Gram-negative rods

Miklos Fuzi
Gram-negative rods

1. Fermentative bacteria: Enterobacteriaceae
   - degrade sugars by fermentation
   - oxidase: negative
   - degrade nitrates to nitrites

2. Non-fermentative bacteria: pseudomonas, acinetobacter
   - degrade sugars by non-fermentative pathways
   - usually oxidase positive
   - fail to degrade nitrates or degrade nitrates to nitrogen
# Pseudomonas Group

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*P = obligate pathogen*
**Pseudomonas aeruginosa**

**Morphology and culture:**
Gram-negative rods 1-2 µm,
no demands; Biofilm forming!
Pseudomonas aeruginosa

Pathogenesis, Infection:

Habitat:
Soil, plants, water (swimming-pools), sewage water
Human GI tract
Respiratory tract: animals

Source of infection:
sick, carriers
contaminated enviroment
Solutions (humid Milieu!) – tap water, variety of solutions

transmission: direct, indirect contact
*Pseudomonas aeruginosa*

**Pigments:**
1. Pyocyanin
2. Fluorescein

Haemolysis (β)
Pseudomonas aeruginosa

VERY RESISTANT!

- to
  Heat
  Light
  Disinfectants
  Antibiotics

www.fiu.edu
**Pseudomonas aeruginosa**

**ANTIGENS AND VIRULENCE FACTORS:**

**Adhaesion and Colonisation**
- "O", "H"
- Pili/Fimbriae
- Capsule = Glycocalyx (adhesion, protection)
- Alginate slime = mucoid Exopolysaccharide ➔ Biofilm

**Invasion, Penetration**
- Extracellular Proteases, Exoenzymes (many!)
- Cytotoxin = Leukocidin and Haemolysins
- Pigments

**Dissemination**
- **Exotoxin A** – inhibit Protein synthesis (EF2 ➔ Diphtheria)
- Exotoxin/Exoenzyme S – cytotoxic
- Endotoxin
Pseudomonas aeruginosa – Structure

- Flagellum (motility)
- Pilus (attachment)
- Alginate (cystic fibrosis isolates) antiphagocytic
- Endotoxin (shock)

Other products:
- Toxin A
- Hemolysin
- Phospholipase C
- Pigments
- Proteases
- Exoenzyme S
- Leukocidin

Medmicro
No single factor is decisive for virulence.
Pseudomonas aeruginosa
Toxin-A – mode of action

Pseudomonas aeruginosa – elmi
**Pseudomonas aeruginosa**

**Clinical findings:**
Nosocomial infections

- **Meningitis, Pneumonia** (Respirator!)
- **Sepsis**
- **Burns!** → skin, wounds
- Urogenital Infections (catheter),
- **GI tract (!), newborn/babies**
- **Otitis externa**
- **EYE + Contact lenses**

Cystic Fibrosis (mucoid strains)
Fig. 13-6 Ecthyma gangrenosum. Necrotic round lesion on the buttock of a child with Pseudomonas septicaemia associated with immunodeficiency.
Fig. 12.46 Bacterial keratitis. Contact lens-associated keratitis due to Pseudomonas aeruginosa. By courtesy of Dr. A.N. Carlson
Fig. 12.47 Bacterial keratitis, in this case due to P. aeruginosa. An infiltrate is seen with corneal thinning. By courtesy of Mr. P.A. Hunter
Fig. 12.48 Bacterial keratitis. A massive inflammatory response in anterior uveitis leads to precipitation of the cells as pus in the anterior chamber. This is called hypopyon. By courtesy of Mr. S. Harding
Fig. 12.49 Bacterial keratitis. *P. aeruginosa* eye infection showing corneal ulceration and hypopyon formation in this rapidly progressive eye infection.
Pseudomonas aeruginosa

- Slime biofilm with embedded bacteria
- Gram-negative bacilli surrounded by halo (capsule)
**Pseudomonas aeruginosa**

**Diagnosis:**
Isolation of pathogen, identification Oxidase +, pigment production, motility

**Therapy and Prophylaxis:**

**Antibiogram!!!**
aminoglycosides
wide spectrum penicillins
antipseudomonas cephalosporines
carbapenems
fluoroquinolones
Panresistance does occur
Pseudomonas aeruginosa

- Major antibiotic resistance mechanisms
  - production of β-lactamases including metallo-β-lactamase
  - efflux systems
  - alteration of membrane proteins

- Vaccine: for patients with cystic fibrosis
Stenotrophomonas (Pseudomonas) maltophilia

• aerobic gram-negative motile rod
• produces pigmented colonies
• found in various aquatic environments
• it is an uncommon pathogen in humans
• has few pathogenic mechanisms and, for this reason, predominantly colonizes rather than infects patients
• naturally resistant to many antibiotics
Stenotrophomonas maltophilia

- *S. maltophilia* usually must bypass normal host defenses to cause human infection
- If an irrigation solution becomes colonized with this organism, irrigating an open wound can cause colonization or infection of the wound
- *S. maltophilia* is usually incapable of causing disease in healthy hosts without the assistance of invasive medical devices that bypass normal host defenses.
- *S. maltophilia* frequently colonizes the respiratory tract in patients with cystic fibrosis
Figure 8. Ultrastructural analysis of *Stenotrophomonas maltophilia* adhering to plastic. (A) Scanning electron micrographs showing the tight adhesion of SMDP92 to the plastic surface. (B) Structures resembling flagella seem to be protruding and interconnecting bacteria (arrowheads) or connecting bacteria to the plastic (arrows). (C) In addition to the flagellalike filaments (arrowheads), high-power magnification shows the presence of thin fibrillar structures connecting bacteria to the abiotic surface. Bars: A 10 mm, B 1 mm, C 2 mm.
Stenotrophomonas maltophilia
Melioidosis – the pathogen

- Burkholderia pseudomallei
- Gram-negative motile rod
- Culture: not fastidious, incubation: for 7 days exclusively in biosafety level 3 laboratory
- Colony morphology: variable depending on the environment; the pathogen is extremely adaptable; „smart bacterium”
Melioidosis – the pathogen

- Habitat: soil, water (in free-living protozoons)
- Genetics: special
  - two chromosomes
  - highly prone to change (the proportion of repetitive sequences is high allowing frequent mutations to occur)
  - commands many genetic islands supporting survival and virulence
- Infects a variety of mammals including humans
Melioidosis

• If the soil becomes infected pathogen is extremely difficult to eradicate
• Floods promote spread of pathogen
• Endemic areas:
  - most of Southern Asia
  - parts of Australia
  - parts of Africa
  - parts of South America
Melioidosis – the disease

• Way of transmission:
  - contact (through skin lesions)
  - air-borne (heavy rain, humidity facilitates transmission)

• Symptoms: variable dependent on way of infection
  - acute form: high fever, pneumonia within hours of infection
  - subacute form
  - chronic tuberculoid form
  - localized to single organ
  - recurrent infection (even after decades)
Cutaneous Melioidosis in a Man Who Was Taken as a Prisoner of War by the Japanese during World War II


“We report a case of a man who was taken as a prisoner of war by the Japanese during World War II who presented with a nonhealing ulcer on his right hand 62 years after the initial exposure.”
Melioiodosis

- Diagnosis:
  - isolate pathogen (long incubation period)
  - demonstrate antigen (latex agglutination)
  - demonstration of antibodies (not sufficiently specific)
  - real-time PCR: not specific
- Therapy: susceptible to a variety of antibiotics
  No vaccine available
Clinical finding: Melioidosis (pseudoglanders)

- Pneumonia, Sepsis

subtropical and tropical area
B. pseudomallei
B. pseudomallei on Ashdown's agar after incubation at 37°C in air for 3 days
Burkholderia sp.
© Dr. med. T. Pietzcker, Ulm

B. cepacia

www.uni-ulm.de
Malleus - pathogen

- Burkholderia mallei
- Gram-negative nonmotile rod
- Genetically closely related to B. pseudomallei
- Culture: not fastidious; incubation 4 days
- Colony morphology: initially white-yellowish later greenish colonies

Biosafety level laboratory 3 required
Malleus – prevalence, symptoms, diagnosis

• No longer present in industrialized countries
• Acut disease in humans and many mammalian species; chronic condition in horses
• Human disease rare
• No environmental reservoir
• Transmission: contact, air-borne
• Inkubation: 1-14 days
• Clinical picture resembles melioidosis; heavy discharge from eyes does occur
• Human infection is always serious
• Diagnosis: culture, demonstration of antibody /not quite specific/, real-time PCR
• Antibiotic resistance: similar to that of B. pseudomallei
• No vaccine
**Burkholderia mallei**

Clinical finding: Malleus (glanders) – horse, donkey
Humans: often fatal!
Occupational disease
**Bioterror category B!**

A horse with glanders and with positive mallein test

- [microbewiki.kenyon.edu/images/3/39/Horse.jpg](microbewiki.kenyon.edu/images/3/39/Horse.jpg)
Burkholderia mallei
Acinetobacter spp.

- Strictly aerobic non-fermentative Gram-negative non-motile bacteria (name hints on inability to move)
- Show coccobacillary forms on solid media
- Rods predominate in fluid media, especially during early growth
- Commands complex metabolism; oxidase negative
Acinetobacter spp.

- Survives for considerable time on dry surfaces
- Infects primarily immunocompromised patients: wound infection, ventilation associated pneumonia; bacteremia, meningitis
- Usually multidrug resistant: innately resistant to chloramphenicol, penicillin, often aminoglycosides
- Treatment of last resort: carbapenems
- Panresistant strains do occur
Acinetobacter spp.

Gram-negative dimorphic rods, coccobacilli
Legionellosis

*Legionella pneumophila*

**Morphology:**
Gram-negative rods

www.lf3.cuni.cz
Legionella
Legionella
Legionella pneumophila

Flagellum, Fimbriae
Legionella
Legionella pneumophila

**Cultivation:** Special Media!

**BCYE** (Buffered, charcoal yeast extract)
(temperature 35°C; 3 days, CO2 atmosphere)

Blood agar
Legionella Culture

BCYE

www.lf3.cuni.cz
Legionella
Pathogenesis-1:
Source of infection, habitat:
ubiquitous
(air-conditioner, water-showers, fountains, humidity (floor),
Biofilms!
transmission:
Airborne - aerosol!
Sources of Legionellosis

- Showers
- Whirlpools
- Respiratory therapy equipment
- Tap water faucets
- Humidifiers
- Cooling towers
Legionella pneumophila

Pathogenesis-2: **Facultative intracellular**!

In water: in protozoon
In humans:
in leukocytes,
Inflammatory reaction,
Proteases,
Phospholipases

www.lifespan.org
FIGURE 40-7
Electron micrograph showing *L. pneumophila* serogroup 1 in the process of dividing (arrows) within a vesicle of an amoeba (Hartmanella veriformis) cell.
Pathogenic cycle of *Legionella*

1. **Coiling phagocytosis**
2. **Phagosome does not acidify**
   - **does not fuse with lysosome**
3. **Host cell lyses**
4. **Bacteria escape**
5. **Phagosome rupture**
6. **Multiply in phagosome**
7. **Phagosome surrounded by ER studded with ribosomes**
macrophages

LEGIONELLA PNEUMOPHILA
silver-positive bacteria (black)
Legionella pneumophila multiplying inside a human monocyte
M. Horwitz
**Legionella pneumophila**

**Clinical findings:**
Legionellosis
1) Pneumonia → Legionaire's disease
2) Pontiac-Fever (non-pneumonia)

**Diagnosis:**
detection – Biopsy! BAL: direct IF
Cultivation
Direct antigen detection – Urine!
Antibody detection – Serology; ELISA
Direct detection of DNA: PCR

**Therapy:**
Macrolides, Tetracyclin, Rifampicin, FQ
Fig. 2.29 Legionnaires’ disease. Chest radiograph showing extensive consolidation affecting parts of all lobes of the lungs.
Fig. 2.28 Legionella pneumophila. Specimen from bronchial biopsy taken through fibreoptic bronchoscope in a patient with fulminant Legionnaires’s disease. The organism can be isolated on selective culture media or by guinea pig inoculation. By courtesy of Dr. S. Fischer-Hoch
Fig. 2.30 Legionnaires’ disease. Autopsy specimen showing consolidation of upper and lower lobes of right lung.
The pharynx and trachea contain primarily those bacterial genera found in the normal oral cavity

- α-hemolytic streptococci (viridans group)
- anaerobes
- apathogenic staphylococci
- apathogenic neisseriae
- apathogenic diphtheroids (corynebacteria)
Potentially pathogenic organisms in the upper respiratory tract

- Haemophilus
- Mycoplasmas
- Streptococcus pneumoniae
- Moraxella catarrhalis
- Neisseria meningitidis
- Streptococcus pyogenes
- Bordetella pertussis

Viral infection often facilitates infection with resident bacteria
The carriage of Streptococcus pyogenes must be eliminated
Normal flora

- The oral flora is involved in dental caries and periodontal disease, which affect about 80 percent of the population in the Western world.
- Anaerobes in the oral flora are responsible for many of the brain, face, and lung infections that are frequently manifested by abscess formation.
Streptococcus viridans group

(S. mutans, S. mitis, S. sanguis,
S. salivarius)

Heterogenous collection of α-haemolytic Streptococci
„viridae” – Latin term for green.
Member of the normal flora of the oral cavity.

1) Morphology: Gram + cocci
2) Cultivation on blood and chocolate agar: α- haemolysis
   • Separate from S. Pneumoniae: normal flora optochin R
   • clinical picture: - endocarditis lenta (subacute)
     - dental caries - S. mutans (mainly serotype c)
     - colonization of the urinary tract