Gram-negative, facultative intracellular zoonotic bacteria

Brucella

Francisella

Pasteurella

Yersinia

Katalin Kristóf MD PhD

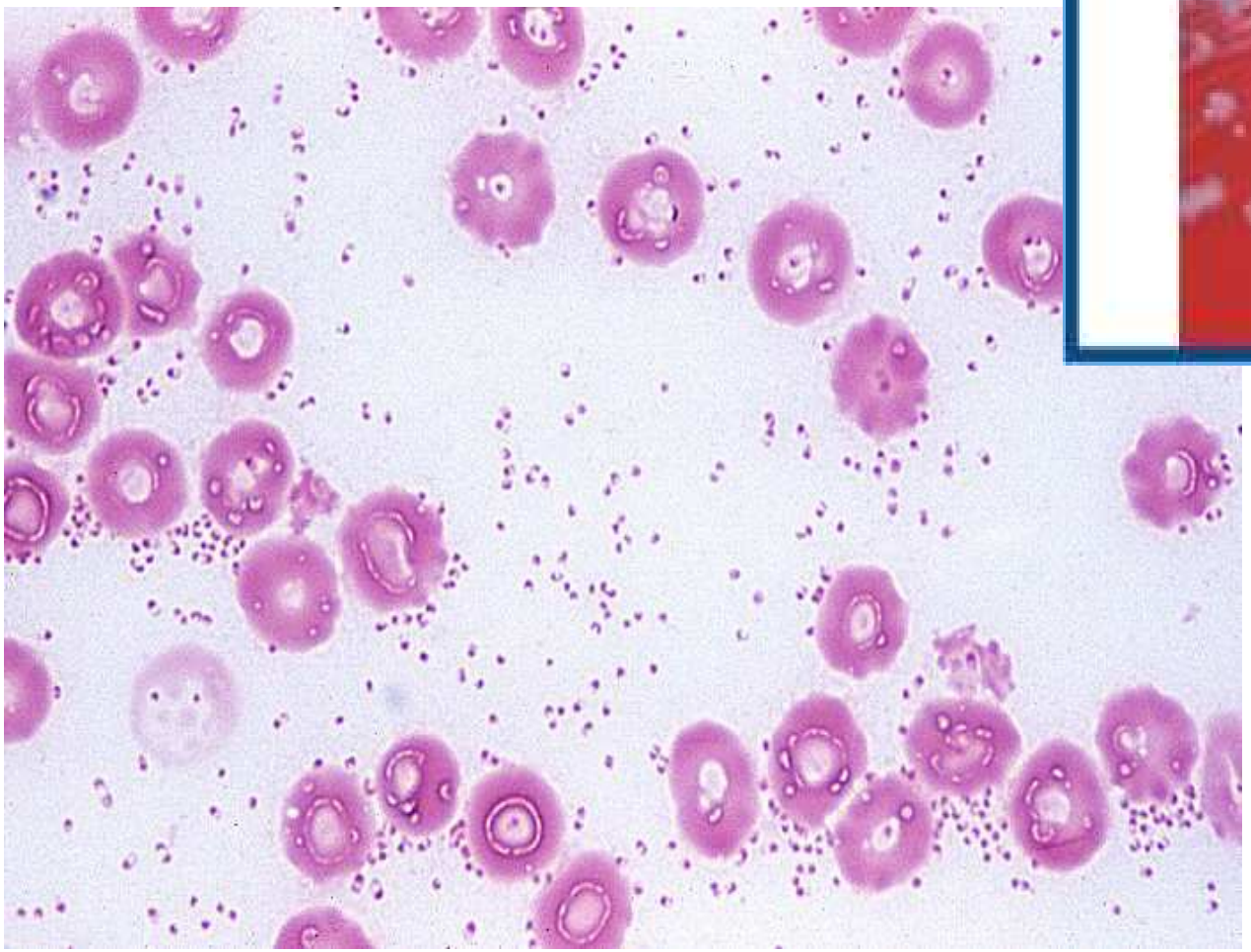
Zoonotic infections

Definition of zoonosis

an infectious disease transmissible under natural conditions between vertebrate animals and human beings

- Animal-human spread
- Rodents, mammalians are the natural hosts
- Direct contact, food or vector bite
- Human disease: severe, for weeks
- High mortality: 1-90%

Pasteurella genus:
Pasteurella multocida



Hialuronic capsule

Culture: blood agar, chocolate agar

Pasteurella multocida

Pasteurella canis

- bite wound infections
  (dog or cat)

- bacteremia
- meningitis
  - predisposing factor: chronic pulmonary disease

Treatment

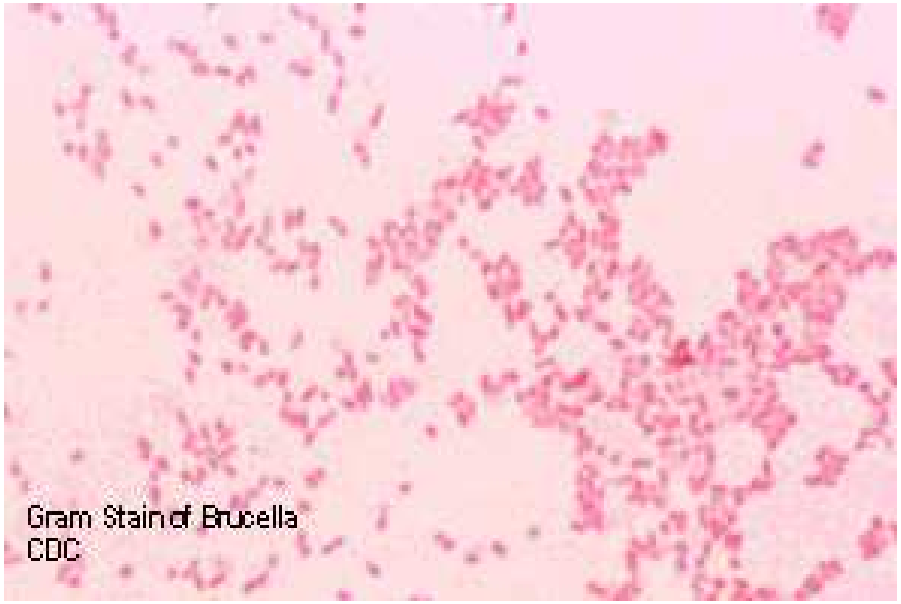
- Primer surgery care!

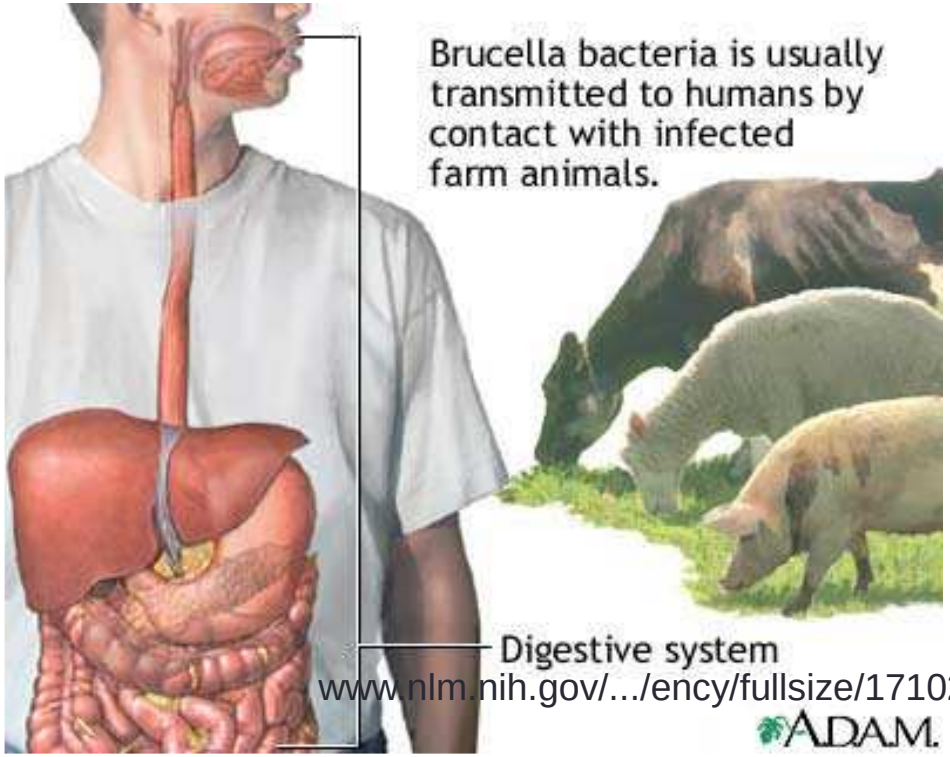
- penicillin

Brucella

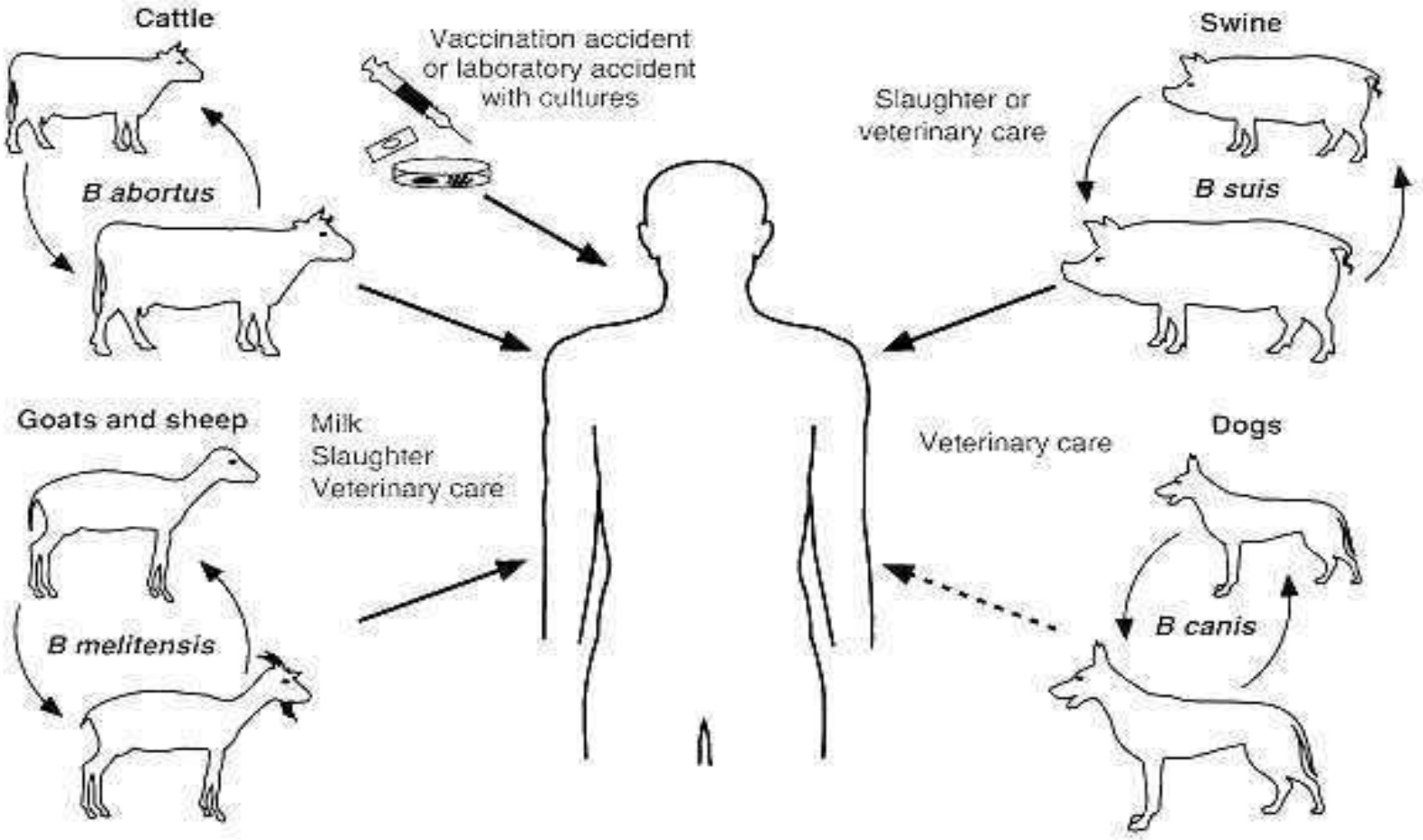
Morphologia:

Gram-negative, aerob, facultatively intracellular coccobacilli





Brucella – natural hosts (abortion)



<u>Only one species with four biotypes!</u>

Brucella causes mild or asymptomatic disease in the natural host infecting organs rich in erythritol (breast, uterus, placenta and epididymis) → sterility, abortus

Brucella-brucellosis

B.abortus /cattle, American bison /

Morbus Bang: mild disease with suppurative complications

B.melitensis / goats, sheep/

Malta fever : severe, acut disease with complications

B.suis / swine, reindeer, caribou /

Swine-brucellosis: chronic, suppurative, destructive disease

B.canis / dogs, foxes, coytes/

Mild disease with suppurative complications

Brucella-brucellosis

Transmission :- ingestion of infected raw milk, meat => mucous membranes, conjunctiva, skin

/ Entry : via lymphatic vessels => regional lymph. node => ductus thoracicus => blood stream => organs: spleen, liver, bone marrow, lymph. tissue… /

Brucellosis

- Undulant fever
- Chronic arthritis
- Orchitis
- Carditis
- RES
- WHO: B bioterror category

Epidemiology

- More than 500 000 documented cases annually
  - In Latin America
  - Africa
  - Middle East
  - Western Asia
- U.S.A. : 104/2003
- Hungary: 4/2001, 1/2005

Microbiological diagnosis

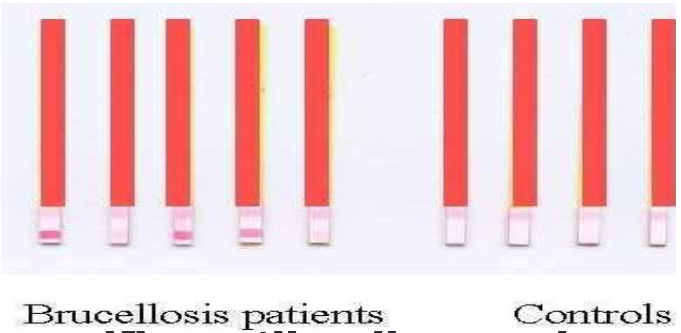
Clinical specimen: sputum, urine, blood-culture, CSF, bone marrow as well as lymph node punctate

Direct smear: Gram negative coccobacillus, located intracellularly

Culture:

Inoculation into enrichment medium, incubation for 21 days.
Subcultures are taken every 5 day onto an agar medium containing serum, glycerol and crystal violet (Brucella-agar) at 37 ^{o}C in 5-10 % CO_2-atmosphere.
Colonies of brucellae are tiny, regular, round shaped, glittering and transparent. No haemolysis is seen.

Serology:



*Rapid test: Direct IF

*Complement fixation (CF), ELISA to test for specific antibodies and establish their titre in the patients` sera.
*IgM chromatographia

*Wright reaction (Widal`s type tube agglutination) to test for specific antibodies and establish their titre in the patients` sera.

Ag = killed brucella cells stained with tryphenyl tetrasolium chlorid.

Ag+Ab complex forms redish agglutinate that sediments onto the bottom of the test tubes.

*Skin test (brucellin reaction)

Treatment and prevention

- doxycycline, rifampicin, streptomycin

- milk, meat proper handling
- Correct control of animal health
    - animal vaccination

Francisella tularensis
- tularemia,
rabbit fever,
glandular fever,
tick fever,
deer fly fever

Francisella tularensis

Morphology

Facultative intracellular, aerob, very small, bipolar stain

Gram-negative coccobacilli

Virulence

Antiphagocytic capsule

intracellularly resistant to killing in serum and by phagocytosis

Epidemiology

- **arthropod vectors (e.g. ticks)**

– small number (10)

- **Inhalation (50), skin (10^8), conjunctiva**

- large numbers of organisms

Disease association: tularemia ⇒ antropo-zoonosis

Key points for the clinical diagnosis:

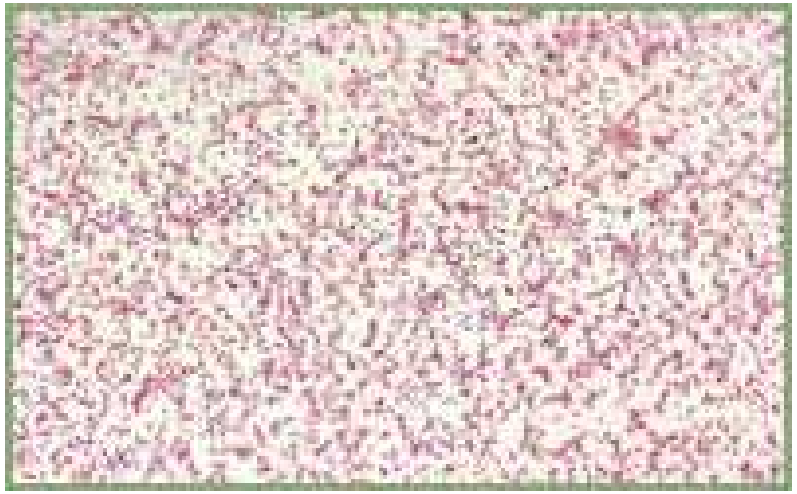
- Fever, headache, nausea, weakness
- Ulcerating papule at the site of the inoculation
- Enlarged regional lymph nodes
- Contact with wild rabbits, or with arthropod vectors (e.g. ticks) at endemic areas in the previous history , inhalation of an infections aerosol

-

Disease association: tularemia

- oculoglandular
- oropharyngeal, gastrointestinal
- ulceroglandular
- pneumonic
- systemic or typhoidal

Microbiological diagnosis



Sample to be taken:
- blood for serology
- (sputum, conjunctival discharge, lymph node punctate)

Direct smear: Gram negative pleomorphic coccobacilli (*Francisella tularensis* is very rarely found in samples therefore direct smear is not contributory to the diagnosis)

Culture:

Avoid!

BCYE

Francis medium

(containing glucose, cystein and rabbit blood at 37 oC, aerobically, for 3-4 days)

Colonies of *Francisella tularensis* appear to be small, regular, round shaped and have a metallic blue colour.

Culture is not routinely done because it requires a special safety level laboratory and it is not contributory to the diagnosis.

WHO: „A" bioterror category

Serology:

Direct IF with FITC-labelled specific antibodies to show presence of *F. tularensis* bacteria in the direct smear.

Antibody detection

passive haemagglutination

complement fixation

Allergic skin (tularin) test

(tuberculin type reaction) often gives a positive reaction long before antibodies in the serum rise to a detectable level.

Treatment

Doxycycline, gentamicin (streptomycin), ciprofloxacin

Prevention

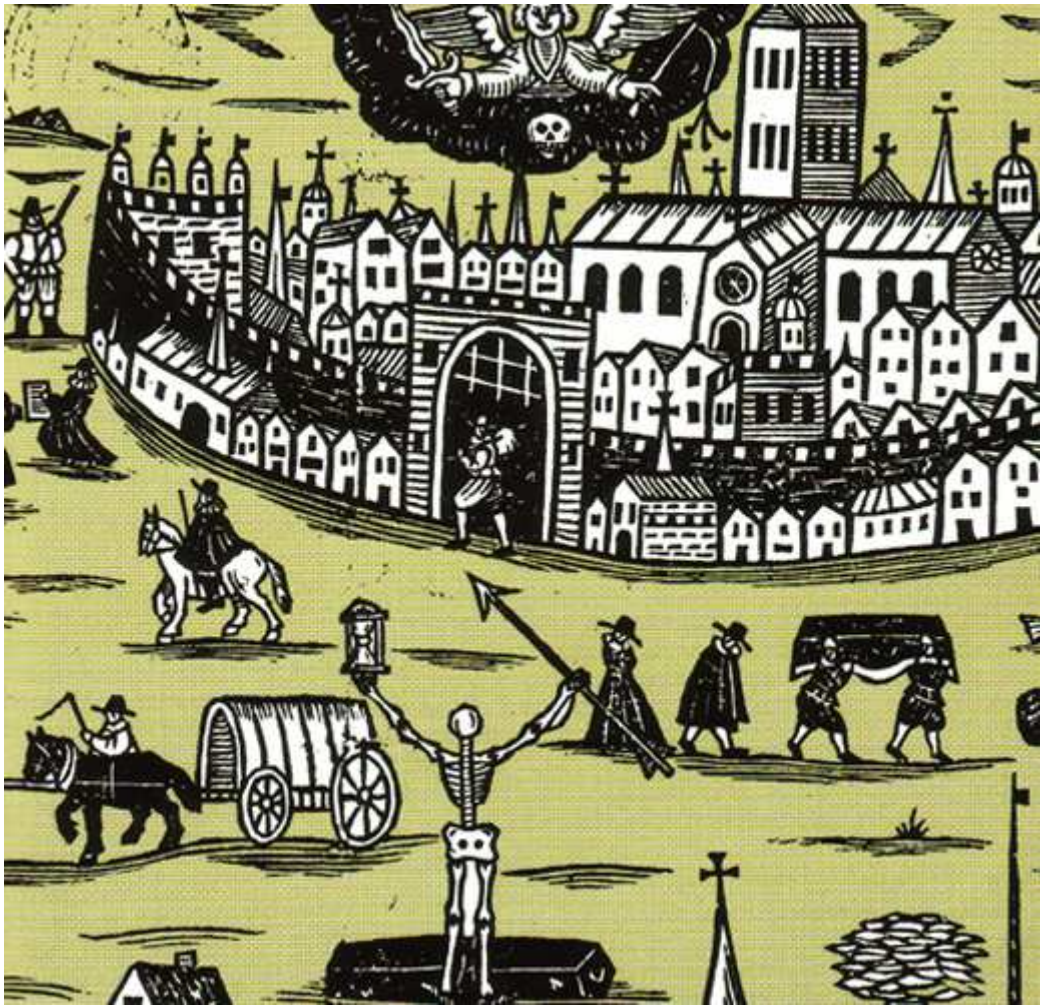
Avoidance of contact with infected animals.
Persons at high risk can be actively vaccinated by live, attenuated strains of *F. tularensis.*

/ Hungarian data:

-2001:86 - 2006:139
-2002:69 - 2007:20
-2003:28 - 2008:25
-2004:36 - 2009:38/
-2005:87

Yersinia pestis

plague, "black death"

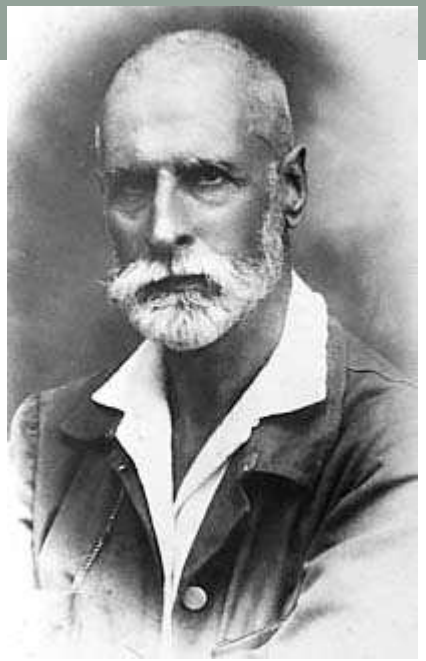


Pestis/plague-history

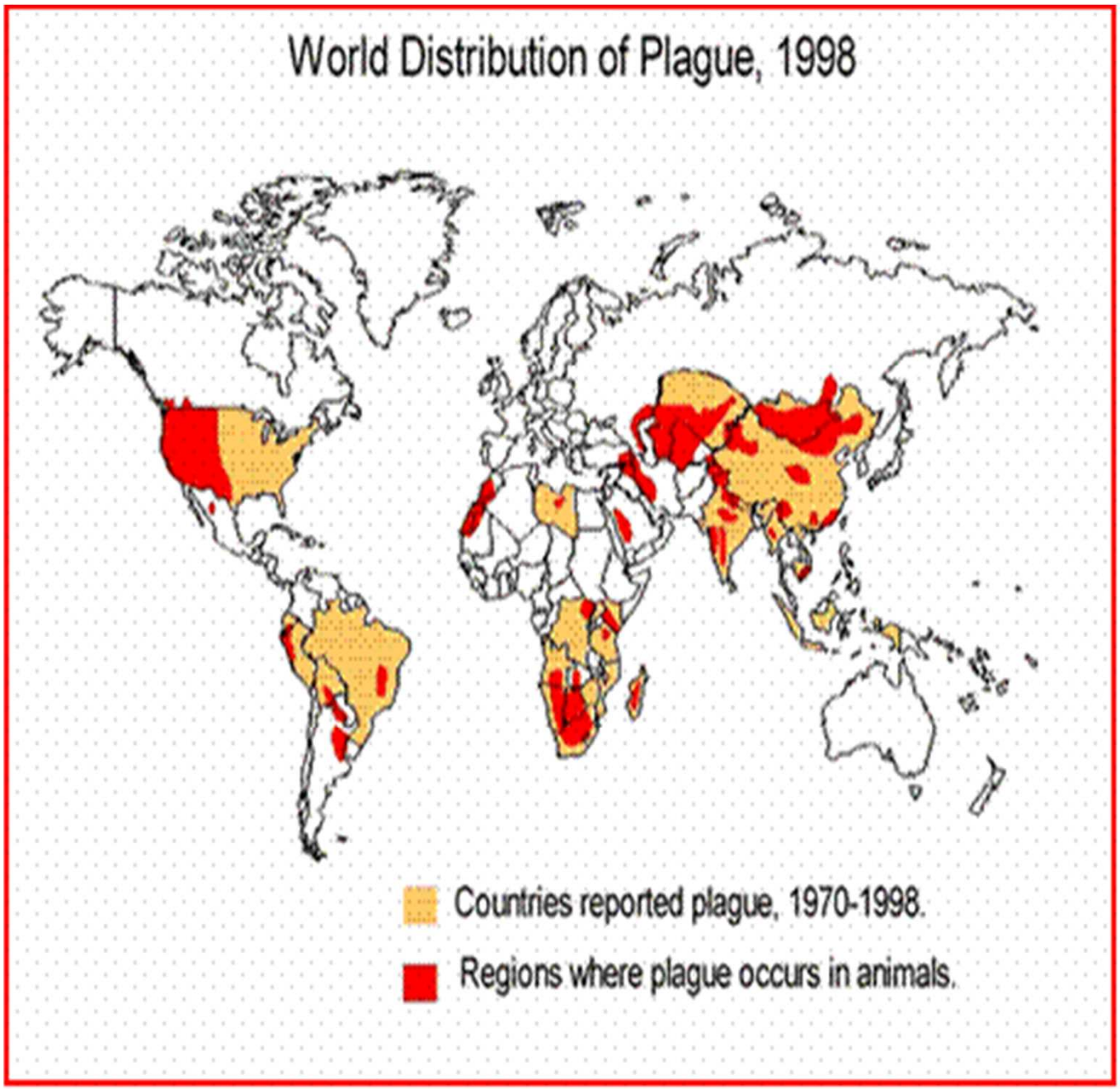
- Old Testament: Egypt in 541 AD

- The **Black Plague** occurred throughout Asia and the Middle East during the 14th Century. This pandemic represents the largest death toll of any known disease to date. With over 34 million fatalities, Europe reduced its population by one third.

- In the late 17th Century (1665-66), London experienced **The Great Plague**. Spread of the plague originated from Dutch trading ships carrying bales of cotton with concentrated flea populations. Estimated losses ranged from 75 to 100 thousand individuals. With a decline in London's population by one fifth, up to 7,000 victims were succumbing each week in the later stages of the outbreak.



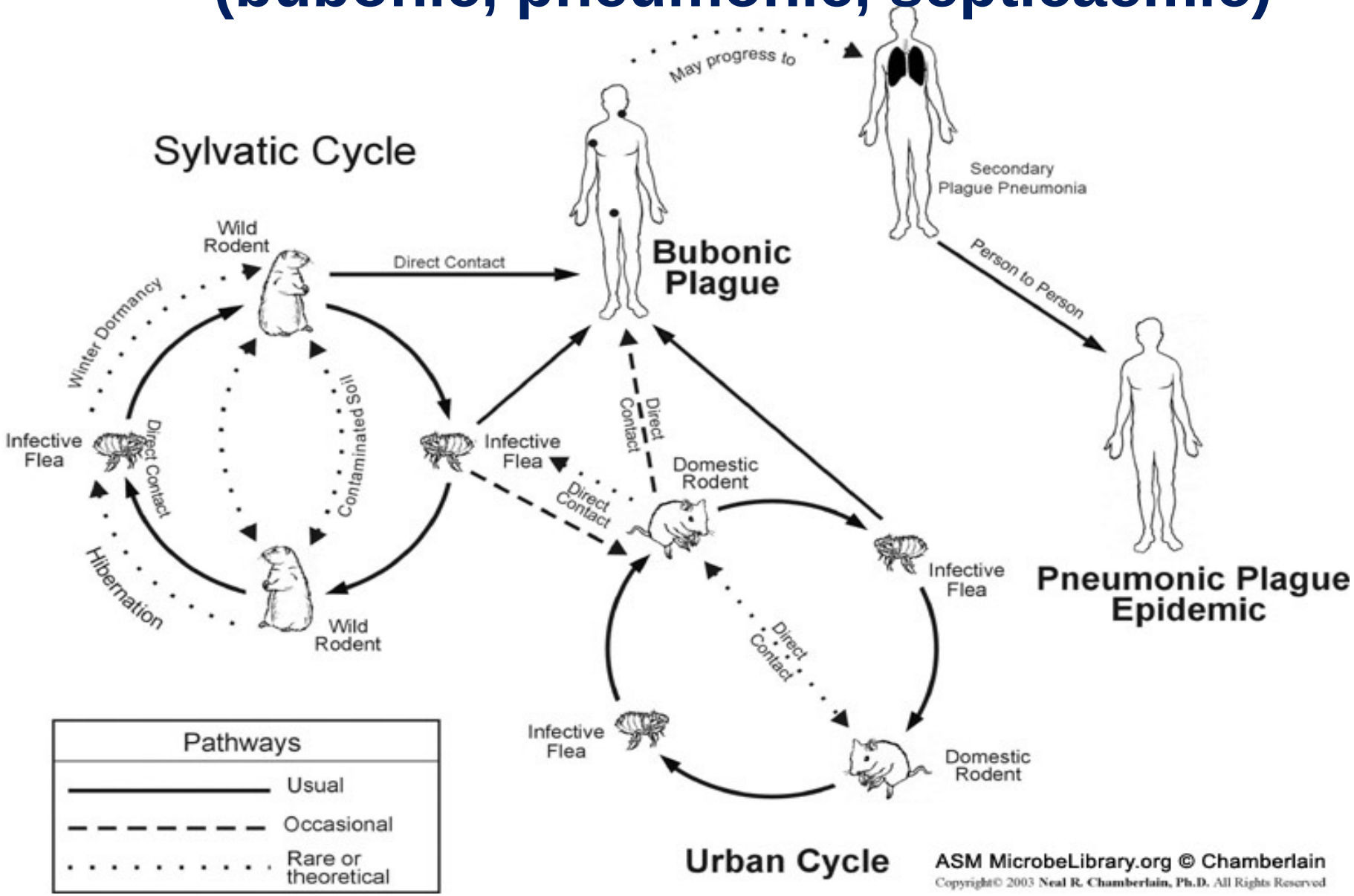
A. Yersin 1884
Asia

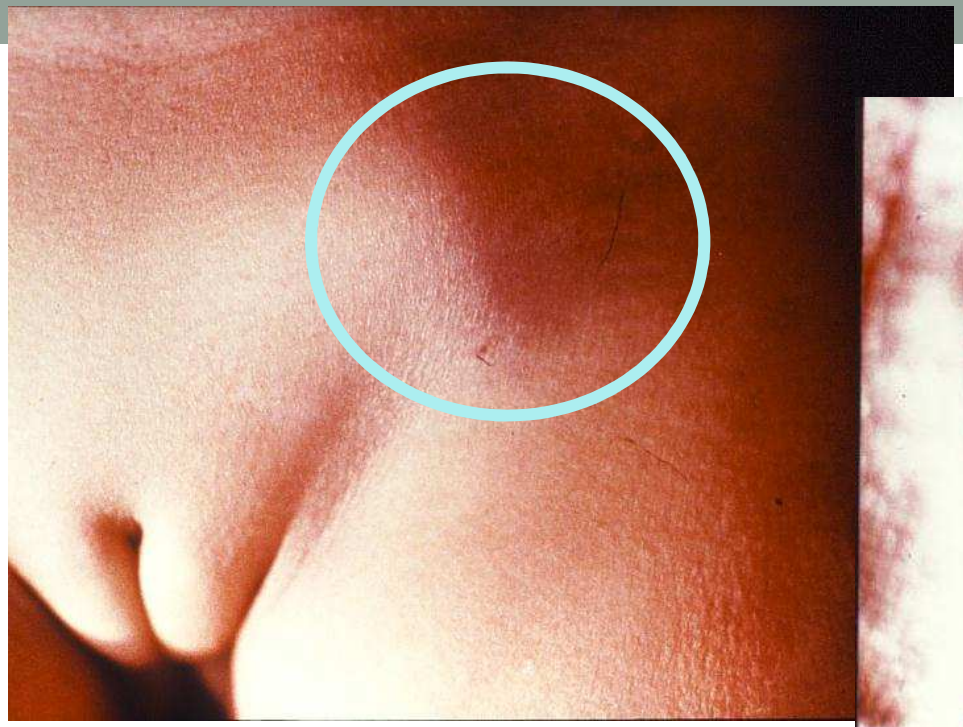


Current Distribution: The WHO reports approximately one to three thousand cases of plague throughout the planet annually.
In 2003: 2,118 (182) cases 98% were from Africa.
1997: In Zambia, 267 cases (26) of plague Since 2001: The Democratic Republic of Congo 1,000 cases of plague each year. Algeria had 11 confirmed cases of bubonic plague in 2003.
The United States averages about 16-18 cases of plague per year. Only about one in seven died from the infection.

Pestis transmission, diseases

(bubonic, pneumonic, septicaemic)





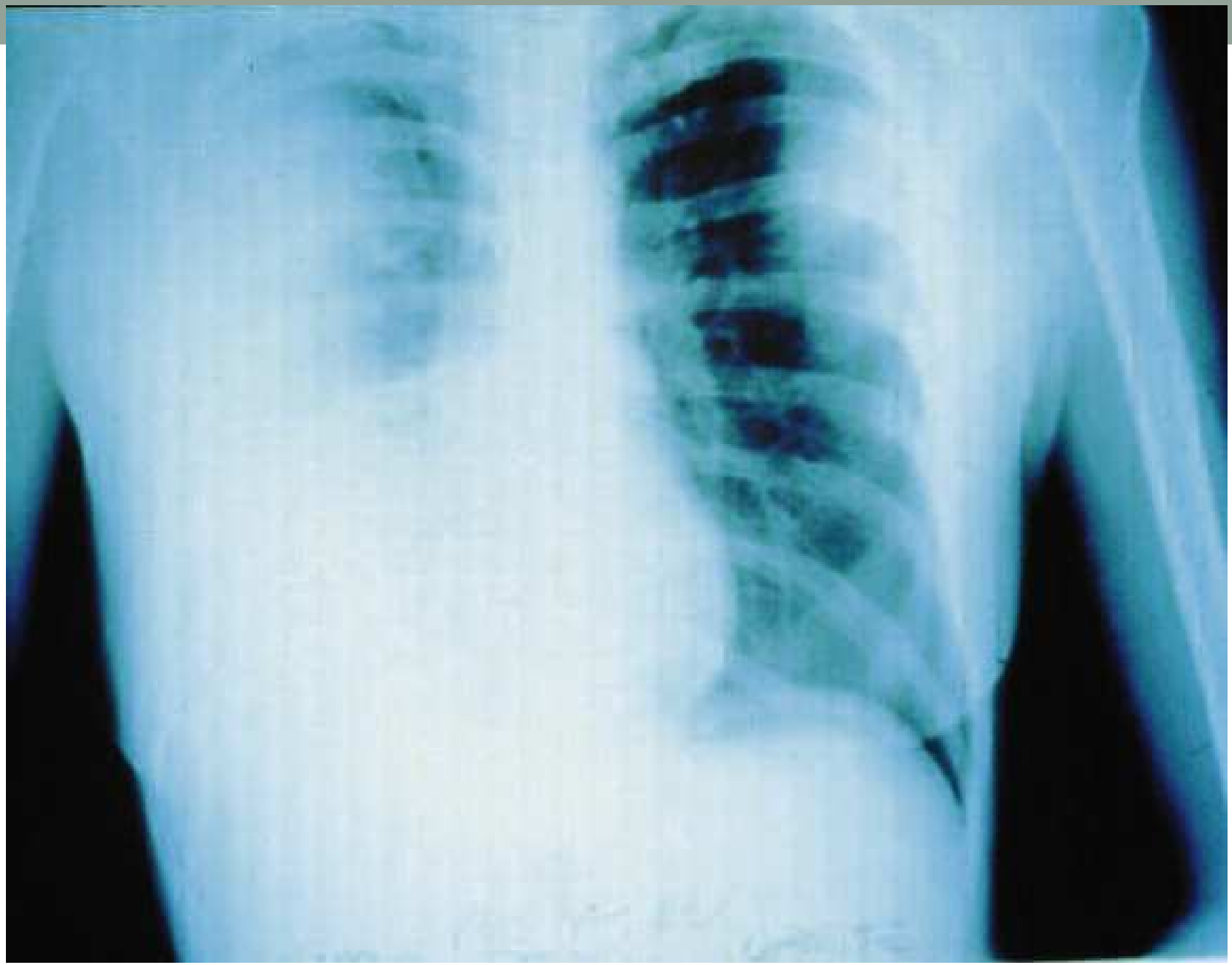
Bubonic

- Enlarged tender inguinal lymphnodes
- Advanced stage of inguinal lymphadenitis in bubonoc plague. The nodes have undergone suppuration and the lesion has drained spontaneously.

Necrosis of finger tips of septicemic plague.

Septic plague

Cutaneous Hemorrhages in Plague.



Lung pestis

Microbiological diagnosis

Sample to be taken: punctate of the enlarged lymph nodes ("buboes"), sputum, blood-culture, CSF

Direct smear:

Giemsa'stain, specific immunofluorescent staining

Wayson`s stain: small rounded 1.5-2.0 μm sized rods with a striking bipolar (hairpin-like) appearance.

Blood serology:

HAG, CF, IDIF

PCR

Treatment

Streptomycine + tetracyclines are powerful combination to treat plague.

Treatment should be started as soon as possible.

Prevention

Active immunization: with formalin killed *Y. pestis* for very high risk groups

Rodents control

Pseudomonads and related nonfermentative Gram-negative rods

Pseudomonas, Burkholderia, Acinetobacter, Stenotrophomonas genus

Nonfermentative Gram negative rods

- pseudomonas and related nonfermentative rods are a complex mixture of opportunistic pathogens of plants, animals and humans

- their taxonomic classification has undergone numerous changes
 in recent years

http://textbook of bacteriology_net_P_aeruginosa_jpeg.htm

Nonfermentative Gram negative rods

I./A Fluorescent group	*Pseudomonas aeruginosa*
	Pseudomonas fluorescens
	Pseudomonas putida
I./B non fluorescent group	*Pseudomonas stutzeri*
	Pseudomonas mendinonica
II.	*Burkholderia pseudomallei*
	Burkholderia mallei
	Burkholderia cepacia
	Burkholderia picketti
III.	*Comamonas species*
	Acidovorax species
IV.	*Brevundimonas species*
V.	*Stenotrophomonas maltophilia*

- **despite the many genera, only a few are isolated commonly**

Opportunistic pathogenic microbes

- are typically members of the normal flora
- do not produce disease in their normal settings

- exploits some break in the host defenses to initiate an infection
    - immuncompromised status
    - age
- introduced into unprotected sites
    - mechanical ventilation
    - catheter

Pseudomonas aeruginosa

Widespread occurence in a variety of habitants

Name:

- Pseudes = false
- Monas =a unit
- Aeruginosa = full of copper rust or green

- first reported case of *P.aeruginosa* infection in 1980

http://textbook of bacteriology_net_P_aeruginosa_jpeg.htm

Pseudomonas aeruginosa

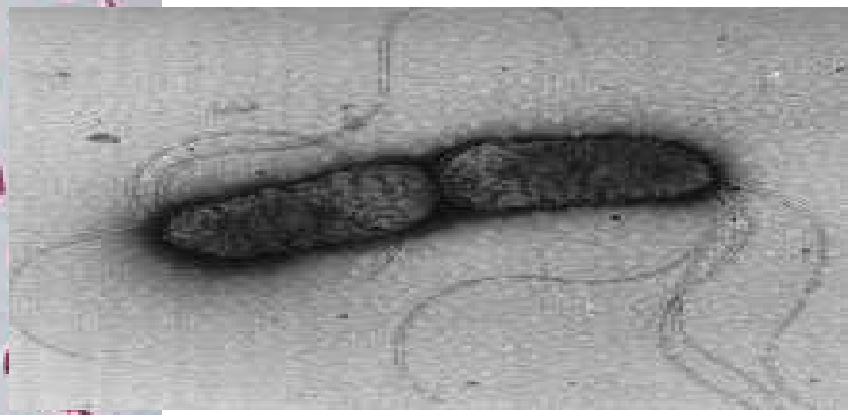
http://textbook of bacteriology_net_P_ aeruginosa_jpeg.htm

Morphology:

Gram negative rods

0,5 to 0,8 µm

by 1,5 to 3,0 µm



motile (single polar flagellum)

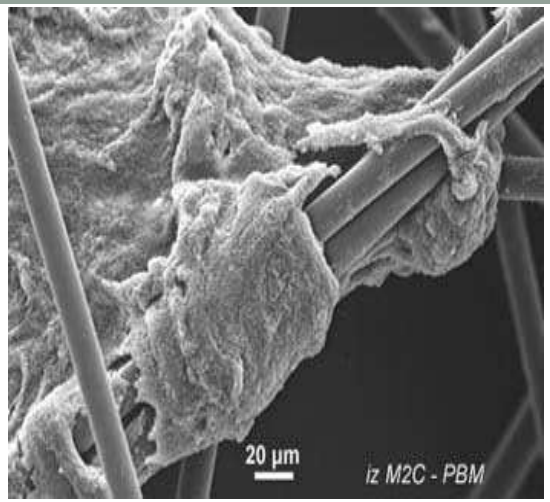
Pseudomonas aeruginosa

Epidemiology:

- **Common in the environment; moist reservoirs**
- **In hospital: disinfectant, respiratory equipment, food, sinks…**
- **Nutritionally very versatile (distillated water), adaptibility to minimal nutritional requirements (metabolic diversity)**

- **Resistant to high concentrations of salts, dyes, weak antiseptics and many commonly used antibiotics**
- **Member of the normal flora (GI)**
- **Colonisation rate is increasing after hospitalization**

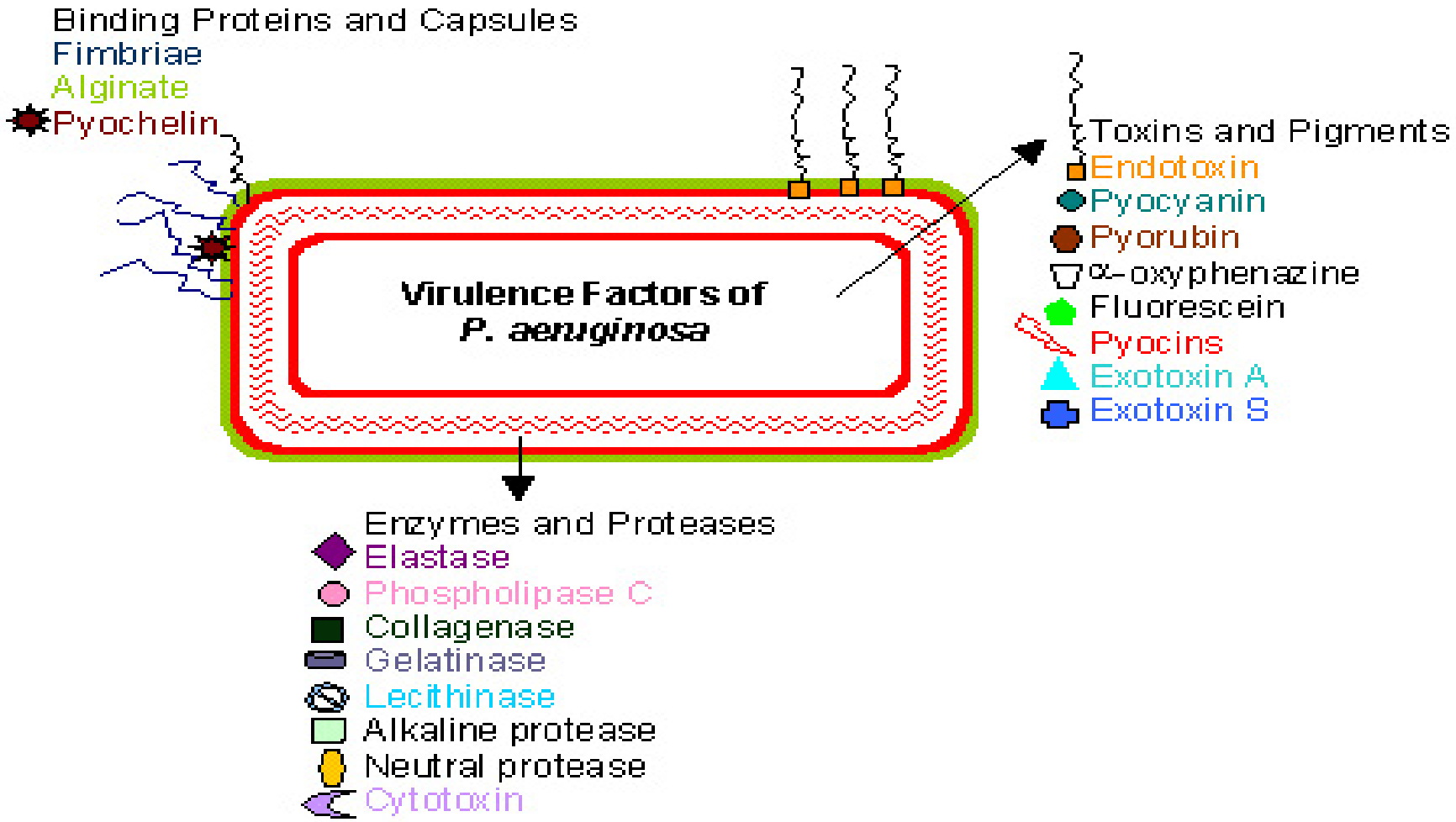
Pseudomonas aeruginosa



- **Pathogenesis**:
  - An **opportunistic pathogen**
  - **Nosocomial pathogen** (4. most commonly-isolated pathogen)
  - the ultimate ~ infection may be seen as composed of three distinct stages:
    - Bacterial attachment and colonization
    - Local invasion
    - Disseminated systemic disease

Pseudomonas aeruginosa

Virulence factors of pathogenic *P. aeruginosa*



Virulence factors of pathogenic *P. aeruginosa*

- Adhesins
  - Fimbriae
  - Polysaccharide capsule (glycocalix)
  - Alginate slime (biofilm)
- Invasins
  - Elastase
  - Alkaline protease
  - Hemolysins (phopholipase and lecitinase)
  - Cytotoxin (leukocidin)
  - Siderophores and siderophore uptake system
  - Pyocyanin diffusible pigment
- Genetic attributes
  - Genetic exchange by transduction and conjugation
  - Inherent (natural) drug resistance
  - R factors and drug resistance plasmids

Motility/chemotaxis
- Flagella

Toxins
- Exoenzyme S
- Exotoxin A
- Lipopolysaccharide

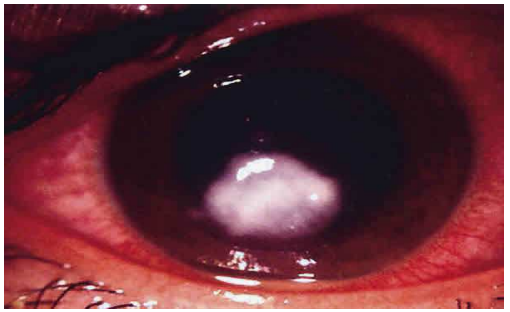
Antiphagocytic surface properties
- Capsules, slime layers
- LPS

Defense against serum bactericidal reaction
- Slime layers, capsules
- LPS
- Protease enzymes

Defense against immune responses
- Slime layers, capsules
- Protease enzymes

Clinical manifestation

- **Endocarditis – i.v. drug abusers; cardiac surgery**
- **Respiratory infections**
  - **Chronic infection/colonisation in cystic fibrosis patients**
  - **Bacteremic pneumonia in neutropenic cancer patients undergoing chemotherapy**



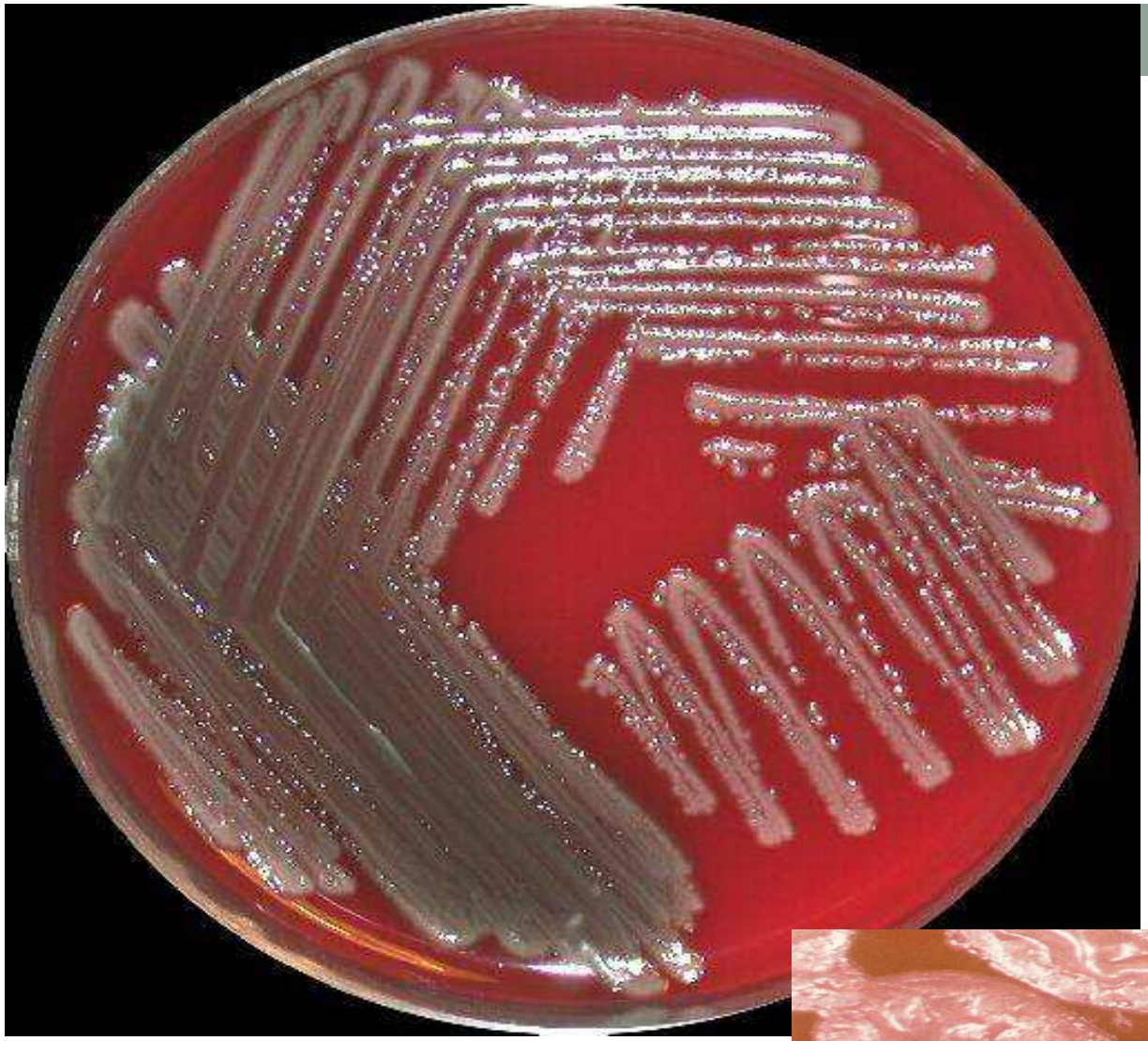
- **Bacteremia and septicemia**

(characteristic skin lesions = ecthyma gangrenosum

- **Eye infections (bacterial keratitis, rapidly destructive infection, neonatal ophthalmia)**

- **Skin and soft tissue infections, pyoderma**
  - **Burn wounds**
  - **Surgical wounds**
  - **Fingernail infection**
  - **Folliculitis, acne**

- **Bone and joint infections**
- **Urinary tract infection**
- **Gastrointestinal infections**
- **Central nervous system infection**
- **Ear infection including external otitis („swimmer's ear")**



microbiological diagnosis

Cultivation

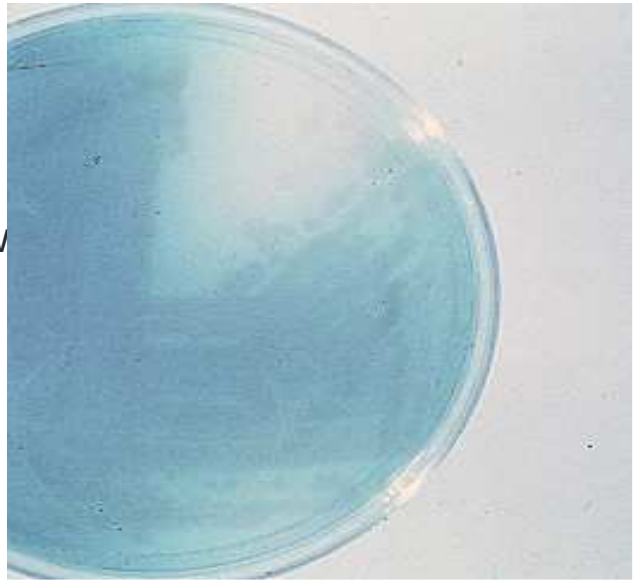
- Blood agar:

beta-hemolysis around smooth, round/ mucoid colonies

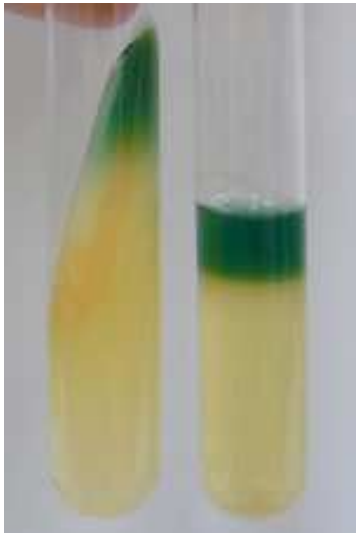
- **EMB: lactose-negative**

Pseudomonas aeruginosa

- **Agar: Characteristic pigments**
  - **fluorescent greenish color from pyoverdin**
  - **nonfluorescent bluish pigment pyocyanin, which diffuses into the agar**
  - **Dark red pigment – pyorubin**
  - **Black pigment – pyomelanin**
- **Sweet or grape-like or corn taco-like odor**



Pseudomonas aeruginosa



Diagnosis – identification based on:

- **Colony morphology**
- **Oxidase positivity**
- **Growth at 42 ºC**
- **Differentiation from other pseudomonads: biochemical activity, molecular technique, MALDI-TOF**

Epidemiological investigation

- **Phage typing**
- **Pyocin-typing**
- **PFGE**

Pseudomonas aeruginosa

Treatment

- Carbapenems, ceftazidime, aminoglycosides
- Inherently resistant to many antibiotics and can mutate to even more resistant strains during therapy
  - decreased permeability
  - beta-lactamases
  - Efflux pumps
- Panresistant strains!

Pseudomonas aeruginosa

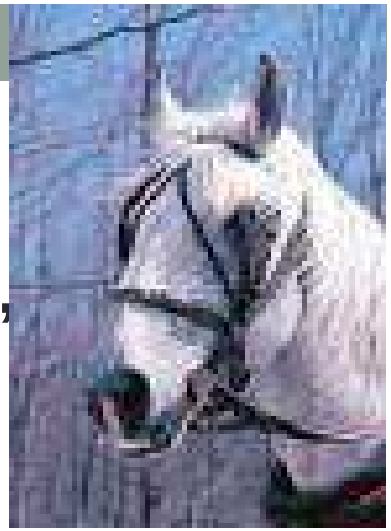
Host defenses

- **Phagocytosis by polymorphonuclear leukocytes**
- **Antibodies to somatic antigens and exotoxins**

Prevention and control

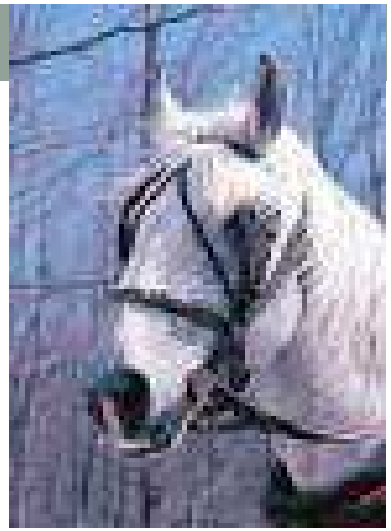
- **By cleaning and disinfecting medical equipment**
- **Topical therapy of the burn with antimicrobial agents**
- **Effective infection-control practices**

Burkholderia mallei



- **Glanders is usually a horse disease (Asia, Africa, East)**
- **Causes glanders (from horses)**
  - **Begins as an ulcer of the skin or mucuos membranes**
  - **Followed by lymphangitis and sepsis**
  - **septicemia is usually fatal**
  - **inhalation of the organisms may lead to primary pneumonia**

Burkholderia mallei



Diagnosis: Very infectiosus!

- Special laboratory equipment

- **Only non-motile Pseudomonad**

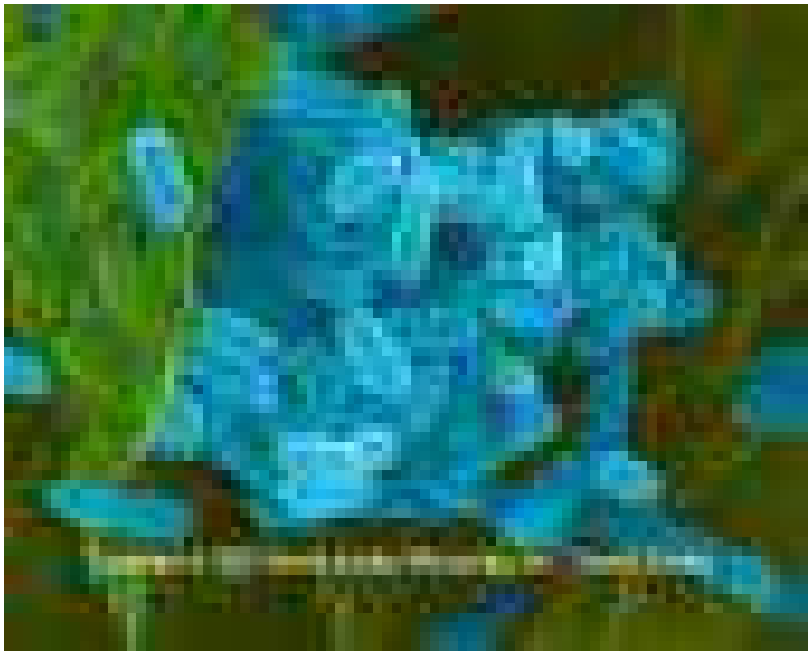
- **Strauss reaction: orchitis, pyogen infection intraperitoneally, death, after injection in a male ginea pig**

Burkholderia pseudomallei

- **In the tropical soil of Southeast Asia, India, Africa and Australia**
- **Infection can be dormant for years, and symptoms can appear under stress**
- **Causes melioidosis (endemic glanders-like disease of animals and humans)**
  - **Chronic lung disease (necrotizing pneumonia -DD: TBC)**
  - **Fulminant upper lobe pulmonary disease**
  - **Acute bacteremia (from localized, suppurative cutaneous infection)**

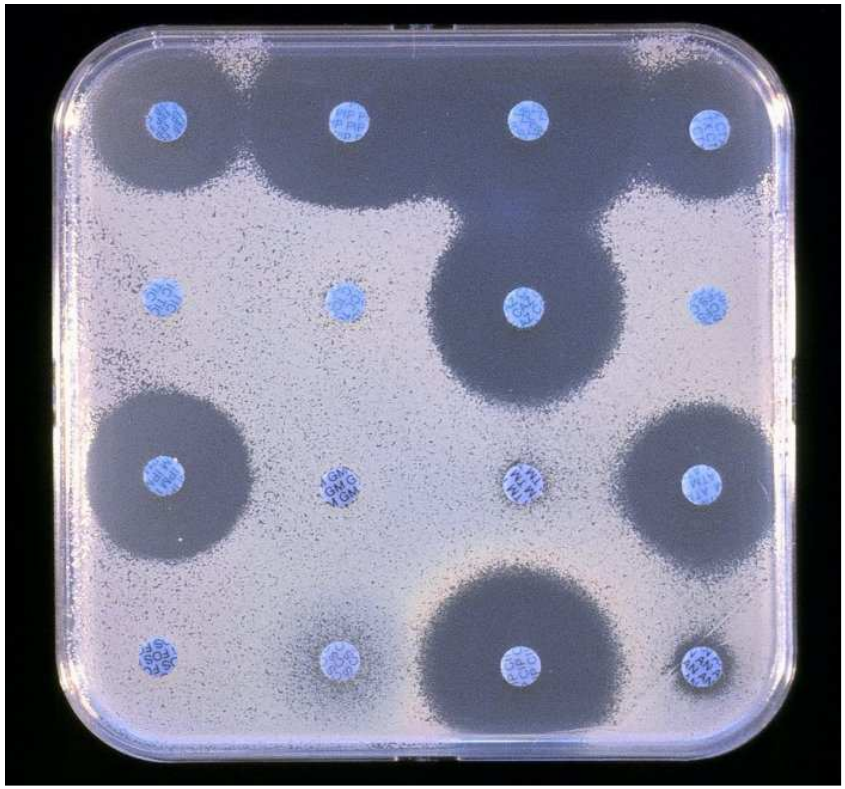
Burkholderia pseudomallei

- **Multitrichous polar flagella**
- **Highly infectious! / biological weapon**
- **Microscopic appearence: hairpine-like, coccobacilli**



Burkholderia cepacia complex

- **Primarily a plant pathogen (onion bulb rot)**
- **Important opportunistic pathogen in CF patients, but otherwise rarely a cause of diseases**
- **Very resistant to antibiotics**
- **It has multitrichous polar flagella**



Stenotrophomonas maltophilia

- **It is the third most common Gram negative pathogen isolated from the sputum of CF patients**
- **Transmission: contaminated equipment**
- **Common nosocomial opportunistic pathogen**
  - **Indwelling plastic intravenous catheters; intravascular devices**
  - **Contaminated disinfectant solutions, ice machines**

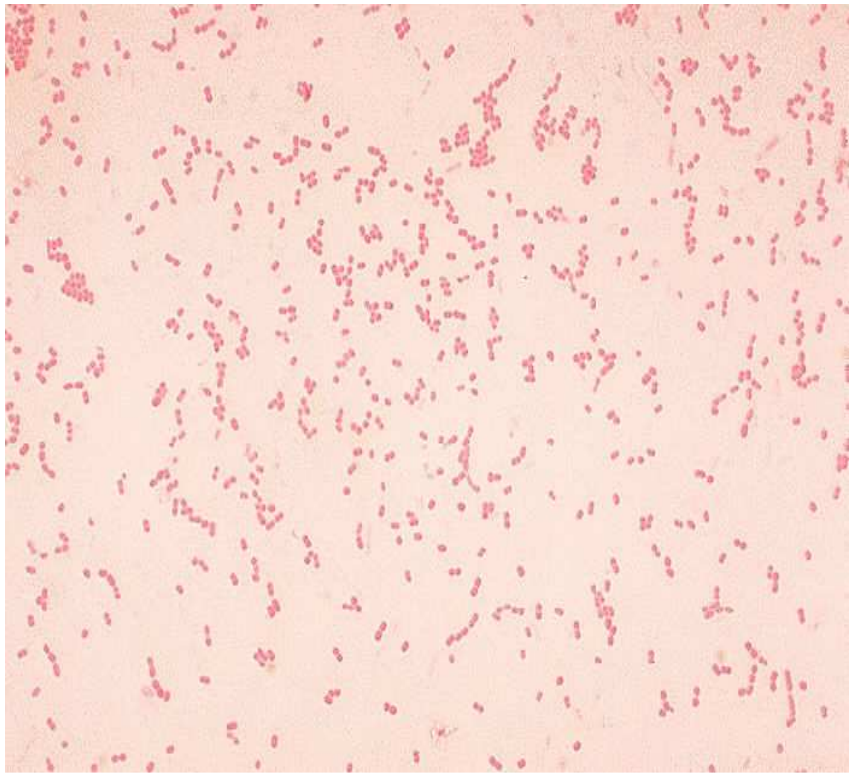
Stenotrophomonas maltophilia

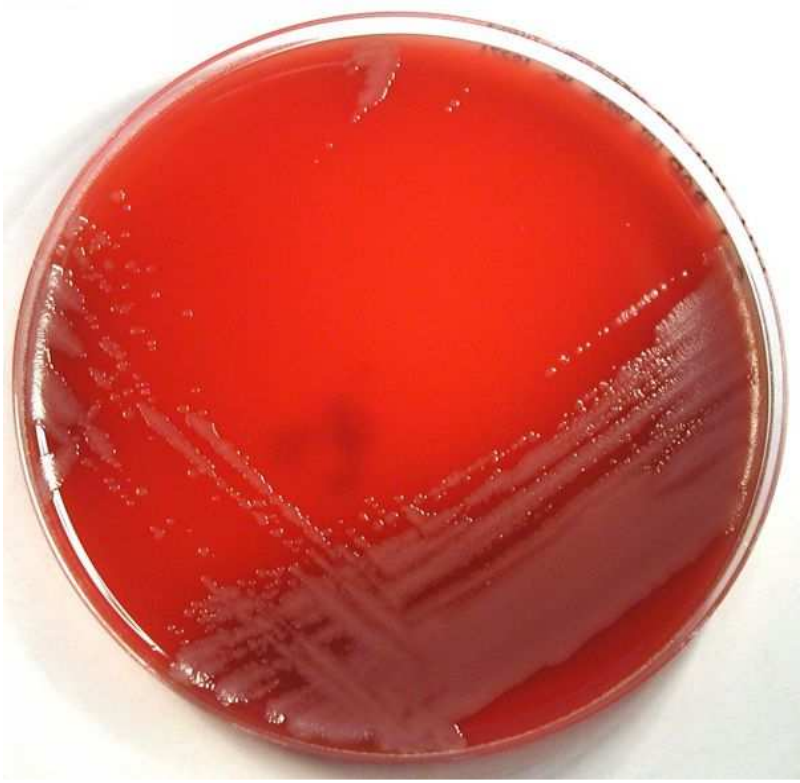
- **oxidase negative**
- **Very resistant to antibiotics including carbapenems**

  - drug of choice:
  trimethoprim/sulfamethoxazole

Acinetobacter baumannii

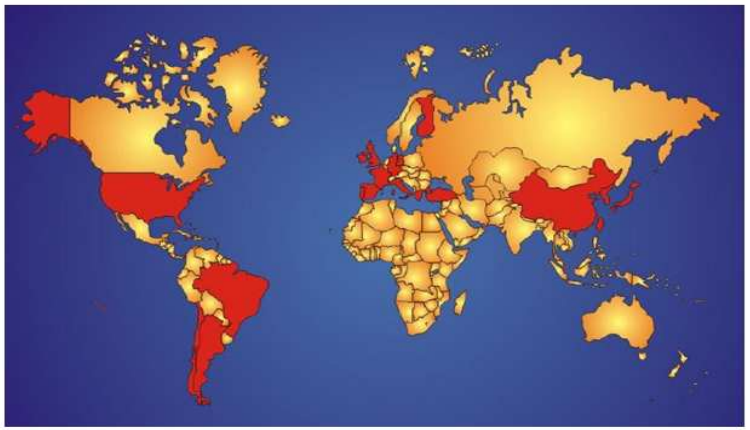
- **Strictly aerobic**
- **Gram-negative plump coccobacilli**
- **Ubiquitous saprophytes (moist or dry surface)**





Acinetobacter baumannii

- **Opportunistic pathogen**
  - **Respiratory tract infection**
  - **UTI**
  - **Wounds**
  - **Sepsis**

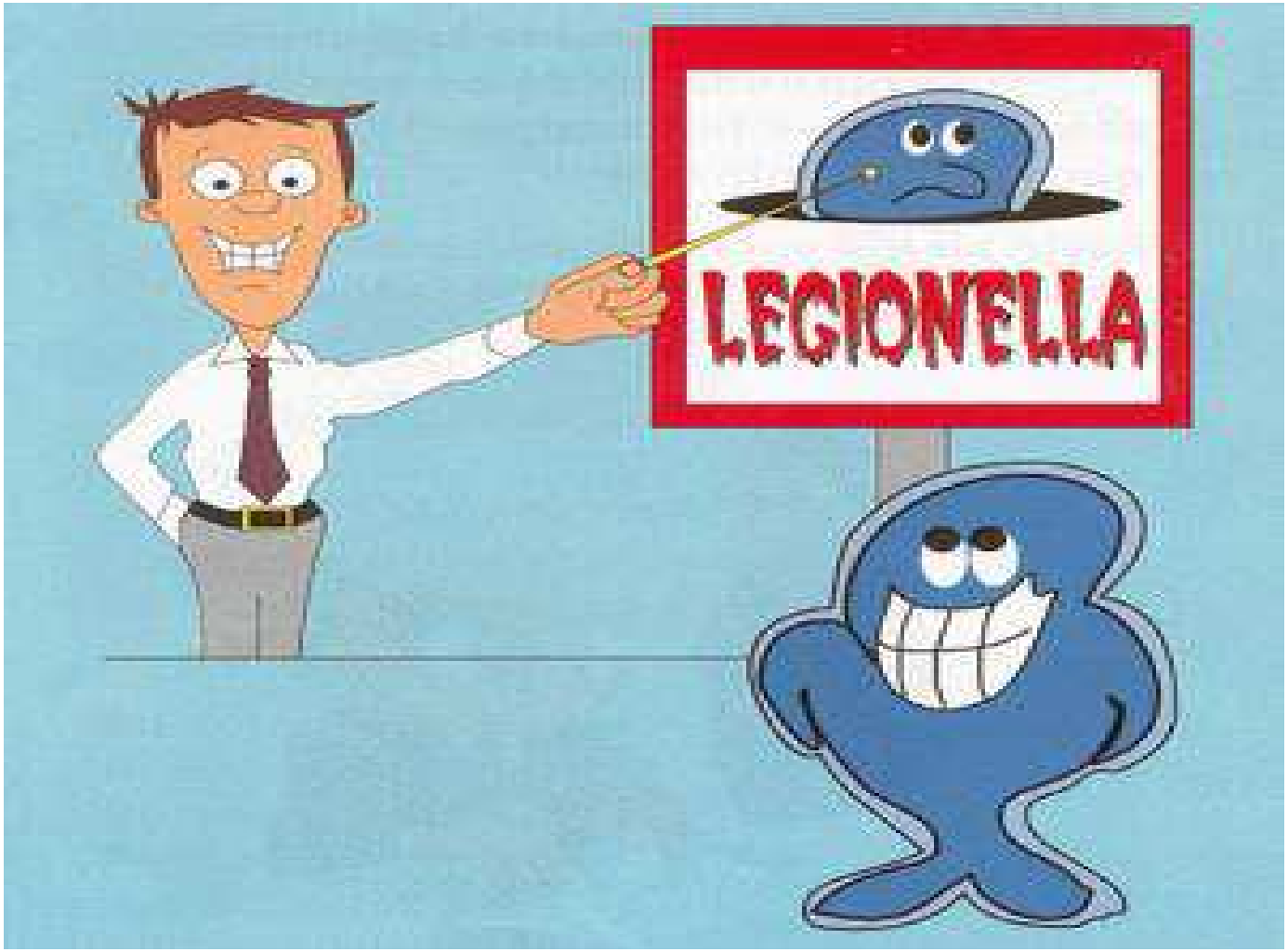


- **Often resistant to antibiotics**

MACI:Multiresistant *Acinetobacter baumannii*

- **Therapy: upon in vitro susceptibility tests**

Legionella pneumophila



Www.ism.gov.tr/haberler/depo/resim/legionella.jpg

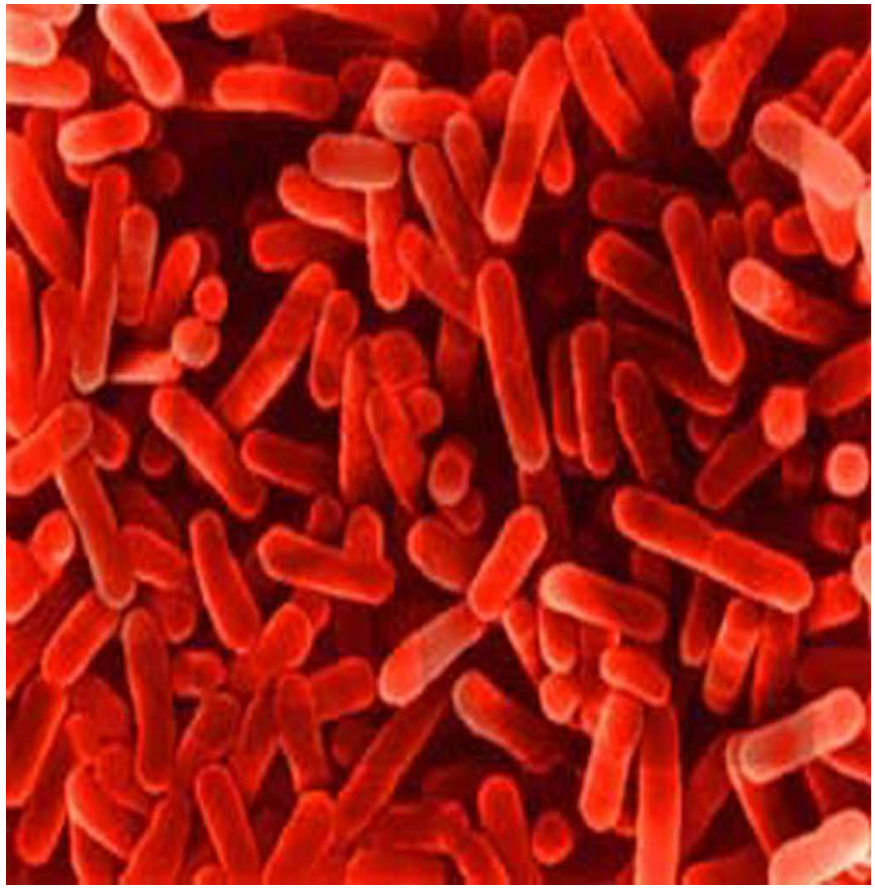
Legionella pneumophila

- **In 1976 a widely published outbreak of pneumonia in persons attending an American Legion convention in Philadelphia**
- **In 1968 caused a self-limited, febrile illness in people working at the Pontiac, Michigan**
- **First case 1947**
- **48 Legionella species and more than 70 serogroups**
- ***Legionella pneumophila* is the major cause of disease (85%); serotypes 1 and 6 are most commonly isolated**

Legionella pneumophila

Morphology:

- **Fastidious, aerobic Gram-negative bacteria (smear from cultivation media, using fuchsin)**
- **0,5-1 μm wide and**
- **2-50 μm long, pleomorphic**



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Legionella pneumophila

Cultivation: special

- **Nutritionally fastidious with requirement for L-cysteine and enhanced growth with iron salts**
- **BCYE (buffered charcoal-yeast extract agar) with α-ketoglutarate; at pH 6,9; 35ºC; 90% humidity)**
  - **3 days incubation period (> 2weeks in blood culture)**
  - **Colonies:round or flat with entire edges; colorless to iridescent, pink or blue; translucent or speckled**

Biochemically:

- **Catalase positive; oxidase positive**
- **Hydrolyzes hippurate**

Legionella pneumophila

cultivation

Www.ism.gov.tr/haberler/depo/resim/legionella jpg

Legionella pneumophila

Epidemiology

- **Ubiquitous in moist environments (water, cooling towers, condensers, water systems, air-conditioning system, shower heads)**
- **Infection of debilitated or immunocompromised hosts (decreased cellular immunity) or compromised pulmonary function commonly follows inhalation of the bacteria from aerosols**
- **Nosocomial, epidemic or sporadic infection**
  - **10,000-20,000 cases of infection in USA annually**
- **No human to human transmission!**

Legionella pneumophila

Histology:

- **Acute purulent pneumonia involving the alveoli; dense intra-alveolar exudate of macrophages, polymorphonuclear leukocytes, red blood cells, and proteinaceous material**
- **Little or no inflammation**

in bronchioles and upper airways

- **Legionellae within phagocytic cells**

Legionella pneumophila

Intracellular Legionellae in phagocytes (and amoebae in nature) are not effectively killed by PMN-s

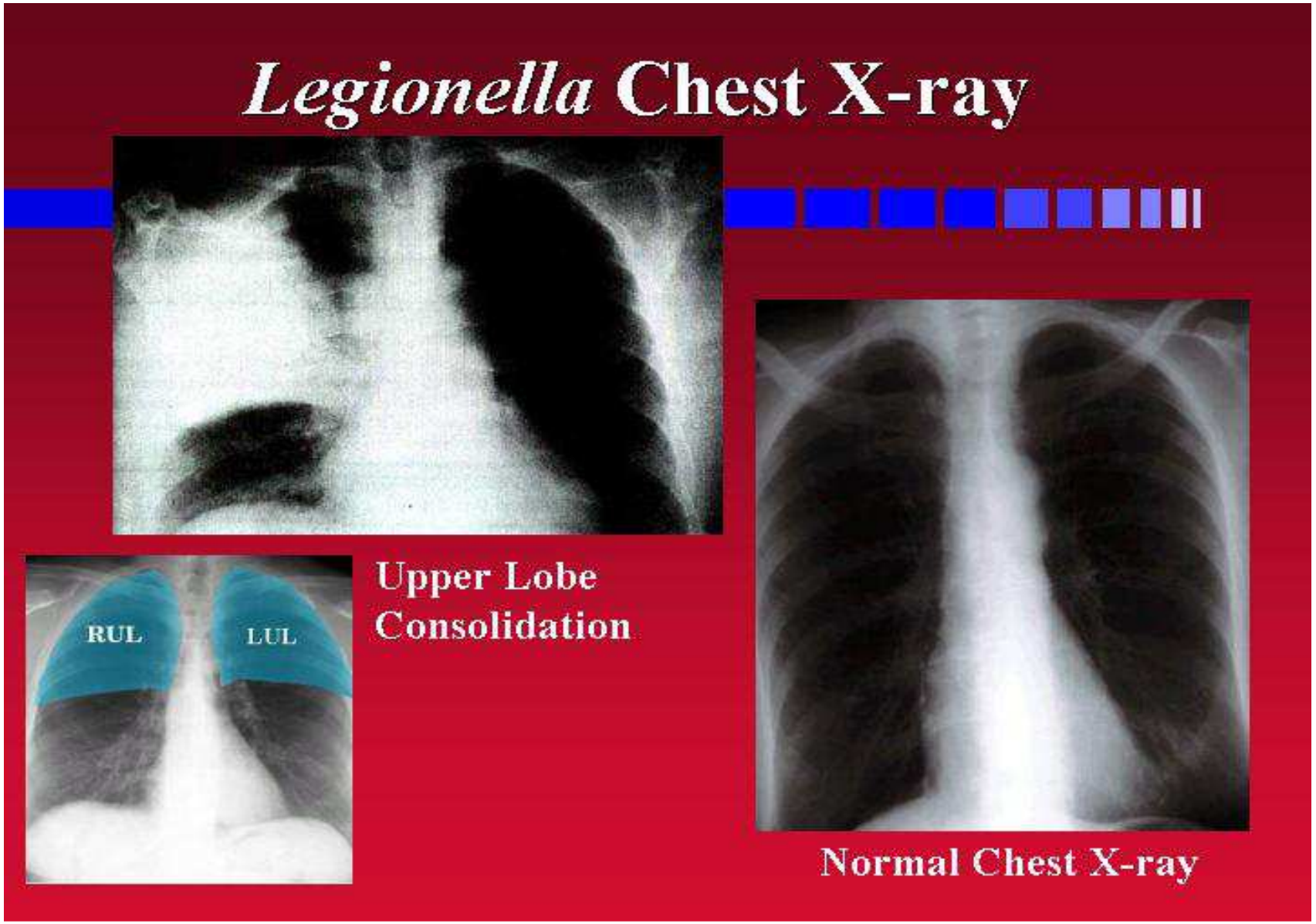
- **Phagosome-lysosome fusion fail**
- **The phagocyte oxidative metabolic burst is reduced; phagosomes do not acidify**
- **The bacteria multiply in the vacuoles until they are numerous ⇒ the cells are destroyed ⇒ the bacteria released ⇒ and infection of other macrophages then occurs**

Legionella pneumophila

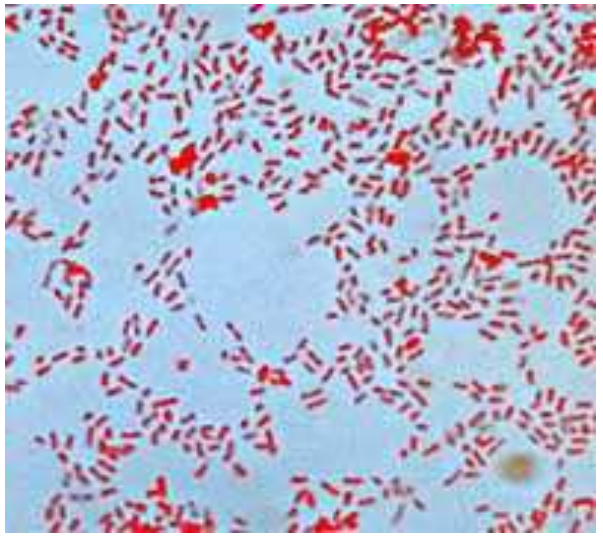
Clinical findings

- **Asymptomatic infection (elevated titer of specific) antibodies)**
- **Pneumonia – Legionnaires'disease**
  - **Predisposing factors! (immunosuppressive therapy, smoking…**
  - **Epidemic disease in late summer or autumn**
  - **Nondescript febrile illness of short duration**
  - **Severe, rapidly progressive illness (high fever, chills, malaise, nonproductive cough, hypoxia, diarrhea, delirium**
  - **Chest X-ray:patchy, often multilobar consolidation**
  - **Leukocytosis, hyponatraemia, hematuria, abnormal liver function**
  - **Mortality rate: 15-20 %**
- **„Pontiac fever"- No pneumonia**
  - **Fever, chills, myalgia,headache, mild cough and sore throat**

Legionella pneumophila

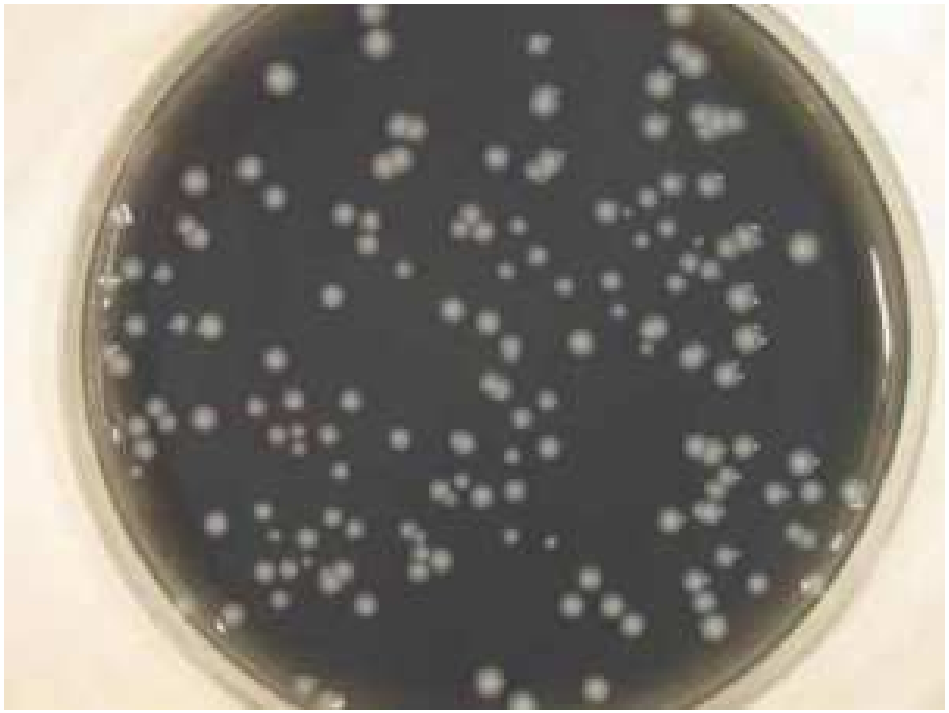


Legionella pneumophila



Laboratory diagnosis:

- **Specimens : bronchial washings, pleural fluid, lung biopsy specimens or blood**
- **Smear:**
  - **Gram ?**
  - **Direct fluorescent antibody tests (low sensitivity)**
- **Cultivation : BCYE + identification with IF**



Legionella pneumophila

- **Specific test: Urine Antigen Test**
  - **Genus specific lipopolysaccharide antigens can be demonstrated in the patient's urine by immunologic reaction – just serogroup 1**
- **Serologic tests: IFA**
  - **Retrospective diagnosis (60-80%sensitivity and 95-99% specificity)**
  - **Persist**
- **Nucleic acid amplification (PCR)**

Legionella pneumophila

Immunity

- **Cell-mediated response is important (IC)**
- **Antibody response may not occur until 4-8 weeks after infection**

Treatment

- **Newer macrolides (azithromycin, clarithromycin)**
- **„Respiratory” Fluoroquinolones**

Legionella pneumophila

Prevention and control

- Decrease environmental exposure
- Hyperchloration, superheating, copper-silver ionization

Normal flora of the upper respiratory tract

Nares

- **With normal breathing many kinds of microbes are inhaled into the nares**
  - **Normal soil inhabitants**
  - **Pathogenic or potentially pathogenic bacteria, viruses, fungi**
- **Some of them filtered out by the hairs in the nose**
- **Others may land on moist surfaces of the nasal passages; subsequently sneezing or blowing $\Rightarrow$ transient colonizers**
- **The external 1 cm of the external nares is lined with squamous epithelium has a flora similar to skin flora**
  - **Colonisation (= carrier state):**
    - **Staphylococcus aureus 25-30 % (~1% MRSA)**

Normal flora of the upper respiratory tract

Nasopharynx

- **Colonization soon after birth following aerosol exposure of microorganisms from the respiratory tract from those individuals who are in close contact with the infant**
- **Establishes itself within several months and generally unchanged throughout life**
- **Site of carriage of potentially pathogenic bacteria**
  - ***N.meningitidis, S.pneumoniae, H.influenzae***

Normal flora of the upper respiratory tract

Bacteria:

- *Acinetobacter*
- *Actinobacillus*
- *Actinomyces*
- *Cardiobacterium*
- ***Corynebacterium***
- *Eikenella*
- *Enterobacteriaceae*
- *Eubacterium*
- *Fusobacterium*
- *Haemophilus*
- *Kingella*
- *Moraxella*
- *Mycoplasma*
- ***Neisseria***
- *- Peptostreptococcus*
- *- Porphyromonas*
- *- Prevotella*
- *- Propionibacterium*
- *- **Staphylococcus***
- *- **Streptococcus***
- *- Stomatococcus*
- *- Treponema*
- *- Veilonella*

Fungi

- *- candida*

Normal flora of the lower respiratory tract

Normally sterile:

- **middle ear**
- **sinuses paranasalis**
- **lung**
- **pleura**

Köszönöm a figyelmet!