

KLEBSIELLA

Klebsiella pneumoniae

- Edwin Klebs – German microbiologist
- Called as Friedlander's bacillus or *Bacillus mucosus capsulatus*.
- First isolated by Carl Friedlander (1883) from fatal cases of pneumonia.
- Widely distributed in nature.
- Commensals in the intestine.
- Saprophytes in soil & water.

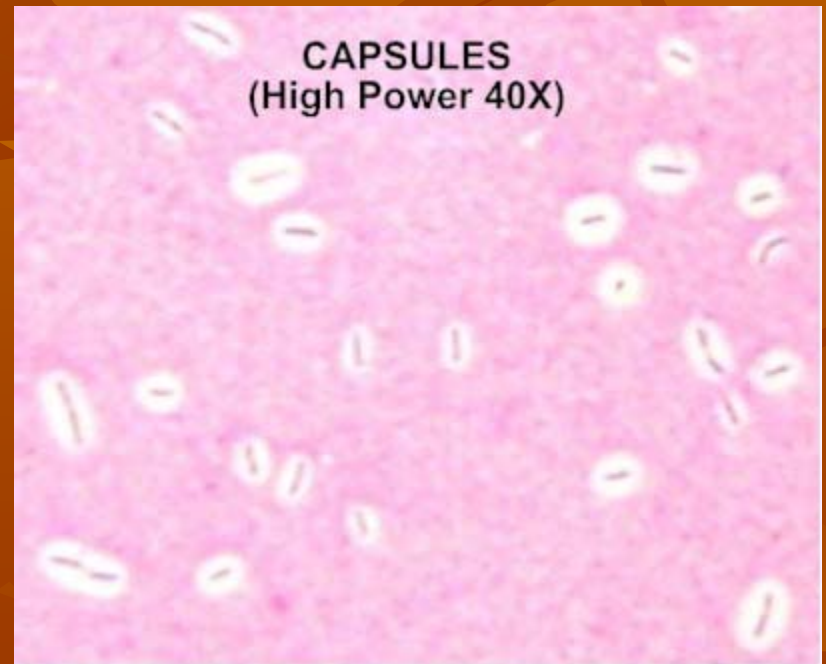
Morphology

**Gram negative bacilli
which are thick & short ,
plump**

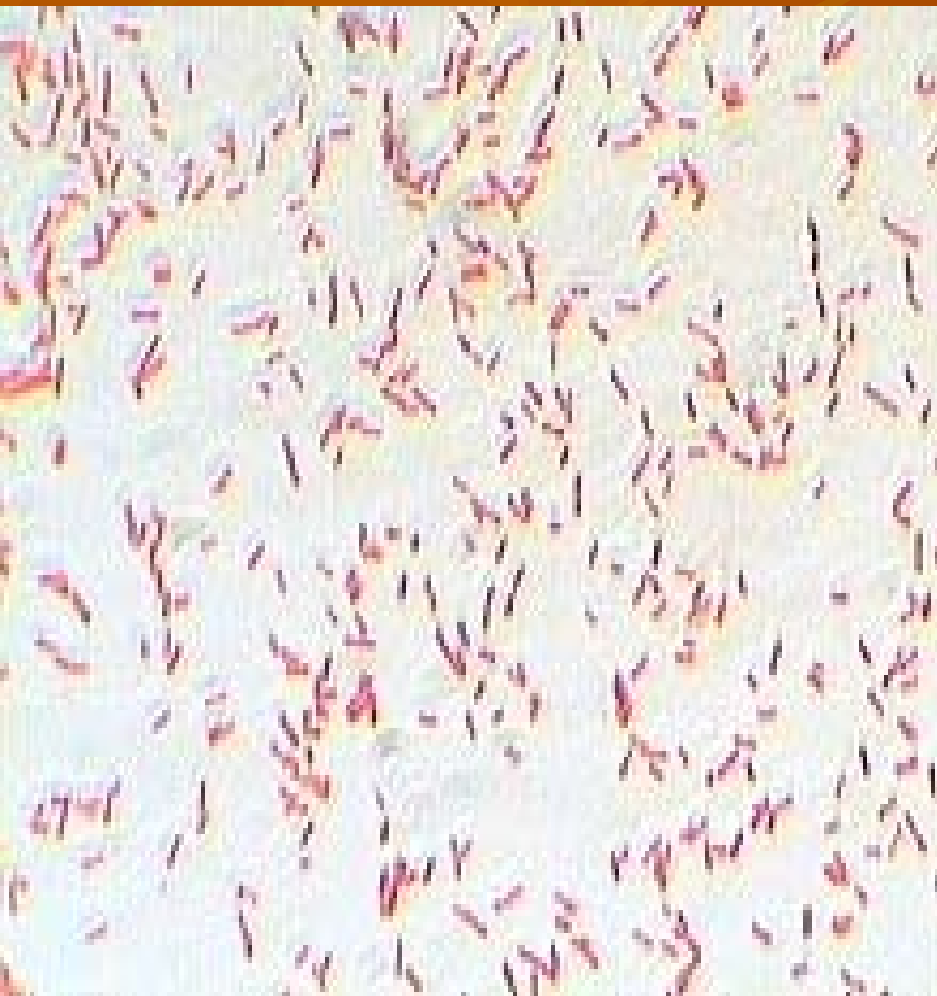
Non motile

Large capsule

1-2 X 0.3-0.8 μm



Morphology /Colonies on Mac Conkey media



Culture characters

- Aerobic, grow well on ordinary culture media
- N.agar : Large, dome shaped, mucoid
- Mac Conkey : Lactose fermenter, mucoid

Biochemical reactions

- Sugar fermentation: Acid & gas in Glucose, Lactose, Sucrose, Mannitol
- IMViC test: - - + +
- Urease test : Positive
- TSI : A/A , gas ++

Species--Types

- *K.pneumoniae*
- *K.ozaenae*
- *K.rhinoscleromatis*
- *K.oxytoca*

Pathogenicity

- Pneumonia (lobar or lobular)
- Urinary tract infection
- Pyogenic infection e.g. abscess, meningitis, septicemia
- Diarrhoea (by some strain)
- Foul smelling nasal discharge (*K. ozaenae*)
- Rhinoscleroma (*K. rhinoscleromatis*)



	E. coli	Klebsiella
Morphology	Slender & Long	Short & Thick
	Motile	Non motile
	Non capsulated	Capsulated
IMViC test	+ + - -	- - + +
Urease test	Negative	Positive
Colony	Moist	Mucoid

Laboratory diagnosis

- Specimen collection
- Culture & gram stain
- Biochemical reactions

Proteus

Introduction

- Normal intestinal commensals
- ‘Proteus’ refers to pleomorphism
- Widely distributed in nature as saprophytes

- Tribe : Proteeae
- Genus : Proteus, Morganella, Providencia

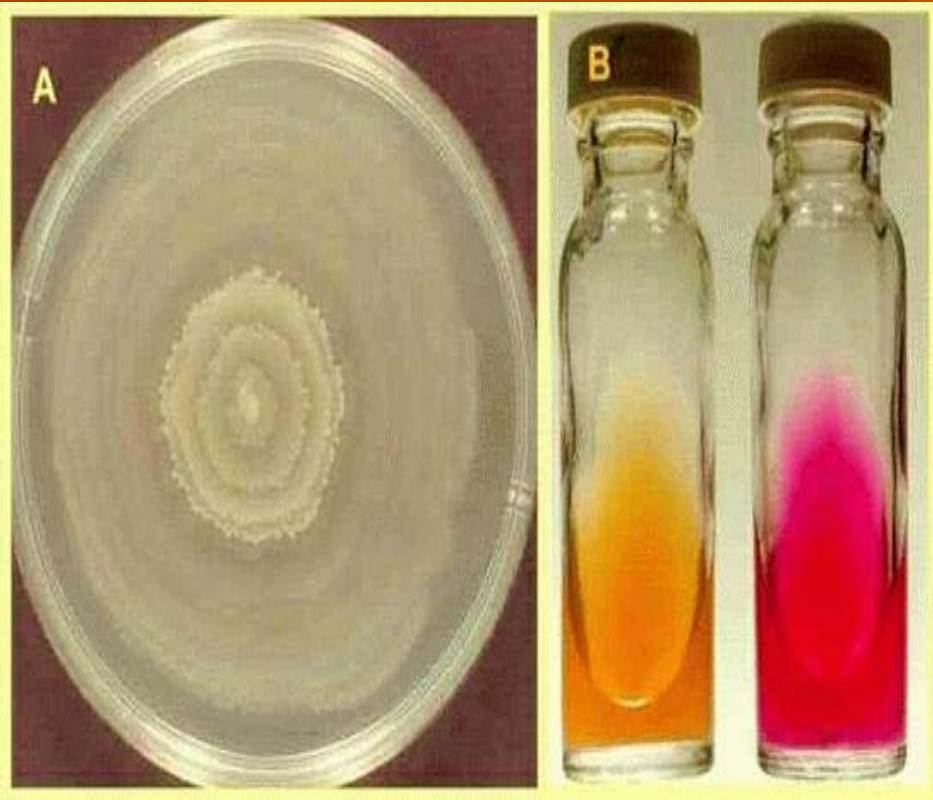
Morphology

- Gram negative, motile, non capsulated, pleomorphic bacilli.
- Non motile strains of Proteus OX2,OX19, OXK are used for Weil Felix reaction in diagnosis of rickettsial diseases.

Gram stain / LNF on Mac Conkey



Culture characters



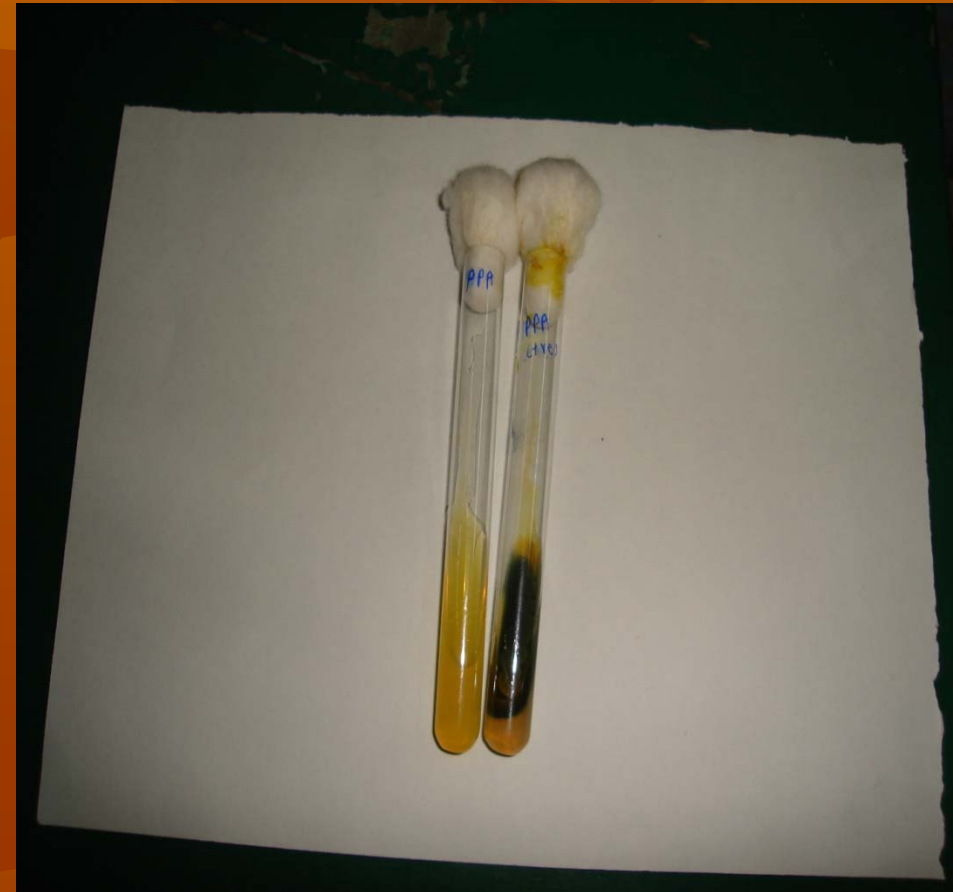
- **N. agar** : Form a thin filmy layer, swarming, putrefactive odour ('fishy' or 'seminal' odour)
- **Mac Conkey agar** : Smooth, colourless, non lactose fermenter

Methods to inhibit swarming

- Increase concentration of agar (6%)
- Incorporation of Chloral hydrate (1:500)
- Sodium azide (1:500)
- Alcohol (5 – 6%)
- Sulphonamide
- Surface active agents or boric acid (1:1000)

Biochemical test

- Enzyme phenyl alanine deaminase which converts phenyl alanine to phenyl pyruvic acid (PPA reaction)
- Fail to acidify lactose, dulcitol or malonate
- IMViC test : - + - -
(Pr.mirabilis)
- Urease test : Positive



Antigenic structure

- Somatic antigen O
- Flagellar antigen H
- Weil & Felix observed that flagellated strains growing on agar formed a thin surface film resembling the mist produced by breathing on glass & named this variety as 'Hauch' form
- Non flagellated is called 'Ohne Hauch' (i.e without film of breath)

Pathogenicity

- *Pr.mirabilis* : Urinary tract infection, nosocomial infection
- *Pr.vulgaris* : Less common to human infection
- *Morganella* (*Pr.morganii*) : No swarming. Cause urinary tract infection & nosocomial wound infection
- *Providencia* (*Pr.alcalifaciens*) Diarrhoeal diseases.

Laboratory diagnosis

- Urine
- Pus swab



YERSINIA

- *Yersinia*

- Three species are important pathogens in man

- *Yersinia pestis* – causes plague

- *Yersinia enterocolitica* – enteritis

- *Yersinia pseudotuberculosis* – resembling tuberculosis lesion

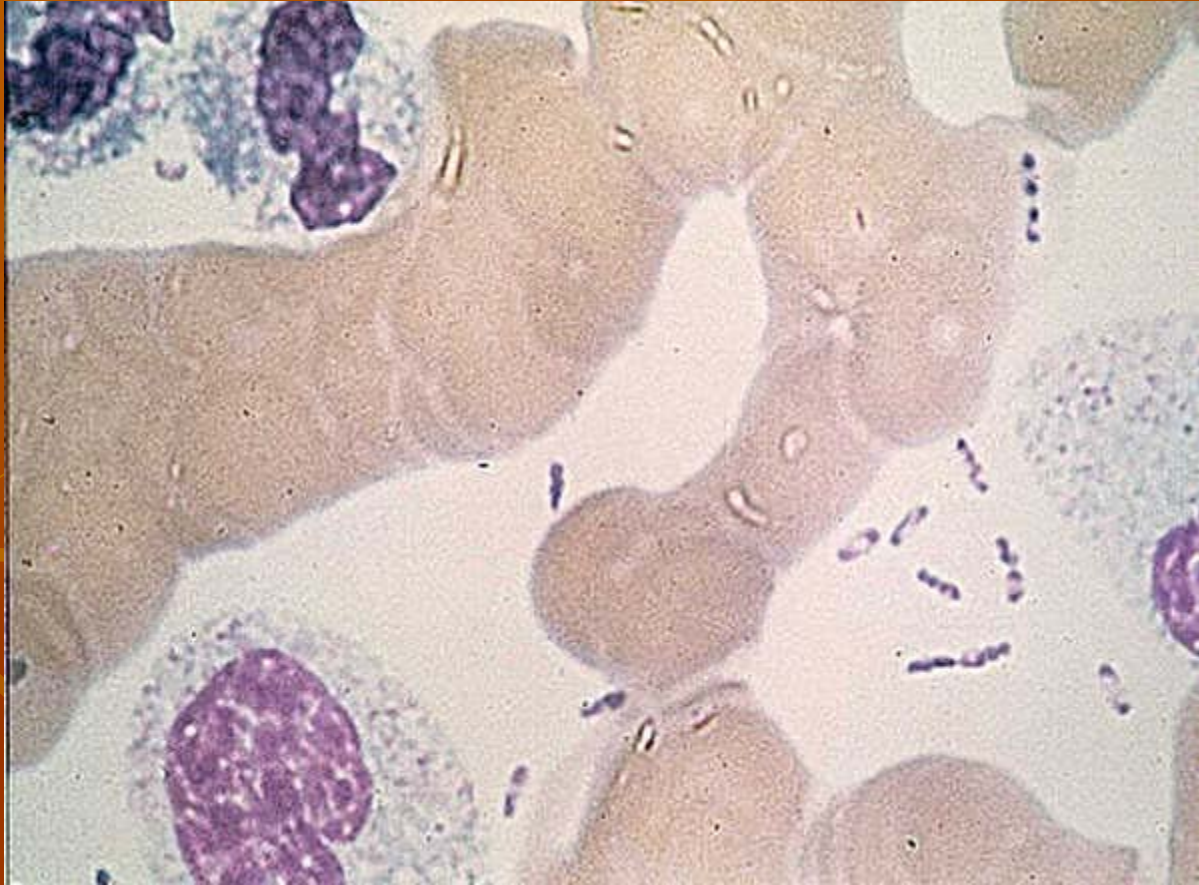
■ Identification

- *Y. pestis* can be separated from *Y. enterocolitica* and *Y. pseudotuberculosis* by the fact that it is non-motile. *Y. enterocolitica* and *Y. pseudotuberculosis* are both non-motile at 37⁰ C, and motile at 22⁰ C.
- *Y. pestis* is identified based on the following:
 - Non-motile, GNB, Small coccobacilli -1.5 X 0.7 um
 - Bipolar staining
 - Slow growth of small colonies on ordinary culture media – it grows better at lower temperature (25-30⁰ C)

Yersinia pestis bipolar staining



Yersinia pestis Wayson's stain



Cultural characteristics

- Aerobic & facultative anaerobic
- pH 5- 9.6 (7.2)
- Temp. 2-45°C
- NA: small, delicate , transparent disc
- BA : dark brown
- MA : colourless
- N broth : Flocculent growth at bottom & along the sides with little or no turbidity
- Ghee broth culture: stalactite growth

- *Yersinia pestis* – virulence characteristics
 - Endotoxin – is responsible for many of the symptoms
 - Murine toxin or plague toxin – causes edema and necrosis in mice and rats, but has not been shown to play a role in human disease

Y. pestis

- Fraction 1 – a protein component of the antiphagocytic protein capsule
- V antigen or W Ag – a secreted protein that controls expression of many of the virulence genes plus it appears to have another unknown function that is essential for virulence

- Pla – a protease that activates plasminogen activator (acts as a fibrinolysin) and degrades C3b (prevents formation of complement membrane attack complex) and C5a (prevents attraction of phagocytes)
- Coagulase
- Psa – a pilus adhesion for attachment
- Iron acquisition and sequestering system

Epidemiology

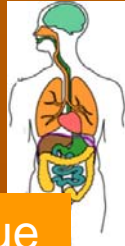
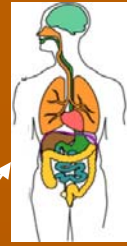
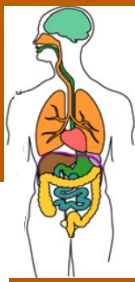
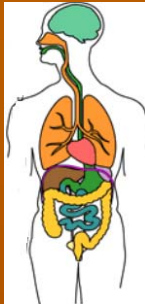
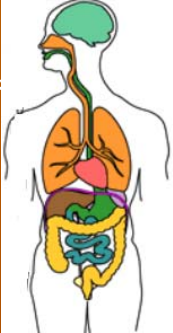
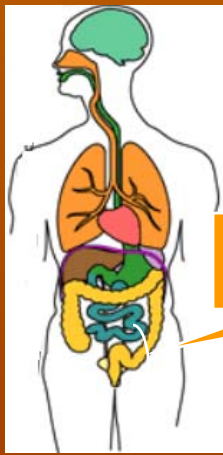
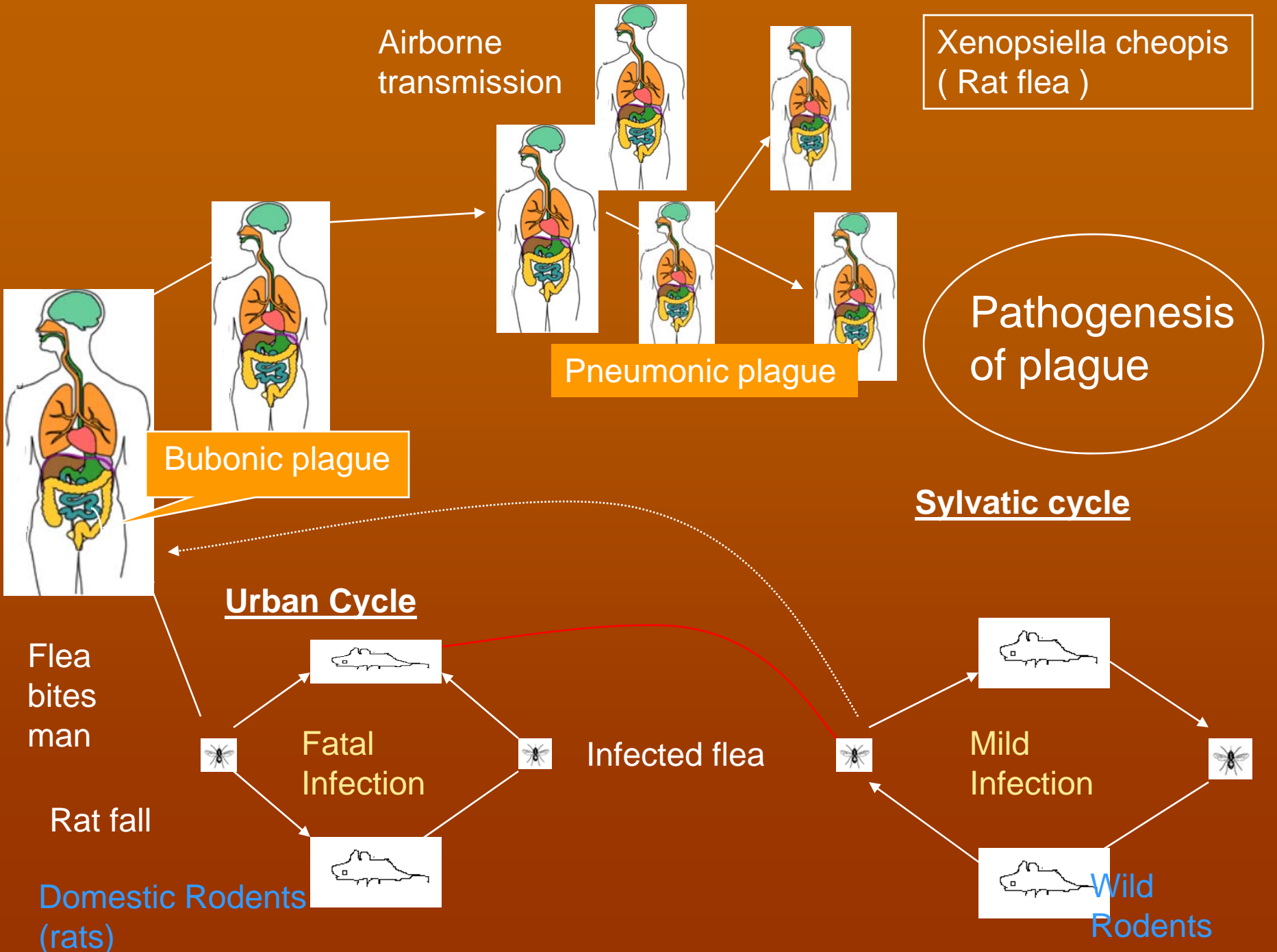
- Outbreak : Sept. 1994- Bombay → Surat
- Sporadic : January 2002 – Northern India

Zoonotic disease

Rodents → Rodents (Rats, squirrels, mice, dogs etc)

Sylvatic plague or wild : occurs in nature & in wild rodents, independent of human beings

Urban or domestic plague : occurs in human beings & rodents, rat - reservoirs



Xenopsiella cheopis
(Rat flea)

Pathogenesis
of plague

Bubonic plague

Pneumonic plague

Sylvatic cycle

Urban Cycle

Flea
bites
man

Rat fall

Domestic Rodents
(rats)

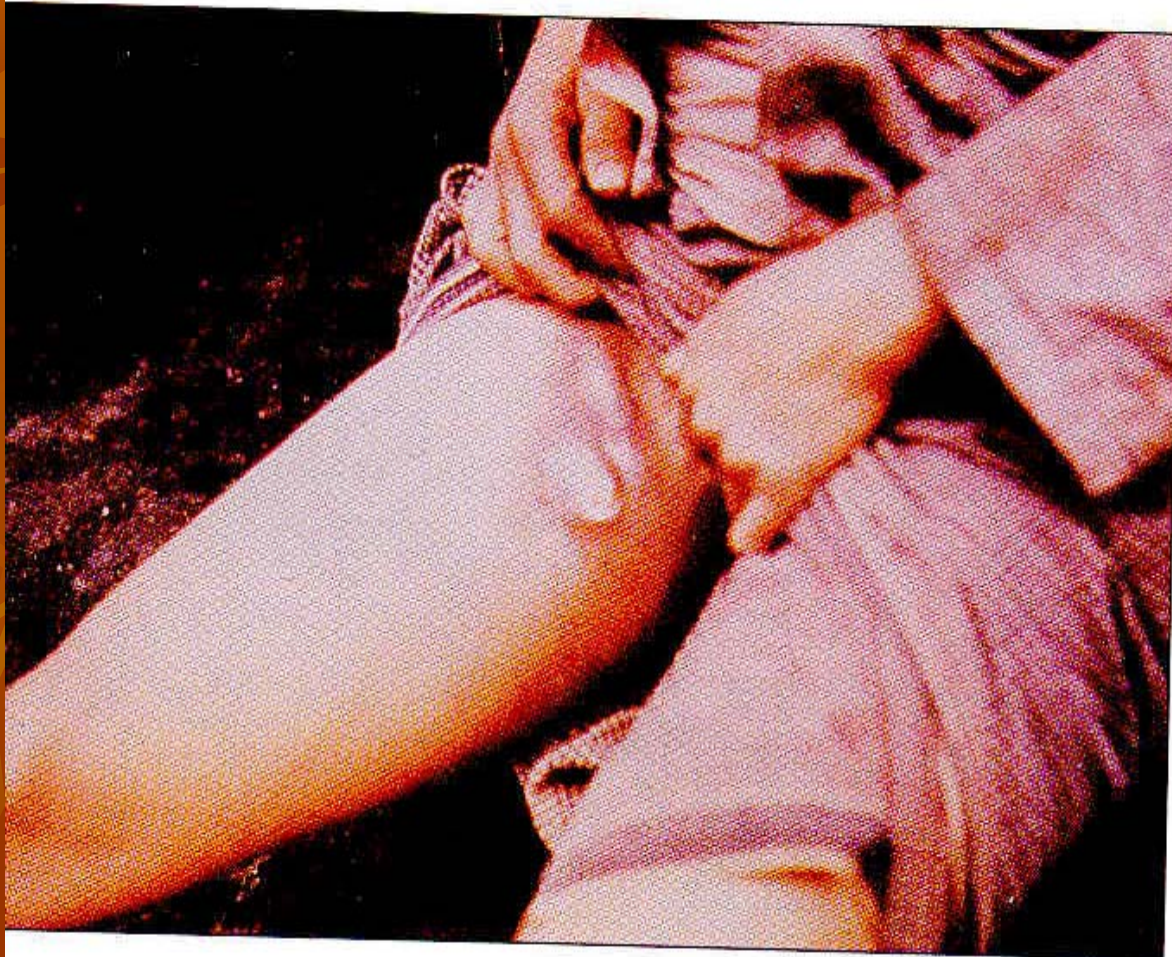
Fatal
Infection

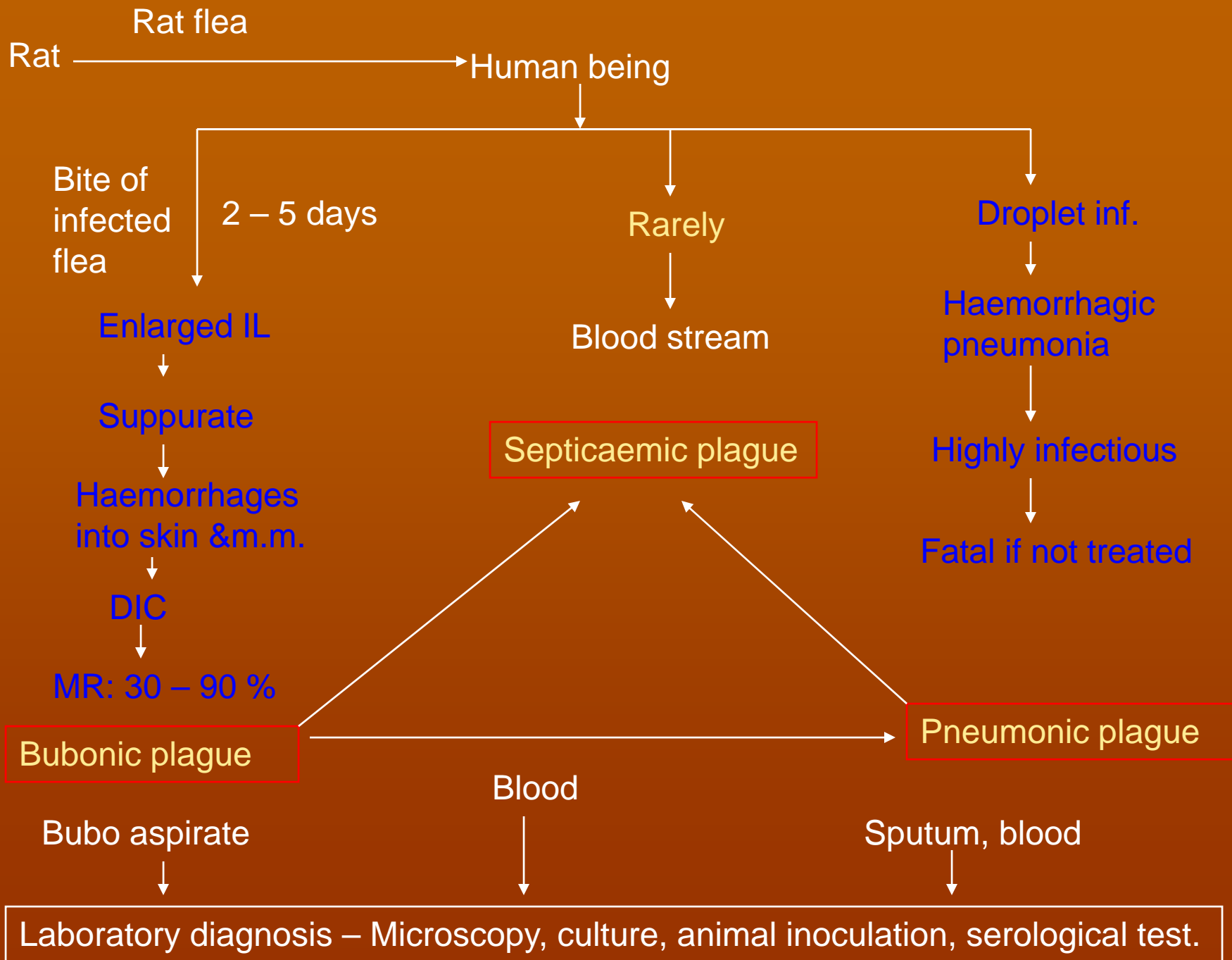
Infected flea

Mild
Infection

Wild
Rodents

Buboes





- *Y. pestis* – clinical significance
- In man plague occurs in three forms; bubonic, pneumonic & septicaemic
- Bubonic plague – transmitted by fleas from an infected rodent (is endemic in our local mountains)
- The bacteria travel in the blood to the nearest lymph node where they are engulfed by fixed macrophages.
- A high fever develops and the lymph nodes in the groin and armpit become enlarged (buboes) as the bacteria proliferate and stimulate an inflammatory response.

- The bacteria growing in the lymph node leak into the bloodstream.
- Lysis of the bacteria releases LPS, causing septic shock.
- Subcutaneous hemorrhages , probably due to LPS causing DIC gave the disease the name, the black death, in the middle ages.
- The untreated mortality rate is quite high.

- Eventually bacteria reach the lungs where they are ingested by lung macrophages to cause pneumonic plague.
 - Pneumonic plague – this can be transmitted directly to others via aerosol. Direct inhalation of aerosols containing the organism produces a form of the disease that progresses much more rapidly and the mortality rate is close to 100%.
 - Septicaemic plague – rarely primary infection occur.
- Treatment for plague
 - Streptomycin or tetracycline are effective



Pseudomonas aeruginosa

Introduction

- Ps. *Pyocyanea*, *Bacillus pyocyaneus*
- Mostly saprophytic, being found in water, soil or other moist environment
- pathogenic to plants, insects or reptiles (some strains)
- Colonizes the tissues & Cause typically opportunistic infections
- Resistant to almost every antibiotics

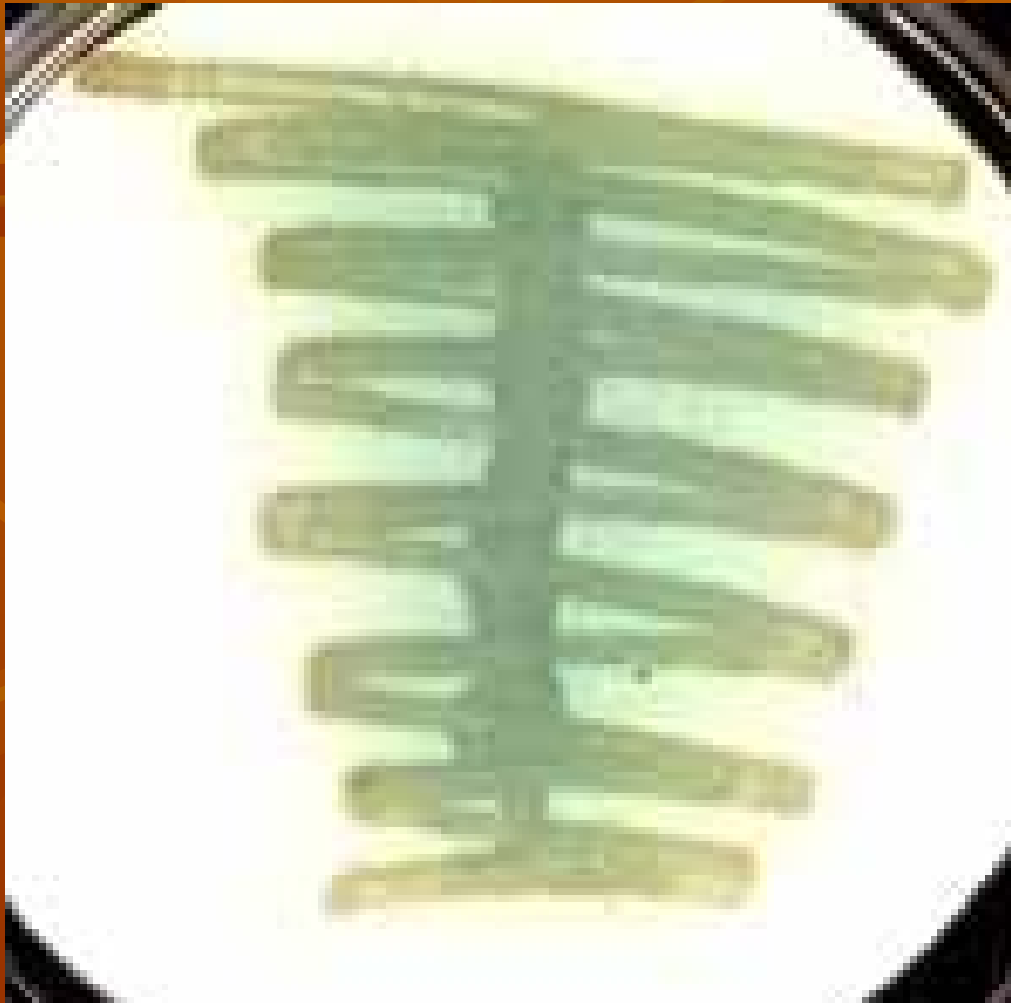
Morphology

- Gram negative slender bacilli
- Actively motile
- Non capsulated (Often form a loose capsule)
- Clinical isolates are often piliated

Culture characters

- Strict aerobes, but can grow anaerobically if nitrate is available.
- N.agar : Large, opaque, irregular with musty or earthy smell. Produce pigments i.e. pyocyanin (bluish green), pyoverdin (greenish yellow), pyorubin (red), pyomelanin (brown)
- Mac Conkey agar: NLF
- Blood agar: hemolytic
- Selective media (Cetrimide agar)

Pigment on N.agar



Pigment by Pseudomonas



Biochemical reactions

- Glucose : Acid only
- IMViC : - - - +
- Catalase test : Positive
- Oxidase test: Positive
- Nitrates are reduced to Nitrite

Virulence factors

- Exotoxin A & S : Mechanism of action is similar to diphtheria toxin & stops protein synthesis
- Protease
- Elastase
- Hemolysin
- Enterotoxin

Wound inf. By Pseudomonas



Pathogenicity

- ‘Blue pus’, the term aeruginosa, meaning verdigris which is bluish green in colour
- Localised lesions : Infection of wounds, bedsores, eye infection
- Iatrogenic infections: Meningitis followed by lumbar puncture, post tracheostomy pulmonary infection
- PUO (Shanghai fever) resembling typhoid fever
- Infantile diarrhoea, sepsis