Gram-negative, facultative intracellular zoonotic bacteria
Brucella
Francisella
Pasteurella
Yersinia

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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

Treatment

- Primer surgery care!
- penicillin

- predisposing factor: chronic pulmonary disease
Brucella

Morphologia: Gram-negative, aerob, facultatively intracellular coccobacilli
Brucella – natural hosts (abortion)

Only one species with four biotypes!
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, acute disease with complications

*B. suis* /swine, reindeer, caribou/

Swine-brucellosis: chronic, suppurative, destructive disease

*B. canis* /dogs, foxes, coyotes/

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
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 °C in 5-10 % CO₂-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.*

\[ \text{Ag} = \text{killed brucella cells stained with tryphenyl tetrascolum chloride}. \]

\[ \text{Ag+Ab complex forms redish agglutinate that sediments onto the bottom of the test tubes}. \]

*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°C, 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
- 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.
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’s 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

<table>
<thead>
<tr>
<th>I./A Fluorescent group</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pseudomonas fluorescens</td>
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<tr>
<td></td>
<td>Pseudomonas putida</td>
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<tr>
<td>I./B non fluorescent group</td>
<td>Pseudomonas stutzeri</td>
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<tr>
<td></td>
<td>Pseudomonas mendinonica</td>
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<tr>
<td>II.</td>
<td>Burkholderia pseudomallei</td>
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<tr>
<td></td>
<td>Burkholderia mallei</td>
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<td></td>
<td>Burkholderia cepacia</td>
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<td></td>
<td>Burkholderia picketti</td>
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<tr>
<td>III.</td>
<td>Comamonas species</td>
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<tr>
<td></td>
<td>Acidovorax species</td>
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<tr>
<td>IV.</td>
<td>Brevundimonas species</td>
</tr>
<tr>
<td>V.</td>
<td>Stenotrophomonas maltophilia</td>
</tr>
</tbody>
</table>

- 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
  - immunocompromised status
  - age
- introduced into unprotected sites
  - mechanical ventilation
  - catheter
Pseudomonas aeruginosa

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

- first reported case of P. aeruginosa infection in 1980

Widespread occurrence in a variety of habitants

http://textbook of bacteriology_net_P_aeruginosa_jpeg.htm
**Pseudomonas aeruginosa**

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 (distilled water), adaptability 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 (phospholipase 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

**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, Middle East)
- Causes glanders (from horses)
  - Begins as an ulcer of the skin or mucuous membranes
  - Followed by lymphangitis and sepsis
  - Septicemia is usually fatal
  - Inhalation of the organisms may lead to primary pneumonia
Burkholderia mallei

Diagnosis: Very infectious!
- Special laboratory equipment
  • Only non-motile Pseudomonad
  • Strauss reaction: orchitis, pyogen infection intraperitoneally, death, after injection in a male guinea 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 appearance: 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
**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

Www.ism.gov.tr/haberler/depo/resim/legionella.jpg
**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; 35°C; 90% humidity)
  - 3 days incubation period (> 2 weeks 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/depol/resim/legionella.jpg
**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
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 Chest X-ray**

Upper Lobe Consolidation

Normal Chest X-ray
**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
- Hyperchlorination, 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 ⇒ 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* - *Peptostreptococcus*
- *Actinobacillus* - *Porphyromonas*
- *Actinomyces* - *Prevotella*
- *Cardiobacterium* - *Propionibacterium*
- *Corynebacterium* - *Staphylococcus*
- *Eikenella* - *Streptococcus*
- *Enterobacteriaceae* - *Stomatococcus*
- *Eubacterium* - *Treponema*
- *Fusobacterium* - *Veilonella*
- *Haemophilus*
- *Kingella*
- *Moraxella*
- *Mycoplasma*
- *Neisseria*  

**Fungi**
- *candida*
Normal flora of the lower respiratory tract

Normally sterile:
• middle ear
• sinuses paranasalis
• lung
• pleura
Köszönöm a figyelmet!