence factors	Biological functions
Capsule	Prevents phagocytosis
LOS endotoxin	Causes damage of the blood vessels associated with meningococcal infections
IgA protease	Destroys IgA immunoglobulin, thereby helps gonococci to attach to the epithelial cells of the upper respiratory tract
Lipooligosaccharides	Stimulates release of TNF-α, which results in host cell damage

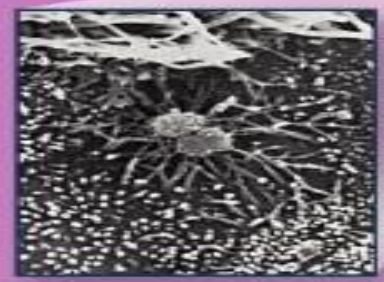
Pathogenesis of meningococcal infections



Nasopharyngeal mucosa

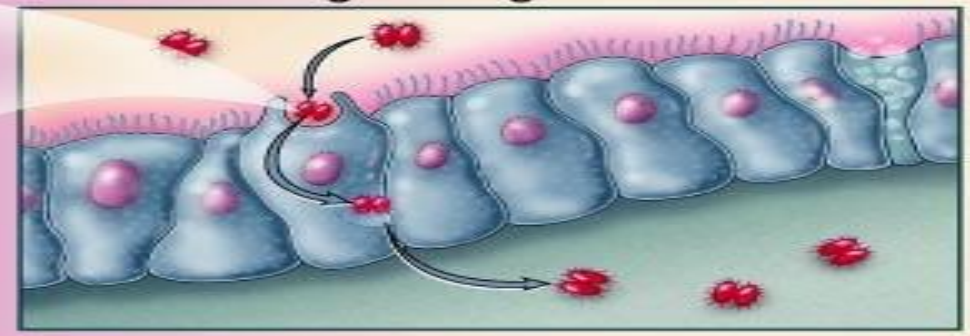
Blood

Cerebrospinal fluid

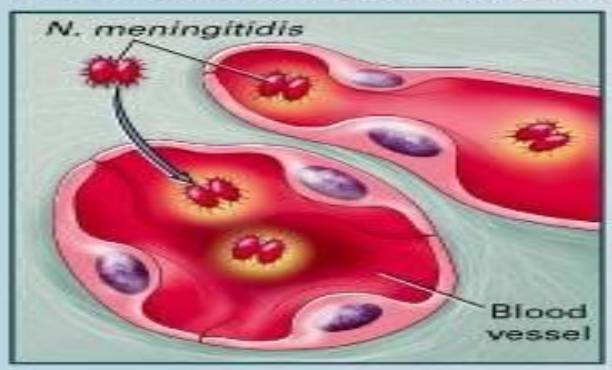


Attachment to and interaction with nasopharyngeal epithelium

Passage through the mucosa



Survival in the bloodstream



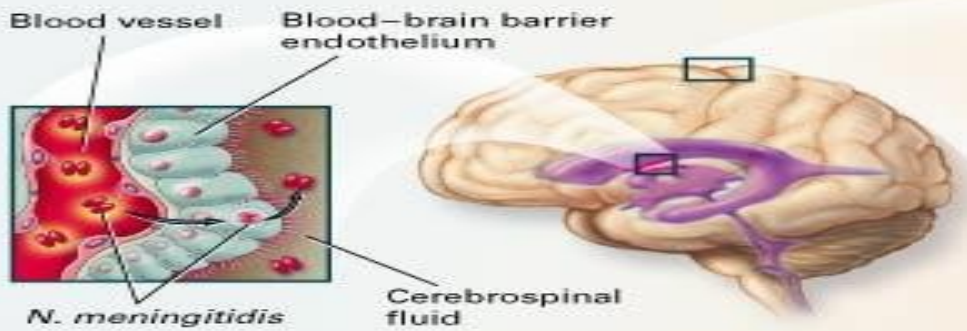
Factors affecting intravascular survival

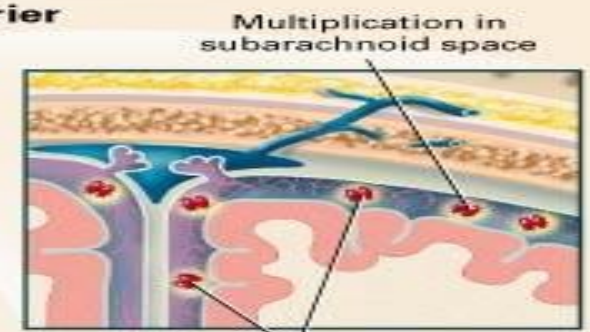
- **Capsule:** protects against complement-mediated bacteriolysis and phagocytosis
- **Acquisition of iron from transferrin**

Endotoxin and other cell components

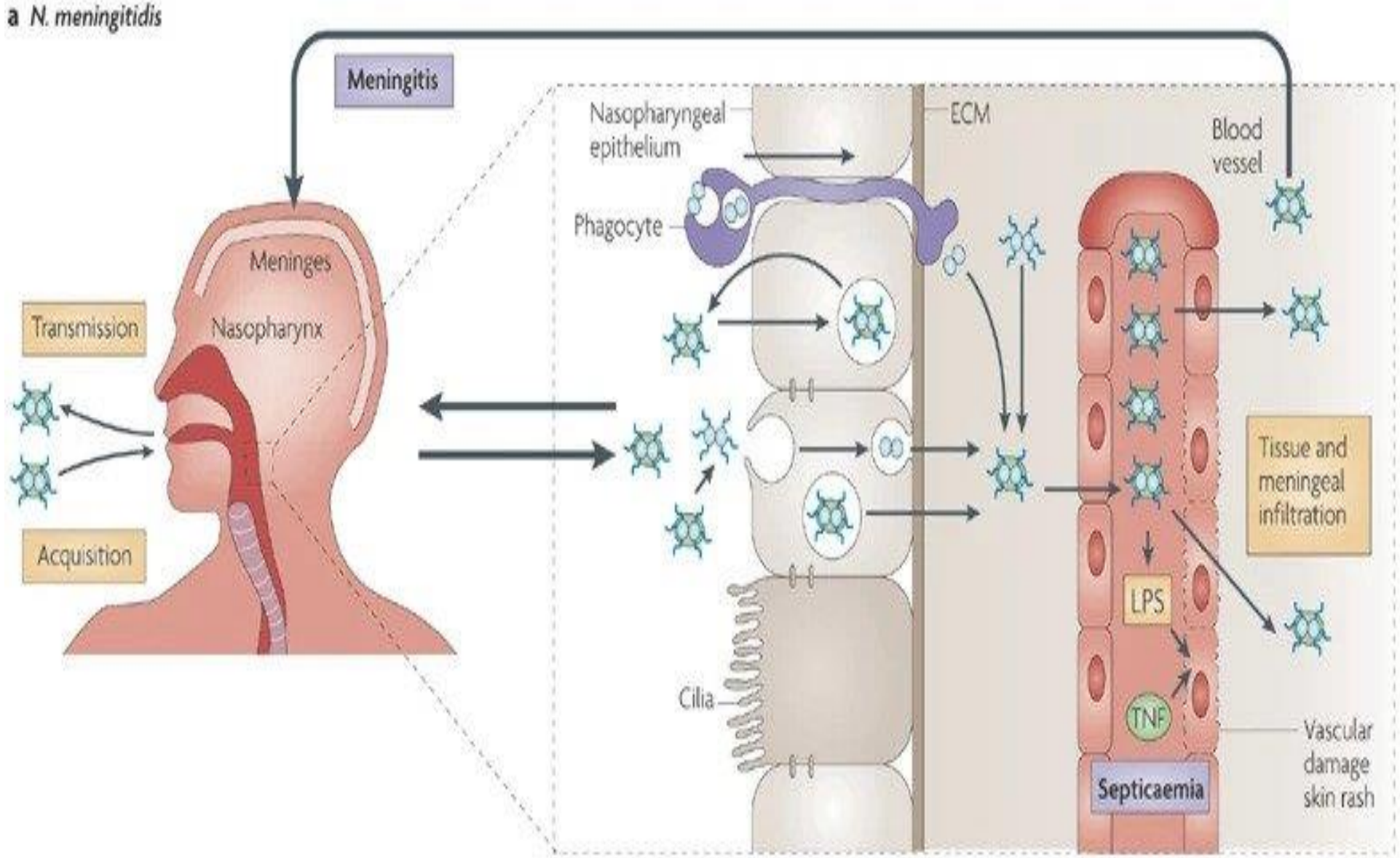
- Host-cell cytokine production
  - **Inflammatory cytokines** (tumor necrosis factor α, interleukin-1β, 6, 8)
  - **Antiinflammatory cytokines** (interleukin-10)
- Alternative complement pathway

Crossing of the blood–brain barrier





Pathogenesis of cerebrospinal meningitis



Epidemiology

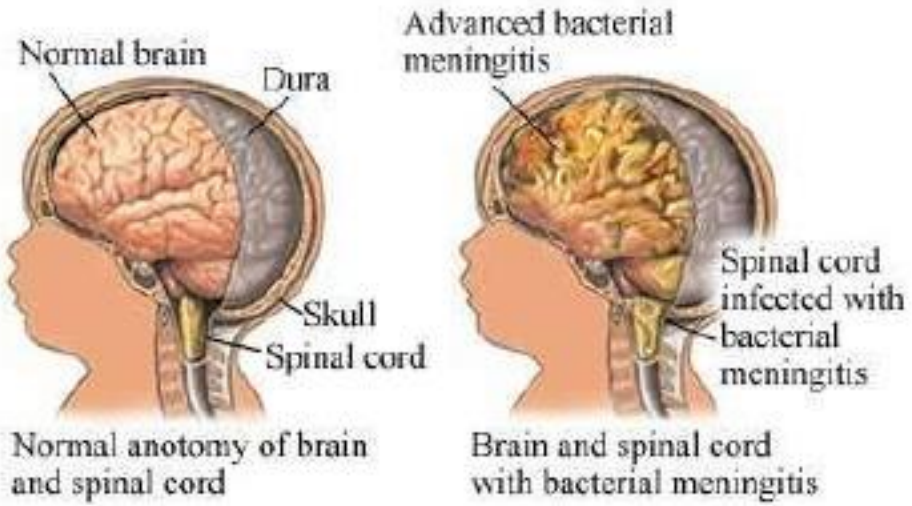
- **Reservoir and Habitat**
  - Upper respiratory tract of humans
- **Transmission**
  - Direct contact and air borne droplets
  - Close contact with infectious person (e.g., family members, day care centers, military barracks, prisons, and other institutional settings)
- **Incubation period:** 1-7 days
- **Carriage**
  - 5-30% of normal persons may harbor meningococci in nasopharynx

Diseases - *N. meningitidis*

- Meningitis
- Meningococcemia
  - Septicemia with or without meningitis
- Meningoencephalitis
- Pneumonia
- Bacteremia
- Arthritis
- Urethritis

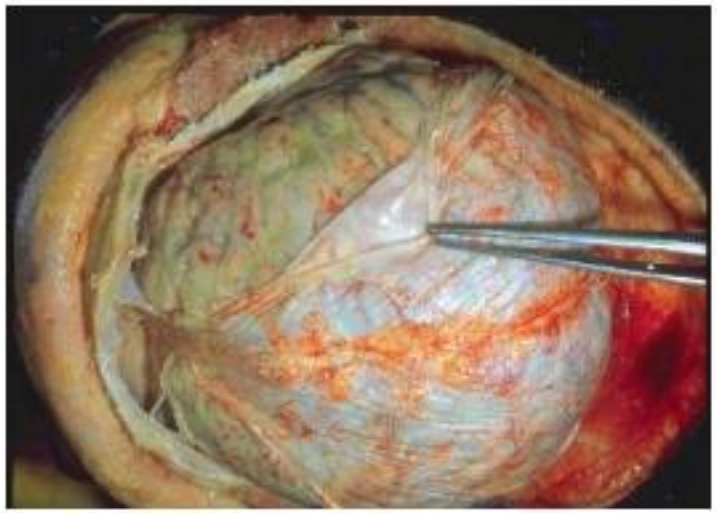
Meningitis

- Inflammation of the membranes of the brain or spinal cord



Normal anotomy of brain and spinal cord

Brain and spinal cord with bacterial meningitis



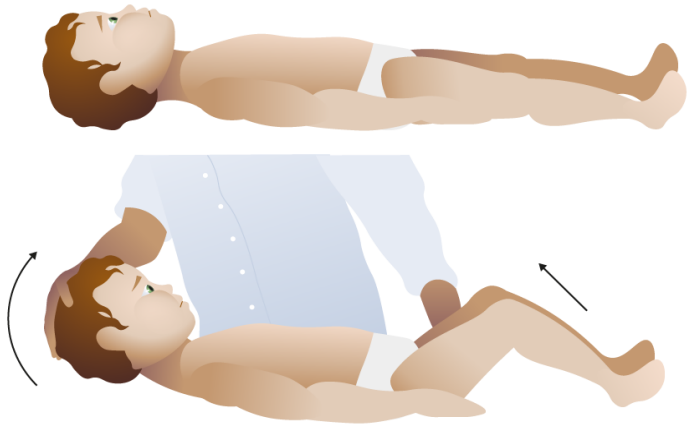
Etiology — Infectious

Organism	Common types	Diagnosis
Bacteria	*Hemophilus influenzae* *Neisseria meningitidis* *Streptococcus pneumoniae*	Leukocyte count and differential Gram stain Acridine orange stain Glucose Protein Culture
Viruses	Enteroviruses Other (mumps, herpes)	Leukocyte count and differential Glucose Protein Culture
Fungi	*Cryptococcus neoformans* *Candida sp.* *Coccidioides immitis*	Gram stain India ink preparation Latex agglutination Culture

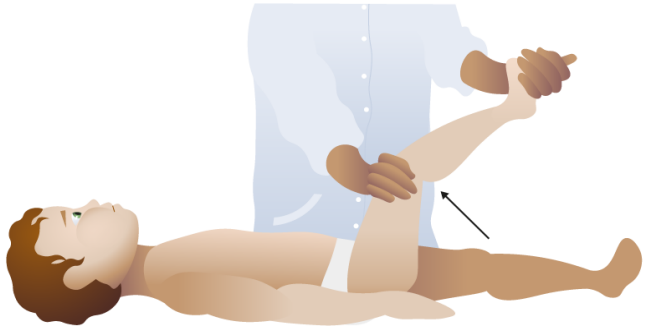
Clinical features Symptoms

IN ADULTS

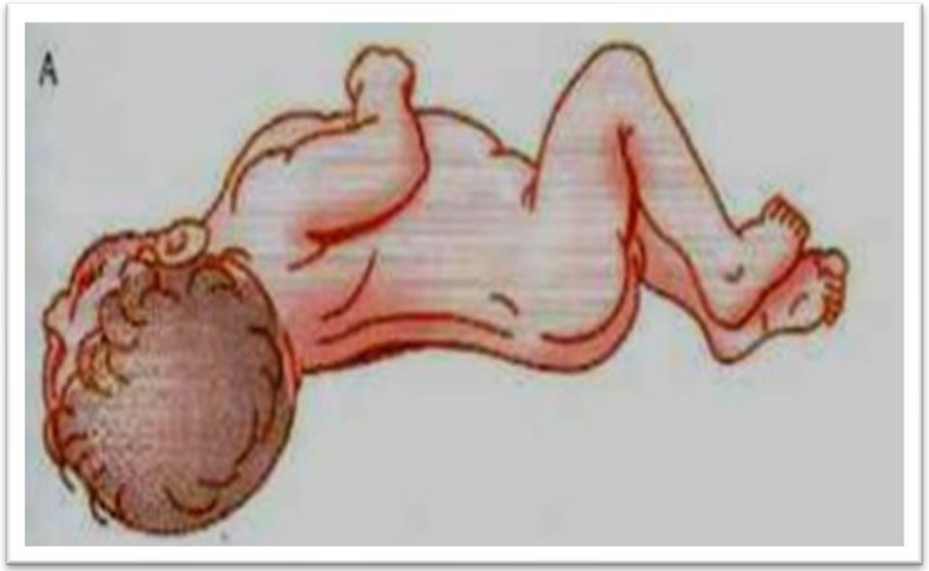
Meningitis – clinical features



Brudzinski's sign:
Flexion of the hips and knees in response to neck flexion

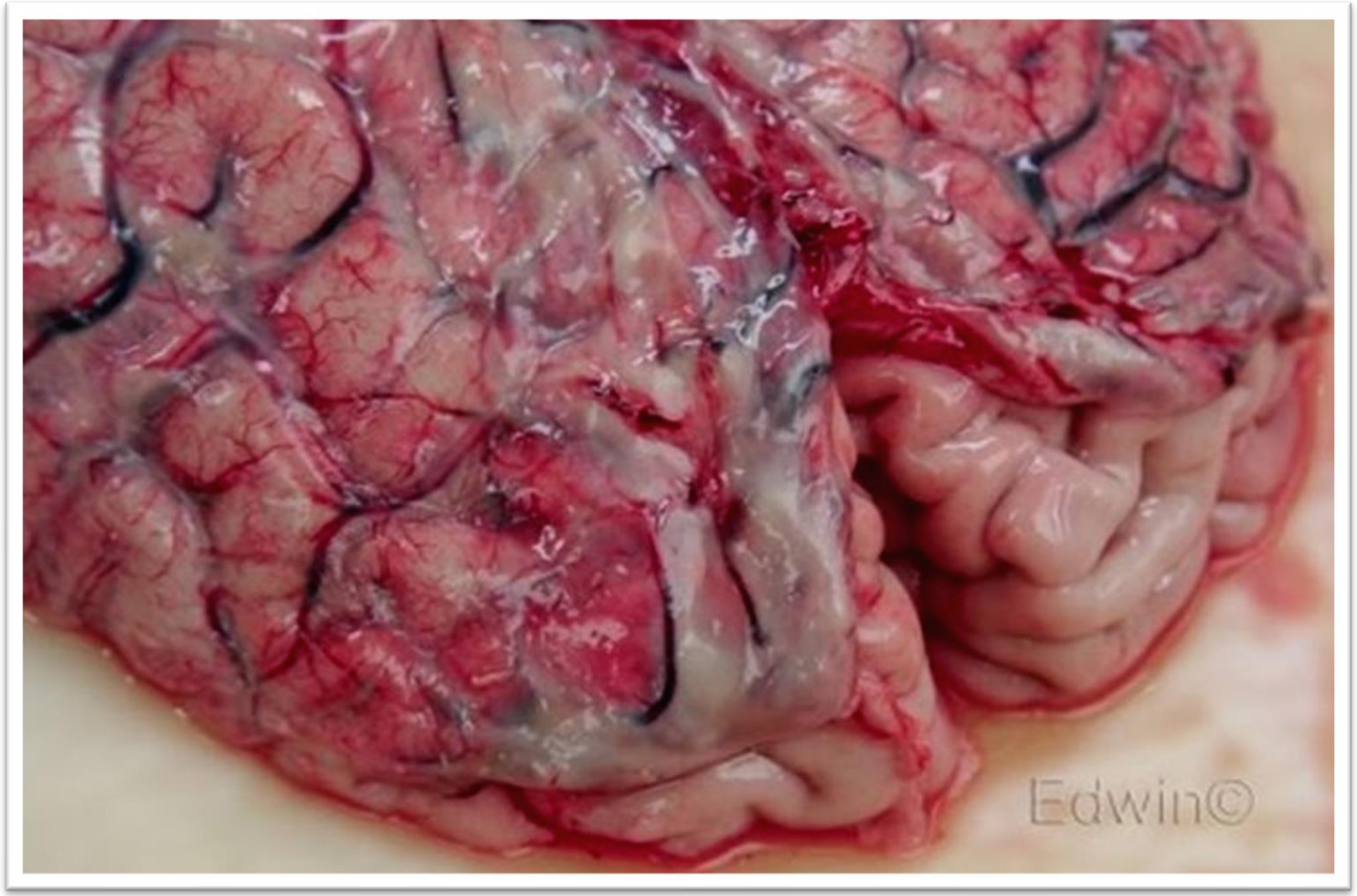


Kernig's sign:
Resistance to extension of leg while the hip is flexed



Pulled trigger position (sleeping form)
(the head of the patient lying on his side is pulled back, his legs are bent at the knee and hip joints and pressed to the stomach)

Epidemic cerebrospinal meningitis (purulent meningitis)

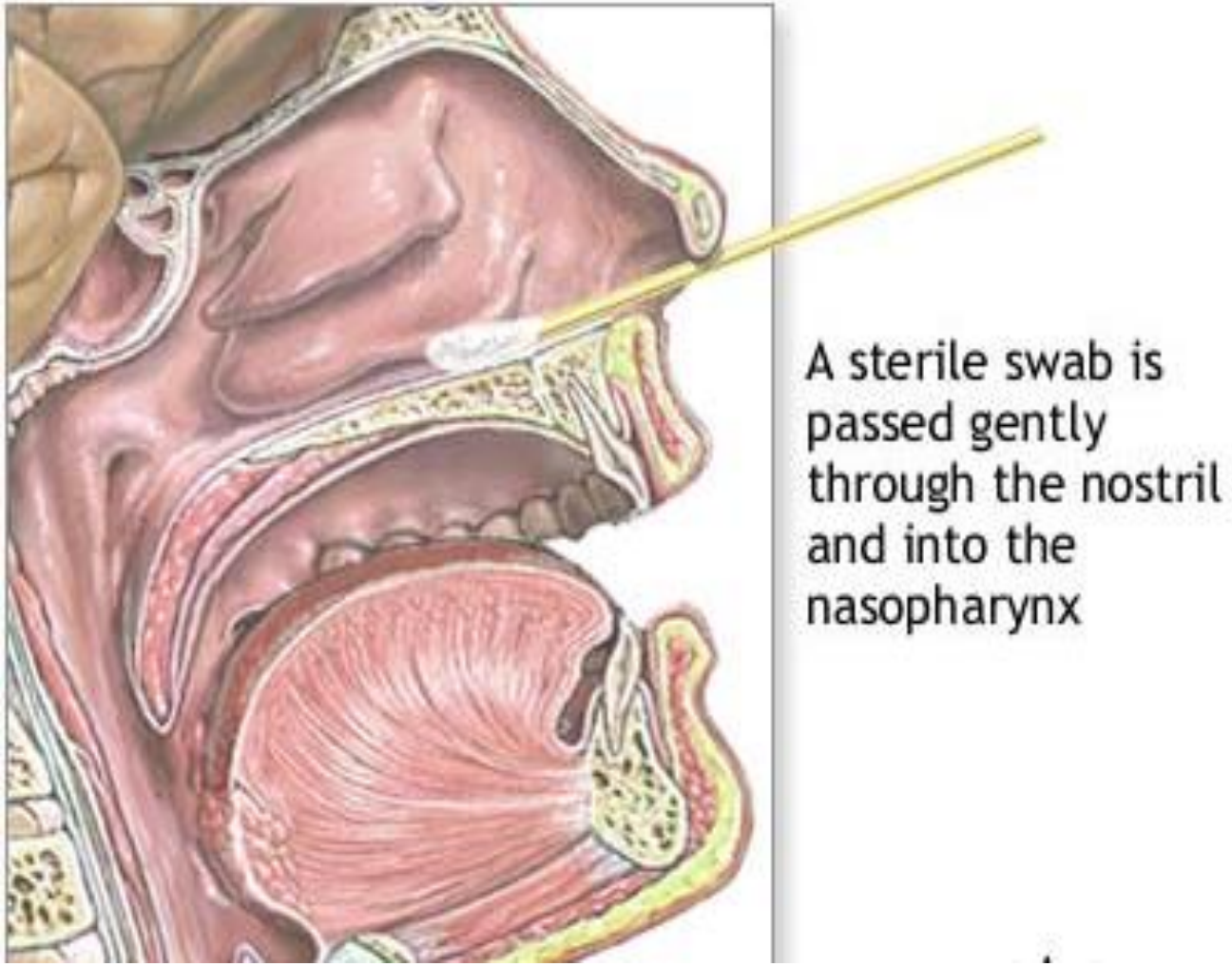


Meningococcemia (rashes)

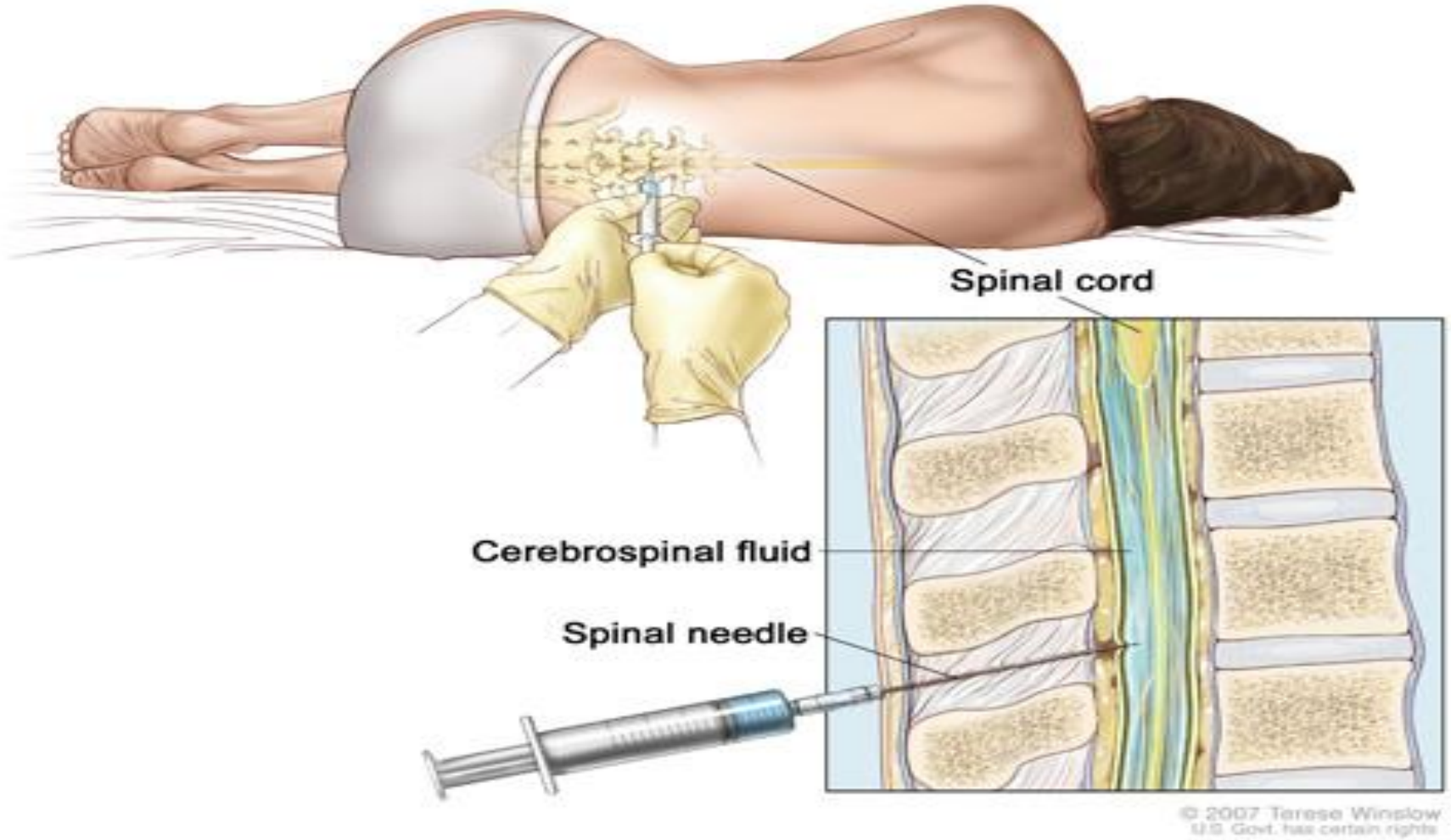
Microbiological diagnostics:

Pathological material:

- *cerebrospinal fluid (CSF)*
- *blood*
- *nasopharyngeal mucus*
- *punctate from rash element*



Collection of cerebrospinal fluid (lumbar puncture)



CSF – Acute Bacterial Meningitis

- **CSF** (Lumbar puncture)
  - Cloudy or purulent
  - Elevated pressure
  - Increased protein
  - Decreased glucose
  - Cell count
    - Usually >1000 cells/μL with Neutrophils predominating
  - Gram stain
    - Gram negative intracellular diplococci

CSF evaluation

	Normal	Bacterial	Viral	TB
Cells	0-5	>1000	<1000	<500
Polymorphs	0	Predominate	Early	+/- increased
Lymphocytes	5	Late	Predominate	Increased
Glucose	60-80	Decreased	Normal	Decreased
CSF : plasma Glucose ratio	66%	<40%	Normal	< 30%
Protein	5-40	Increased	+/- Increased	Increased
Culture	Negative	Positive	Negative	Positive (MTB)

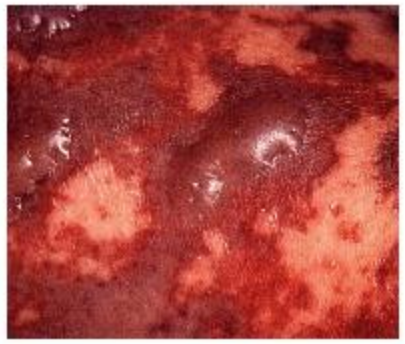
Meningococcemia

Clinical features

- **Intravascular multiplication of *N. meningitidis***
  - Abrupt onset of spiking fevers, chills, arthralgia, joint and muscle pains
  - Abrupt onset of hypotension and tachycardia
  - Rapidly enlarging petechial lesions
  - Wide spread purpura
  - Shock
  - DIC
  - Coma
  - Death ensues within hours

Skin Lesions of Meningococcemia

NOTE: Petechiae have coalesced into hemorrhagic bullae



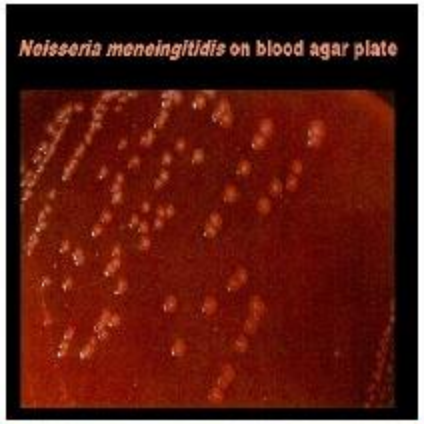
Laboratory diagnosis

- **Specimens**
  - Blood and CSF for smear and culture
  - Nasophyrangeal swab for carrier state
- **Culture media**
  - Blood agar
  - Chocolate agar
  - Selective medium

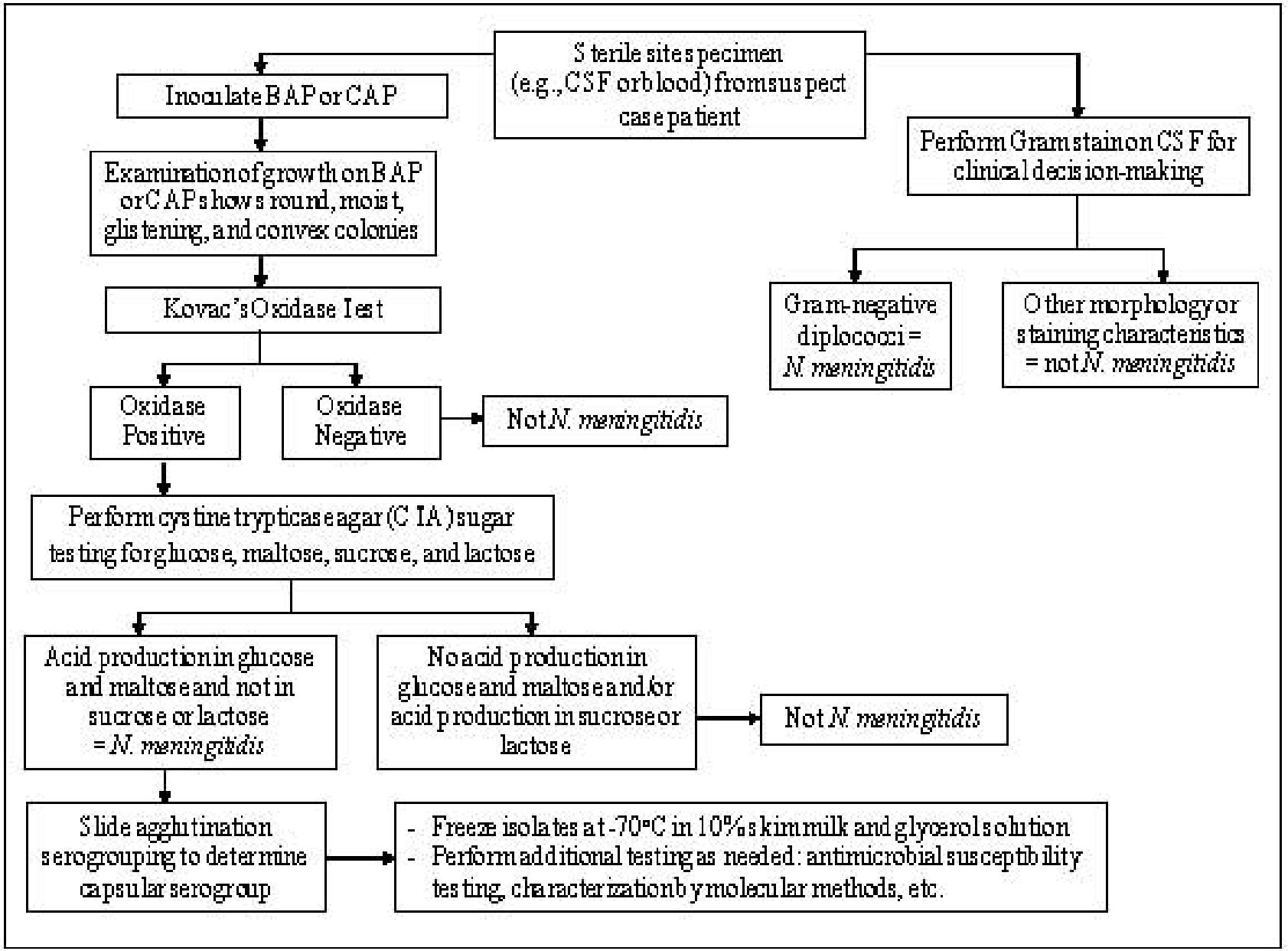
    (Modified Thayer-Martin medium)
    - To avoid contamination vancomycin, amphotericin B and colistin are added

Growth characteristics

- Oxygen Requirement
  - Aerobic or facultative anaerobic
- Temperature
  - 37^0C
- Growth promoted by
  - 5-10% CO_2
- Colony morphology
  - 1-2 mm dia, convex, grey, translucent, non-pigmented and non-hemolytic
  - After 48 hours, colonies are larger with an opaque raised centre and transparent margins



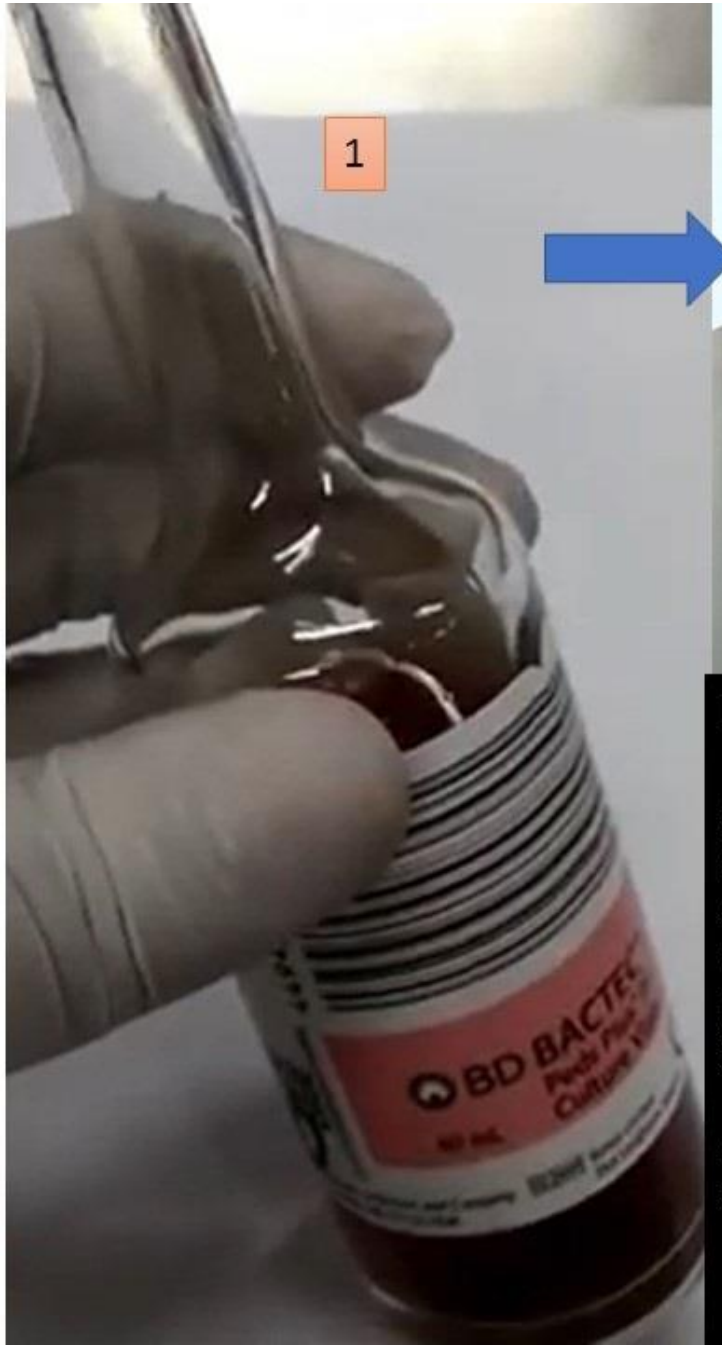
N. meningitidis – cultural identification

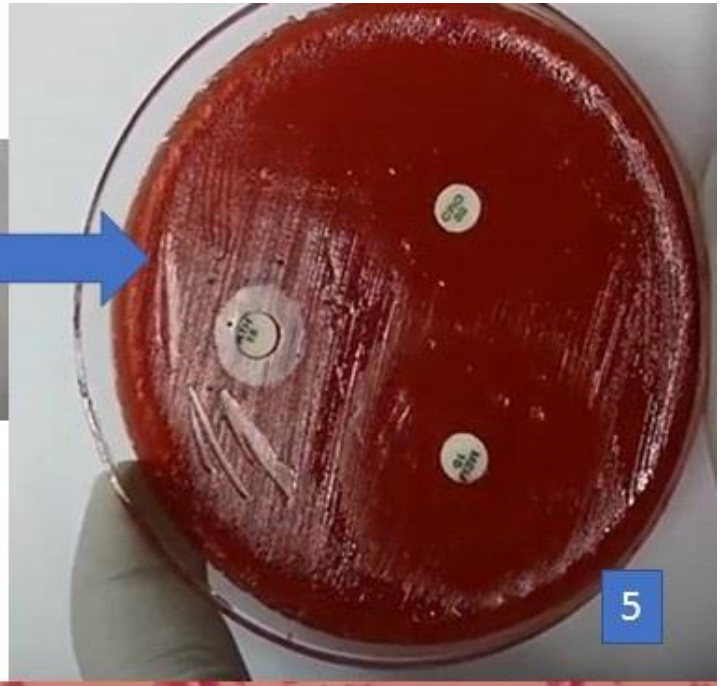


Biochemical reactions

- Oxidase positive
- Ferments **glucose** and **maltose** but not sucrose or lactose

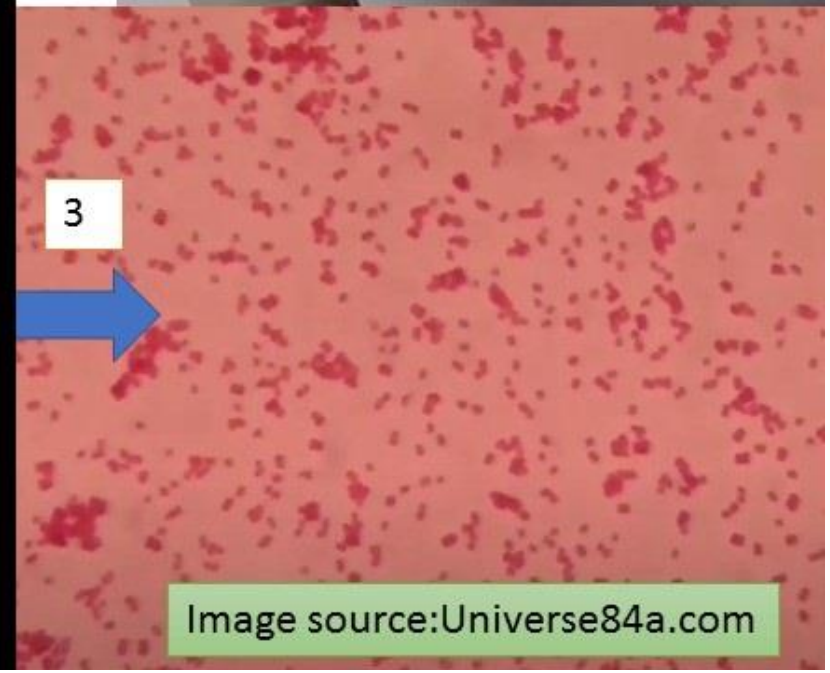
Characteristic	*N. gonorrhoeae*	*N. meningitidis*	*N. lactamica*	*N. sicca*	*N. mucosa*	*N. flavescens*
Growth on:						
CHOC, BA (22°C)	0	0	V	+	+	+
MTM, ML (35°C)	+	+	+	0	0	0
Nutrient agar (35°C)	0	V	+	+	+	+
Acid from:						
Glucose	+	+	+	+	+	0
Maltose	0	+	+	+	+	0
Lactose	0	0	+	0	0	0
Sucrose	0	0	0	+	+	0
Fructose	0	0	0	+	+	0
Nitrate reduction	0	0	0	0	+	0





Neisseria meningitidis (Meningococcus)

1. Blood culture Positive (growth in Bactec bottle)
2. Subcultured into chocolate agar (growth), blood agar (growth as shown above) and MacConkey agar(no growth-Gram negative bacilli out)
3. Gram stain from blood agar: Gram negative cocci in pairs (as shown here)
4. Oxidase test: Positive (as shown above)
5. Antimicrobial Susceptibility Testing (AST) plate



Antibiotic sensitivity testing

- Ampicillin/Penicillin
- Ceftriaxone
- Chloramphenicol
- Rifampicin
- Ciprofloxacin
- Meropenem

Immunity and Prevention

- Infants - passive immunity from mothers
- Under 2 years of age - do not reliably produce antibodies with bacterial polysaccharides
- Quadrivalent menigococcal polysaccharide vaccine **(A,C,Y & W135)**
- The use of meningococcal vaccine should be strongly advised if an outbreak occurs

Neisseria gonorrhoeae (Gonococcus)

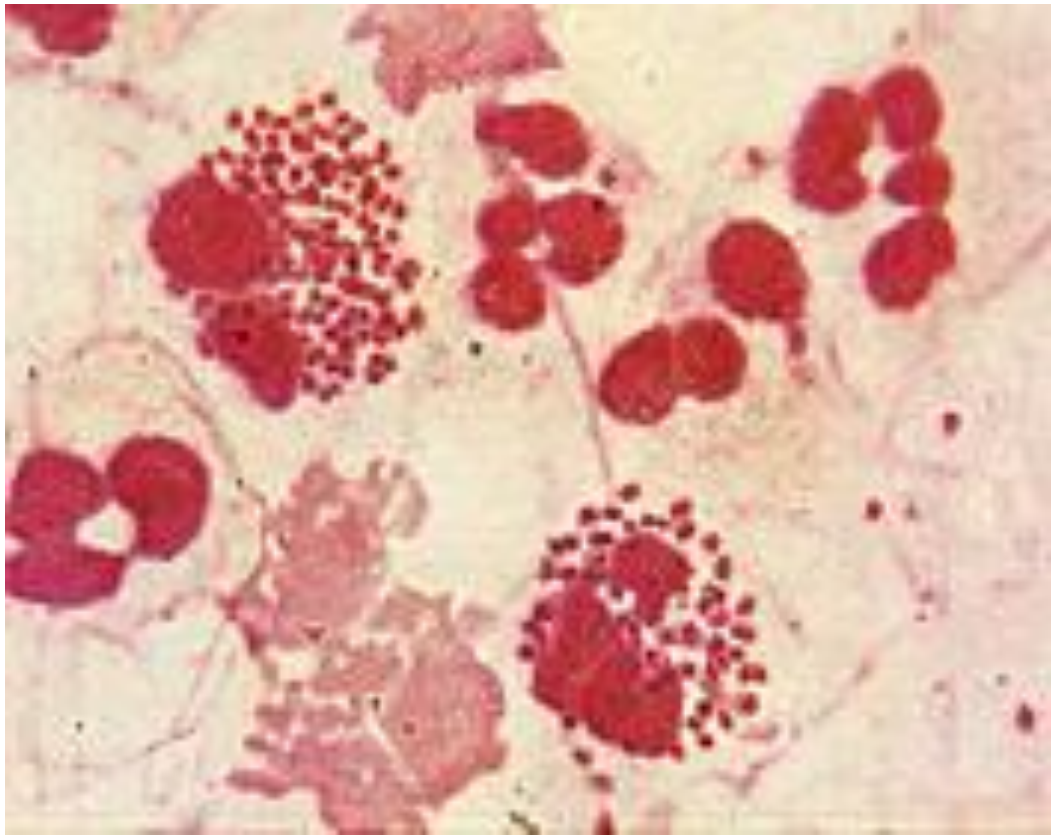
- *N. gonorrhoeae* causes the sexually transmitted disease **gonorrhoea**.
- first described by Neisser in 1879 in gonorrheal pus.
- resembles meningococci very closely in many properties.

MORPHOLOGY:

Morphology

- **Gram negative**
- oval/spherical **cocci**
- usually found with in the polymorphs
- Arranged in pairs (**adjacent sides concave**)
- **Kidney shaped**
- possess pili on their surface

Neisseria gonorrhoeae
(smear from urethral discharge)



CULTURE & CULTURAL CHARACTERISTICS:

- fastidious organisms do not grow on ordinary culture media.
- aerobic but may grow anaerobically also
- The optimum temperature for growth is 35-36℃ & optimum pH is 7.2-7.6.
- It is essential to provide 5-10% CO_2.

Media used:

a) Non selective media: Chocolate agar,
Mueller-Hinton agar
Modified New York City medium

b) Selective media: Thayer Martin medium with antibiotics (Vancomycin, Colistin & Nystatin)

Colony morphology: Colonies are

small

round

translucent

convex or slightly umbonat

finely granular surface

lobate margins.

Biochemical reactions:

1) Oxidase test: Positive

2) Ferments only glucose but not maltose.

BIOCHEMICAL TESTS

1. **Catalase: positive (+ve)**
2. **Oxidase: positive (+ve)**
3. **Carbohydrate utilization:**

Specie	Glucose	Maltose
N. gonorrhoeae	Acid (+ve)	(– ve)
N. meningitidis	Acid (+ve)	Acid (+ve)

Antigenic structure & virulence factors:

1. **Pili**

2. **Lipooligosaccharide:** Endotoxic.

3. **Outer membrane proteins:** 3 types
   a) Protein I (por)- it is a porin & helps in adherence.
   b) Protein II (opa)- helps in adherence.
   c) Protein III (rmp)- it is associated with protein I.

4. **IgA1 protease:** Splits & inactivates IgA.

GONOCOCCI

VIRULENCE FACTORS

1) *Outer membrane protein*

2) *Pili : Virulence*

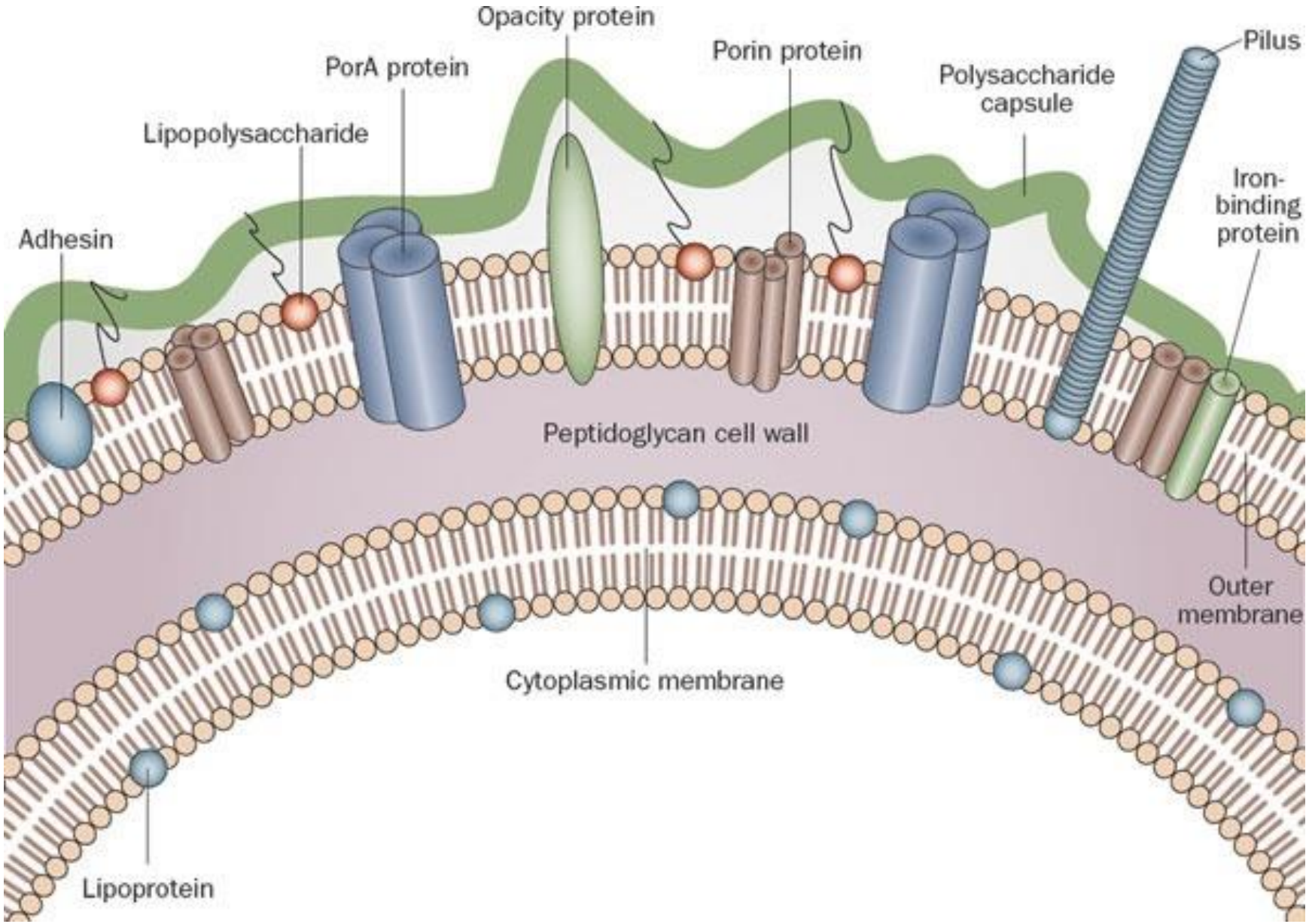
3) *Lipopolysaccharide*

4) *Capsular polysaccharide : Not responsible for symptoms*

5) *Lactoferin and transferrin*

Same as Meningococci

Gonococci - virulence factors



Gonorrhea: *Pathogenesis*

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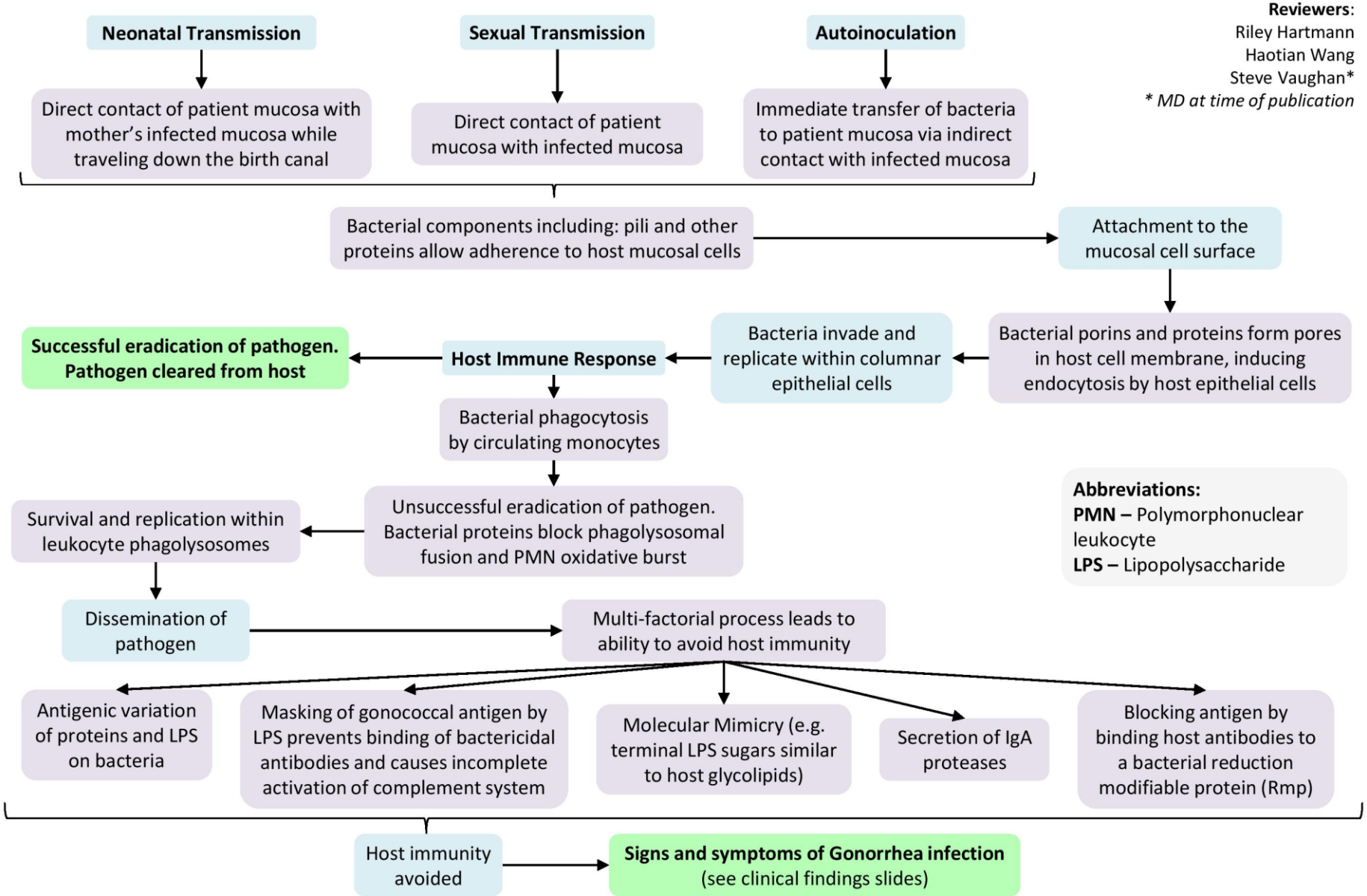

Abbreviations:
PMN – Polymorphonuclear leukocyte
LPS – Lipopolysaccharide

Legend: Pathophysiology | Mechanism | Sign/Symptom/Lab Finding | Complications

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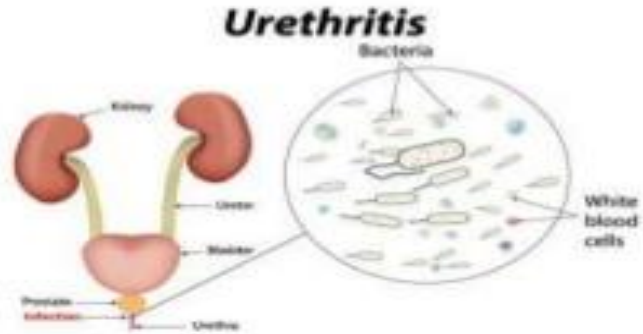
GONOCOCCI

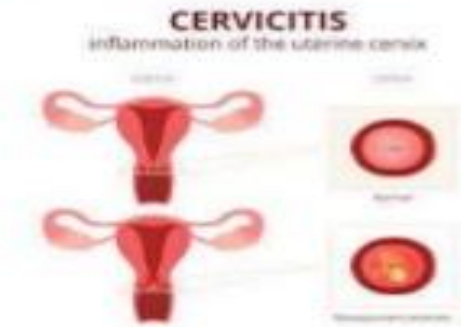
DISEASES

Ophthalmia neonatorum in newborns

*MC:**Urethritis** in **males***

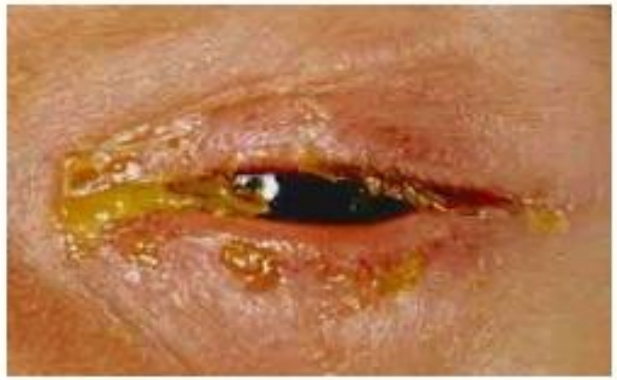
*MC: **cervicitis** in **females***





OPTHALMIA NEONATORUM:

- **EYE INFECTION IN THE NEWBORN.**
- **RESULTS DUE TO DIRECT INFECTION DURING PASSAGE THROUGH THE BIRTH CANAL.**



GONOCCOCCI

LAB DIAGNOSIS

SAMPLES

- *Eye swab*
- *Urethral swab*
- *Endocervical swab*

Media : ***Thayer Martin media***

Modified new York media

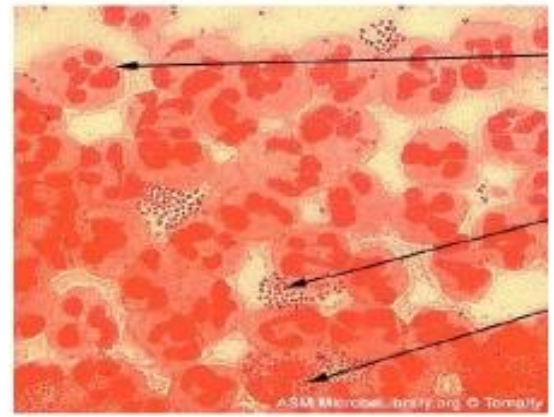
LAB DIAGNOSIS

SPECIMEN:

- **PUS EXUDATES - URETHRAL AND VAGINAL.**
- **VAGINAL AND CERVICAL SWABS.**
- **TRANSPORT MEDIUM: STUART'S MEDIUM.**

DIRECT MICROSCOPY:

- **INTRACELLULAR GRAM NEGATIVE KIDNEY SHAPED DIPLOCOCCI.**
- **FLUORESCENT ANTIBOBY TECHNIQUE –RAPID, SENSITIVE AND SPECIFIC DIAGNOSIS.**



INTRACELLULAR GRAM NEGATIVE DIPLOCOCCI

LAB DIAGNOSIS- CONT.,

CULTURE:

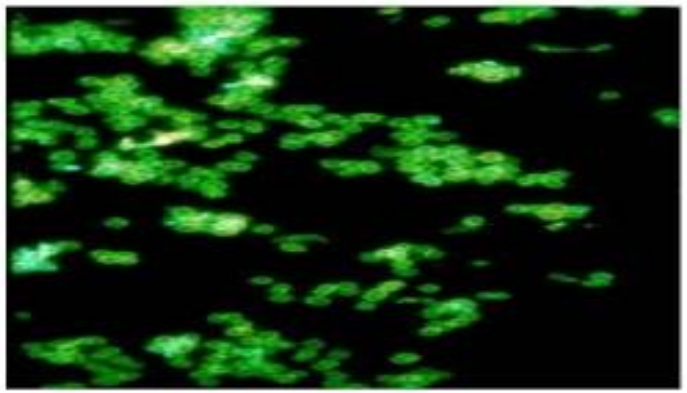
- **CHOCOLATE AGAR.**
- **SELECTIVE MEDIA: THAYER MARTIN.**
- **INCUBATION AT 37° C IN THE PRESENCE OF 5-10% OF CARBON DIOXIDE.**

BIOCHEMICAL REACTION:

- **GLUCOSE FERMENTATION.**
- **OXIDASE POSITIVE.**

SEROLOGY:

- **IMMUNOFLUORESCENCE.**
- **RIA.**
- **ELISA**



IMMUNOFLUORESCENCE

Treatment and prevention

- Treatment - beta-lactamase-resistant cephalosporins (ceftriaxone), new generation macrolides (azithromycin) are used.
- Specific treatment - antibiotic therapy in chronic complicated gonorrhea is carried out against the background of specific (gonovaxin) or non-specific (pyrogenal) immunotherapy.
- **There is no specific prevention.**

Microbiology diagnosis of diseases, caused by opportunistic bacteria (klebsiella, proteus, acinetobacter, pseudomonas)

Klebsiella spp.

Taxonomy :

| Domain = Bacteria
| Phylum = Proteobacteria
| Class = Gammaproteobacteria
| Order = Enterobacteriales
| Family = Enterobacteriaceae
| Genus = *Klebsiella*
| Species =k.pneumonia , k.ozaenae
, k.rhinoscleromatis.

Klebsiella

K. pneumoniae complex (KpSC):

- *K. pneumoniae*
- *K. quasipneumoniae*
- *K.variicola*
- *K. quasivariicola*
- *K. africana*

Others

- *K. indica,*
- *K. terrigena,*
- *K. spallanzanii,*
- *K. huaxiensis,*
- *K. oxytoca,*
- *K. grimontii,*
- *K. pasteurii*
- *K. michiganensis*

Characteristics:

1. gram-negative
2. Non motile
3. Lactose fermenting
4. Oxidase negative
5. Rod shaped organism
6. Facultative anaerobe
7. Surrounded by thick capsule
8. Act as oppurtunistic human pathogen

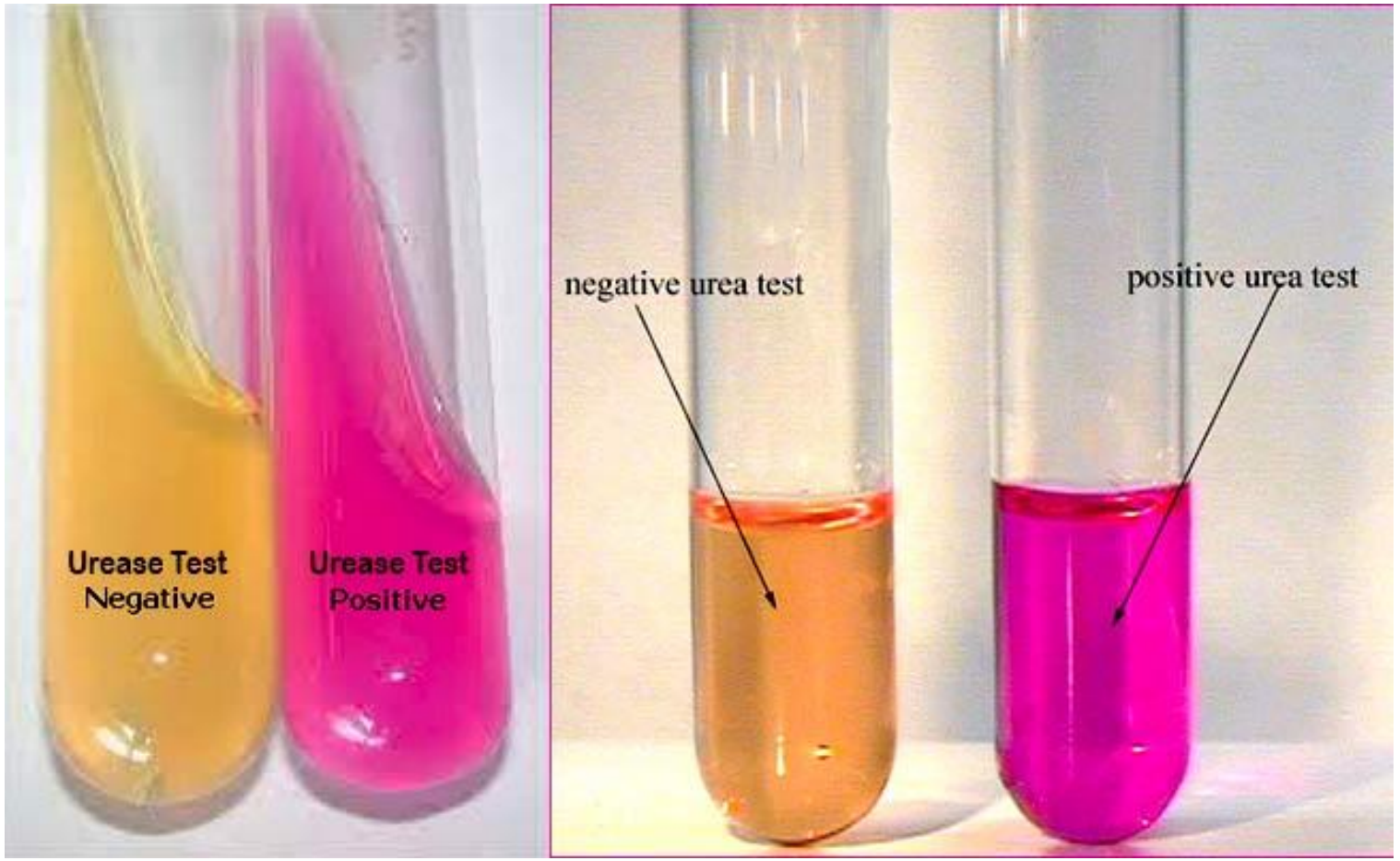
Where it is found?

1)Found in the normal flora of the nose, mouth, skin, GI tract and intestines.
2)It is also found in soil and water.

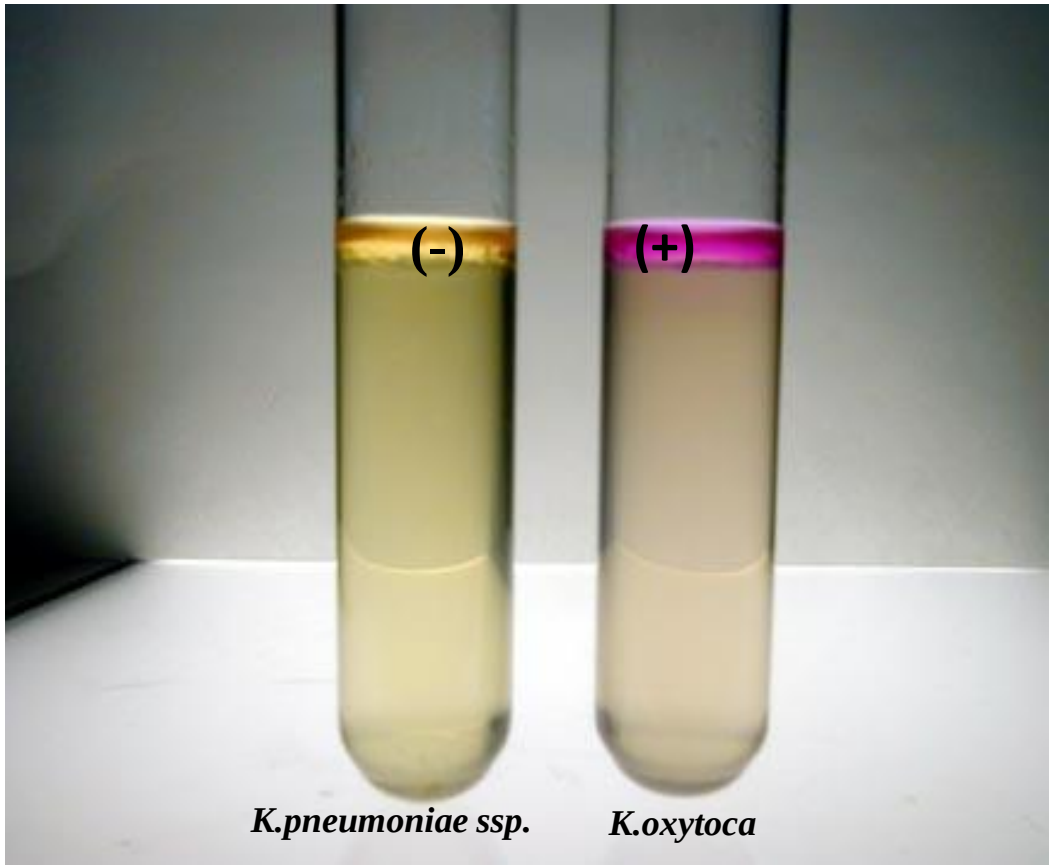
Generally, Klebsiella infections are seen mostly in people with a weakened immune system.

Tests	*K. pneumonia*	*K. oxytoca*	*K. terrigena*
Capsule	+	+	+
Oxidase	-	-	-
Catalase	+	+	+
Indole	-	+	-
Methyl red	-	-	+
Voges proskauer	+	+	+
Citrate utilization	+	+	+
Urease production	+	+	-
Motility	-	-	-

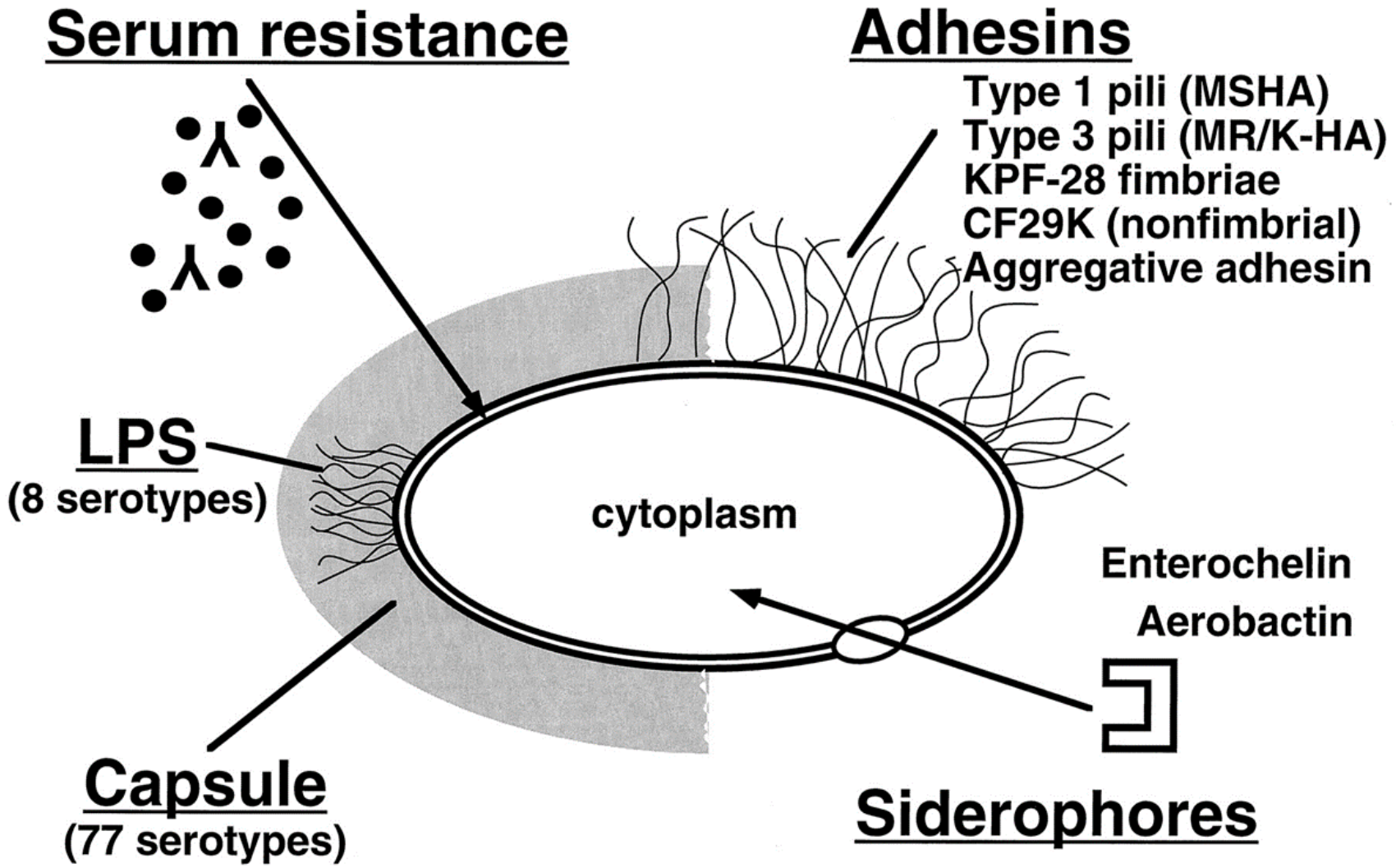
Identification of *Klebsiella* bacteria (positive urease test)



Identification of *Klebsiella* bacteria (indole test)



VİRULENCE FACTORS

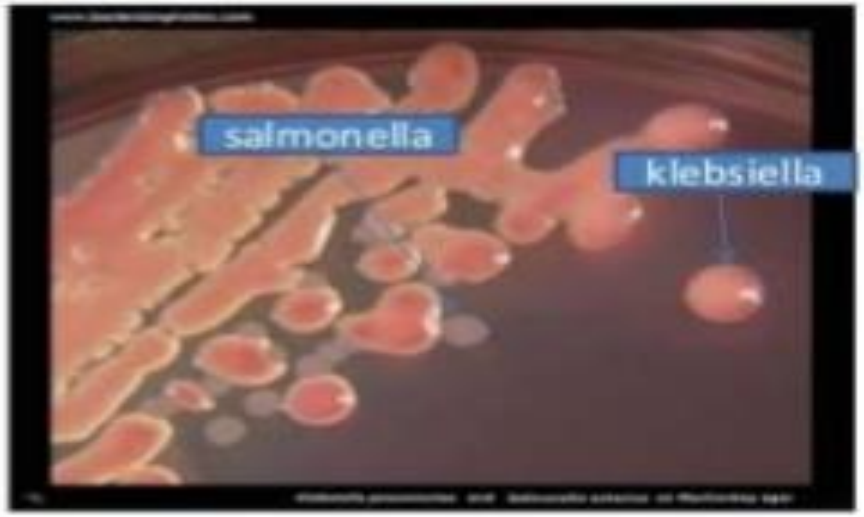


On blood agar

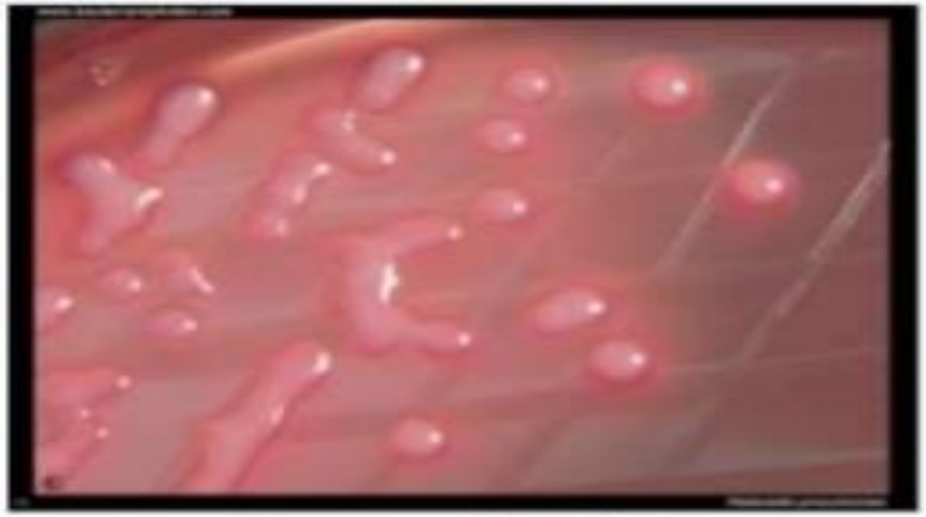
-slimy appearance of the colonies

On MacConkey agar

- red/pink colonies



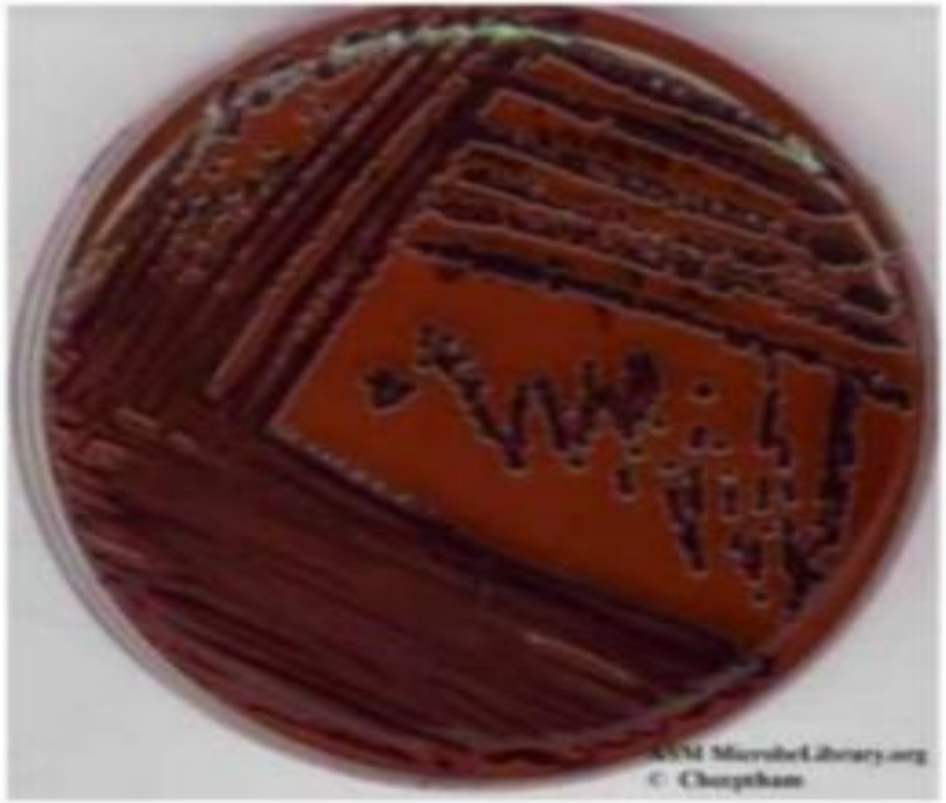
Klebsiella pneumoniae and *Salmonella enterica* on MacConkey agar:
lactose + and -



Mucous, lactose positive colonies of *Klebsiella pneumoniae* on MacConkey agar. Cultivation 37°C, 24 hours.

On EMB

- *Klebsiella* species produces large, mucoid, pink to purple colonies with no metallic green sheen on EMB agar.



India ink capsule stain

-The background will be dark.
-The bacterial cells will be stained purple.
-The capsule (if present) will appear clear against the dark background.

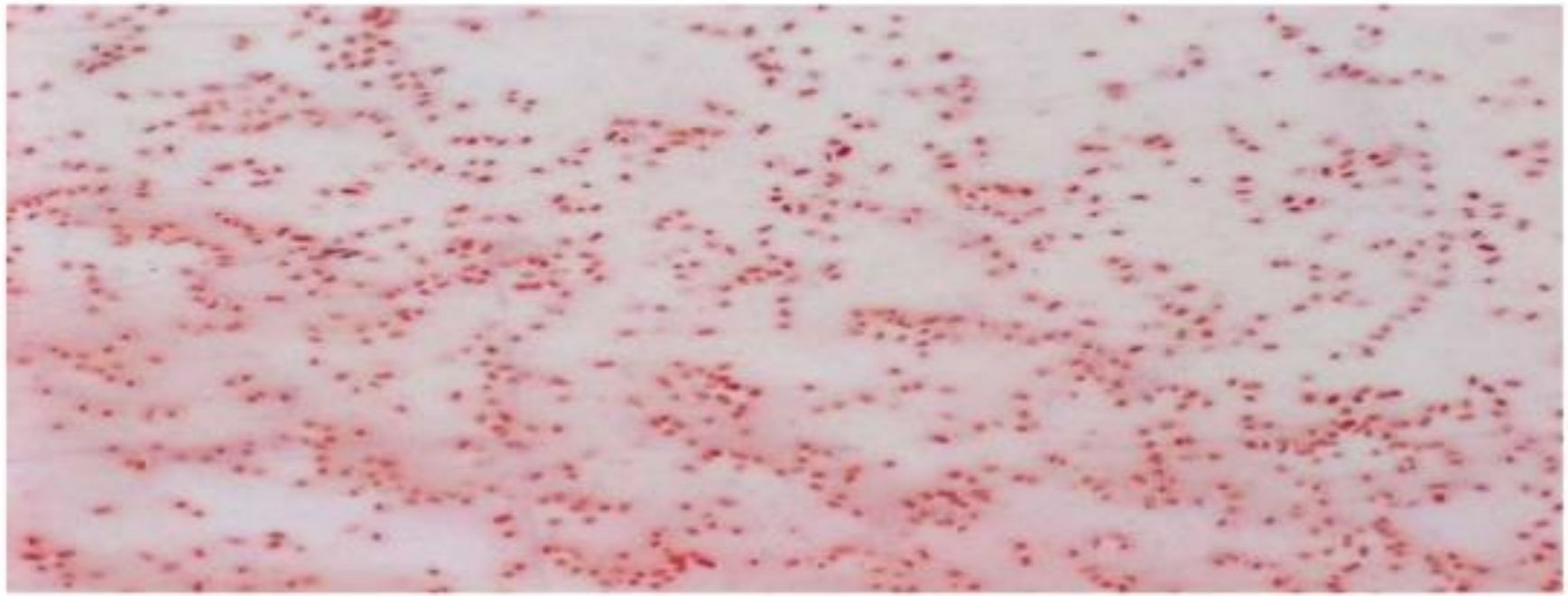
India Ink Capsule Stain of *Klebsiella pneumoniae* showing white capsules (Glycocalyx) surrounding purple cells

Laboratory diagnosis

- **Specimen**
- Sputum.
- Urine.
- pus.
- CSF.

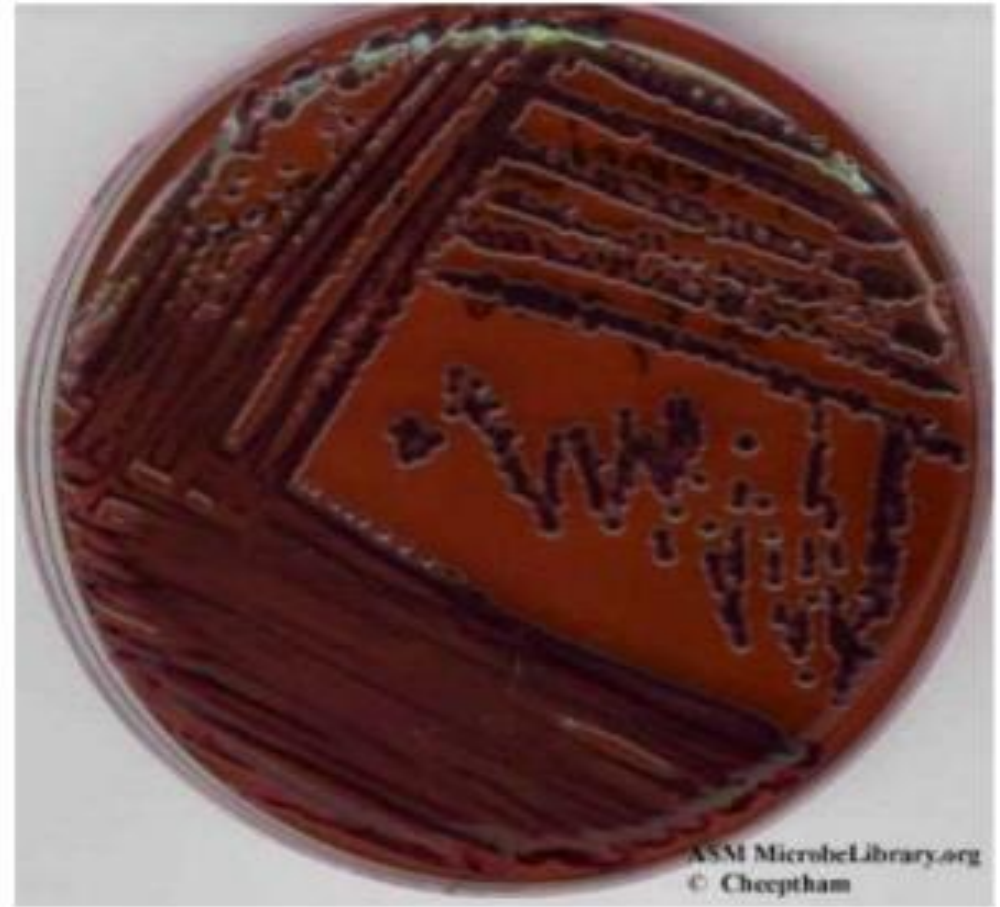
- **Gram stain:**

gram-negative rods



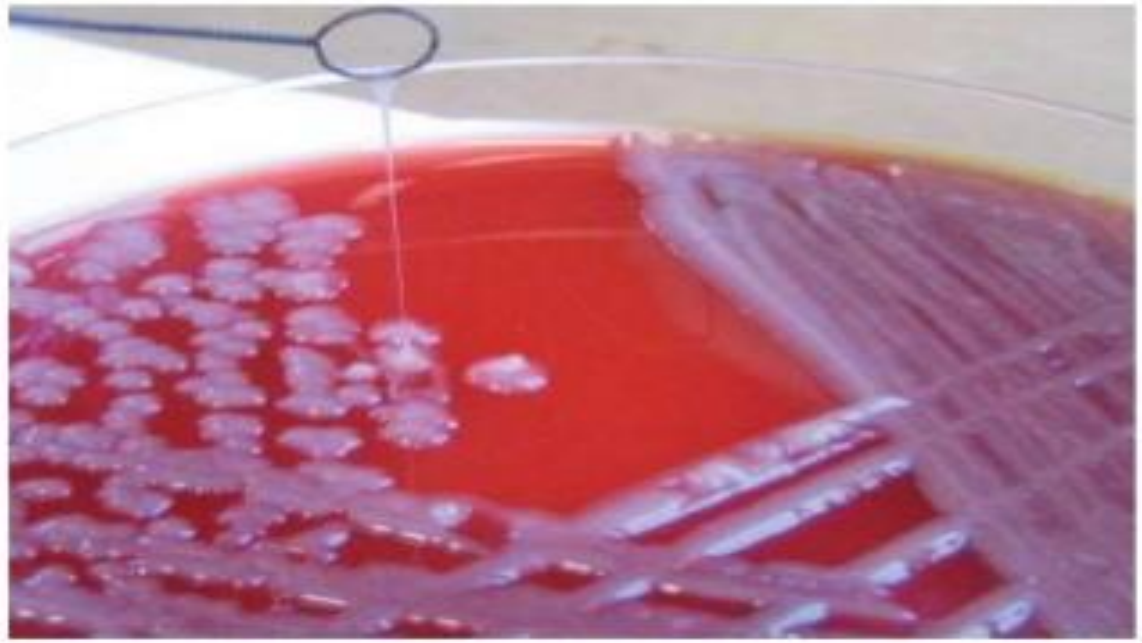
On EMB

- *Klebsiella* species produces large, mucoid, pink to purple colonies with no metallic green sheen on EMB agar.

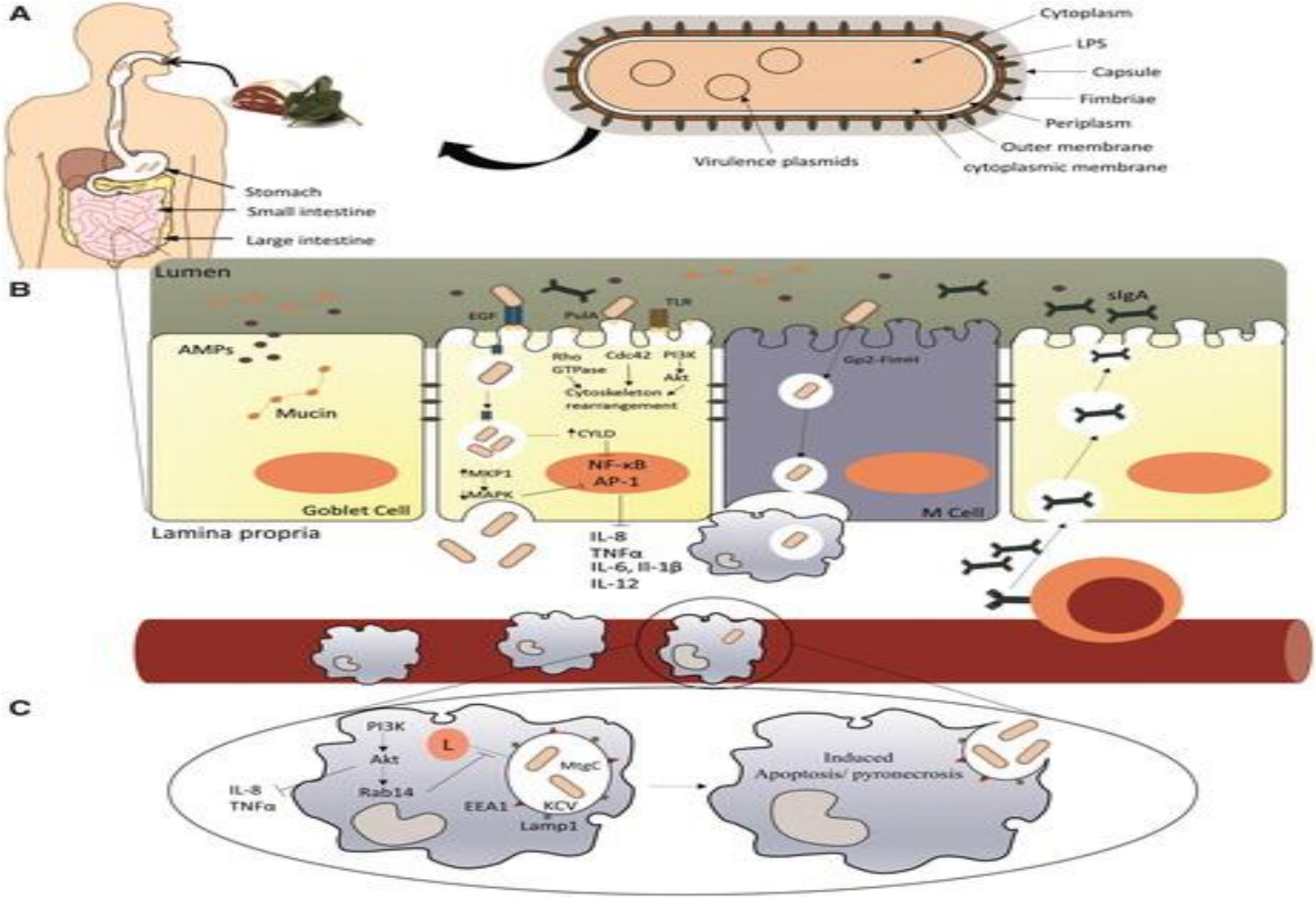


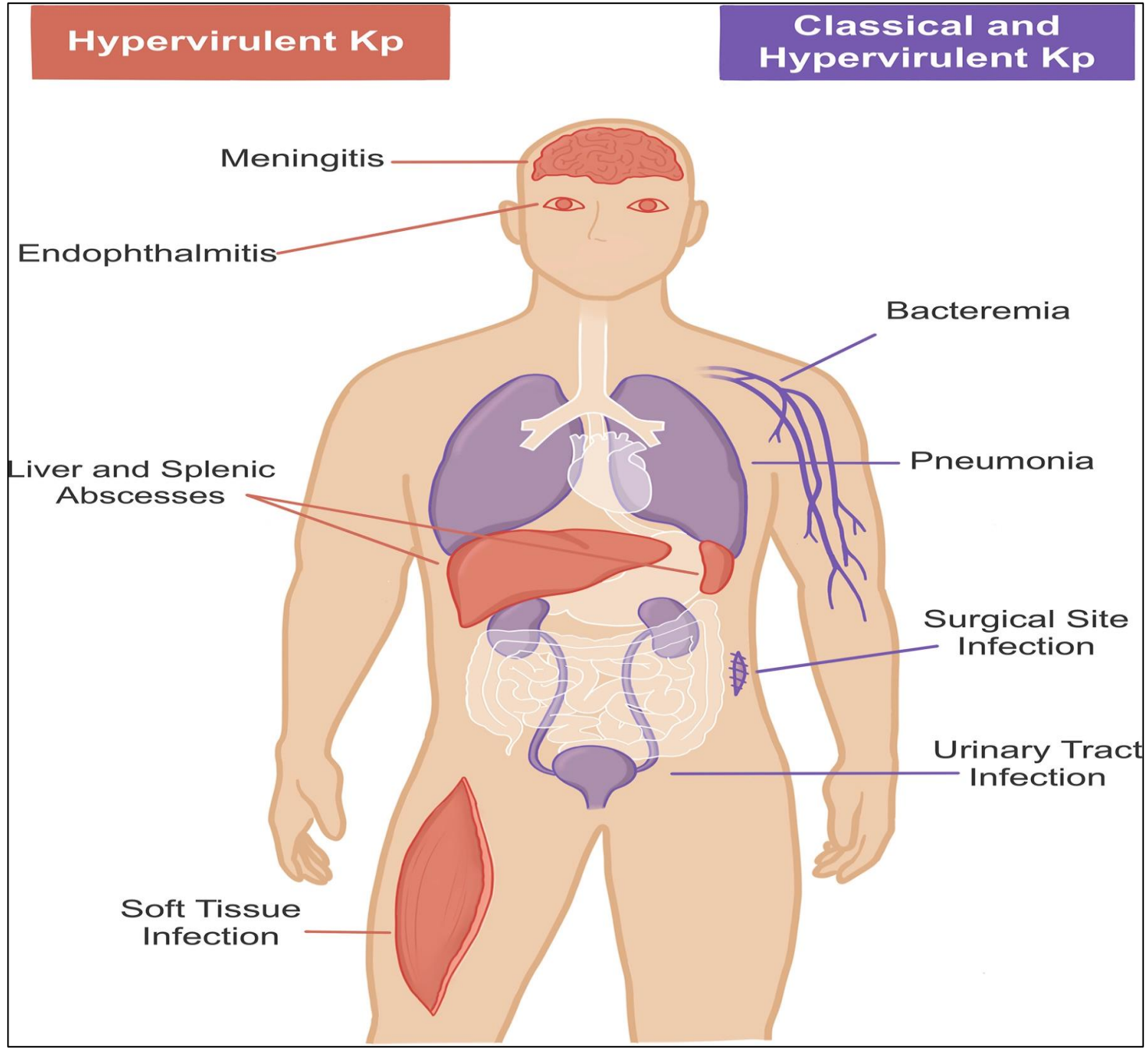
String test

- A colony that stretches more than 5 mm using a standard inoculation loop tests positive for hypermucoviscosity.



Mucoid colony of *Klebsiella pneumoniae* . When colonies were touched with a loop and the loop lifted vertically from the surface of the agar plate, mucoid isolates adhered to the loop as it was lifted from the plate .

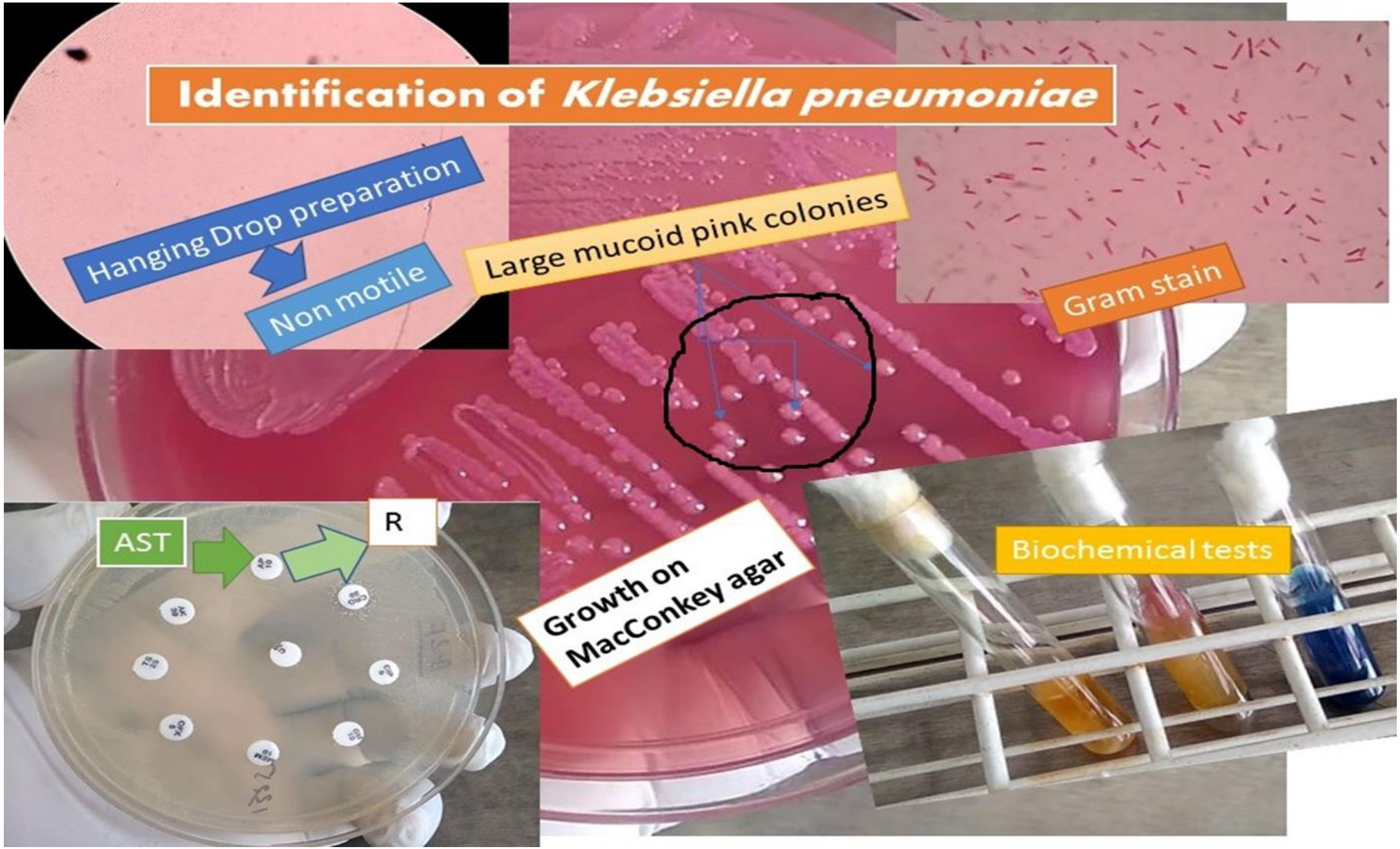

A
Stomach
Small intestine
Large intestine
Cytoplasm
LPS
Capsule
Fimbriae
Periplasm
Outer membrane
cytoplasmic membrane
Virulence plasmids
B
Lumen
sIgA
EGF
PulA
TLR
AMPs
Mucin
Goblet Cell
Rho GTPase
Cdc42
PI3K
Akt
Cytoskeleton rearrangement
Gp2-Fimet
CYLD
MKP1
MAPK
NF-κB
AP-1
M Cell
Lamina propria
IL-8
TNFα
IL-6, Il-1β
IL-12
C
PI3K
Akt
L
Rab14
IL-8
TNFα
MtgC
EEA1
KCV
Lamp1
Induced
Apoptosis/ pyronecrosis


Hypervirulent Kp
Classical and Hypervirulent Kp
Meningitis
Endophthalmitis
Bacteremia
Pneumonia
Liver and Splenic Abscesses
Surgical Site Infection
Urinary Tract Infection
Soft Tissue Infection

Diseases Caused by Klebsiella :

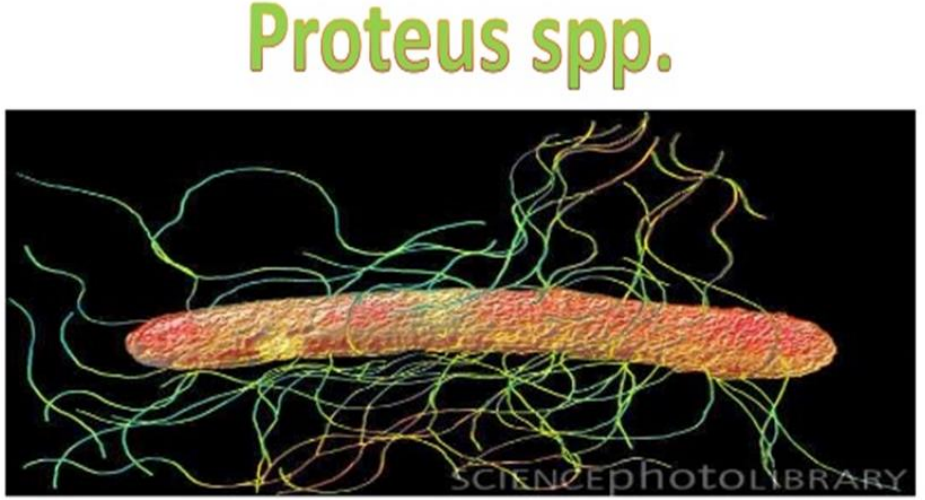
1) urinary tract infections
2)pneumonia
3) Specticaemia
4) nosocomial infections
5) soft tissue infections.

K.rhinoscleromatis


Identification of *Klebsiella pneumoniae*
Hanging Drop preparation
Non motile
Large mucoid pink colonies
Gram stain
AST
R
Growth on MacConkey agar
Biochemical tests

PROTEUS - Taxonomy

- (Domain): Bacteria
- (Kingdom): Proteobacteria
- (Class): Gammaproteobacteria
- (Order): Enterobacteriales
- (Family): Enterobacteriaceae
- (Genus): Proteus
- (Species): ***P.vulgaris, P.mirabilis***



General charachteristic:

Gram negative rods, facultative anaerobics.

Motile they have peritrichous flagella

Non capsulated

Non spore forming

Proteus sp. are most commonly found in the human intestinal tract as part of normal human intestinal flora.

Non-lactose fermenting

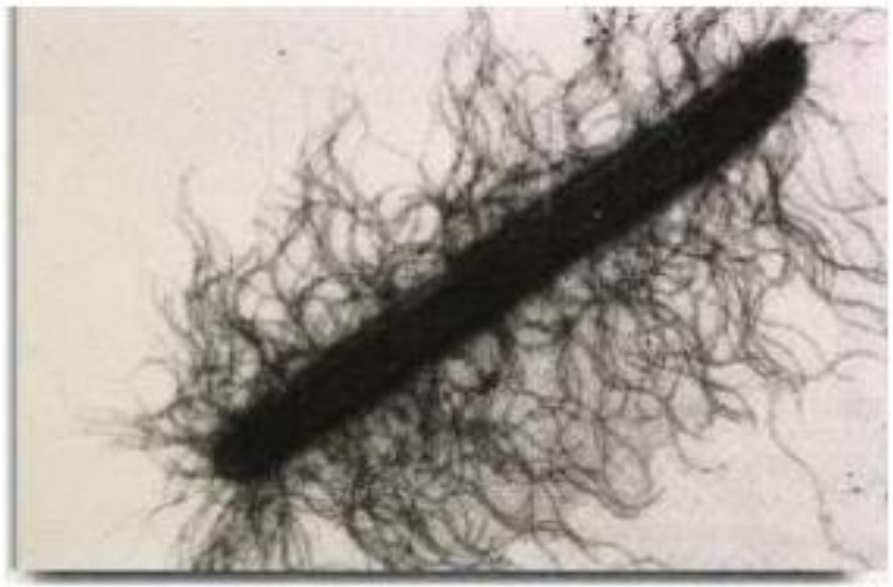
Gram stain

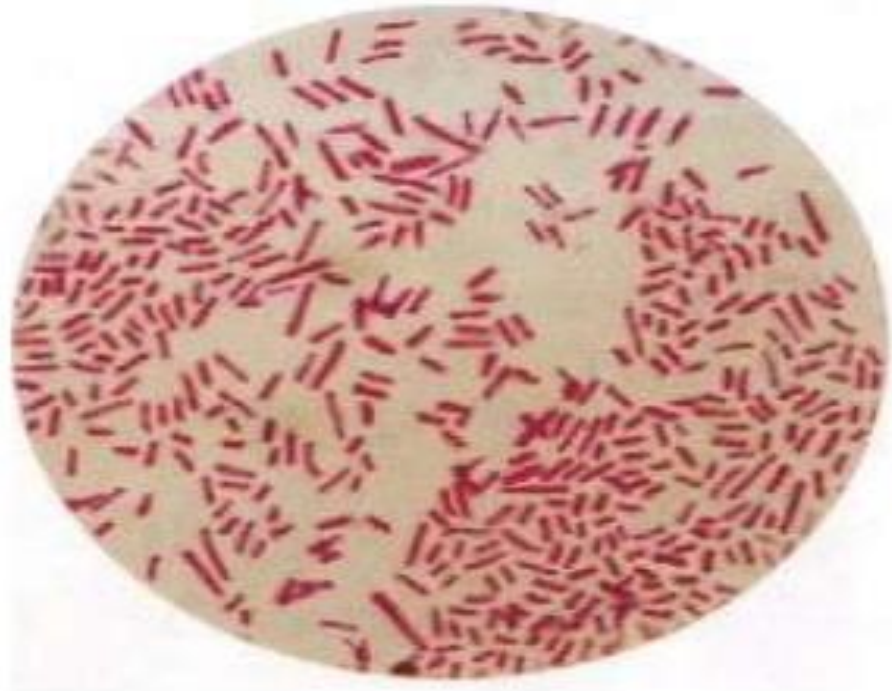
- Gram-negative rods

Morphology of proteus spp

Microscopical morphology

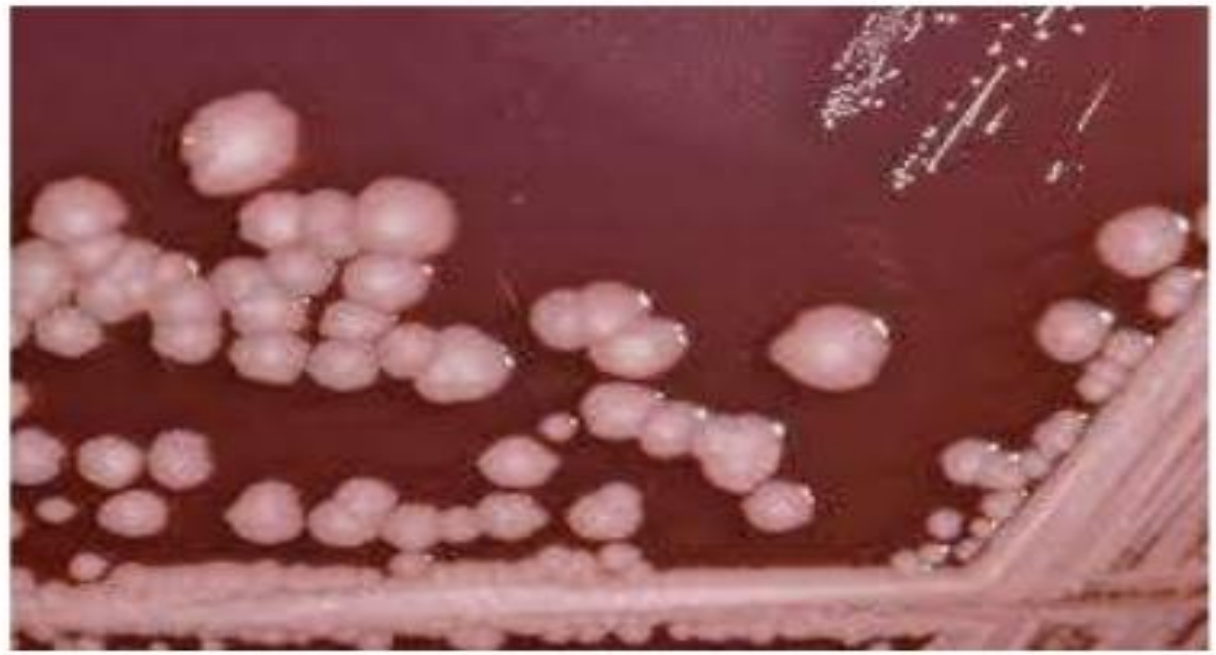
Gram negative bacilli, motile has flagella





Colony morphology:

Large ,circular,gray ,smooth colonies



The main species of medical importance are:

P. mirabilis

P. vulgaris

Proteus spp. are opportunist pathogens and may cause many types of infection.

P. mirabilis

causes 90% of all *Proteus* infections in humans.

Clinical features:

- Urinary tract infection
- Septicemia
- Abdominal and wound infection
- Secondary invader of ulcer, burn and chronic discharging ear.

Diagnosis method of proteus spp.

Specimens:

Urine, pus and ear

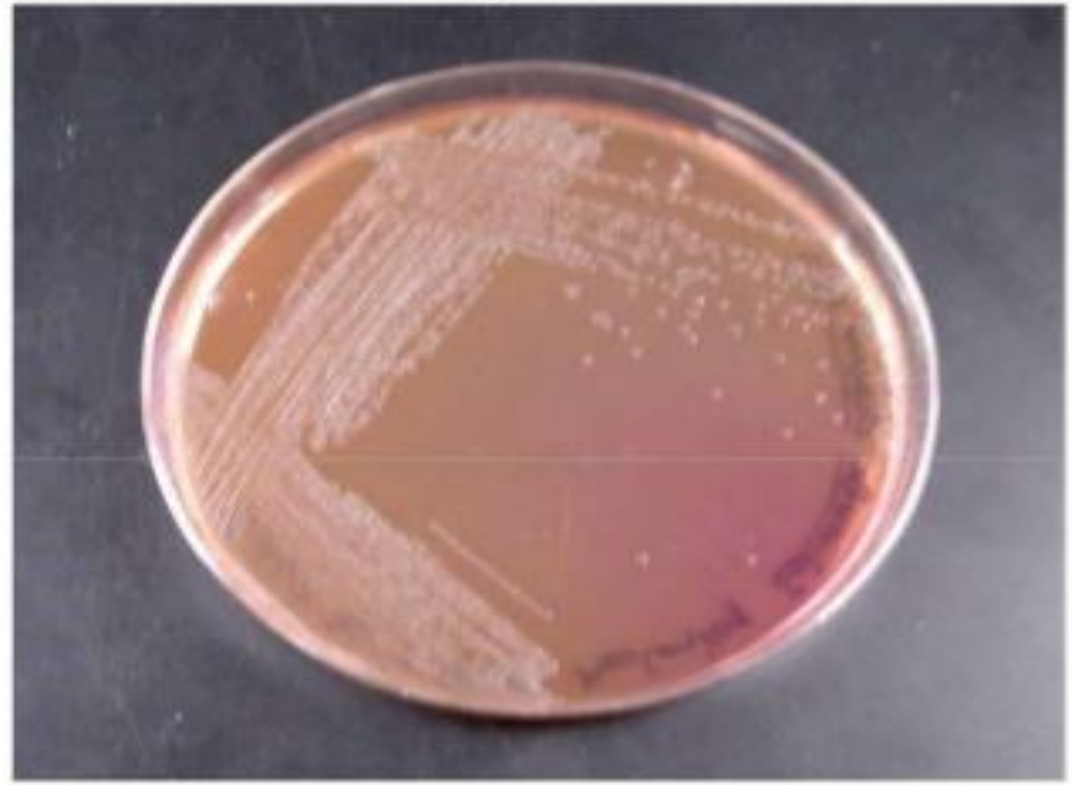
Gram stain:

Rod shaped gram negative

Culture:

Blood agar: Swarming effect over blood agar plate as a consequence of the organisms active motility.

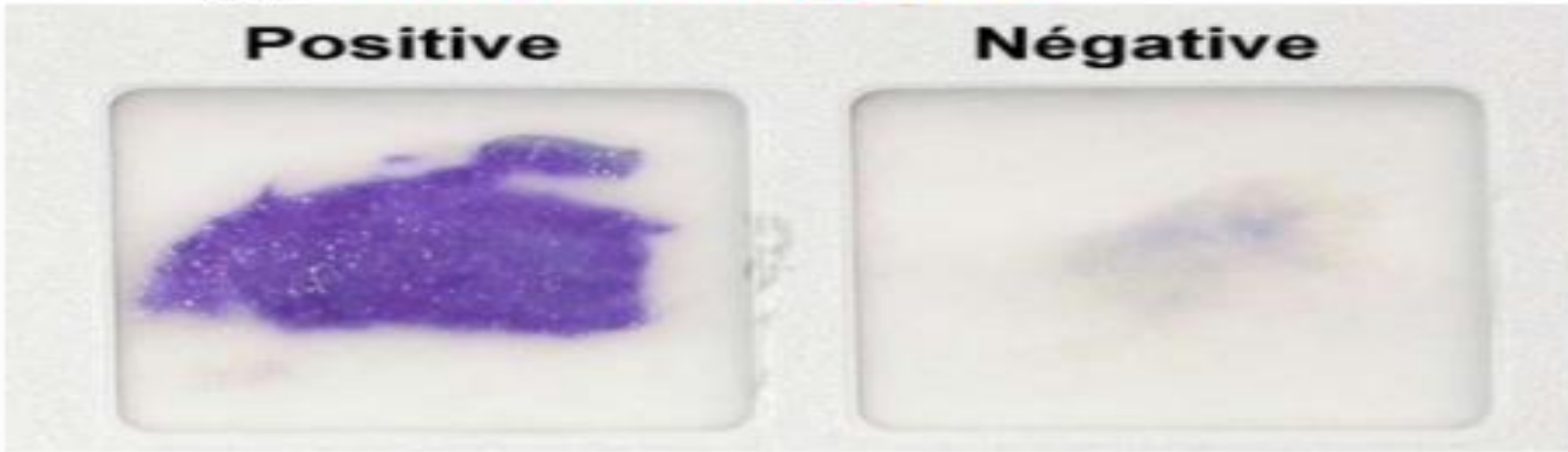
Macconkey agar: Cultures give out an odour described as fishy, Non- lactose fermenting colonies .



Oxidase test:

The oxidase test is used to determine if a bacterium produces certain cytochrome c oxidases. The reagent turns dark blue when oxidized (oxidase positive). The reagent is colorless when reduced (oxidase-negative)

Proteus spp. Oxidase negative



IMViC test

1-indole test:

is used to determine the ability of bacteria to convert tryptophan into indole.

P. mirabilis can be differentiated from *p.vulgaris* by indole test.

P. mirabilis → **negative**

P. vulgaris → **positive**

Methyl red test:

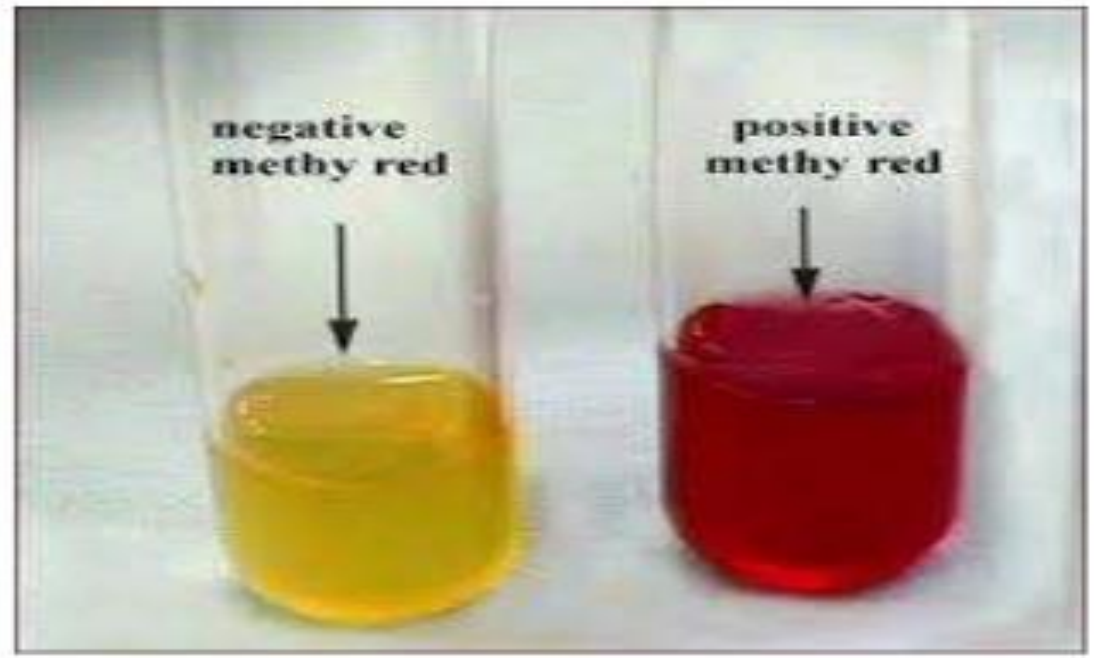
. The methyl red test is used to identify bacteria to produce pyruvic acid from glucose metabolism.

Proteus vulgaris:

Methyl red: **posative**

Proteus mirabils:

Methyl red: **posative**

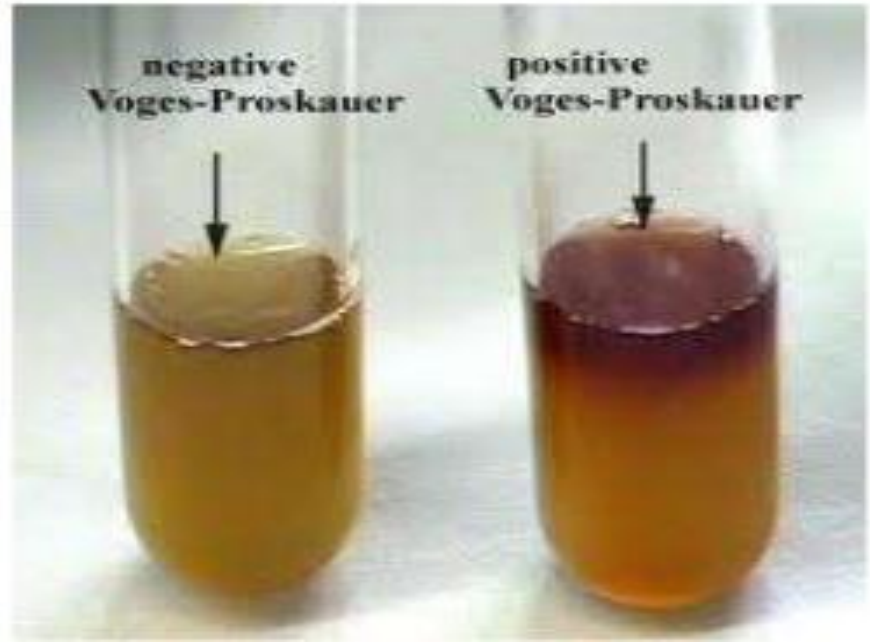


Voges–Proskauer:

is a test used to detect acetoin in • a bacterial broth culture. A red-brown color indicates a positive result, while a yellow-brown color indicates a negative result.

p.Vulgaris : **Negative**

p.mirabilis:**negative**

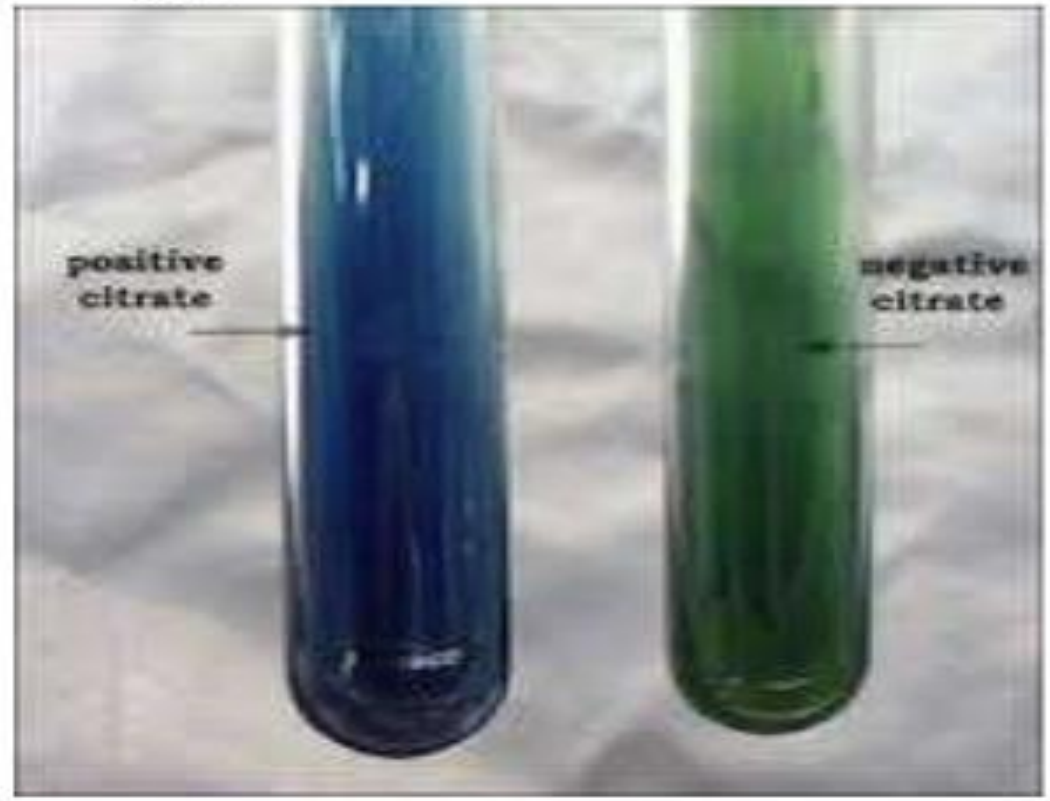


Citrate test:

Ability of an organism to use citrate as the sole source of carbon and energy.

p.Vulgaris: **Negative**

p.mirabilis: **posative**



TSI test
Triple Sugar Iron test

This test is used to determine the ability of bacteria to ferment sugars and to produce hydrogen sulfide (H2S) or other gases

Proteus spp.

(red/red with H2S production)

(black)

Motility test

used to determine whether an organism is equipped with flagella and thus capable of swimming away from a stab mark.

Left tube •

shows positive motility test

for *Proteus spp.*

Right tube •

negative for *S.aureus*

Swarming phenomenon

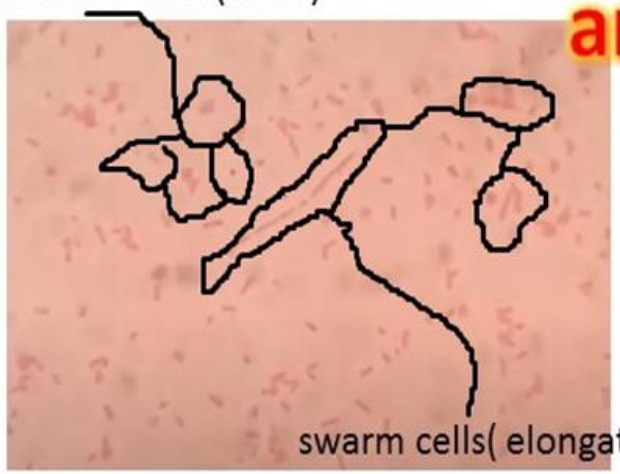
swarming is described as the formation of concentric zones of bacterial growth, able to cover the whole surface of solid culture medium.

P.mirabilis & P.vulgaris are known for their swarming ability over(sheep blood agar)

Proteus: Growth on Various media, Gram Stain and Biochemical Reactions

MacConkey agar: Lon-lactose fermenting (NLF) colonies and swarming prevented due to bile salt present in the medium.

Blue grey translucent colonies on CLED agar



TSI Test:
Red slope/Yellow butt
H_2S : Positive
No gas formation

MIU Test:
Motile
Indole Negative
Urease positive

Citrate Utilization Test Positive

Pseudomonas – Taxonomy

- (Kingdom): Bacteria
- (Class): Gammaproteobacteria
- (Order): Pseudomonadales
- (Family): Pseudomonadaceae
- (Genus): **Pseudomonas**
- (Species): ***P.aeruginosa,***

  P.fluorescens,

  P.putida, P.cepacia, P.stutzeri,

  P.maltophilia, P.putrefaciens.

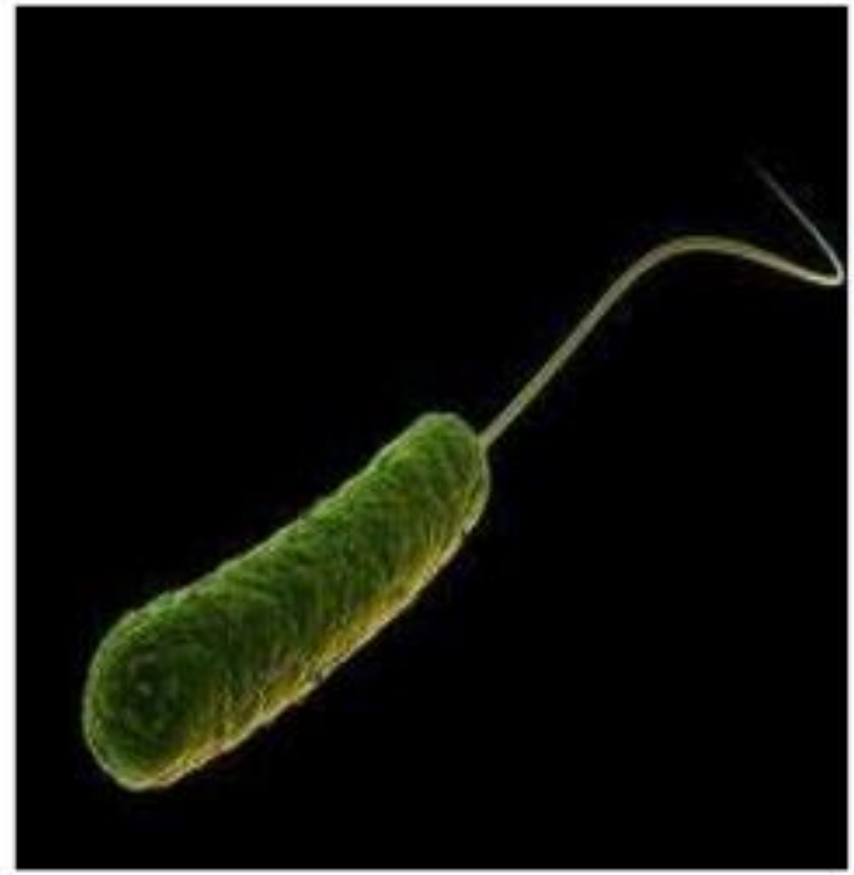
- Medically important *Pseudomonas*:

P. aeruginosa.

- present in small numbers in the normal intestine flora and on the skin.
- Commonly present in moist environments in hospitals.
- It is primarily a nosocomial pathogen.

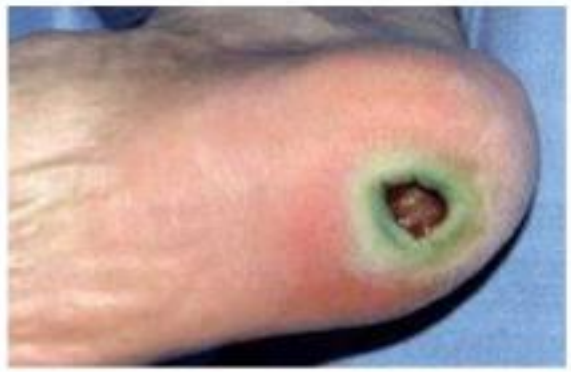
General character

- Gram-negative rods.
- Motile by polar flagella.
- aerobics.
- Grow well at 42°C.
- Non-lactose fermenting



Pigment production

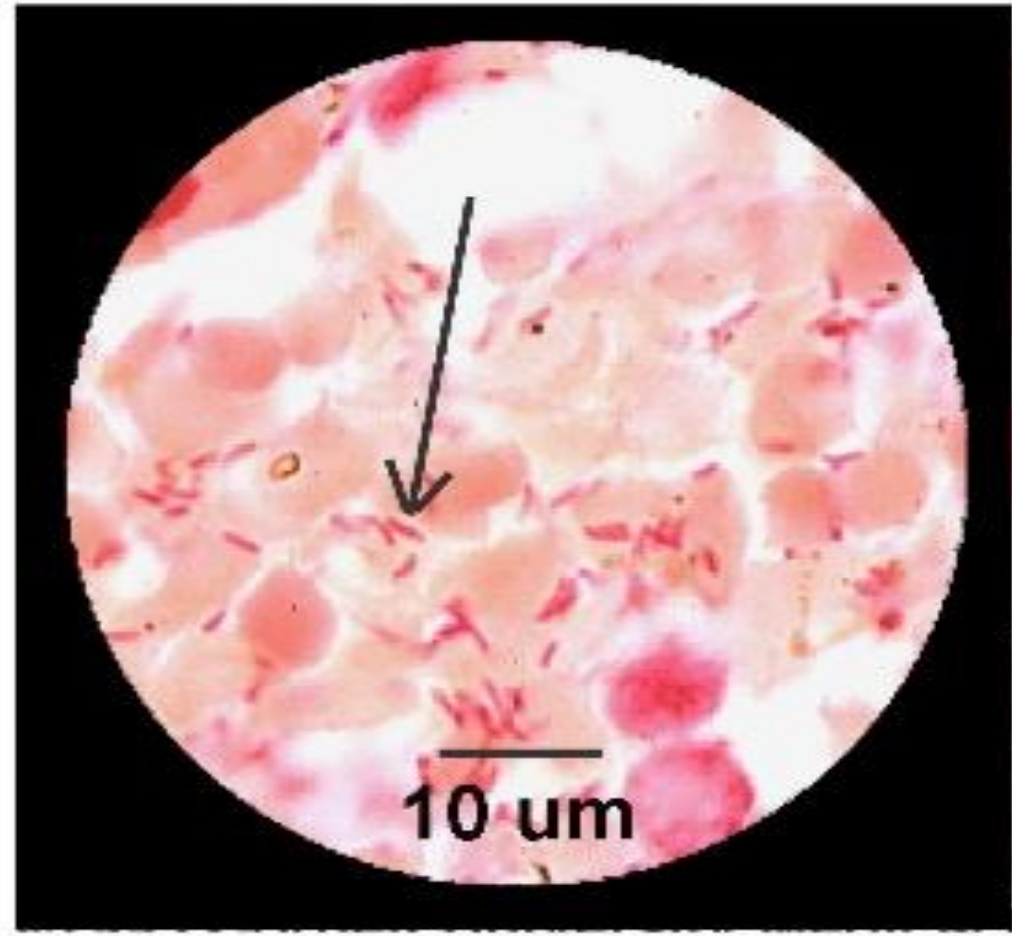
- can produce pigments, such as:
- Pyocyanine (blue-green)
- Pyoverdin (fluorescent yellow- greenish pigment)
- Pyorubrin (red)
- Pyomelanin (brown)



Microscopy

-**Gram stain**

Gram negative rod undistinguished from enerobacteriaceae.



Cornell University Medical College

Culture character

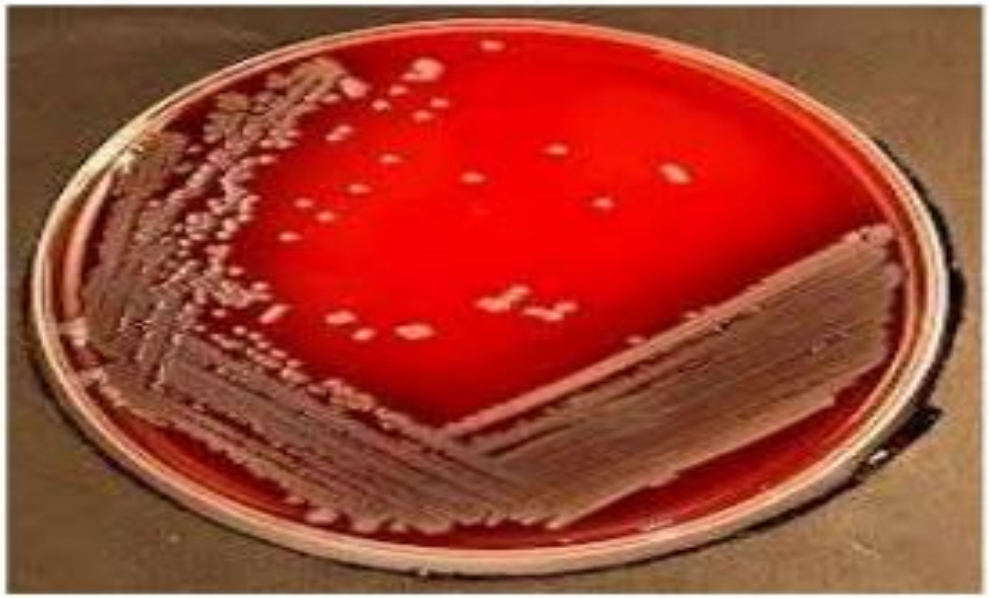
- Form smooth and round colonies.
- **Fluorescent greenish colour.**
- production of fruity odor (grape-like) .
- Inability to ferment lactose.

On nutrient agar

- Colonies are smooth, large, translucent
- Greenish blue diffusible pigment

On blood agar

- Grayish colonies
- Many are haemolytic (beta hemolysis).



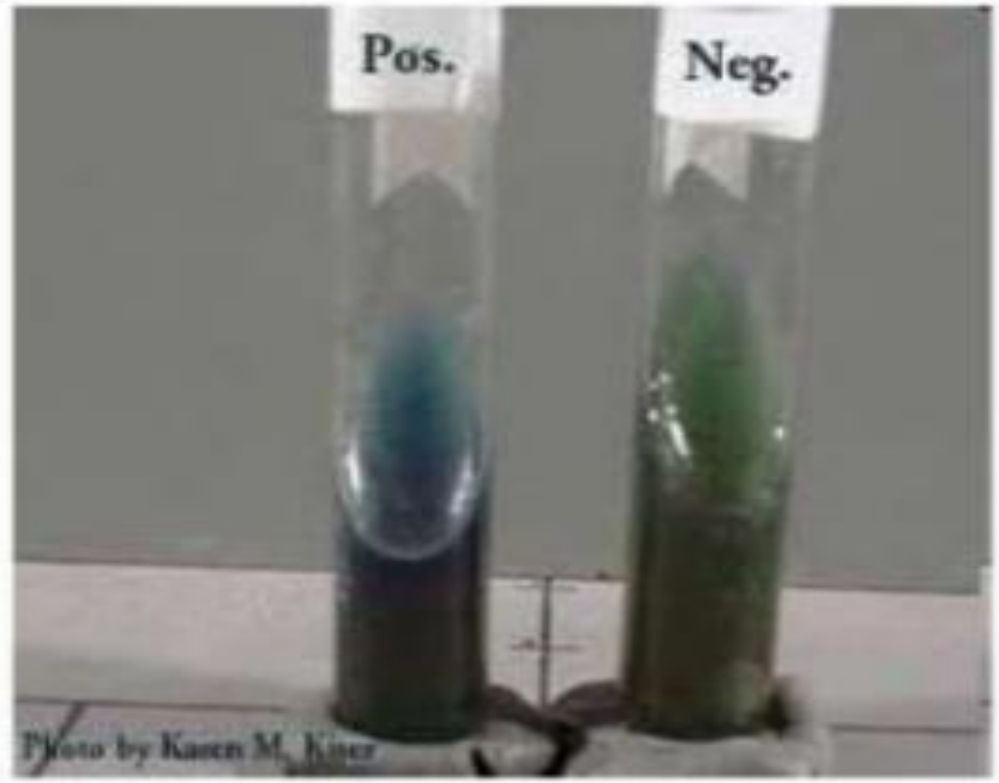
This picture shows *Pseudomonas aeruginosa* (on the right) and *Shigella dysenteriae* (on the left) in blood agar.

On MacConkey agar

- Non-lactose fermenting (colourless colonies)

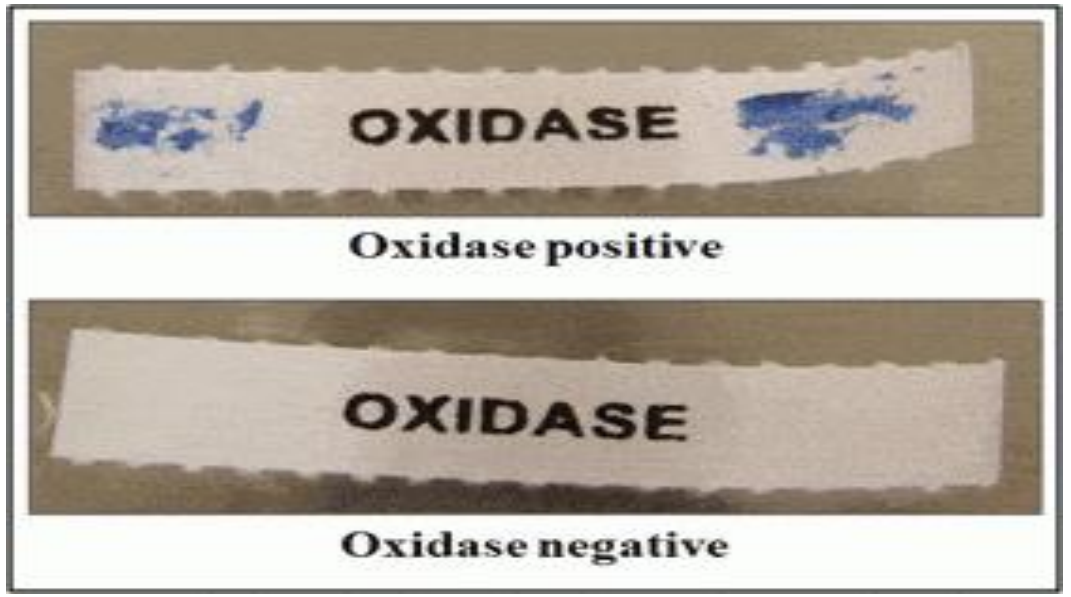
Biochemical Tests

- Indole test-negative
- Methyl red test-negative
- Vp test-negative
- Citrate test-positive
- Urease test-negative



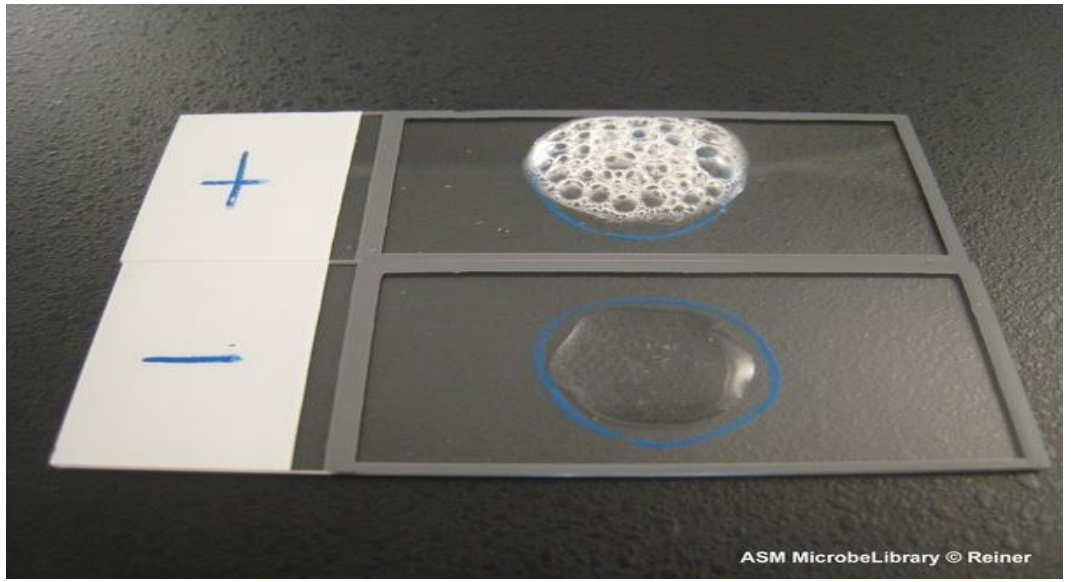
Oxidase test

- **Oxidase positive**

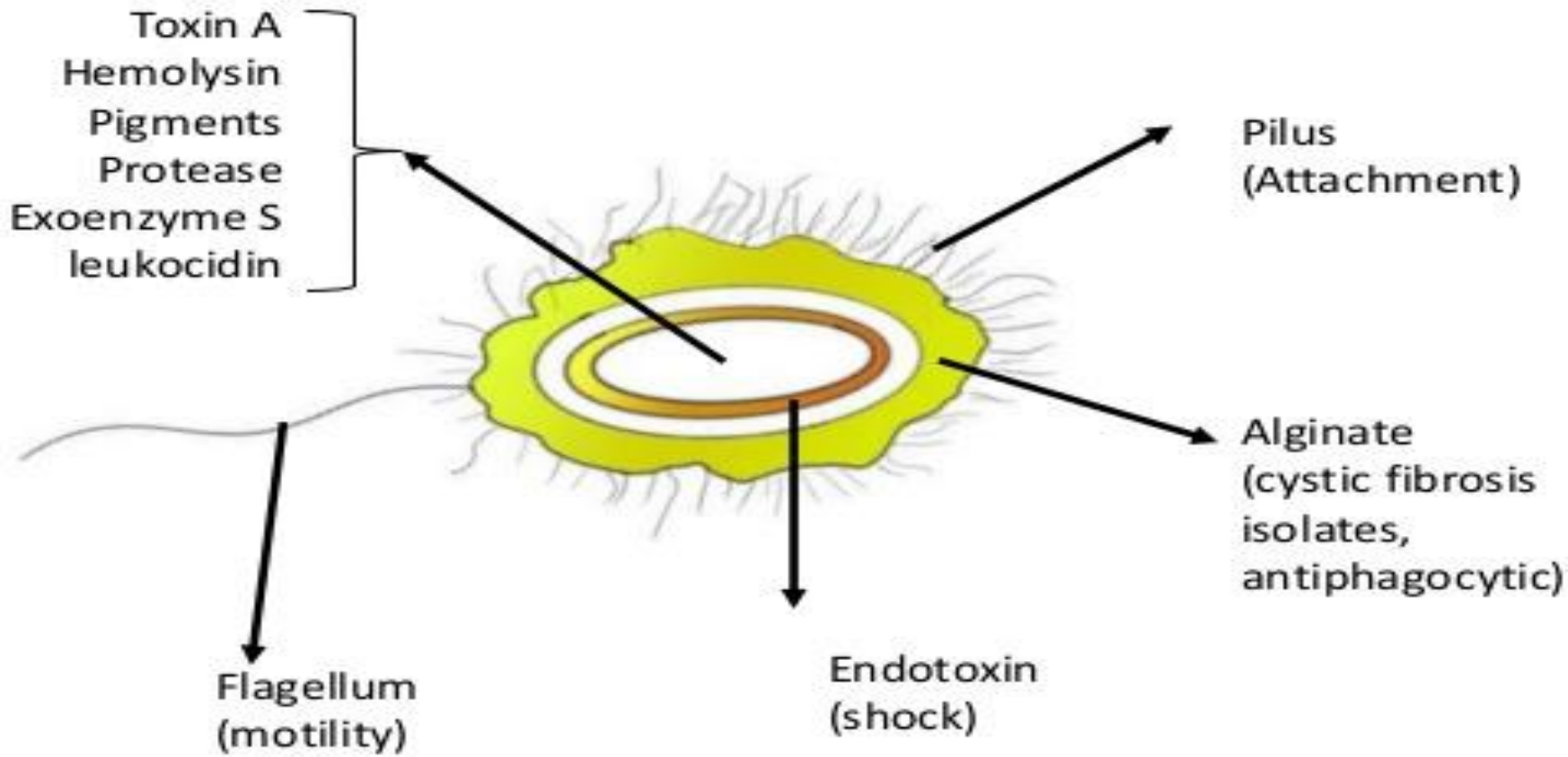


Catalase test

- Catalase positive



Pseudomonas aeruginosa



Structure and pathogenic mechanisms of *P aeruginosa*.

Pathogenesis

- P. aeruginosa can produce a lot of antigens some of them are

1. Endotoxin
2. Hemolysin
3. Leukocidin
4. Pigments: fluorescein, pycocyanin
5. Proteases: elastase
6. Toxin A
7. Exoenzyme S
8. Phospholipase c

- Almost all strains of **P aeruginosa are hemolytic** on blood agar plates
- **Hemolysin** produced is **toxic to alveolar macrophages** and play a role in pulmonary infections
- This **leukocidin** (also called cytotoxin) damages lymphocyte

Pathogenesis

- P. aeruginosa can produce a lot of antigens some of them are

1. Endotoxin
2. Hemolysin
3. Leukocidin
4. Pigments: fluorescein, pycocyanin
5. Proteases: elastase
6. Toxin A
7. Exoenzyme S
8. Phospholipase c

- **extracellular polysaccharide** impede phagocytosis and impair diffusion of antibiotics.
- **Proteases** induce formation of hemorrhagic lesions, which become necrotic within 24 hours. It contribute to the tissue destruction that accompanies P aeruginosa **eye or lung infections** and may aid bacteria in tissue invasion

Pathogenesis

Toxin A

- **most toxic known extracellular protein** of P aeruginosa
- Toxicity has been attributed to its ability to **inhibit protein synthesis**
- by catalyzing the transfer of the **ADP-ribosyl** moiety of nicotinamide adenine dinucleotide (**NAD**) onto **elongation factor 2 (EF-2)**. The resultant ADP-ribosyl-EF-2 complex is inactive in protein synthesis
- most patients surviving P aeruginosa sepsis have **elevated levels of antitoxin A antibody**
- toxin A may be a **major virulence factor** of P aeruginosa
- **toxin A-deficient mutants are less virulent**

Clinical Manifestations

- P aeruginosa causes various infections

1. **Infections on skin** and skeletal tissues, Burn wounds, Surgical wounds
2. **Respiratory tract:** Pneumonia and chronic infection in cystic fibrosis patients
3. **CNS infections**
4. **Endocarditis**
5. **UTI**
6. **Bacteremia**

- most cystic fibrosis patients ultimately die of localized P aeruginosa infections
- **Necrotizing P aeruginosa pneumonia** may occur in other patients following the use of contaminated respirators

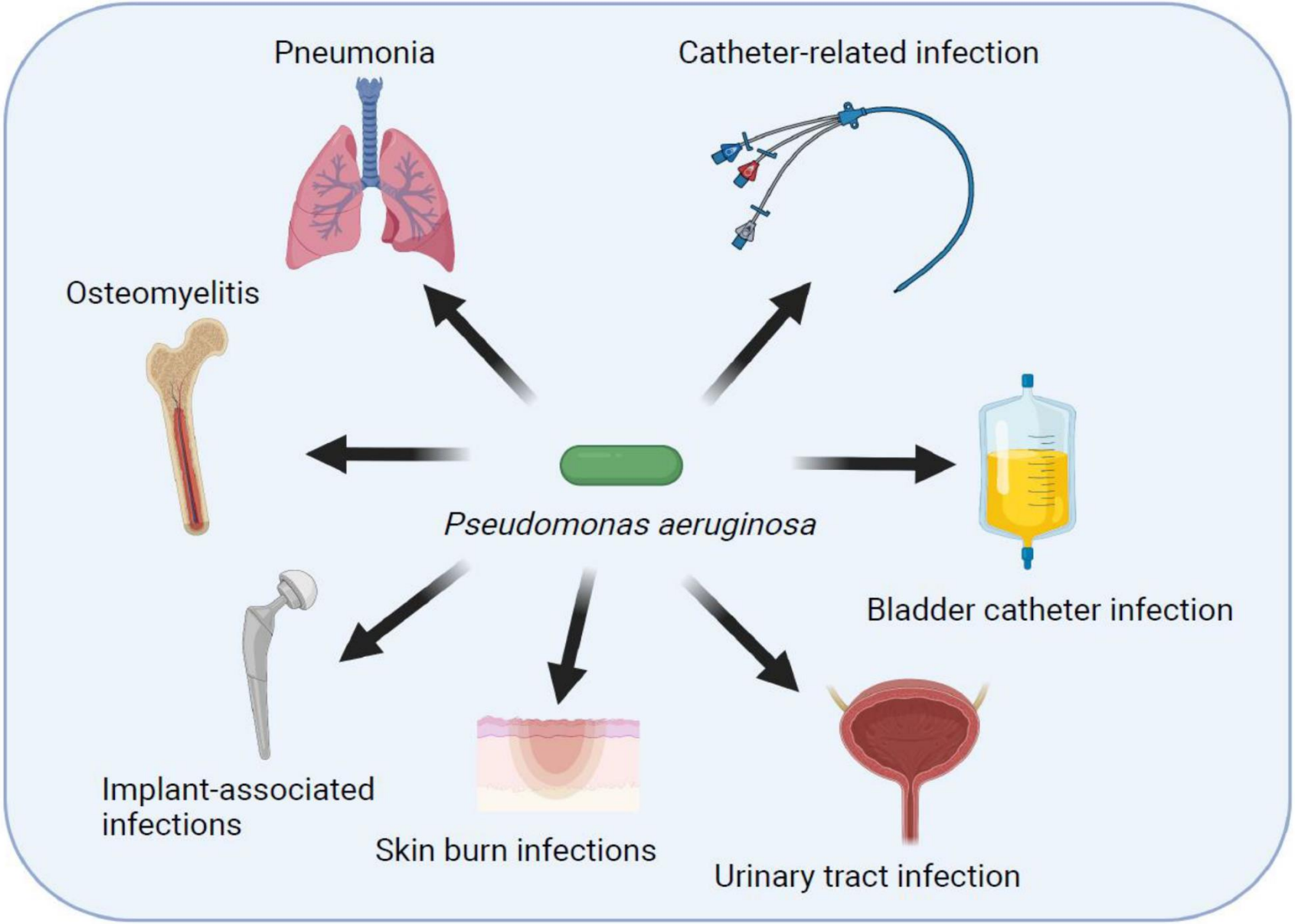
Clinical Manifestations

- Cause severe **corneal infections** following eye surgery or injury
- It is found in pure culture, especially in children with **middle ear infections**
- It occasionally causes **meningitis** following lumbar puncture and **endocarditis** following cardiac surgery
- It has been associated with some **diarrheal disease episodes**

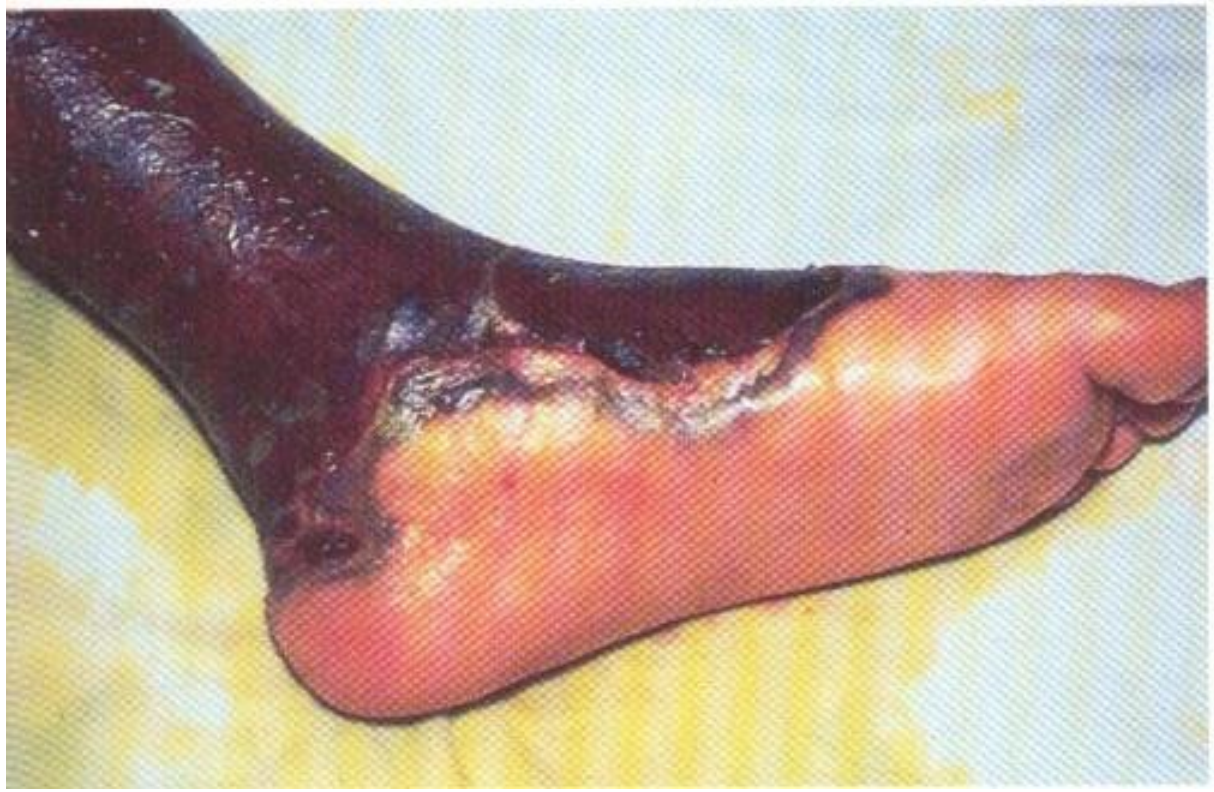
Nosocomial infection *(healthcare-associated infections (HAI))*

- *P.aeruginosa* is the second most common etiological agent of nosocomial infections. It is considered the main causative agent of burn wound infections. It is the third most common etiological factor in hospital-acquired pneumonias.
- Invasive manipulations, instruments used in intensive care units, patients with immunodeficiency, hands of hospital workers, surgical and medical waste, antiseptic solutions, cleaning solutions of contact lenses play an important role in the epidemiology of this microorganism.

Pseudomonas aeruginosa



Pseudomonas aeruginosa - wound infection



Pseudomonas aeruginosa («ecthyma gangrenosa»)

Laboratory diagnosis

Specimen:

- Wound discharge
- sputum
- Blood
- Urine
- CSF

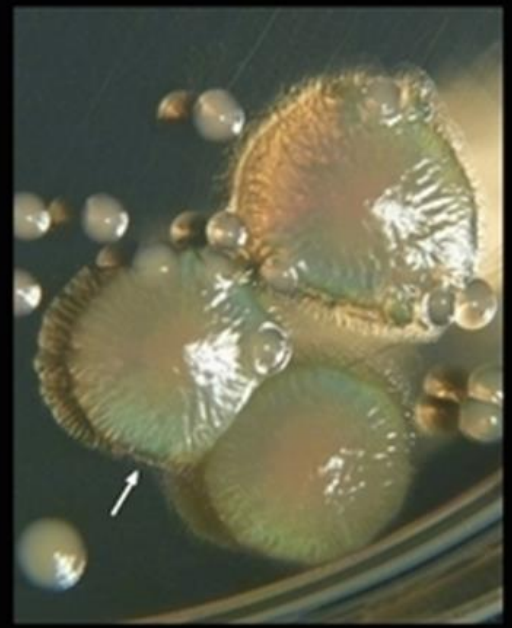
Diagnosis

- isolation and laboratory identification
- identified on the basis of its **Gram morphology**, **inability to ferment lactose, a positive oxidase reaction, its fruity odor, and its ability to grow at 4 2° C**
- **Fluorescence under ultraviolet radiation** helps in early identification of P aeruginosa colonies and also is useful in suggesting its presence in wounds

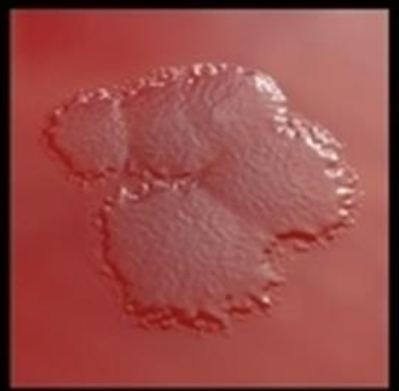
Tryptic soy agar

Gram-negative rods

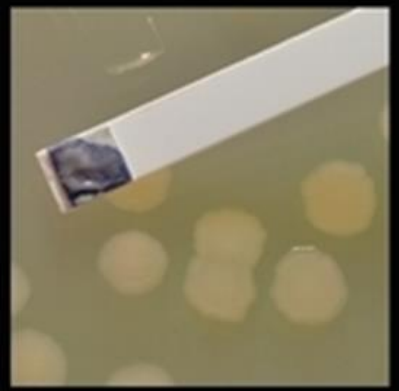
Blood agar



Pseudomonas aeruginosa



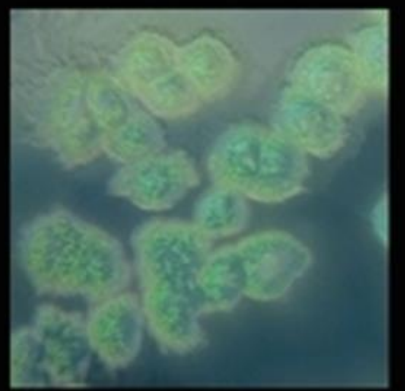
Blood agar



Positive oxidase test

Pseudomonas aeruginosa and *S.aureus* on TSA

P.aeruginosa on TSA



Cetrimide agar

HansN.

Acinetobacter

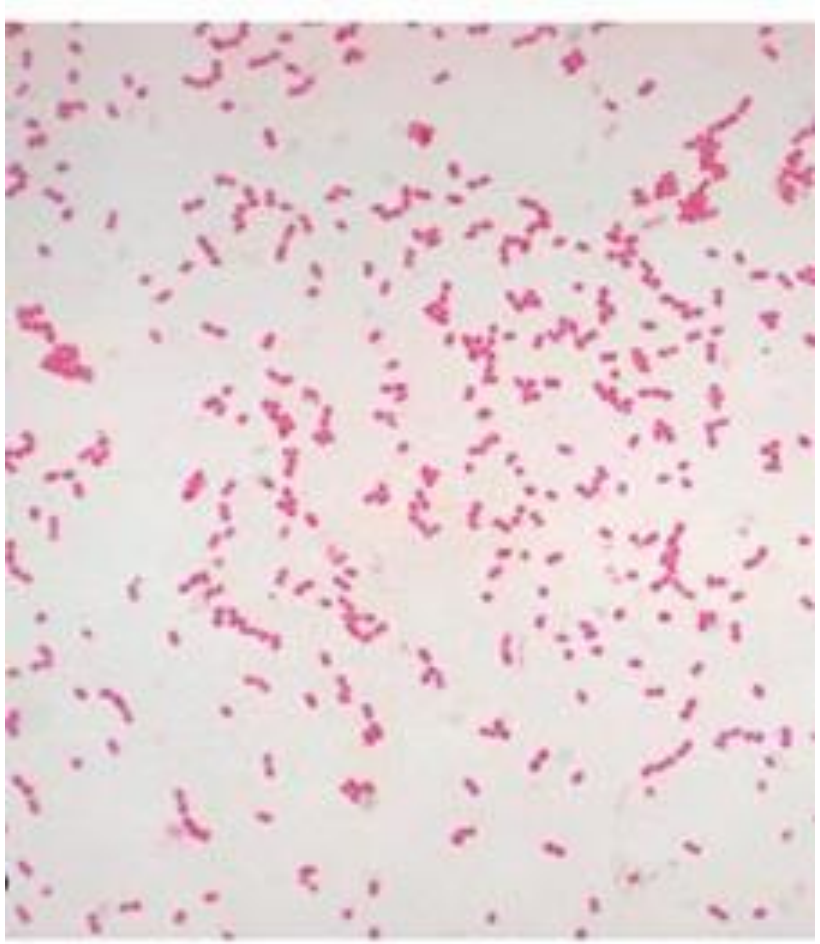
Acinetobacter

Morphological characteristics:

- Acinetobacter are **Gram negative**, cocci. In smears made from pathological materials, as well as from colonies developing in solid nutrient media, it is located as a diplococci and resembles neisseria. Sometimes they can be thick, short, polymorphous, 1.5-2.5 µm long, in the shape of a rod. In smears, they are found in mixed states, sometimes in the form of short chains. They are non-motile, they do not form spores. They have piles. They can form a capsule.

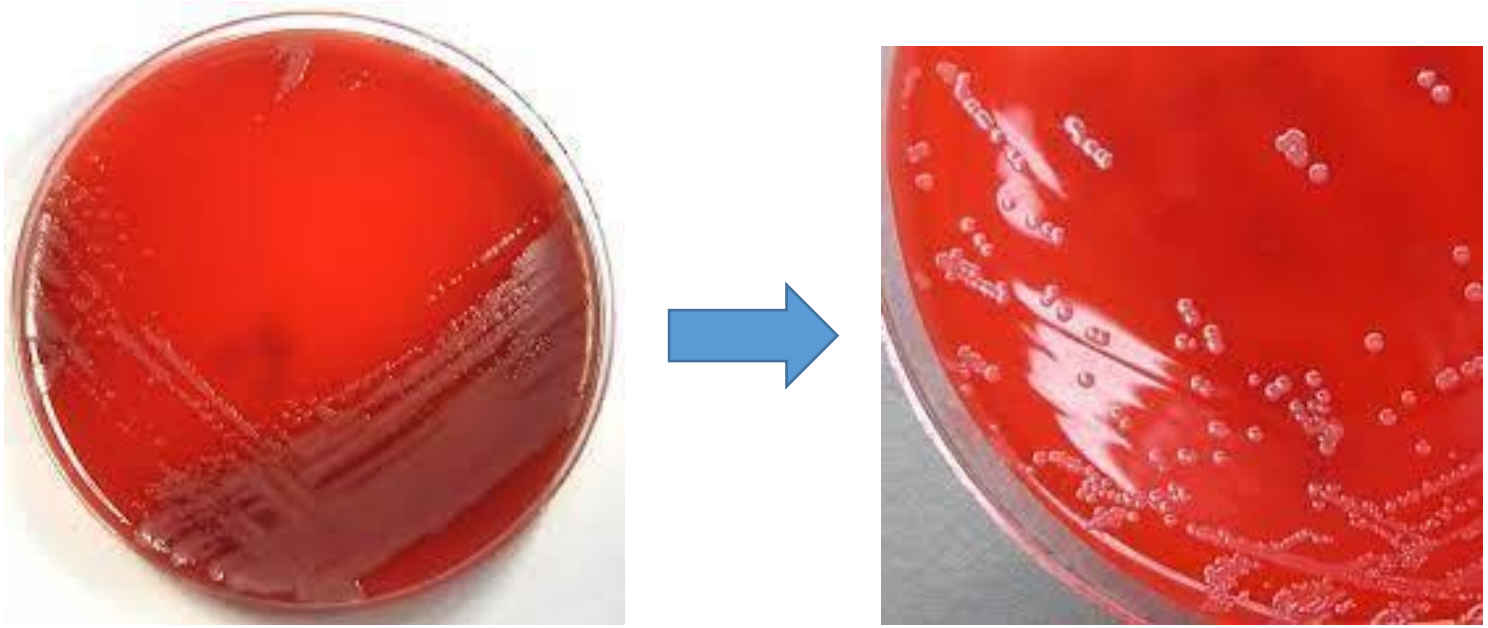
Acinetobacter

(Gram stain, x100)



Acinetobacter
cultural characteristics:

- They are obligate aerobes. They grow in normal nutrient media with neutral pH, at 30-35^{0}C. Small, glistening colonies on solid nutrient media, sometimes forming a zone of alpha-hemolysis on blood agar.



Acinetobacter spp. **(Blood agar)**

Acinetobacter
biochemical properties:

- Biochemical activity is weak.
- It does not break down polysaccharides, some species ferment monosaccharides with acid formation, which allows them to be separated into species. They do not form indole and hydrogen sulfide.

Acinetobacter

- Acinetobacter is widespread in the environment - soil and water. They are included in the normal human microflora, they are found as commensals in the skin of healthy people, in the mucous membrane of the nasopharynx. Acinetobacter baumannii and A.johnsonii species cause nosocomial infections. Among the causative agents of nosocomial infections, acinetobacter, which ranks second after pseudomonads, causes sepsis, peritonitis, endocarditis, wound and burn infections, especially in children and middle-aged people. It is found in the mucous membranes of the urogenital and respiratory tracts, in lesions of the skin surface. Infections are mainly observed in immunocompromised individuals.

The risk group includes:

- Acinetobacter infections usually occur in people in health care settings. People most at risk include patients in hospitals, especially:
- those in breathing apparatus (ventilators).
- those with a catheter
- those with open wounds from surgery
- those in intensive care units
- long hospital stays
- people with weakened immunity, chronic lung disease or diabetes

Acinetobacter
microbiological diagnostics

- Materials such as blood, pus, and wound contents are used for examination.
- Identification of the culture is carried out based on its biochemical properties. Acinetobacter obtained in meningitis and sepsis should be differentiated from N.meningitidis and acinetobacter obtained from female genitalia should be distinguished from N.gonorrheae. Unlike Neisseria, Acinetobacter are oxidase negative.

Acinetobacter
treatment

- As Acinetobacter isolates are quite resistant to antibiotics, treatment is carried out taking into account the sensitivity to antibiotics. Acinetobacter are usually sensitive to gentamicin, amikacin, tobramycin, III generation cephalosporins.
- Carbapenem-resistant Acinetobacter is usually multidrug resistant.