LECTURE VIII

Introduction to special microbiology.Causative agents of pus-inflammatory diseases. Pathogenic cocci(genus *Staphylococcus*,*Streptococcus*,*Neisseria*). Opportunistic bacteria (genus *Pseudomonas*,*Proteus*, *Klebsiella*).

- *The cocci as a causative agents*
- *of pus inflammation proses*

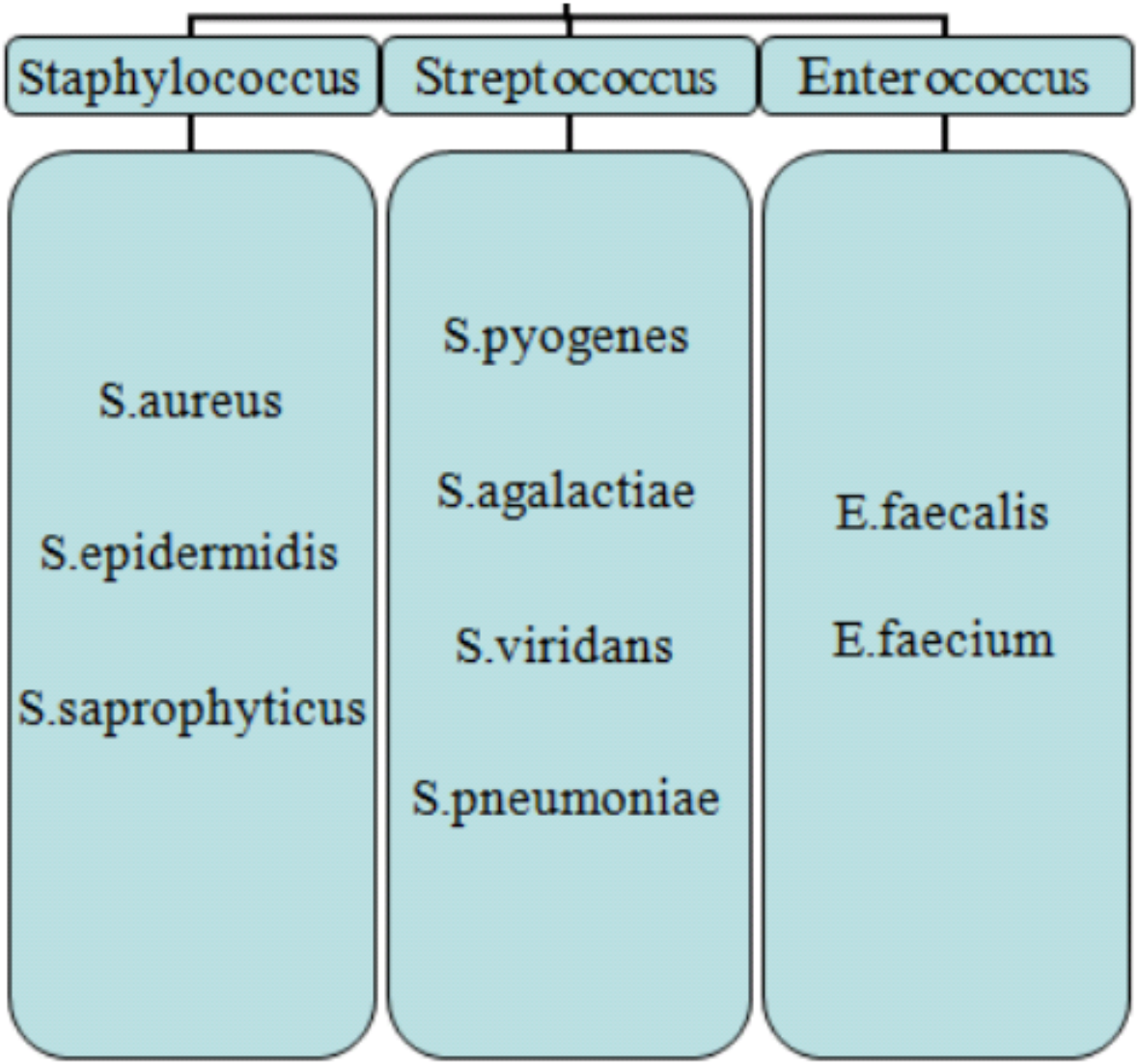
- *Aerobic gram-positive cocci:*

- *- family Micrococcaceae (genes Staphylococcus,*
- *Micrococcus, Stomatococcus)*

- *- Streptococcaceae family (genes Streptococcus,*
- *Enterococcus, Aerococcus, Leuconostoc,*
- *Pediococcus, Lactococcus)*

- *Aerobic gram-negative cocci:*
- *- cocci of the family Neisseriaceae (genus*
- *Neisseria)*

Gram positive cocci



Staphylococcus

- Most important genus in Family *Micrococcaceae*
- *Other genera*
  - *Stomatococcus*
  - *Micrococcus*

Staphylococci

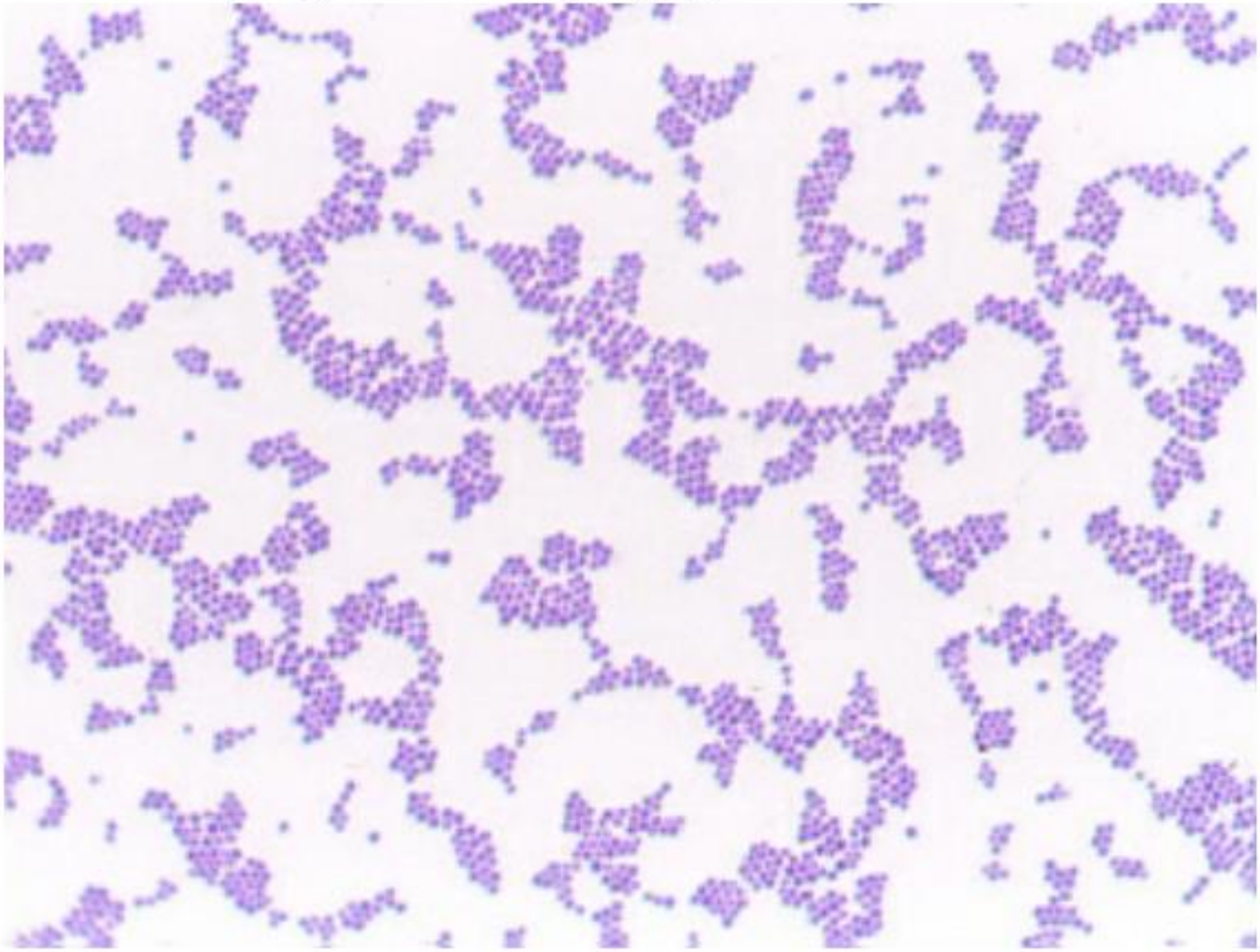
- Family - *Micrococcaceae*
- Genus - *Staphylococcus*
- Species - 27 species are known, 14 species are found in the human body, 3 of them are pathogenic: *S.aureus, S.epidermidis, S.saprophyticus*

- The greatest clinical value is *S.aureus*.

Habitat

- ***S. aureus***
  - anterior nares 50-75% healthy people
  - skin & mucous membranes
  - hospital environment
- ***S. epidermidis* & others**
  - resident skin flora, gut, upper respiratory tract
- ***S. saprophyticus***
  - Urinary tract in young women

Staphylococci, by gram stained

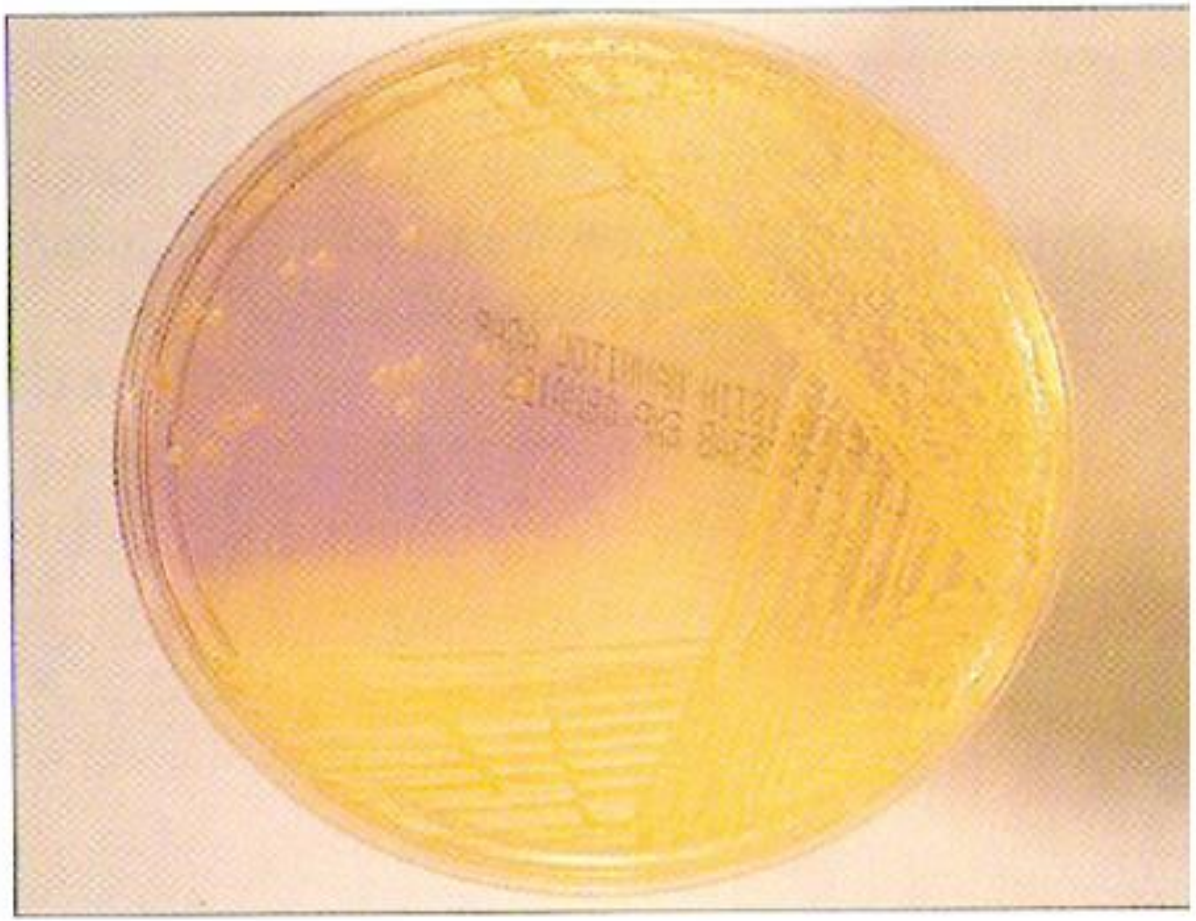


Staphylococcus aureus

culture on blood agar

Staphylococcus aureus

lecithinase test on yolk-salt agar



Differential signs of *staphylococci*

Oxidase	Negative							
Novobiocin	Susceptible							
Coagulase	Negative	Positive[1] – variable[2] – negative[3]		Negative				
Species group	Hyicus-Intermedius			Epidermidis-Aureus				
Cluster group	Muscae	Hyicus	Intermedius	Aureus	Epidermidis	Warneri	Haemolyticus	Lugdunensis
Species	*S. muscae* *S. microti* *S. rostri*	*S. hyicus*[2] *S. agnetis*[2] *S. chromogenes*[3] *S. felis*[3]	*S. intermedius*[1] *S. delphini*[1] *S. lutrae*[1] *S. pseudintermedius*[1] *S. schleiferi* *ssp. schleiferi*[3] *ssp. coagulans*[1]	*S. aureus* *ssp. aureus*[1] *ssp. anaerobius*[1] *S. simiae*[1]	*S. epidermidis* *S. capitis* *ssp. capitis* *ssp. urealyticus* *S. caprae* *S. saccharolyticus*	*S. warneri* *S. pasteuri*	*S. haemolyticus* *S. devriesei* *S. hominis* *ssp. hominis* *ssp. novobiosepticus* *S. jettensis* *S. petrasii* *ssp. croceilyticus* *ssp. petrasii*	*S. lugdunensis*

Oxidase	Negative							Positive
Novobiocin	Susceptible			Resistant				
Coagulase	Negative							
Species group	Auricularis	Simulans	Saprophyticus					Sciuri
Cluster group	Auricularis	Simulans-Carnosus	Pettenkoferi-Massiliensis	Saprophyticus	Cohnii-Nepalensis	Arlettae-Kloosii		Sciuri
Species	*S. auricularis*	*S. simulans* *S. carnosus* *ssp. carnosus* *ssp. utilis* *S. condimenti* *S. piscifermentans*	*S. pettenkoferi* *S. massiliensis*	*S. saprophyticus* *ssp. saprophyticus* *ssp. bovis* *S. equorum* *ssp. equorum* *ssp. linens* *S. gallinarum* *S. succinus* *ssp. succinus* *ssp. casei* *S. xylosus*	*S. cohnii* *ssp. cohnii* *ssp. urealyticus* *S. nepalensis*	*S. arlettae* *S. kloosii*		*S. sciuri* *ssp. sciuri* *ssp. carnaticus* *ssp. rodentium* *S. fleurettii* *S. lentus* *S. stepanovicii* *S. vitulinus*

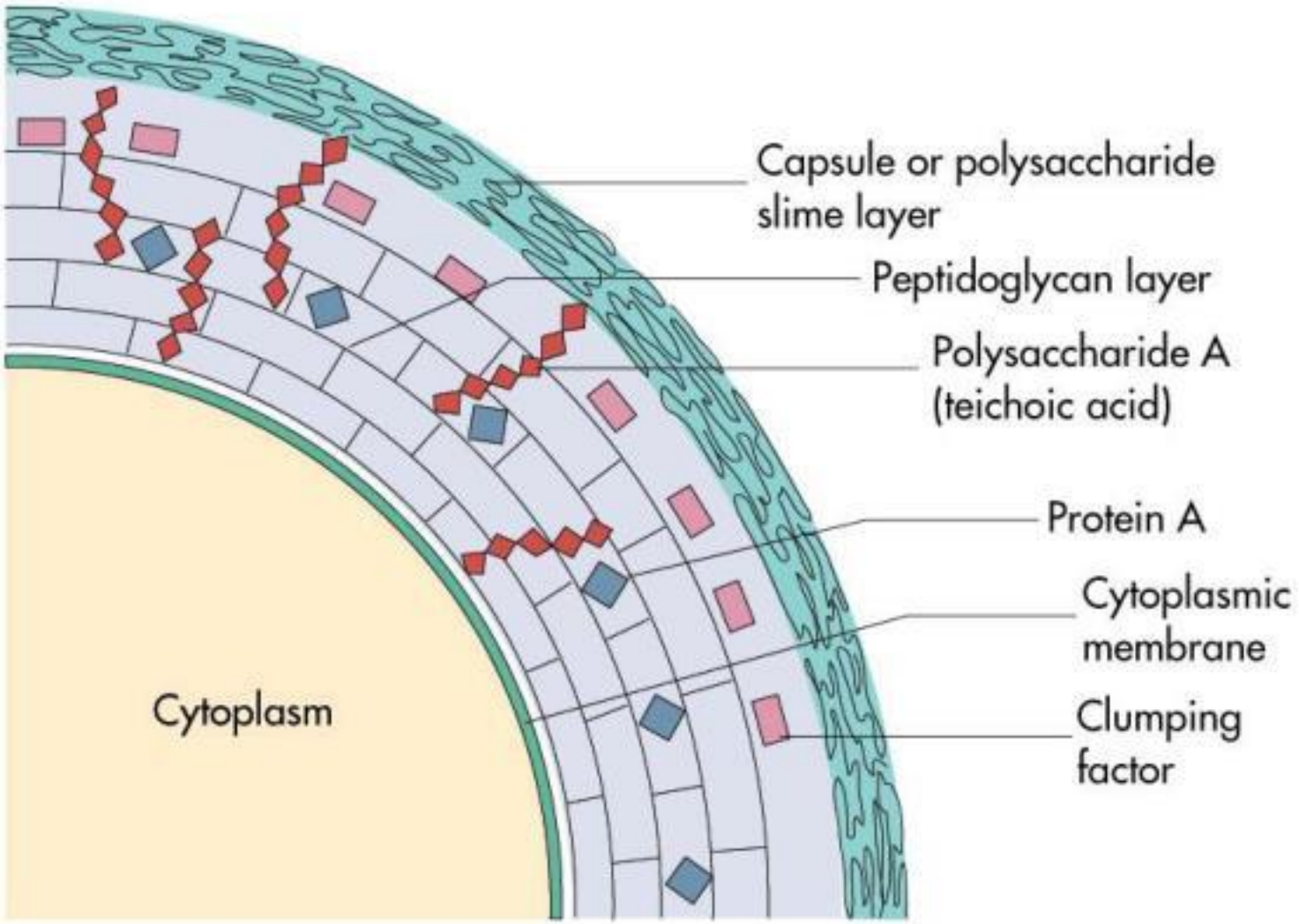
Antigenic structure

- About 30 antigens
- Common for all types of protein antigen
- Protein A is species-specific for *S.aureus*.
- Polysaccharide antigens for serological specificity
- Polysaccharide A - ribitteichoic acid, found *in S.aureus*
- Polysaccharide B - glycerine-teichoic acid, found in *S.epidermidis*.

Antigenic Structures & Virulence Factors of *S. aureus*

- Cell wall peptidoglycan
  - elicits production of IL-1 and opsonic antibody
  - PMN chemotaxis “pyogenic”
  - induces sepsis
  - activates complement
  - teichoic acid binds fibronectin on host cells
- Protein A - binds Fc of IgG
- Capsule (some strains) antiphagocytic

Cell Wall of S. aureus



S. aureus Soluble Virulence Factors

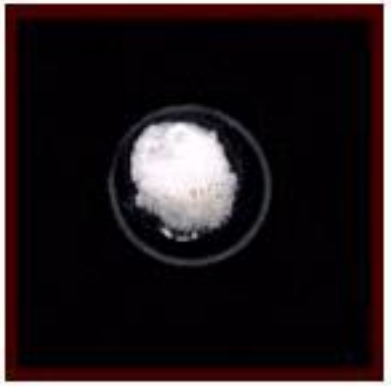
- Catalase - reduce phagocyte killing - remove H_2O_2
- Coagulase - clots plasma (free & bound)
- Hyaluronidase - destroys connective tissue
- Beta lactamase - destroys beta lactam drugs
- Altered Penicillin binding proteins (PBP2')
- Fibrinolysin
- Lipases
- Nucleases

Catalase Test for Distinguishing Staphylococci from Streptococci

$$H_2O_2 \xrightarrow{catalase} O_2 + H_2O$$

Bubbles

Strep. Negative



Staph. positive

Tube Coagulase Test

- Free coagulase secreted by *S. aureus* but not CNS
- Clots rabbit plasma

S. aureus Soluble Virulence Factors

- Cytotoxins & leukocidins
  - lyse white blood cells (Panton-Valentine)
  - release lysosomal enzymes → damage tissue
- Exfoliatin
  - interrupts intercellular skin junctions
  - "Scalded Skin Syndrome"
- Toxic Shock Toxin
  - stimulates T cells → cytokines,
  - endothelial damage → rash
  - "Toxic Shock Syndrome"
- Enterotoxins
  - stimulate vomiting by interaction with GI neural receptors (food poisoning)

S. aureus Diseases

Skin and soft tissue infections
- Furuncles
- Carbuncles
- Wound infections
- Cellulitis
- Impetigo
- Bacteremia

Endocarditis

CNS Infections
- Brain abscess
- Meningitis - rare
- Epidural abscess

Impetigo

S. aureus Diseases

Pulmonary Infections
- embolic
- aspiration

Musculoskeletal
- osteomyelitis
- septic arthritis

Genitourinary Tract
- renal carbuncle
- lower UTI

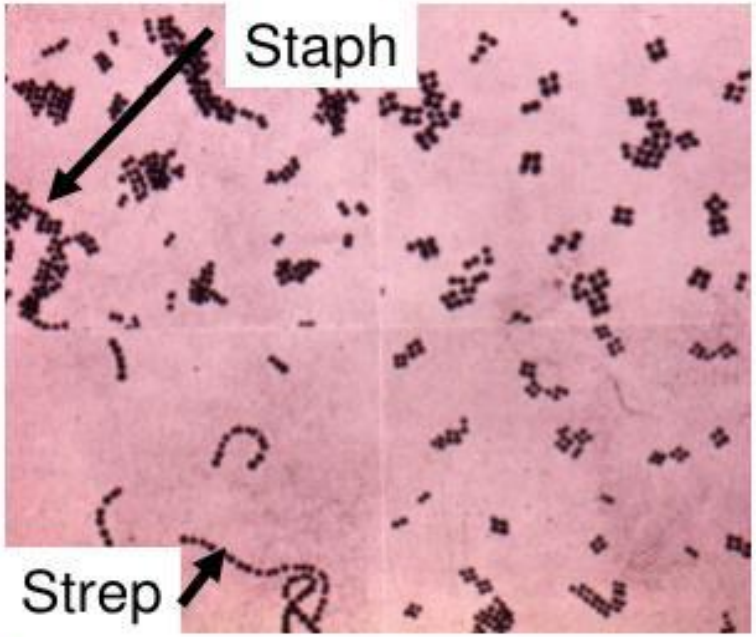
Toxin mediated diseases
- scalded skin syndrome
- food poisoning
- toxic shock disease

Toxic shock

Scalded skin

Streptococcaceae

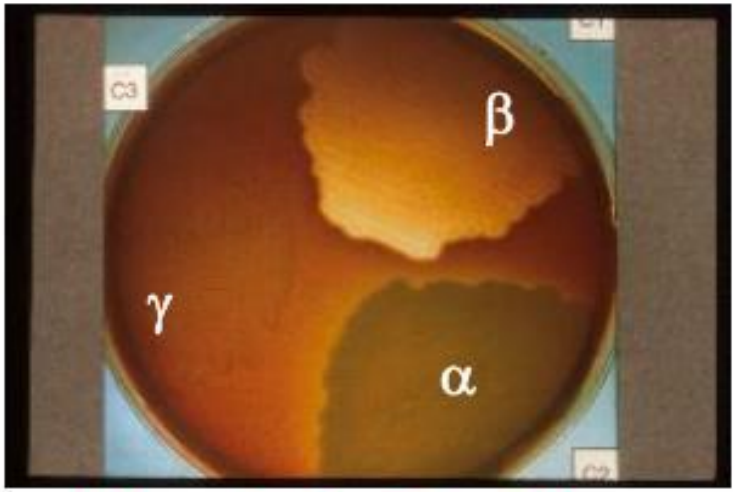
- *Streptococcus*
- *Enterococcus*



Gram stain of staph (clusters) and strep (chains)

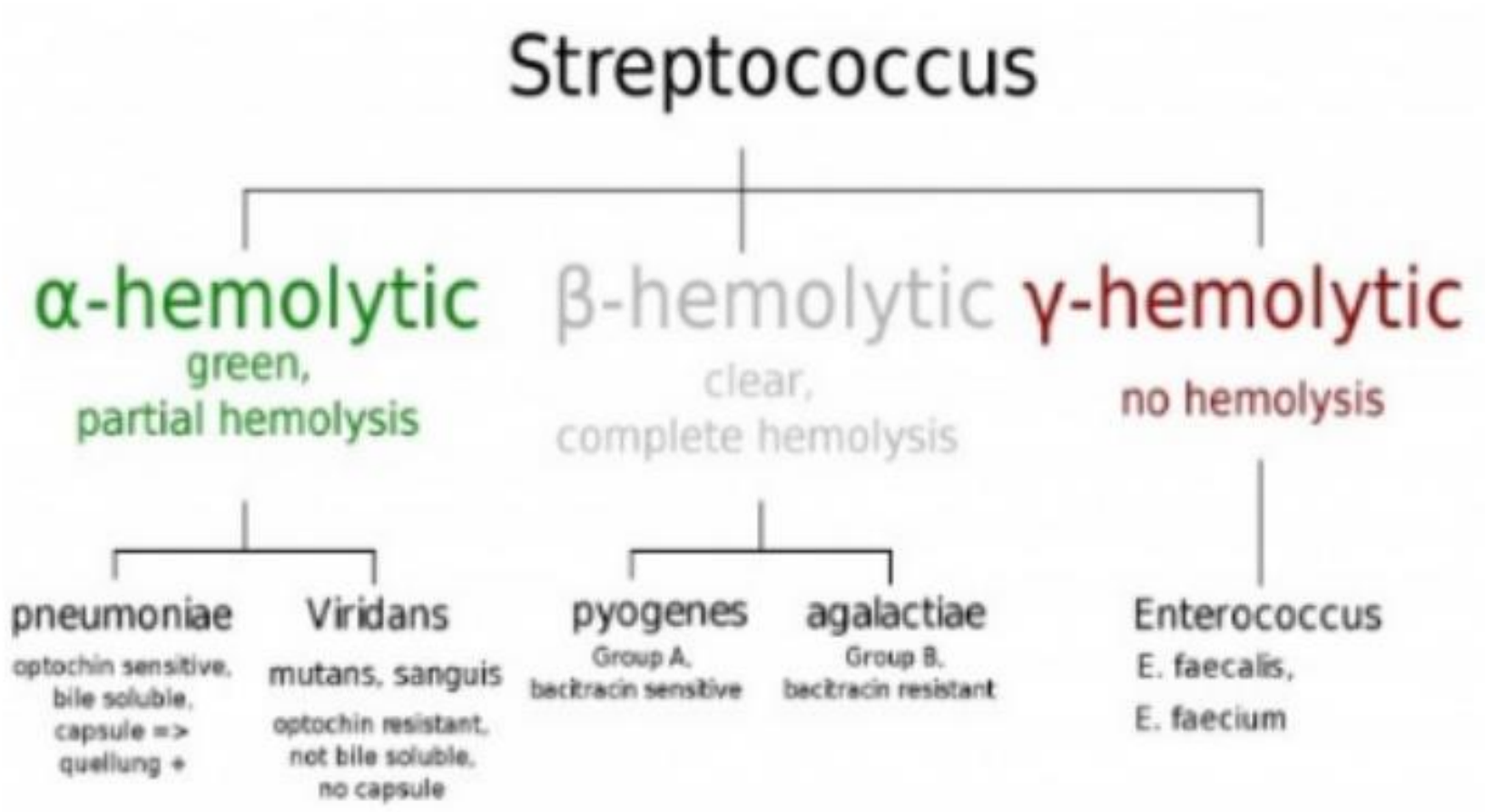
Streptococcus Classification

- Hemolysis
  - beta
  - alpha
  - gamma
- Lancefield Groups
  - *(*A-T- β hemolytic*)*
  - group-specific cell wall polysaccharide
- Species
  - phenotypic biochemical reactions



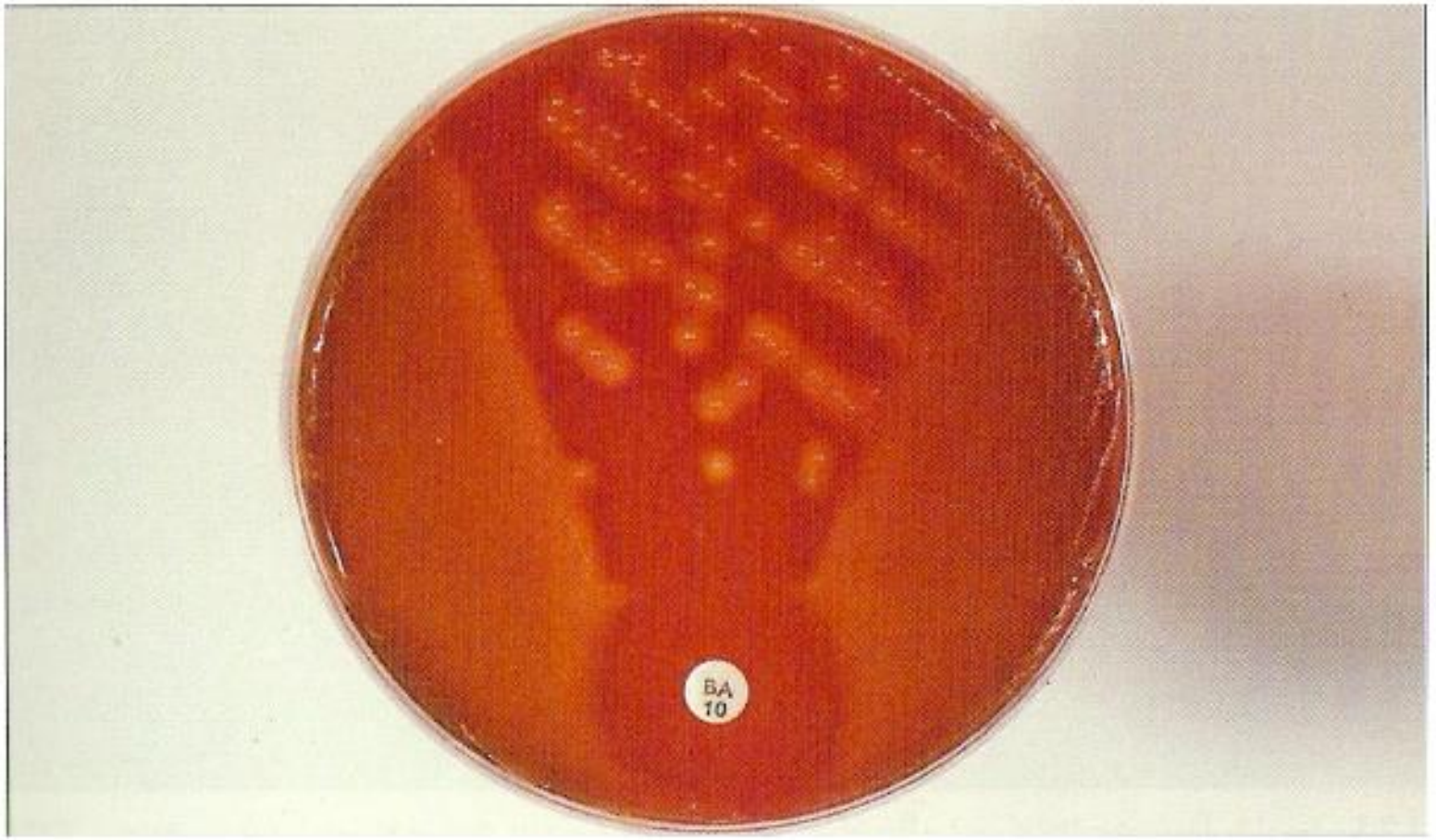
Hemolytic Reactions

Classification of streptococci

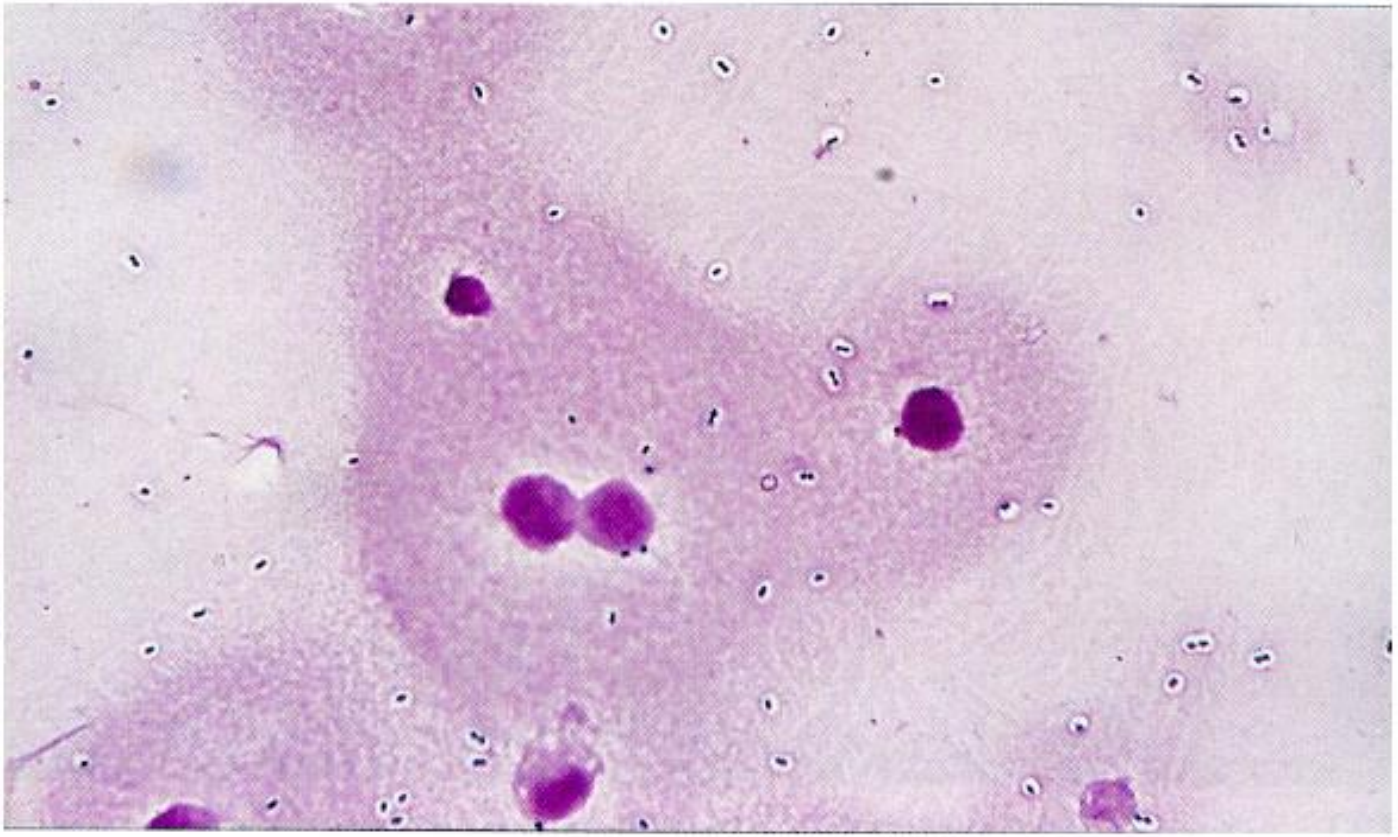


Streptococcus pyogenes

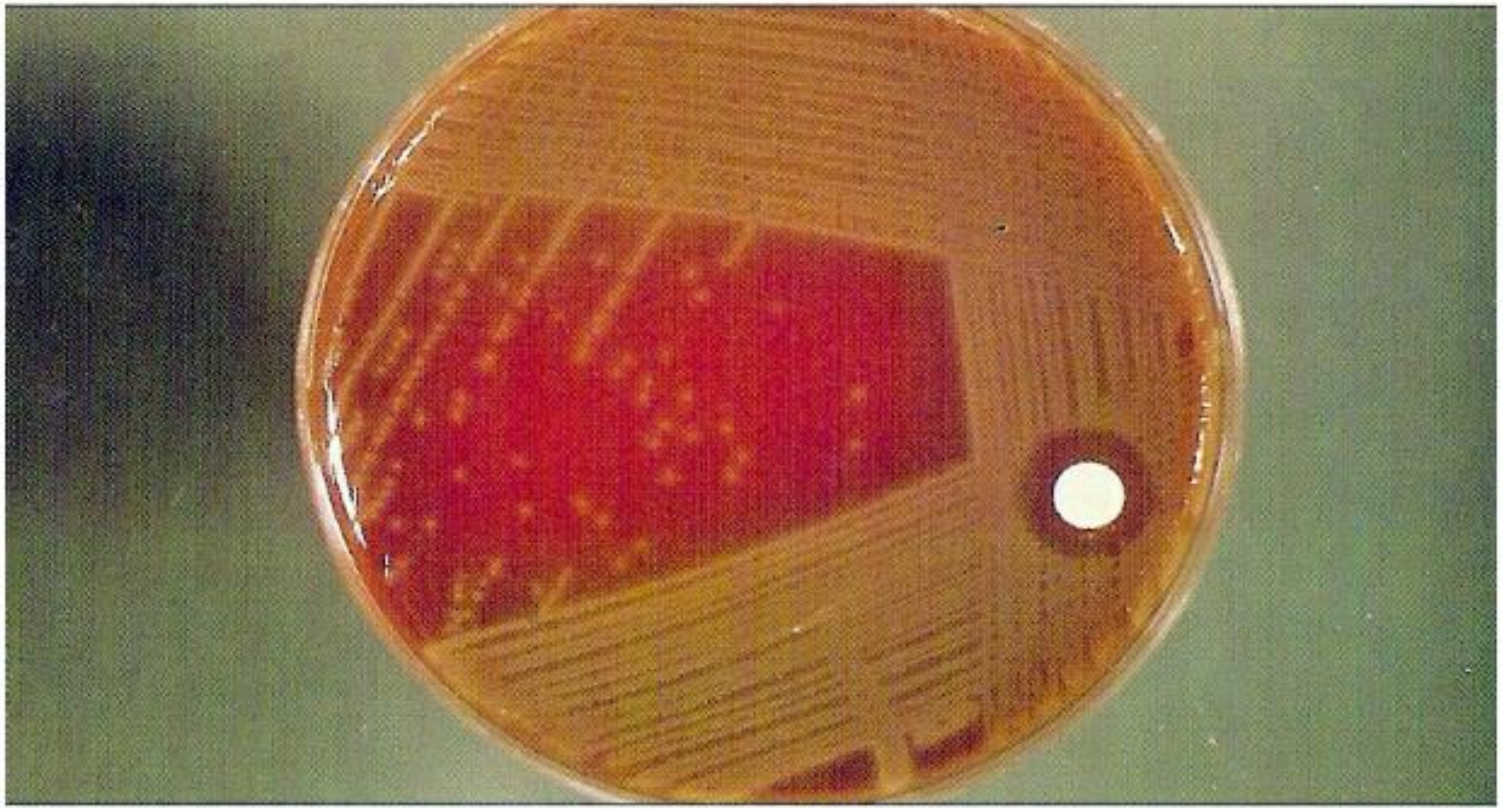
Streptococcus pyogenes on blood agar



Streptococcus pneumoniae



Streptococcus pneumoniae on blood agar

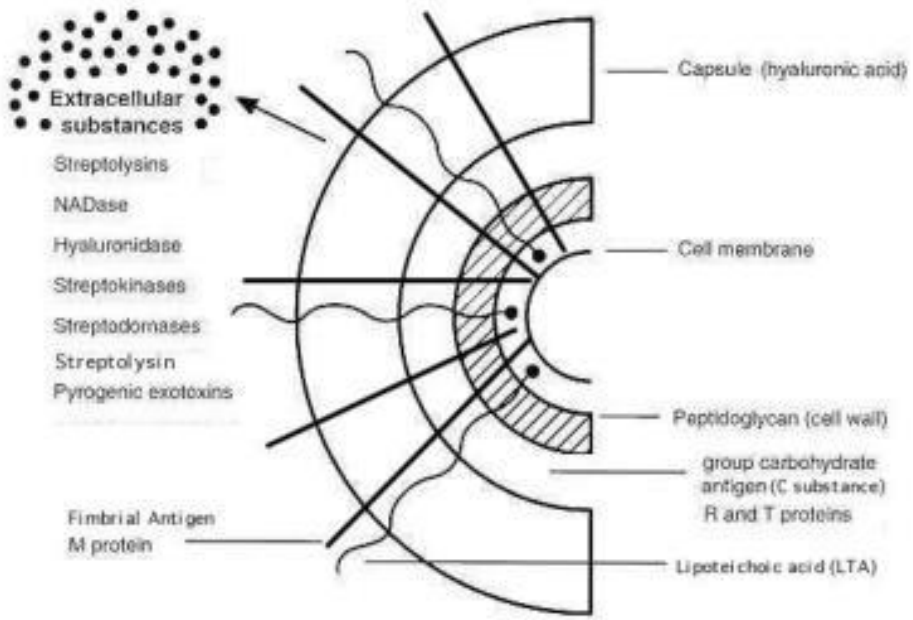


Streptococcus Habitat

- Skin, mucous membranes, respiratory tract and GI/GU tracts, depending on species
- 20% of children may carry GAS in their pharynx during winter months.
- *S. pneumoniae* is commonly isolated from the respiratory tract of asymptomatic carriers.
- Enterococci in gut flora are are important pathogens in hospitals where they are selected by high antibiotic usage.
- Organisms spread by droplets, direct contact and fomites.

Antigenic Structure & Virulence Factors of *S. pyogenes*

- Hyaluronic acid capsule - antiphagocytic
- Hyaluronidase - tissue penetration
- Group specific cell wall antigen distinguishes from B,C,D,F,G, etc.
- Beta hemolytic



Antigenic Structure & Virulence Factors of *S. pyogenes*

- **M Protein**
  - Virulence factor present on pilus with teichoic acid
  - Organisms lacking it are readily opsonized and phagocytized
  - Binds fibrinogen, fibrin & degradation products forming dense coating on the organism's surface, blocking complement
  - Antibody against M protein is an important protective mechanism, but repeated infections with strains possessing one of over 80 different serotypes can occur
  - Autoantibody target-Acute Rheumatic Fever

Antigenic Structure & Virulence Factors of *S. pyogenes*

- Protein F - facilitates attachment by binding fibronectin
- Protein G - binds Fc portion of antibody
- Diphosphopyridine nucleotidase (DPNase) – enzyme kills WBCs
- C5a peptidase

Erysipelas

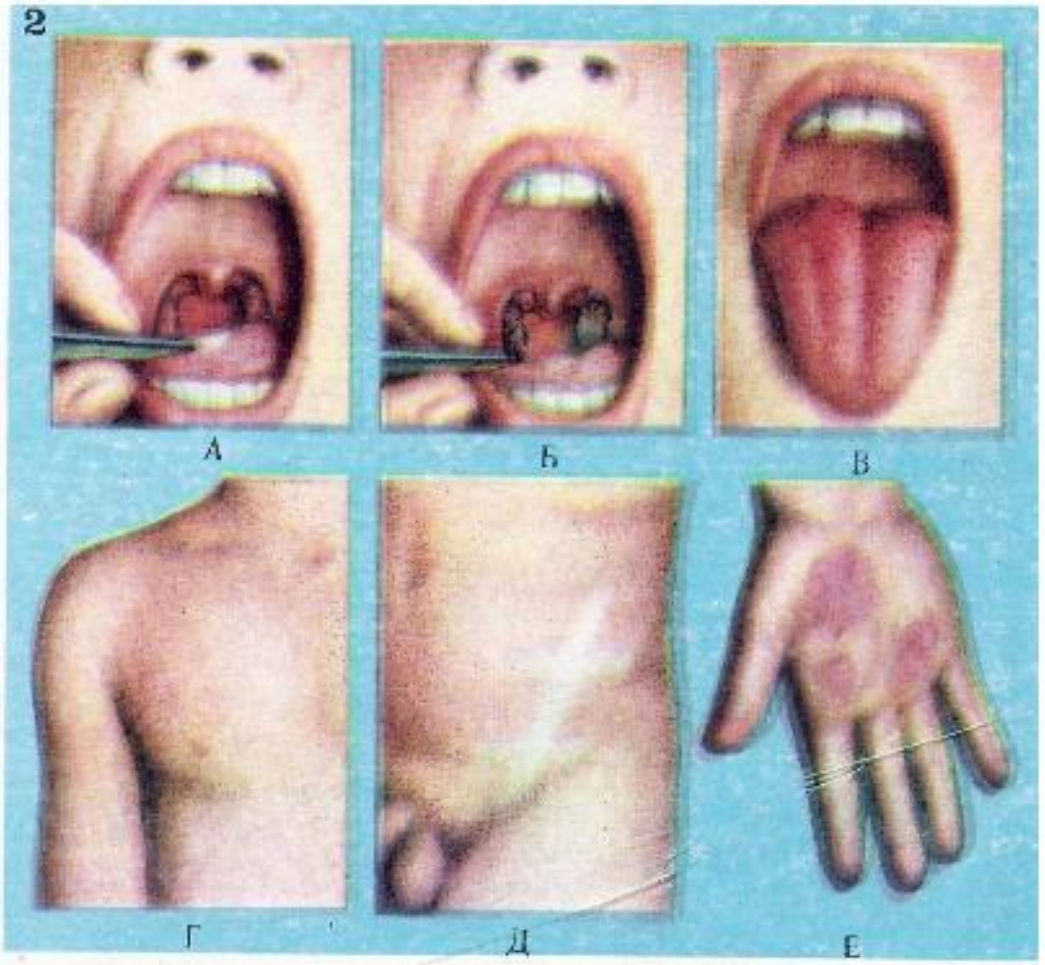
Antigenic Structure & Virulence Factors of *S. pyogenes*

- Erythrogenic Toxin "Scarlet Fever"
- Streptokinases
  - transform plasminogen to plasmin
  - digest fibrin
- DNAase
  - depolymerizes DNA
    antibody used to follow pyoderma
- Hemolysins "Streptolysins"
  - Important immunogens
  - Antibody against streptolysin O used to follow course of pyoderma
  - Streptolysin S - β hemolysis

Strep. cellulitis

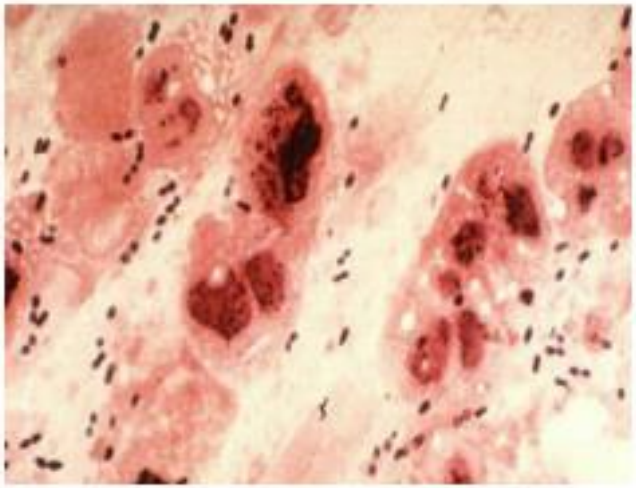
Erysipeloid

Scarlet fever

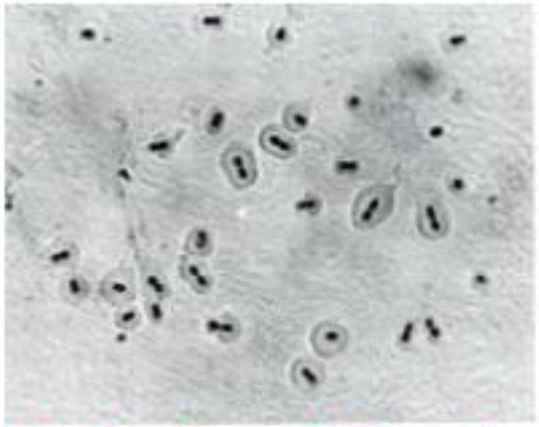


S. pneumoniae Virulence Factors

- Antiphagocytic capsule – immunogen
- PspA: inhibits opsonization
- Autolysin – release cell components
- Pneumolysin
  - Cytotoxic – inhibit cilia, wbcs
  - lyses RBCs
  - activates classic complement path.
  - stimulates cytokines → tissue damage & purulent inflammation
- Hydrogen peroxide - tissue damage
- Surface protein adhesins
- Neuraminidase
- IgA protease
- Peptidoglycan
  - activate alternate complement
  - cytokine release
- Transformation– antibiotic resistance
- Intracellular invasion

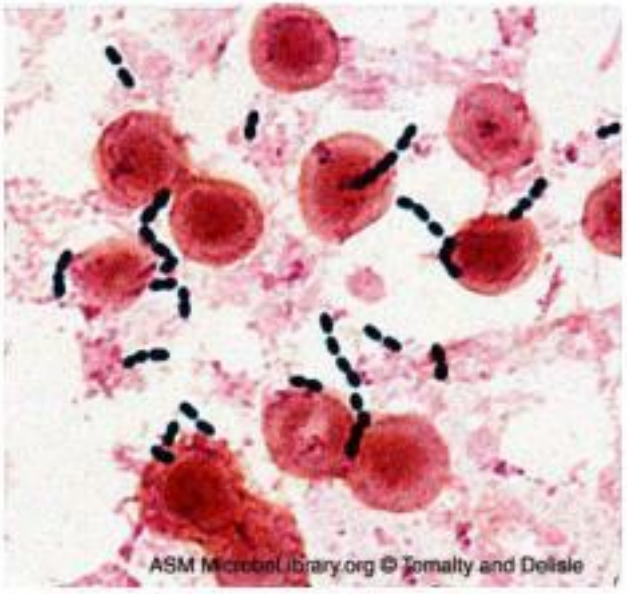


sputum - pneumonia



Capsule Quellung Reaction

Enterococcus

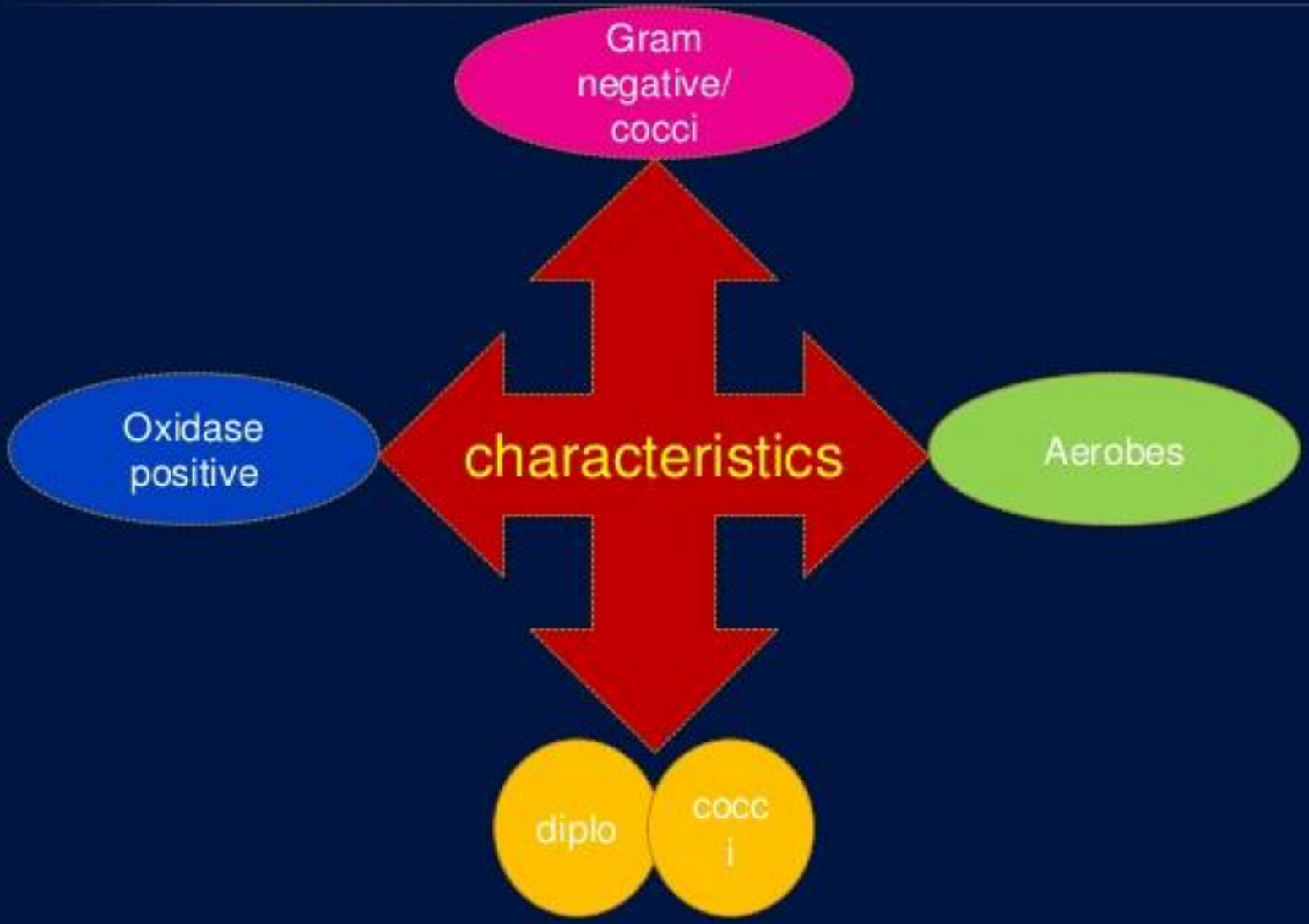


- At least 12 species
- Usually non-hemolytic
- *E. faecalis* most common
- Distinguish from streptococci by:
  - esculin hydrolysis
  - growth in 6.5% NaCl
  - PYR hydrolysis (Group A β strep. are +)
- Enteric flora
- Opportunist - nosocomial pathogen
- Intrinsic antimicrobial resistance
- *E. faecium* - vancomycin-resistance
- Abscesses, urinary tract, endocarditis, abdominal/pelvic, bacteremia, wound infections

Aerobic Gram-negative cocci

- Family - *Neisseriaceae*
- Genus - ***Neisseria***
- Species – ***Neisseria meningitidis, Neisseria gonorrooeae,*** *N.flava, N.subflava, N.perflava, N.sicca, N.mucosa* etc.

CHARACTERISTICS:



CONTAINS TWO IMPORTANT PATHOGENS

- *Nesseria meningitidis*
- *Nesseria gonorrhoeae*

The intracellular presence of these bacteria inside polymorphs in patient sample is a characteristics finding

IMPORTANT DIFFERENCE BETWEEN
N.gonorrhoeae & N. meningitidis

N. gonrrhoeae

N. meningitidis

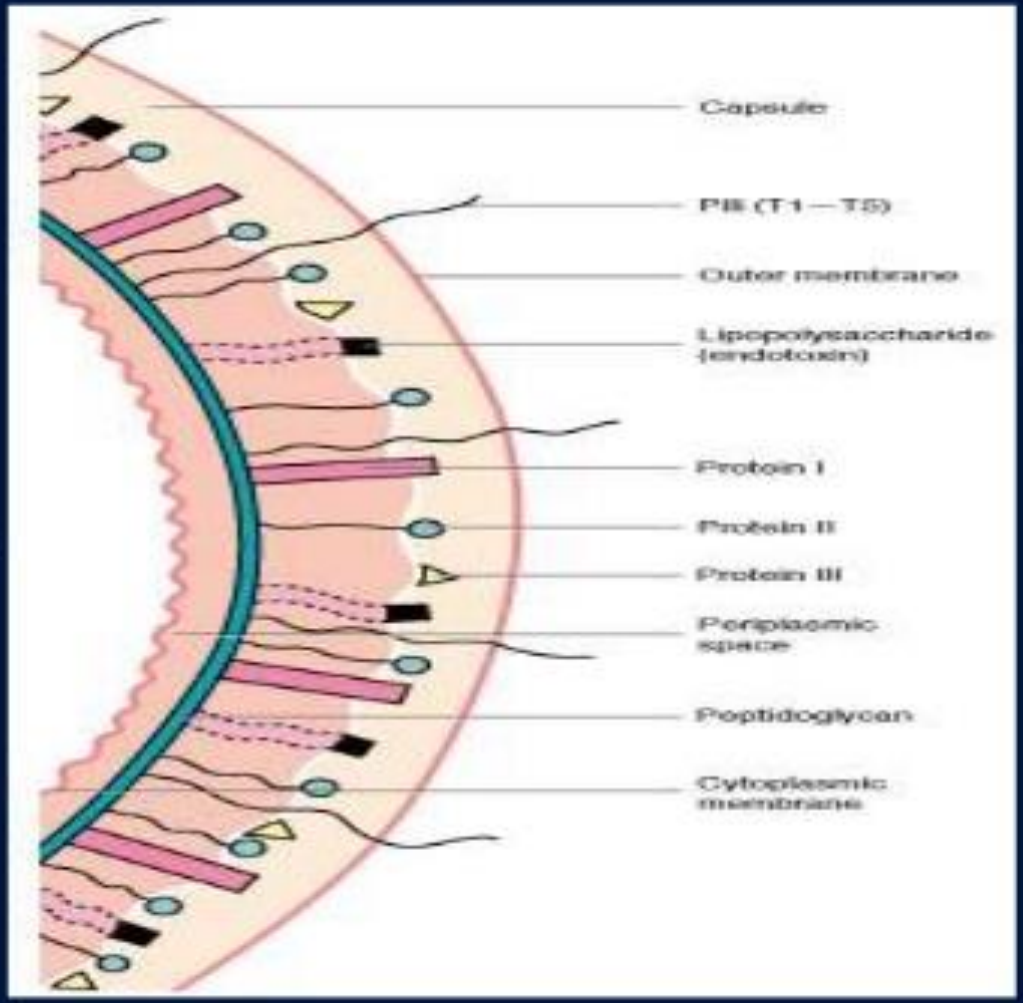
Note:both can be differentiated by biochemical tests using serum sugar

Neisseria meningitidis growing on chocolate agar

Neisseria meningitidis growing on sheep blood agar

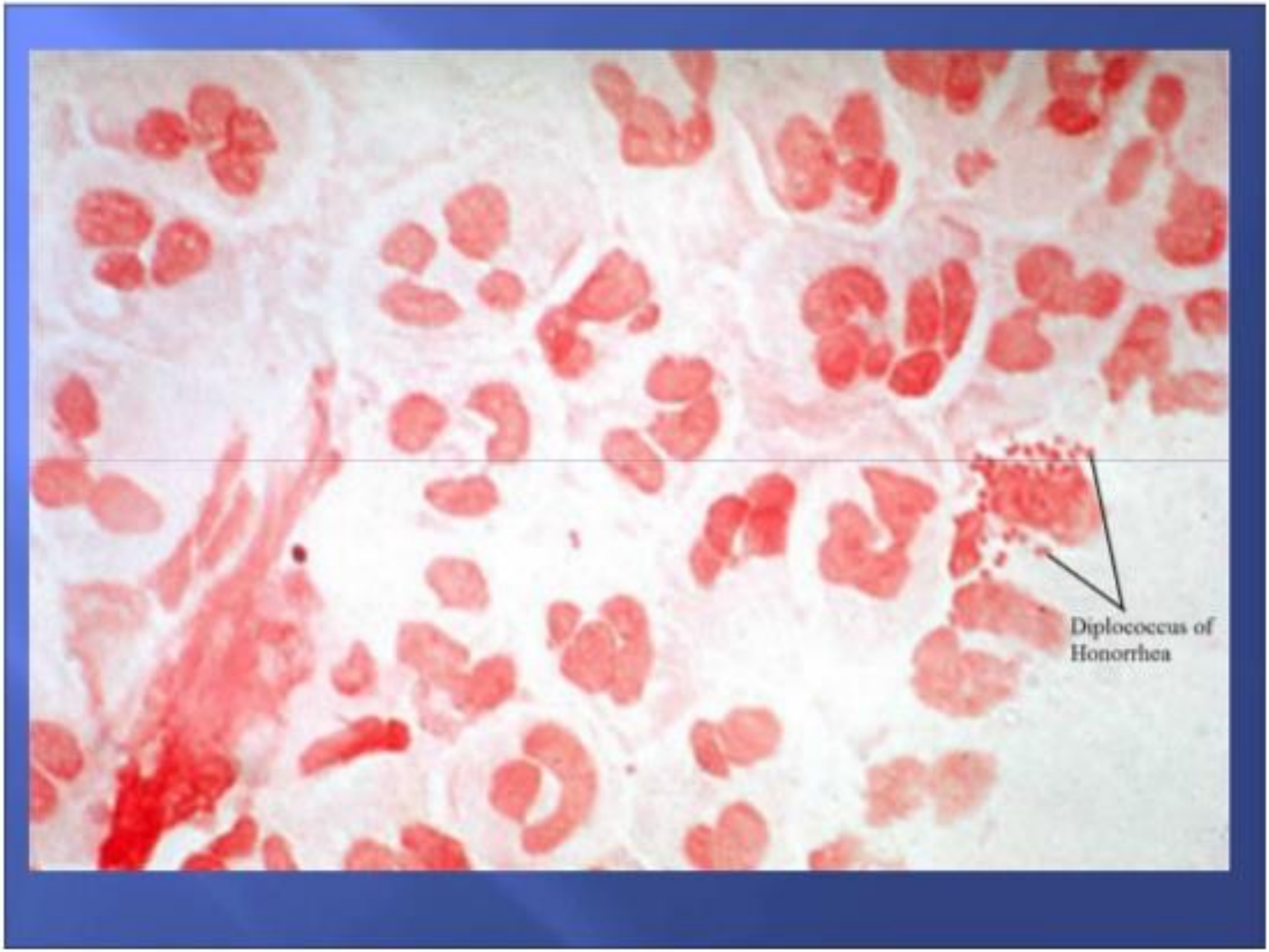
VIRULENCE FACTORS

- **Fimbrae** (common pili)-
- **Lipooligosaccharide**:
- **Capsule**
- **Cell membrane proteins**
- **IgA protease-**



Hemorrhage in the adrenal glands in Waterhouse-Fridericksen syndrome

Meningococcal disease is favoured by defieciency of the terminal complement components (C_5-C_9)


Diplococcus of
Honorrhea

Meningococcemia showing striking involvement of the extremities with relative sparing of the skin of the child's body surface.

Taxonomy :

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

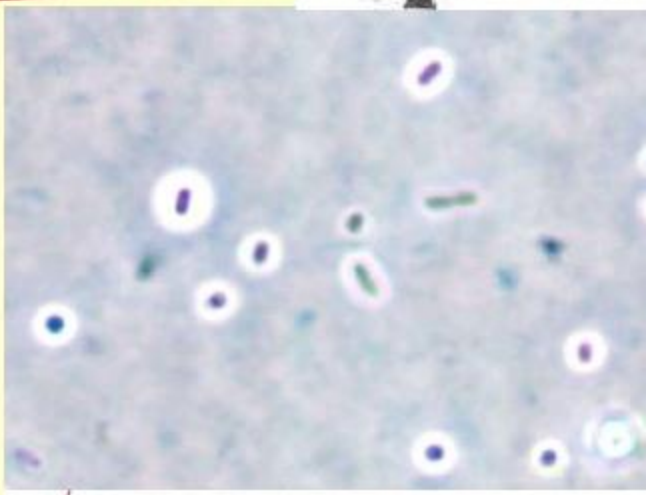
Klebsiella pneumoniae

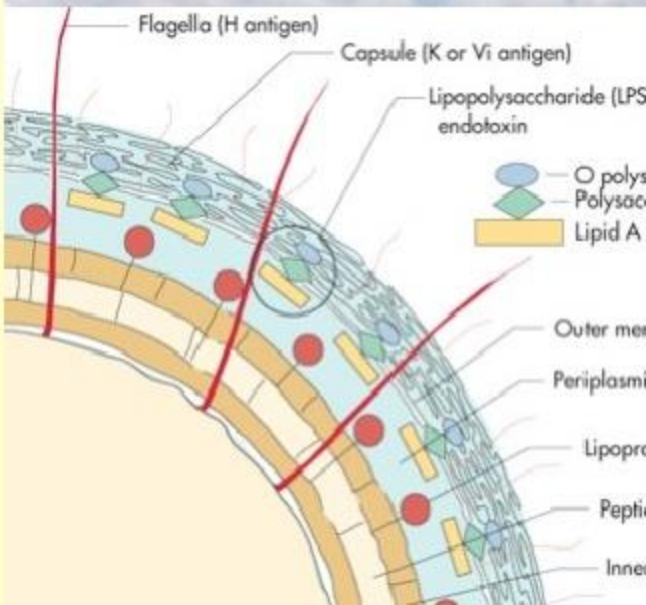
Characteristics:

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

Antigenic structure

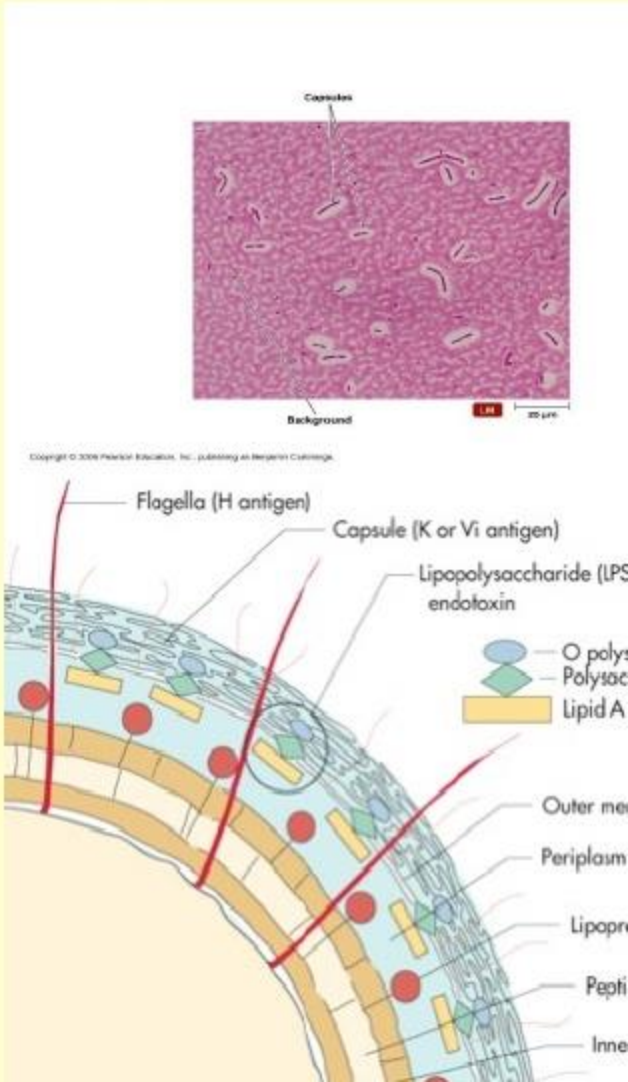
- 80 Capsular (K) antigens
  - Gram stain
  - Capsular ‘swelling’ reactiion
  - CCIE
  - ELISA
- 5 Somatic (O) antigens



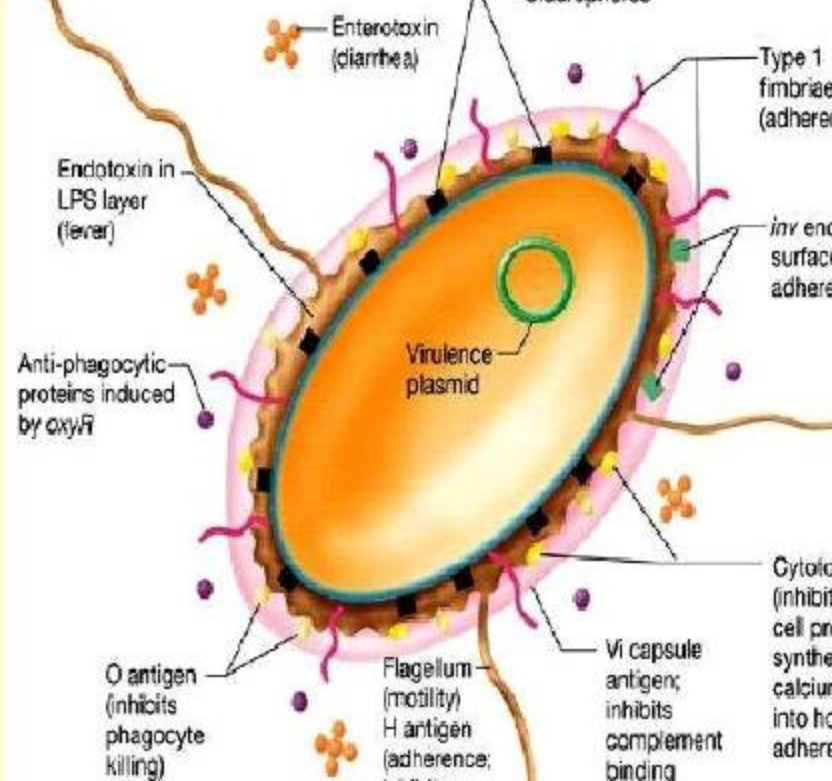


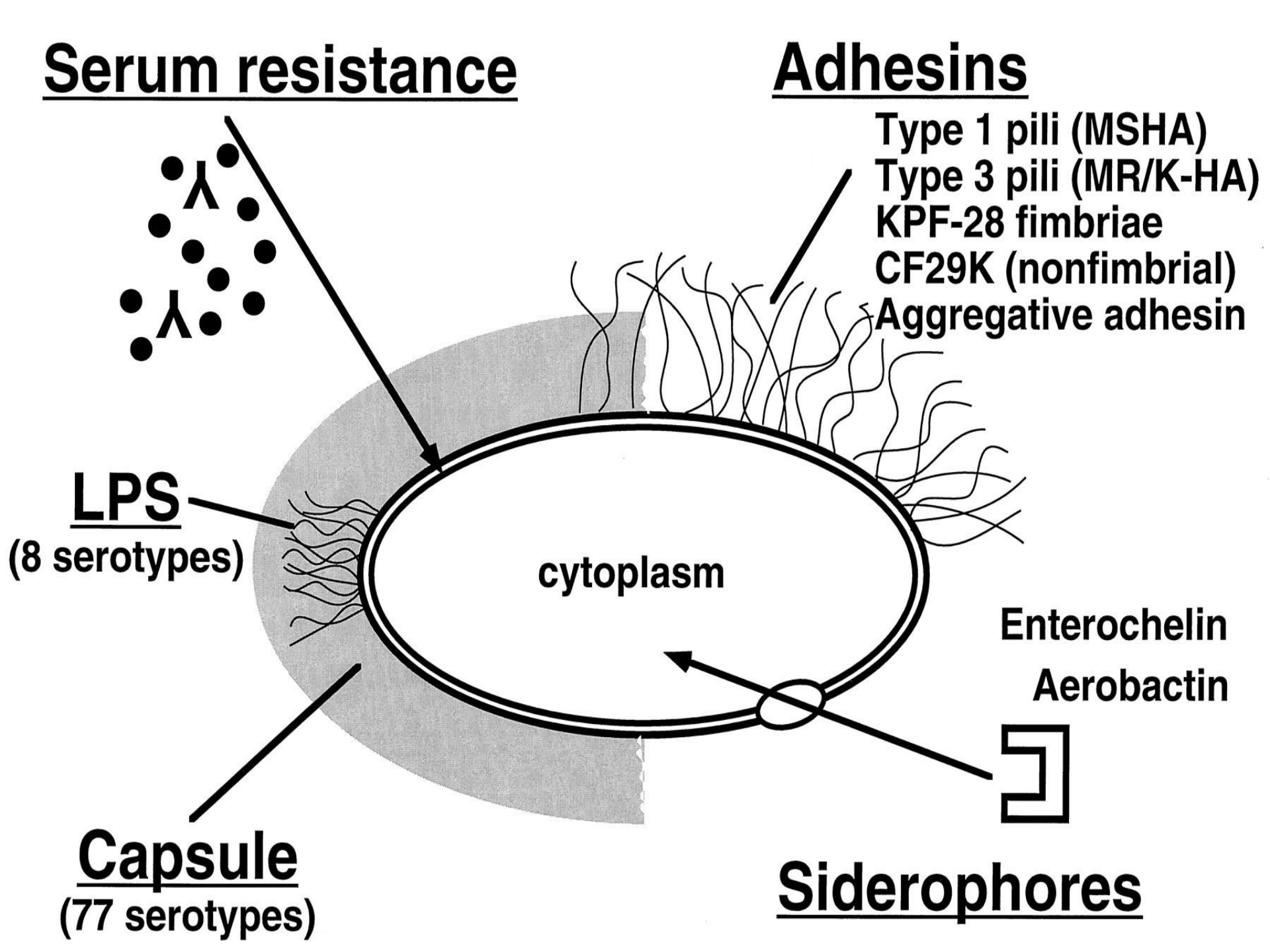
Virulence factors and Pathogenesis

- Capsule
  - Anti phagocytic
  - Prevents from complement mediated bacteriolysis
- LPS
  - Prevent from complement mediated bacteriolysis
- Adhesins (Fimbrial and non-fimbrial)
  - Type-I and Type-III
  - Adhesion to host tissues



- Toxins
  - Heat labile and heat stable toxins
  - Role not well defined
- Enzymes
  - β-lactamase and ESBL




Serum resistance
Adhesins
Type 1 pili (MSHA)
Type 3 pili (MR/K-HA)
KPF-28 fimbriae
CF29K (nonfimbrial)
Aggregative adhesin
LPS
(8 serotypes)
cytoplasm
Enterochelin
Aerobactin
Capsule
(77 serotypes)
Siderophores

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.

Diseases Caused by Klebsiella :

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

Some Klebsiella bacteria have become highly resistant to antibiotics.

Klebsiella pneumoniae produce an enzyme known as a carbapenemase (referred to as KPC-producing organisms).

APPEARANCE OF KLEBSIELLA ON CULTURE MEDIA

On blood agar

-slimy appearance of the colonies

On MacConkey agar

- red/pink colonies

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

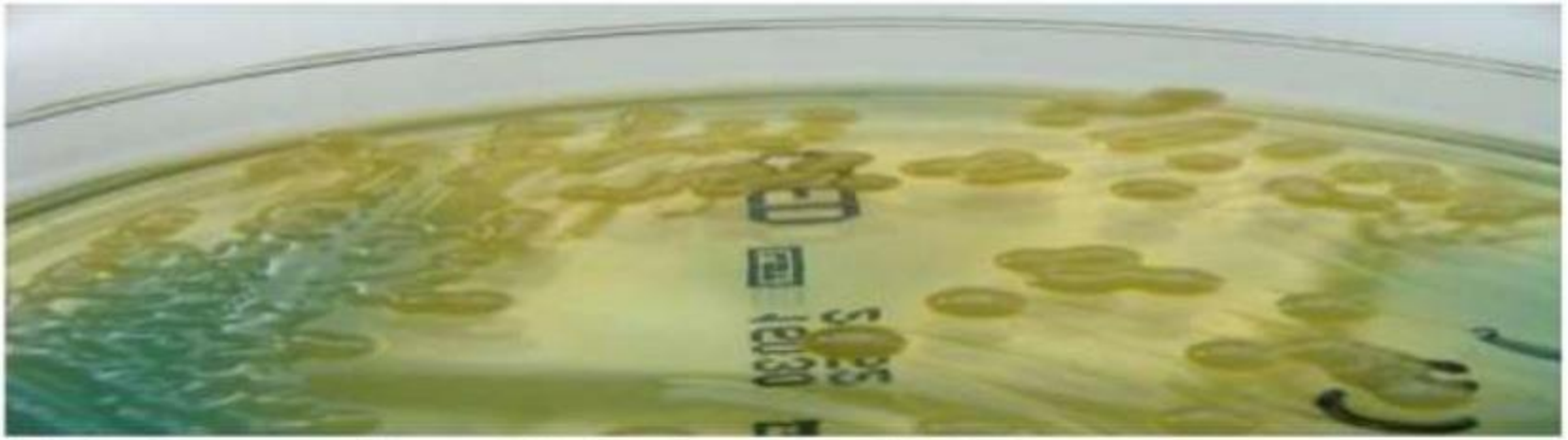
Mucous, lactose positive colonies of *Klebsiella pneumoniae* on MacConkey agar. Cultivation 37°C, 24 hours.

On EMB

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

On CLED AGAR

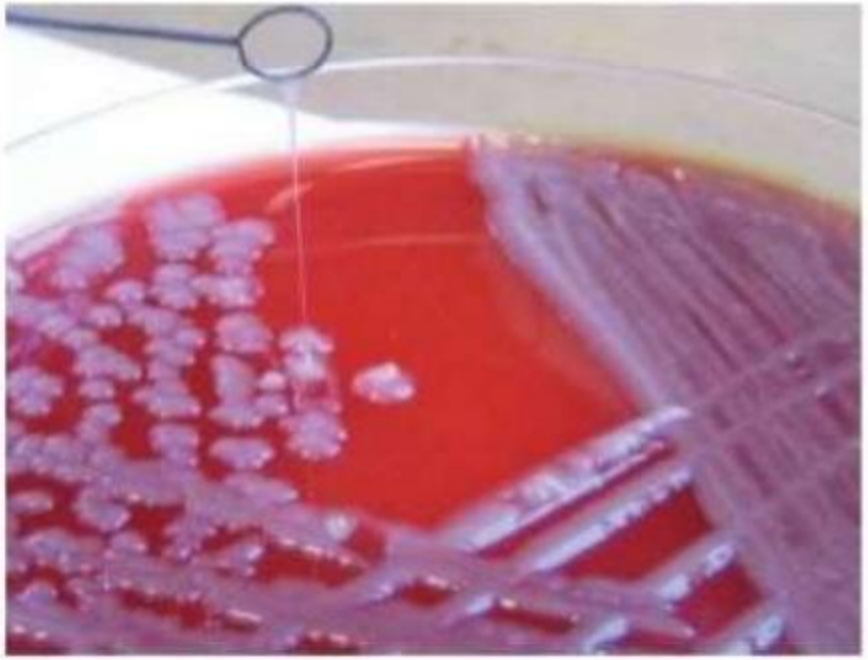
- This medium supports the growth of urinary pathogens and provides distinct colony morphology.



Klebsiella pneumoniae on CLED Agar. Large, mucoid colonies.
Bromothymol blue indicator in the agar changes to yellow due to acidification of the medium due to lactose fermentation by bacterial growth.
Lactose fermenters appear yellow. Non Lactose fermenters remain a translucent blue.

String test

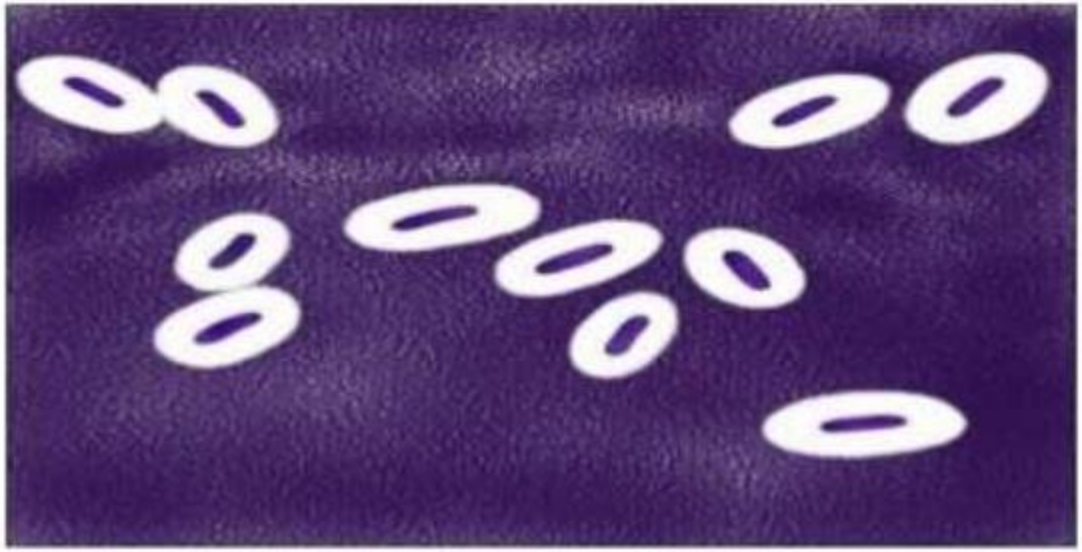
- A colony that stretches more than 5 mm using a standard inoculation loop tests positive for hypermucoviscosity.



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 .

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

IMViC Reactions

	I	M	Vi	C
Escherichia coli	+	+	–	–
Proteus vulgaris	+	+	–	–
Klebsiella pneumoniae	–	–	+	+
Klebsiella oxytoca	+	–	+	+
Enterobacter spp.	–	–	+	+
Serratia marcescens	–	–	+	+
Citrobacter freundii	–	+	–	+
Citrobacter koseri	+	+	–	+

PSEUDOMONAS

- A large group of aerobic, non sporing gram negative bacteria motile by polar flagella
- Found I nature water, soil, other moist environments
- Some of them are pathogenic to plants
- Creation of new genera such as Burkholderia. Stenotrophomnonas

GENERAL CHARACTERISTICS

- Widely distributed in soil and water
- Gram negative rods
- Aerobic
- Motile
- Produce water-soluble pigments
- Opportunistic pathogens

Pseudomonas aeruginosa

Pseudomonas aeruginosa causing :-

- Skin infections (wound swab)*

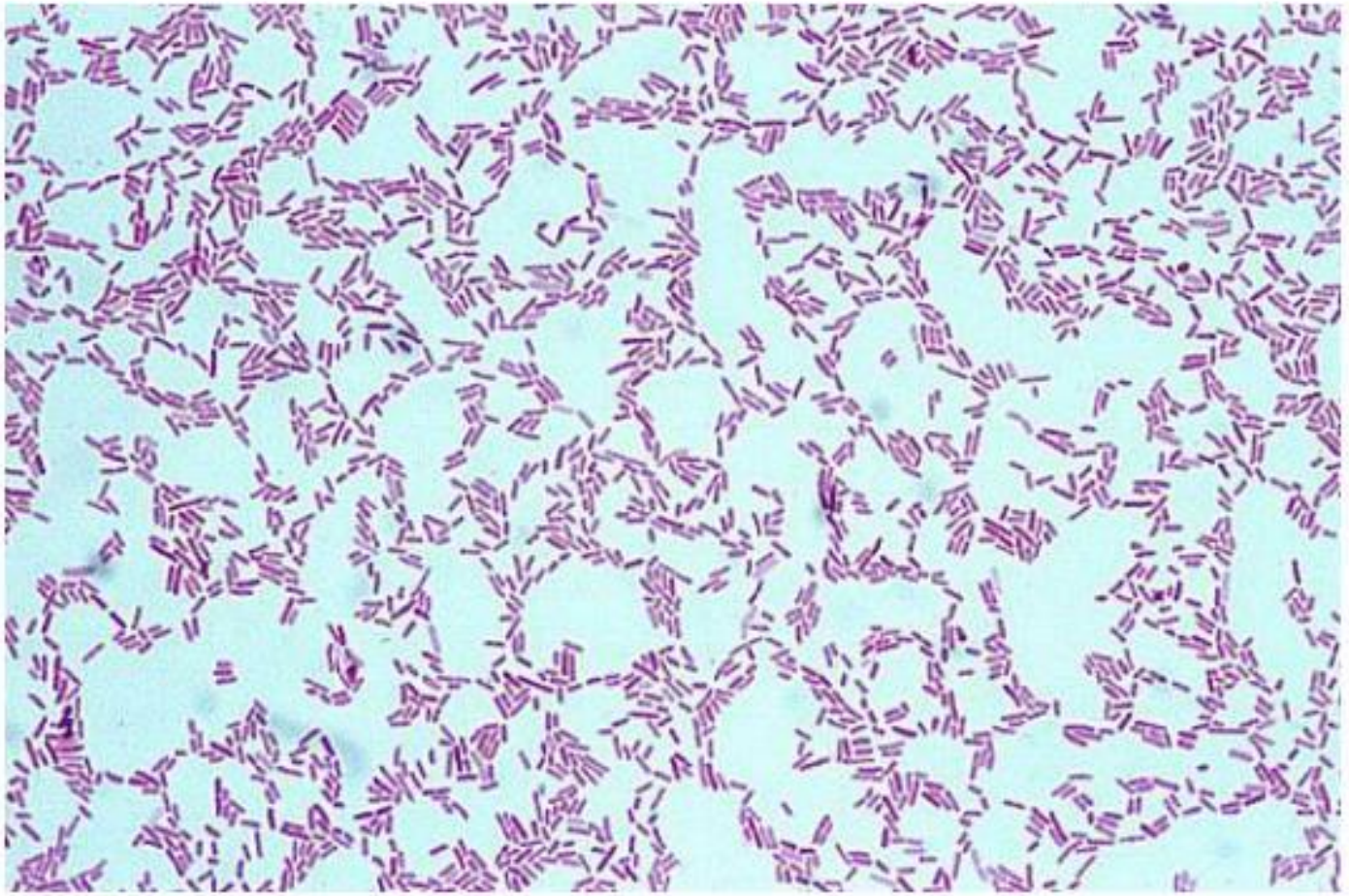
* Specimens depending on the site of infection.

- Urinary Tract infection (urine)*

- Respiratory infections (sputum & effusions)*

- Otitis Externa (ear swab)*

* Specimens depending on the site of infection.



Pseudomonas aeruginosa is a Gram negative, non-sporing motile rod

P. aeruginosa produces large, flat, spreading colonies which are often haemolytic and usually pigment-producing.

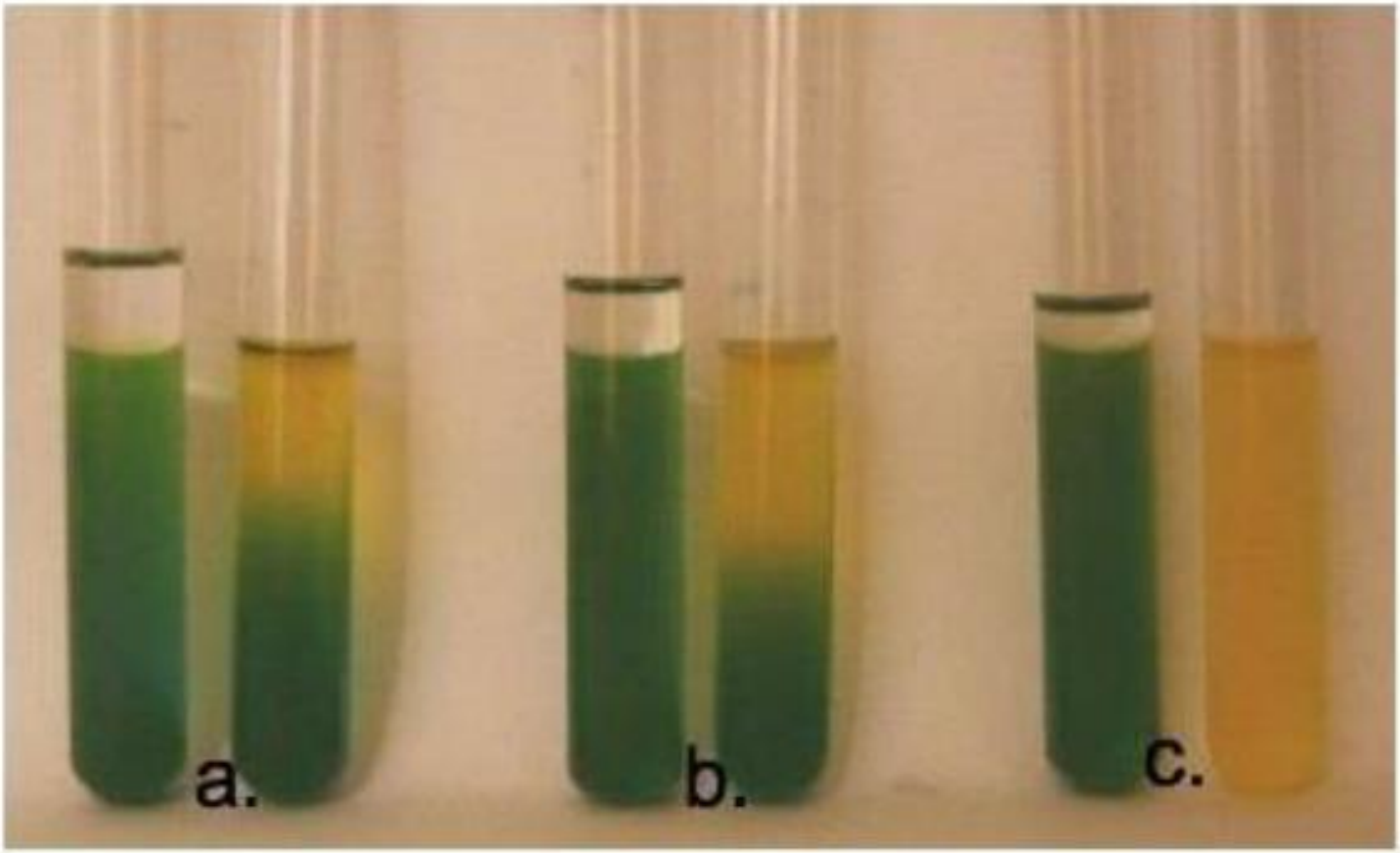
The pigments diffuse into the medium giving it a dark greenish-blue colour

P. aeruginosa produces pale coloured colonies on MacConkey agar

On Nutrient agar *P. aeruginosa* can be recognized by the pigments, it produces a blue-green pigment (pyocyanin).

O-F TEST

The oxidative-fermentative test determines if certain gram-negative rods metabolize glucose by fermentation or aerobic respiration (oxidation). During the anaerobic process of glucose fermentation, the high concentration of acid produced will turn the bromthymol blue indicator in OF media from green to yellow in the presence or absence of oxygen

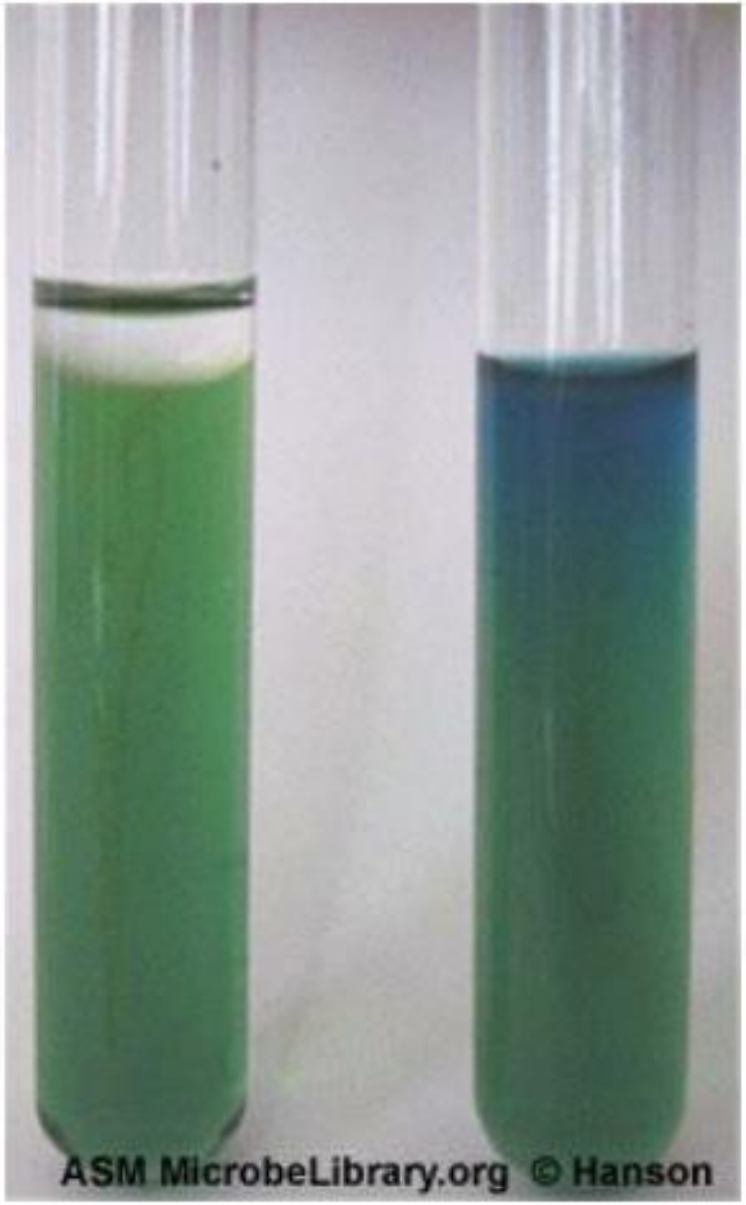


Acid production in the open tube and not the oil-covered tube indicates an oxidative result.

(a) *P. aeruginosa* incubated for 24 hours.

(b) *P. aeruginosa* incubated for 48 hours.

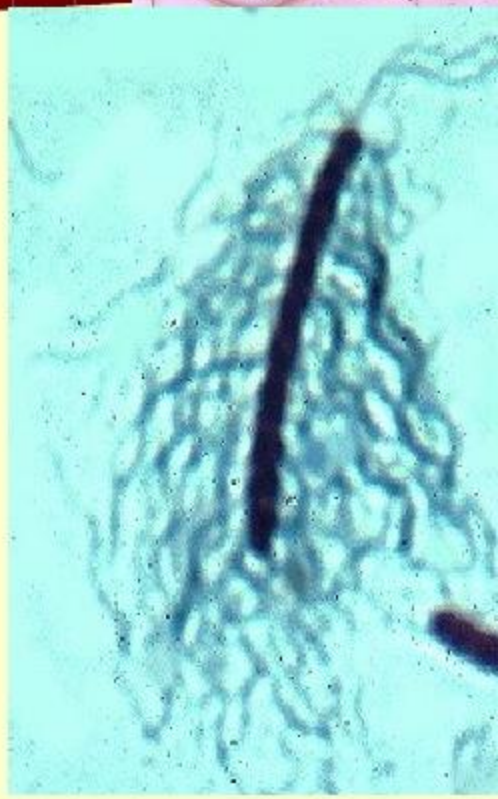
(c) *P. aeruginosa* incubated for 5 days.



No color change in the oil-covered tube and color change to alkaline in the open tube indicates a negative result. *A. faecalis* cannot use glucose fermentatively or oxidatively. The blue at the top of the open tube is due to amine production resulting from the metabolism of protein in the media.

Species of Proteus

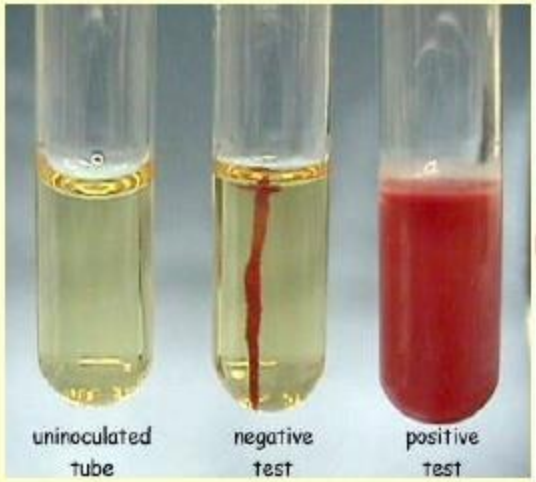
- ***Proteus mirabilis***
- ***Proteus vulgaris***
- *Proteus myxofaciens*
- *Proteus penneri*



Virulence Factors

- Urease activity
- Protease
- Fimbriae
- Haemolysins
- Motility
- Swarming

Motility test



Pathogenicity

- *P. mirabilis* -70-90 %
- UTI – Commonest site
  - Young / elderly patients
  - High concentration of Urea in urine
- Superficial septic lesions
- Meningitis
- Osteomyelitis
- Septicemia
- Otitis media

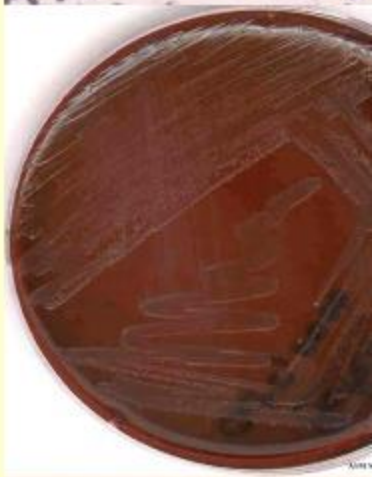
Lab Isolation and Identification

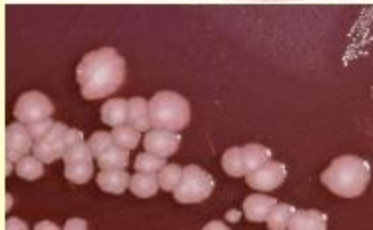
Morphology

- GNR, 1 – 3 um
- Motile-peritrichate flagella

Cultural Characteristics

- Grow well on ordinary media
- Swarming
  - Continuous
  - Discontinuous
- Faint ammonia / fishy odor





Swarming

- Characteristic but not unique
  - *Serratia marcescens*
  - *Vibrio parahaemolyticus*
  - Bacillus
- Continuous swarming
- Discontinuous swarming
- Ascending infection

Anti-swarming Agents

- Increasing Agar concentration 3-4 %
- Incorporation into media of a polyvalent-H anti-sera
- Incorporating growth inhibitors
  - Sulphonamides
  - Chloral Hydrate
  - p-Nitrophenyl Glycerol
  - Neomycin
  - Barbiturates
- Incorporation of
  - Detergents
  - Bile Salts-MacConkey Agar
- Electrolyte Deficiency- CLED