Pseudomonas, Legionella and other nonfermentative bacilli

Gram negative nonfermenting bacilli

- Heterogeneous group of numerous genera of gram negative bacilli with the **lack of glucose fermentation**
- **General characteristics:**
- Low pathogenicity, infections caused mostly in immunodefficient persons
- Common occurance in outer environment
- Low nutritional requirements, prolonged cultivation time
- The most important genera:
  - **genus *Pseudomonas***
  - **genus *Burkholderia***
  - **genus *Acinetobacter***
  - **genus *Stenotrophomonas***

Pseudomonas and other nonfermentative bacilli

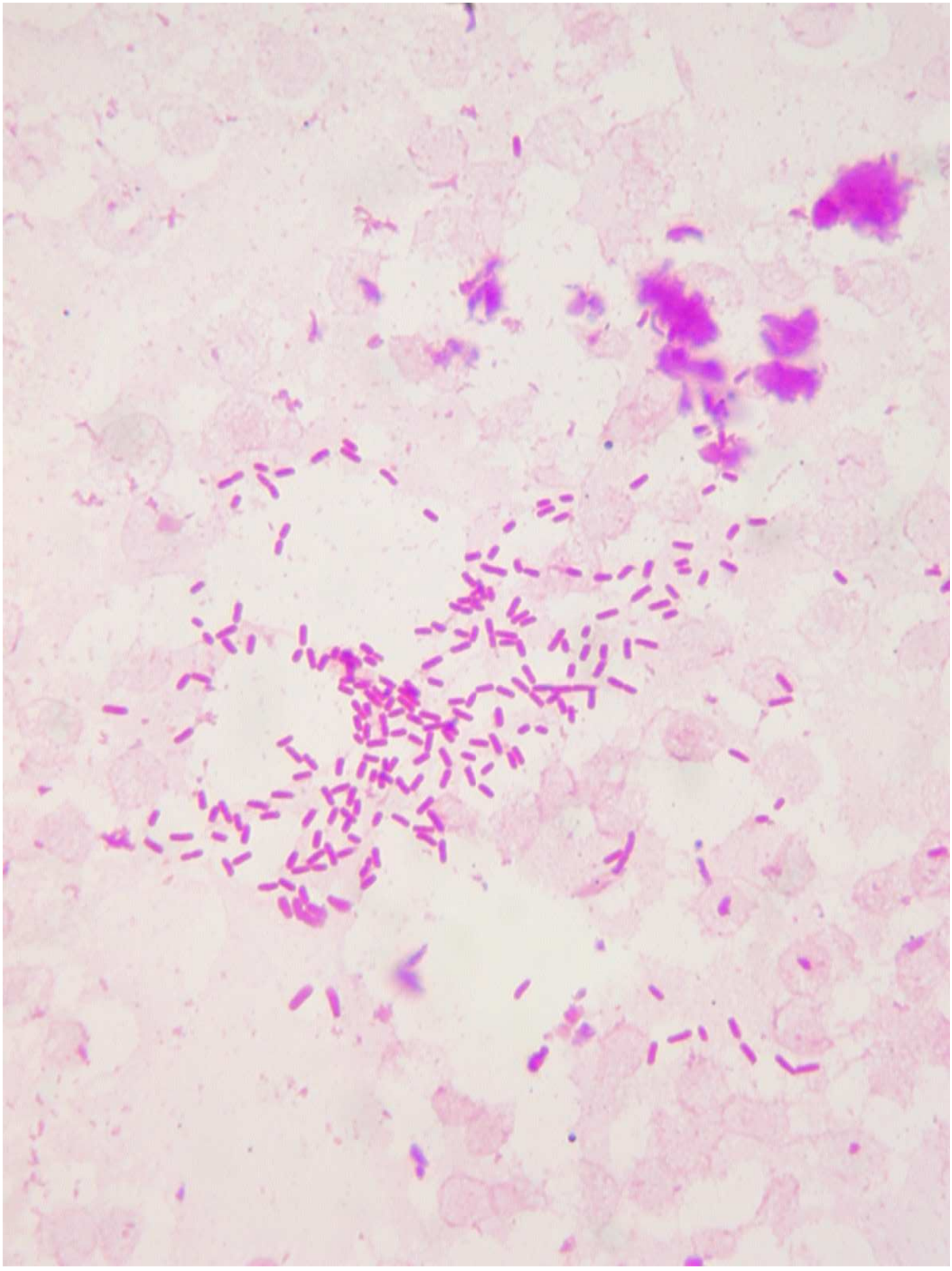
- **Diagnosis of clinically significant nonfermenting rods, importance in opportunistic / hospital infections**

- *Pseudomonas aeruginosa*
- *Pseudomonas spp.*
- *Stenotrophomonas maltophilia*
- *Acinetobacter spp.*
- *Burkholderia cepacia* complex

Pseudomonas species

- Aerobic, non-spore forming, gram-negative rods
- Usually motile (one or several polar flagella)
- They are strict aerobes (non-fermenting metabolism – aerobic respiration) but strains can grow anaerobically using nitrate as electron acceptor
- Oxidase positive

- Ubiquitous microorganism – wide variety of environmental niches (soil, vegetation, waste water, moist reservoirs)



Pseudomonas aeruginosa

http://www.microregistrar.com

- Sometimes covered with mucous layer
- Respiratory and urinary tract mucous epithelium colonization
- Occurance in hospital environment, important source of nosocomial infections – catheter, breathing apparatus contamination
- Minimal nutritional requirements, grows well on the basic culture media at 30-37 °C, in room temperature (wide range of temperature 4 -42°C)

P. aeruginosa - epidemiology

- Hydrophilic – recovered from moist environment (sink drains, vegetables, river water, antiseptic solutions)
- Colonisation of healthy individuals is rare (GIT)
- *P. aeruginosa* could be recovered from moist body sites (outer ear in swimmers, endotracheal tube in ventilated patients, upper-respiratory tract of patients at ICU (Ventilator-associated pneumonia)
- Neutropenic patienst (repeatedly treated with ATB) are at risk of GIT colonisation – source for sepsis/Ventilator-associated pneumonia
- Burned skin – support of colonisation – *P. aeruginosa* is leading cause of sepsis in patients with burn trauma
- Cystic fibrosis – *P. aeruginosa* is leading cause of respiratory tract infections – „CF phenotype“ (LPS rough, mucoid, nonmotile)

Pseudomons aeruginosa strain covered with mucous layer

P. aeruginosa – clinical significance

INFECTIONS MAINLY IN

- neutropenic patients (hemoblastoses, cancer chemotherapy, patients after transplantations)
- patients with burns - breached dermal barrier
- patients on mechanical ventilation – compromised mucociliary clearence
- cystic fibrosis patients – persistent infections (CF phenotype – biofilm, Small Colony Variants – dwarf colonies)

P. aeruginosa – clinical significance

- penetrating wound infection
  - Osteochondritis of the dorsum of the foot (propensity to survive of rubber of running shoes)
- Folliculitis in hot tub users
- otitis externa („swimmers ear“)
- Conjunctivitis in contact lens users
- Meningitis (trauma or surgery)
- Sepsis and meningitis in newborns
- soft tissues infections (ecthyma gangrenosum)
- Osteomyelitis (diabetes mellitus; iv drug users)
- urinary tract infections
- ventilator-associated pneumonia
- peritonitis

P. aeruginosa - identification

<u>Low nutritional requirements – standard media (nutrient agar, blood agar, McConkey agar</u>

Typical appearance of bacterial colony:

- β-hemolysis on blood agar
- Pigmentation:
  - Blue-green pigment – pyocyanin (pyo – pus, kyaneos – blue)
  - Yellow-green pigment – fluorescein - siderophore
  - Red-brown pigment – pyorubrin
- Odor:
  - younger colonies – yasmin and lime flower odor (even violet)
  - Older colonies – corn taco-like odor
- Pearle to metal sheen of colonies
- Oxidase positive

Pseudomonas aeruginosa pigmentation

1. Oxidase production test

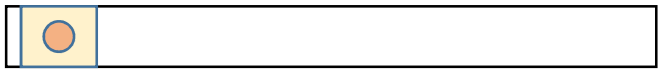
- **Background:**
- Enzyme **cytochrome c oxidase detection** (last enzyme in the respiratory chain) takes part in electron transmission to oxygen in electron transport chain of aerobic bacteria
- Oxidase reagent contains chromogenic oxidoreductase substrate, its colour is changed during oxidisation into dark red-violet

Procedure:

- Impress selected bacterial colony with the stripe with filter paper saturated with oxidase reagent (press against the bacterial colony)

Evaluation:

1. Positive reaction: intensive blue colour within 30 sec
2. Late positive reaction: intensive blue colour within 2 min
3. Negative reaction: no change or reaction later than 2 min



1. Oxidase production test

Use in G- bacteria

1. **Enterobacteriacae**

OXI -: *Escherichia, Klebsiella, Salmonella, Enterobacter spp.*

2. OXI +: *Vibrio spp., Plesiomonas shigeloides, Aeromonas hydrophila* identification of less common causative agents of diarrhoea

3. G- nonfermentative bacilli

Differentiation of genera:

OXI +: *Pseudomonas spp., Flavobacterium spp., Chryseobacterium spp.*

OXI + late: *Burkholderia cepacia complex*

OXI -: *Acinetobacter spp., Stenotrophomonas maltophilia*

P. aeruginosa - pathogenicity

Factors of pathogenicity:

extracelular polysacharides (adhesins), proteolytic enzymes, cytotoxin, hemolysins

Adhesins

flagella, pili,

alginate – mucoid colonies,

LPS – lipid A – endotoxin

Toxins

exotoxin A – disrupt protein synthesis

pyocyanin – catalyse production of toxic forms of oxygen

Proteolytic enzymes

phospholipase C - hemolysin,

elastases – degrades elastin-containing tissues

alkaline protease

P. aeruginosa - treatment

- intrinsic resistance (inducible chromosomal *Amp*C betalactamase, efflux pump systems)
- Acquired resistence – upregulation of intrinsic resistence mechanism, loss of porins, mutations in gyrase, carbapenemases
- Antibiotic tolerance – biofilm, CF phenotype

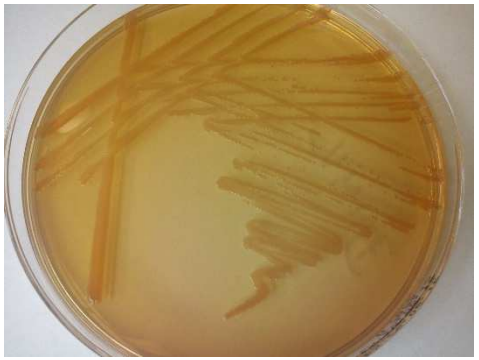
Antipseudomonal ATB:

- Betalactams: **piperacilin, ceftazidim**, cefepim, imipenem, meropenem
- Aminoglycosides: gentamicin, amikacin, **tobramycin**
- Quinolones: ciprofloxacin, levofloxacin
- Colistin

Local therapy: wound desinfection, eye/ear drops (tobramycin, gentamicin), inhalation

Pseudomonas aeruginosa – antibiogram

Burkholderia cepacia complex

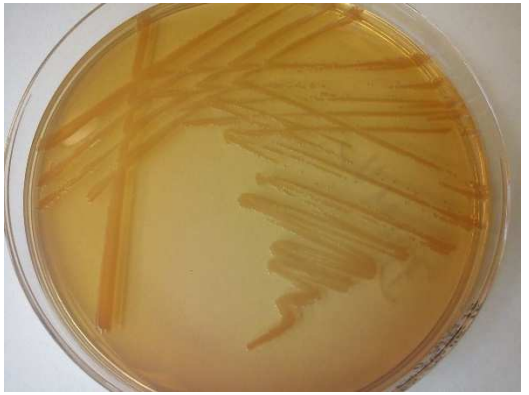


- Complex of 9 species
- ***B. cepacia, B. multivorans, B. cenocepacia*** and others
- Ocurrance in water, soil, longtime survival in moist environment
- Interpersonal transmission reported
- Discovered by American botanist Walter Burkholder in 1949 - the cause of onion rot

Cultivation:

- Slowly growing bacteria on MacConkey agar
- Within 3-5 days tiny, smooth or rough colonies
- Selective culture media

Burkholderia cepacia complex



Clinical significance - infections:

1. Infection in patients with cystic fibrosis (first reported in the 80th of 20th century)
2. Nosocomial infections and colonisation (mostly of respiratory tract, VAP)

- Reported nosocomial transmission by means of contamination of nasal sprays, medicaments for gargling and oral desinfection, oral probes

Burkholderia cepacia complex

- **ATB resistance:**

- Natural resistance to various ATB aminoglycosides, colistin included
- Clinical efect of cotrimoxazol, frequently used in combination with other ATB

Burkholderia mallei

Mostly occuring in tropical and subtropical countries

Animal disease (donkey, horse, mule) - malleus

Transmission: direct contact with infected animal, aspiration of contaminated dust or aerosol

Clinical symtoms: small knots and ulcus formation on mucous surfaces, skin and inner organs

Burkholderia pseudomallei

saprophyte

The causative agent of melioidosis – disease similar to maleus

Source of infection – contaminated water, food, inhalation, injury, athropode transmission

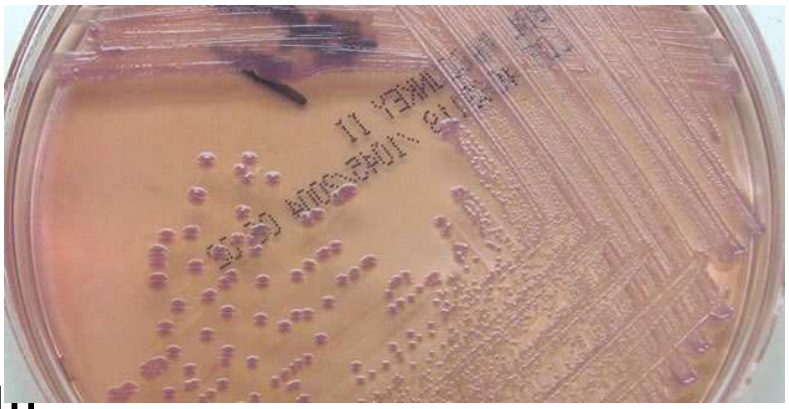
Fever, septic status, skin lesions, diarrhoea, pneumonia, abscesses formation

Stenotrophomonas maltophilia

https://www.flickr.com

- Aerobic, nonfermenting, G- bacilli
- Growth on McConkey agar, tiny colonies, production of light yellow pigment
- Widespread in nature, soil, water, plants
- In humans: nosocomial infections (respiratory, ventilatory pneumonia, cystic fibrosis, wound infections less frequently)
- Highly resistant to majority of ATB (aminoglycosides, carbapenems, cefalosporins) – selection of resistant strains during the therapy
- Susceptible to cotrimoxazol

Acinetobacter spp.



http://www.medical-labs.net

- G-nonfermenting aerobic short bacilli

Rods, sometimes of coccoid shape or in pairs

- Medically important species: *A. baumannii, (A. lwoffi, A. junii* more likely commensal bacteria)
- Smooth, rounded, mucoid colonies
- Growth on MacConkey agar (some strains of *A. lwoffii* except)
- Widespread in nature, soil
- Long time survival on moist and dry surfaces, in hospital environment either (even up to several weeks)
- Commensal bacteria on skin and respiratory mucous epithelium in humans

Acinetobacter spp.

Medical significance:

- cause of nosocomial infections, maily in intensive care wards (sporadic cases and outbreaks)
- **Major pathogen: *A. baumannii***

1. nosocomial pneumonia (late source > 4 days of hospitalization
2. skin, wound, burns infections
3. bacteremia (high mortality)
4. meningitis (frequently connected with CSF drain)
5. urinary tract infections

Acinetobacter spp.

Therapy:

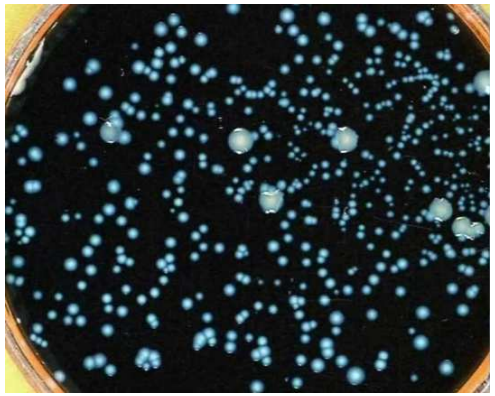
- Natural resistance to many groups of ATB
- Clinical efficacy of sulbactam
- Aminoglycosides, carbapenems, cotrimoxazol
- Lately the dramatic increase of resistance (multiresistant, panresistant strains)

4. Antibiogram

Evaluation of natural resistance:

Natural resistance (always resistant)	Reduced susceptibility	G-nonfermentative bacilli
Cotrimoxazol	Any ATB	*Pseudomonas aeruginosa*
Colistin	Any ATB	*Burkholderia cepacia complex*
Carbapenems (imipenem, meropenem)	Any ATB	*Stenotrophomonas maltophilia*
	Any ATB	*Acinetobacter spp.*

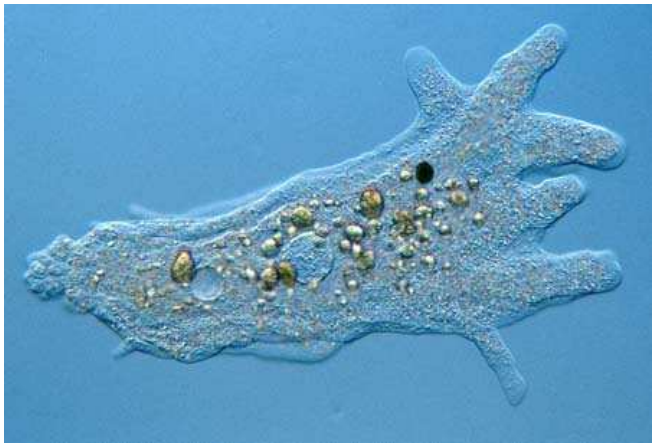
Legionella



- The disease was first diagnosed in 1976 after causing pneumonia-like symptoms (referred to as Legionnaire's disease) in the attendees of a convention in Philadelphia
- includes more than 50 the species
- the most important species *is L. pneumophila*
- Related to *Coxiellaceae* (*Coxiella burnetii* – Q-fever) – intracellular parasitism
- cause of legionellosis (all illnesses caused by *Legionella*)
  - pneumonia-type illness called **Legionnaires' disease**
  - mild flu-like illness called **Pontiac fever**

Legionella - description

- Mesophilic, motile, asaccharolytic, obligately aerobic, fastidious gram-negative pleomorphic rods
- Growth dependent on presence of cysteine, enhanced by iron
- Legionella – environmental facultative intracellular parasite of free-living **amoebae**
- Humans – accidental host of the bacterium
- it is common in environments, including soil and **aquatic systems (air-conditions, showers, whirpools, fountains...)**



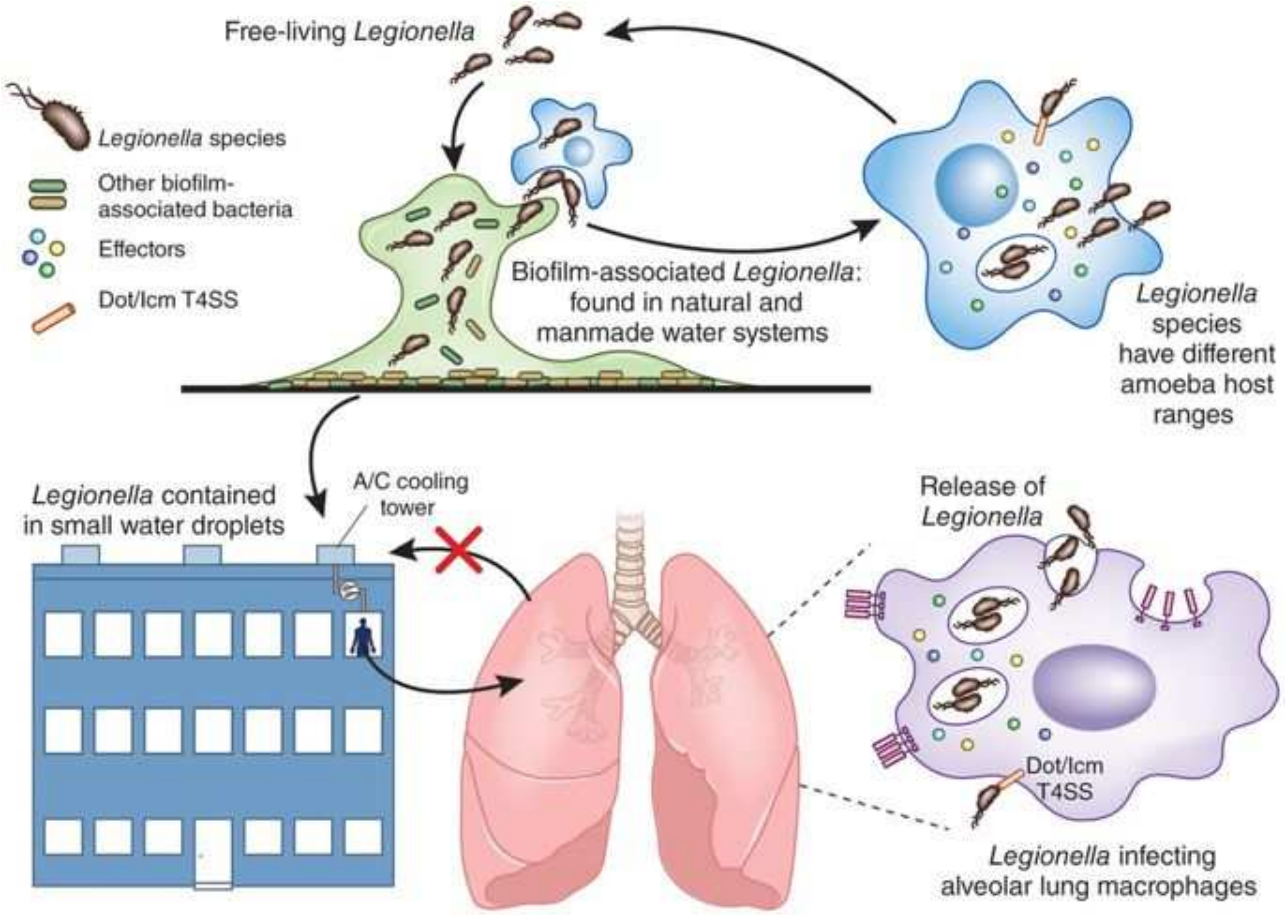
Legionella

- oxidase positive
- difficult to visualise by Gram stain
- could be stained using silver stain in tissues
- facultative intracellular pathogen (monocytes, macrophages, epithelial cells)
- inhibits phagolysosome fusion
- cell mediated immunity, and interferons neccessary for clearance
- fastidious – growth on **BCYE** agar (**B**uffered **C**harchoal **Y**east **E**xtract)
- *Legionella* requires the presence of **cysteine** and **iron** to grow

Legionella - pathogenesis

- In the natural environment *Legionella* lives within amoebae
- upon inhalation of water droplets from a contaminated source *Legionella* replicates incide of alveolar macrophages
- inhibits phagolysosome fusion
- cell mediated immunity, and interferons neccessary for clearance

Legionella – living cycle



Source: *Nature Genetics* 2016; 48: 115–116.

Legionella - epidemiology

- Source – contaminated cooling towers or aerosol-generating devices
- Disease incidence is relatively low (up to 5% cases of community-acquired pneumonia requiring hospitalization)
- Incubation period 2 -14 days
- patients at risk
  - middle-aged, elderly
  - Immunocompromised (cellular immunity)
  - organ transplant recipients
  - alcoholic, smokers
  - COPD disease history
- travellers disease
- hospital-acquired disease
- professional disease

Legionella - clinical disease

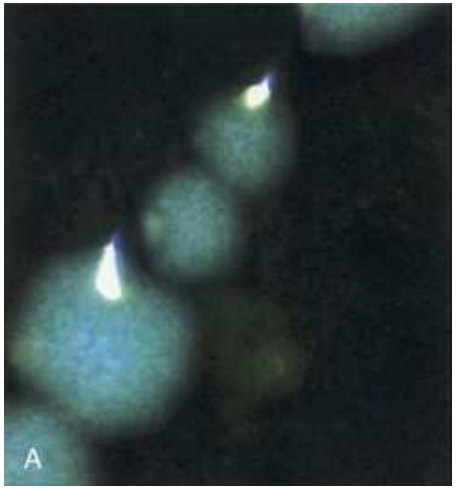
Pontiac fever

- sel-limited
- flu-like illness
- 2 – 5 days duration of symptoms
- no therapy is neccessary

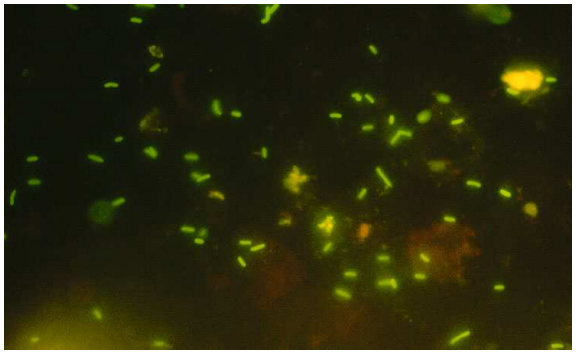
Legionnaires' disease

- severe pneumonia
- „atypical" pneumonia-disproportion between subjective and objective symptoms
- ~15% mortality
- multilobular pneumonia (microabscesses)
- prodromal symptoms - flu-like, including fever, chills, and dry cough
- advanced symptoms – GIT (nausea, diarrhoea, nervous system-deterioration, hepato-renal dysfunction)

Legionella - diagnosis



- Specimen – sputum, BAL (respiratory specimens)
- gram stain – rarely seen
- Immunofluorescent microscopy (monoclonal antibodies) – false positivity with other bacterial pathogens
- cultivation on BCYE (Buffered Charcoal Yeast Extract) medium (consult microbiologists!!)
  - lenght of cultivation – up to 10 days – medium 3 days
  - Small colonies, ground-glass appearance, sticky (form string touched by loop)
  - temperature 25-45°C; optimum 35°C
- urine antigen detection - specific only for *L. pneumophila* serogroup 1!!
- PCR based detection
- Identification
  - biochemically inert
  - cysteine dependence
  - serotyping (monoclonal/polyclonal antibodies)



Legionella – treatment, prevention

- Antibiotics with intracellular penetration (macrolides, fluoroquinolones)
- antibiotic of choice: **erytromycine** (iv)
- alternatives: **ciprofloxacin** (fluoroquinolone)

- prevention: maintaining and cleaning of possible sources
  avoid to stagnate water

  - heat desinfection (60°C)
  - cooper-silver ionization
  - chlorine

Bacterial metabolism

- Medically important bacilli gain the energy, carbon and reduction equivalents from the organic compounds
- Classification according to thier ability to perform aerobic respiration, anaerobic respiration or fermentation

1. **Fermentation:** process of liberating energy from saccharides, organic acids, purine or pyrimidine without the need of oxygen

2. **Aerobic respiration:** system of processes – **glycolysis, Krebs cycle, respiration circle –** with ATP production

3. **Anaerobic respiration:** not so common**, process of use of electron-transport system** with substance another than oxygen as a final electrons acceptor in its end (NO_3, SO_4, CO_2)

Clasification of bacteria on the basis of metabolism type

- **G- rods**
  - Obligatory **aerobic bacteria** – grow only in oxygen presence
    lack fermentative metabolism (*Pseudomonas, Neisseria, Vibrio, Bordetella*)
  - Obligatory **anaerobic bacteria** – grow only in oxygen absence
    have only fermentation metabolism (*Bacteroides*)
  - **Facultative anaerobic bacteria** – able to perform both oxidative and fermentative metabolism (enterobacterales)
  - Microaerophilic bacteria – fermentative metabolisms

2. Test O/F - glucose (oxidation/fermentation)

Background: Glucose uptake into the cell, then metabolised

- Some microorganisms catabolise glucose by oxidative reaction
  - With CO_2 and H_2O production
- Majority of microorganisms catabolise glucose by fermentation without oxygen use
- End products of fermentation are small organic molecules, usually organic acids (e.g. lactic acid)
  - Gas (oxygen, CO_2) is generated by some microorganisms during fermentation

- For laboratory testing medium containing glucose and acidobasic indicator (bromothymol blue) is used
- **Results**: yellow colour in resulted acidic reaction; blue colour in alkaline reaction; green colour in neutral reaction

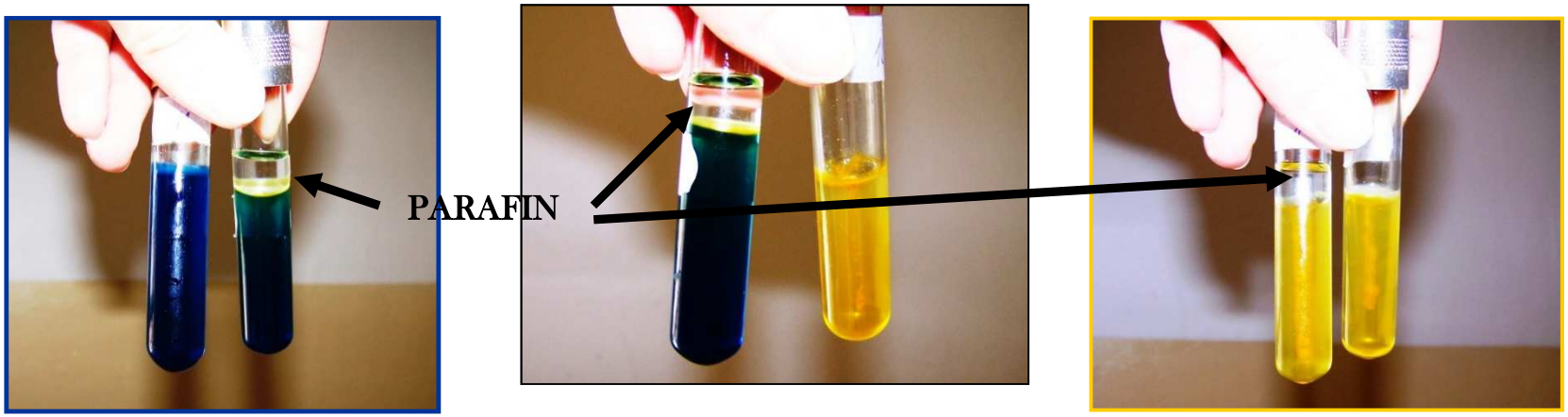
2. Test O/F - glucose (oxidation/fermentation)

Procedure:

1. 2 tubes with semisolid culture medium
2. Inoculum of pure culture is applied by puncture
3. Tube No 1. is cultivated without the layer of paraffin (oxidation)
4. Tube No. 2 is covered with a thin layer of paraffin (anaerobic setting = fermentation)
5. Incubation in thermostat at +37 °C
6. Reading the results after 24 hours

2. Test O/F - glucose (oxidation/fermentation)

Positive reaction – oxidation or fermentation of GLUCOSE result in yellow colour change of medium in tube



I.Both tubes stay blue
oxidation negative
fermentation negative

II. Tube No 1. yellow
oxidation positive
fermentation negative

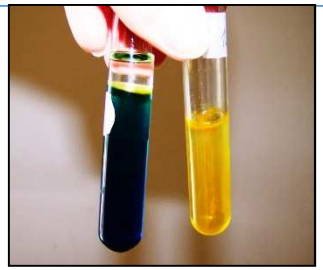
III. Both tubes yellow
oxidation positive
fermentation positive

2. Test O/F - glucose (oxidation/fermentation)

O/F +/+

Enterobacteriaceae

Escherichia spp.,
Klebsiella spp.,
Enterobacter spp.,
etc.



O/F +/-

Nonfermentative bacteria aerobic

Pseudomonas spp.

Vibrio spp.

Aeromonas spp.

O/F -/-

Nonfermentative bacteria

Acinetobacter spp.

Stenotrophomonas

Burkholderia