Genus *Proteus*

Clinical Significance

The microorganisms of the genus *Proteus* are commensal to the human body and colonize the intestinal tract. Furthermore, they are found in soil, trash, sewage, water and altered meat (involved in putrefaction processes). Thus, they may become facultative pathogens causing UTIs, otitis, sinusitis, pneumonia, meningitis and sepsis in the setting of a community acquired or nosocomial infections.

The four species important in human pathology include:

- ***Proteus vulgaris***
- ***Proteus mirabilis***
- ***Proteus penneri***
- ***Proteus myxofaciens***

Bacteriological Diagnosis

Collection

Collection of specimens depends on the site of infection, i.e. urine, faeces, pus, sputum, CSF and blood, etc.

Microscopic examination

Microscopic examination is only performed for naturally sterile sites, such as CSF.

Culture media

Because *Proteus* spp. have peritrichous cilia, they are highly motile. After an overnight incubation, after multiplication and growth has occurred on culture media, a **swarming phenomenon** can be noticed with the naked eye, meaning that concentric growth waves are visible.

On blood agar, the swarming phenomenon accounts for visible concentric waves, with invasion into other colonies and without distinctive, isolated colonies.

On selective media for *Enterobacteriaceae* (MacConkey, Hektoen enteric agar, XLD) round colonies with a same colour as the media (colourless) and black centres (H_2S production) are visible.

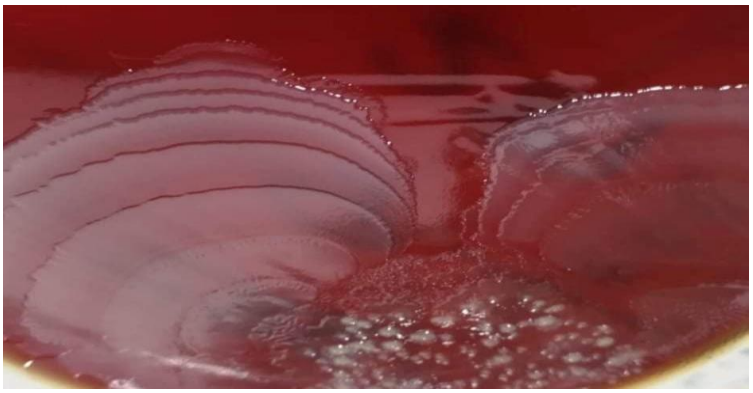

Figure 27. *Proteus* sp.- colonies on blood agar medium (invasive phenomenon)

Antimicrobial susceptibility testing (Antibiogram)

Proteus spp. are generally sensitive to beta-lactams, aminoglycosides, fluoroquinolones, Trimethoprim, sulfamethoxazole, and are naturally resistant to furans, cyclins and polymyxins. Multidrug-resistant *Proteus* isolates represent an important public health problem in both hospital- and community-acquired infections.

Essential to remember:

- Gram-negative bacilli
- Non-lactose fermenting (colourless colonies)
- Swarming phenomenon
- Causative agents for various nosocomial and community acquired infections

Gram-negative bacilli: Pseudomonas

Pseudomonas spp. are Gram-negative bacilli that are **obligate aerobic**, **motile**, **non-sporulating** and **oxidase positive**.

An important characteristic of *Pseudomonas*, opposed to the Gram-negative bacilli of the *Enterobacteriaceae* family is that they lack the enzymes responsible for catabolizing carbohydrates, i.e. they are **nonfermenters** – an important differential tool to distinguish *Pseudomonas spp.* from *Enterobacteriaceae*, in which all the members are able to ferment **glucose**.

Members of the Pseudomonas family are found in water, soil, air and human skin.

The clinically most significant member is *Pseudomonas aeruginosa*, especially found within hospitals, where it produces a biofilm under humid conditions, i.e. in toilets, humidifiers, respiratory equipment, plants, etc. – a germ extensively associated with **hospital-acquired infections**.

Pseudomonas aeruginosa

Clinical Significance

Pseudomonas aeruginosa is a commensal microorganism of the human body, colonizing mucous membranes. An isolation of *Pseudomonas aeruginosa*, in the absence of a clinical context, does not implicate an aetiological diagnosis.

Thus, *Pseudomonas aeruginosa* is an opportunistic pathogen infecting impaired tissues and organs, i.e. in patients with tissue lesions and immunosuppression.

The nosocomial infections include **respiratory** infections, **urinary tract** infections, **eye** infections, infections of **burns** and **surgical wounds**, **meningitis** and **septicaemia**. *P. aeruginosa* infections are especially common in patients with cystic fibrosis, accounting for the primary cause of death in these patients.

Bacteriological Diagnosis

Collection

Collection depends on the site of infection, i.e. pus, wound secretion, CSF, blood, urine, etc.

Microscopic examination

Microscopic examination of *P. aeruginosa* reveals **Gram-negative bacilli**, without being able to differentiate between other non-fermenting Gram-negative bacilli and *Enterobacteriaceae*. A Gram-stained smear is only relevant for naturally sterile specimens and especially in patients with cystic fibrosis, where Gram-negative bacilli surrounded by amorphous material can be observed.

Culture media and Identification

P. aeruginosa is a nonfastidious germ and can grow on any media. Naturally sterile specimens are inoculated on **blood agar**, whereas specimens from other sites of infection can be inoculated on selective media already discussed in the chapter of the *Enterobacteriaceae* family.

P. aeruginosa is also known as **pyocyanic bacillus**, because it secretes several pigments, the most characteristic of which are termed **pyocyanin** and **pyoverdin.** When one examines the culture media *P. aeruginosa* was inoculated on, a characteristic greenish colour of the colonies can be observed, due to the formation of a complex between pyocyanin and pyoverdin. These pigments also give the characteristic, gross appearance of greenish pus in the areas of infection.

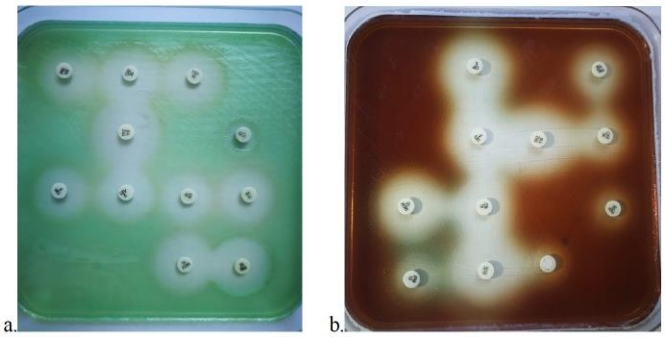

Figure 28: *P.aeruginosa* diffusimetric antibiogram, (a) pyoverine secreting strain, (b) pyomelanin secreting strain

On Blood agar *P.aeruginosa* colonies appear large and haemolytic, sometimes with a characteristic metallic sheen. On the selective culture media with lactose such as MacConkey, *P. aeruginosa* appears **lactose negative.** Furthermore, *P. aeruginosa* has a characteristic, flowery or fruity smell.

Identification is based on colonial morphology and biochemical characteristics: nonfermenter of glucose, catalase and oxidase positive.

Antimicrobial susceptibility testing (Antibiogram)

Performing AMS testing is essential in case of *P. aeruginosa* infections, because it rapidly acquires antibiotic resistance - especially dangerous strains emerge in the setting of hospital-acquired infections.

P. aeruginosa is naturally resistant to aminopenicillins, amoxicillin + clavulanic acid and 1st and 2nd generation cephalosporins.

Antibiotics active on *P. aeruginosa* include aminoglycosides, fluoroquinolones, 3rd and 4th generation cephalosporins and carbapenems.

In severe infections, an aminoglycoside (gentamycin, amikacin, tobramycin) is associated with a beta-lactam (ticarcillin, piperacillin, mezlocillin).

Essential to remember:

- Gram-negative bacillus
- Nonfermenter of sugars (glucose-, lactose-negative)
- Produces pigments (Green colonies; metallic sheen on blood agar)
- Flowery or fruity odour

- Catalase and oxidase positive
- Associated with hospital-acquired infections (habitat in humid areas of hospital)

Gram-positive, anaerobic bacilli

Recall from theory, that bacterial growth is to be seen in relation with the enzymatic equipment of the bacteria and in an environmental (atmosphere) context, with respiratory processes, i.e. the use of oxygen and carbon dioxide. Thus, different categories of bacterial growth in relation with those respiratory processes were established:

- **Obligate aerobes** are **dependent on oxygen** in the atmosphere and cannot respire anaerobically, as is true for *Mycobacterium tuberculosis* as an example.
- **Facultative anaerobes** can grow in atmospheres with or without oxygen, as is true for *Staphylococcus, Streptococcus* and *E. coli.*
- **Microaerophiles** are dependent on **small concentrations** of oxygen, but **are poisoned by high concentrations of oxygen**, as is true for *Campylobacter, Helicobacter, Neisseria gonorrhoeae.*
- **Obligate anaerobes** on the other hand are poisoned by oxygen, because they lack enzymes known as **superoxide dismutases**, which are **antioxidant factors** that **catalyse the breakdown of superoxide radicals** (i.e. oxygen anions; by-products of oxygen metabolism with high cellular toxicity) into **molecular oxygen** and **hydrogen peroxide**. Hydrogen peroxide is also toxic to these microorganisms and requires the enzyme **catalase** for breakdown, which obligate anaerobes also lack. Therefore, they can **only grow in atmospheres devoid of oxygen**, i.e. anaerobic atmospheres.

Thus, special precautions and methods have to be implemented in order for **anaerobic** microorganisms to be transported and processed for growth to occur.

After collection of the specimens, transport has to occur as fast as possible and in anaerobic tubes, e.g. “Hungate tubes” designed to maintain anaerobic conditions with specific transportation media, i.e. **modified Cary Blair** and **Stuart transport** media. These modified transportation media are designed to provide:

- Minimal nutrients to increase survival of organisms without multiplication
- Sodium thioglycollate to provide low oxidation-reduction potential
- Alkaline pH to minimize bacterial destruction by acid production
- Phenol red indicator (red at alkaline pH, yellow at acidic pH)
- Redox indicator: **resazurin** that turns pink in presence of oxygen

Inoculation has to be performed as fast as possible, in order for the obligate anaerobic microorganisms not to be exposed to oxygen for too long.

Suggestive **macroscopic** signs of anaerobic infections on patients include **fetid odour**, **purulent aspect**, **necrotic tissue** and **gas gangrene**.

Isolation may be performed on nonselective media, as is blood agar – with incubation in an anaerobic atmosphere by special incubators for three days in strict anaerobic conditions.

Genus Clostridium

The natural habitat of bacteria of the genus Clostridium is the intestine of animals. An important general character of this genus is the capacity to form spores. Bacterial spores represent a form of resistance when the conditions become unfavourable. Spore-forming bacteria such as those of the genus *Clostridium* use this form of resistance whenever the environmental conditions become unfavourable (as is the case when they are eliminated from animal intestines on the soil). Spores may survive in this "dormant" form for long periods of time (years) and whenever the conditions become favourable again (e.g. spore-contamination of a wound, ingestion of spores) they will germinate and turn into vegetative forms, capable of causing infections.

Clostridium tetani

Clinical Significance

Clostridium tetani is found in the intestinal tract of sheep and cattle, which eliminate the vegetative bacteria through faeces into soil, where they form spores.

Infection with *C. tetani* occurs via skin lesions contaminated with spores, e.g. wounds highly contaminated with dirt and dust or through extensive wounds with crushed tissue and foreign bodies, as is in accidents. An important condition required for an infection with *Clostridium tetani* is the so-called "tetanigenic potential" of a skin lesion i.e. the combination of high contamination of the wound with dirt, foreign bodies, etc. and the lack of oxygen at the site of the wound which is provided in cases of profound and narrow wounds with a small entry orifice (such as puncture wounds) and/or extensive lesions with crushed and necrotic tissues where the blood supply is severely impaired. All these conditions create the local oxygen deprivation providing the anaerobic conditions, which support the germination of bacterial spores. The spores germinate into vegetative bacteria that multiply at the site of entry, where they produce the tetanic toxin, composed of **tetanospasmin** (**neurotoxin**), leading to muscular spasms and **tetanolysin**, which is cardiotoxic.

The disease is known as **tetanus**, which is marked by a rigid, spastic paralysis (as opposed to botulism marked by flaccid paralysis). The onset of tetanus is associated with spasms of the face and chewing muscles (**"lock jaw"**), leading to a characteristic facial expression known as **risus sardonicus** or **sardonic grin**. Further evolution is marked by **opisthotonos** = generalised spastic contracture of all body muscles (severe spastic hyperextension of head, neck and spine). In about one in ten cases, the outcome is lethal, due to spastic paralysis of respiratory muscles.

It is important to know, that tetanus is a **vaccine preventable** disease, thus several vaccines have been developed to prevent tetanus among children, adolescents and adults. Immunization schedules include a trivalent immunization for diphtheria, pertussis and tetanus.

In patients with potentially tetanigenic wounds (e.g. puncture wounds caused by dirty instruments, soil-contaminated crushed wounds with necrotic tissues, animal bites, etc.) the tetanus prophylaxis is extremely important and it includes the administration of anti-tetanus vaccine associated with specific tetanus immune globulin. The exact protocol for wound management depends on the vaccine status of the patient and on the assessment of the wound (tetanigenic potential assessment).

Bacteriological Diagnosis

The laboratory diagnosis of *C. tetani* is only required in suspicion of iatrogenic infections, e.g. infection of umbilical cord stump, post-partum infections, etc. In most cases diagnosis relies on the clinical context, e.g. symptoms, history and tetanigenic circumstances, such as wounds contaminated with dirt, faeces, saliva and soil or puncture wounds, animal bites, burns and frostbite.

Microscopic examination

Microscopic examination reveals Gram-positive bacilli, single or in pairs, with the resemblance of a drumstick, due to terminal spore formation.

Essential to remember:

- Anaerobic
- Gram-positive bacillus with a terminal spore (drumstick appearance)
- Neurotoxin secretion: tetanospasmin that causes tetanus
- Vaccine preventable
- No human-to-human transmission

Clostridium botulinum

Clinical Significance

C. botulinum is the aetiological agent for the **foodborne** disease **botulism**, with a potentially fatal course, due to the production of the **botulinic toxin** (**neurotoxin**). Ingestion of this toxin is associated with ingestion of canned vegetables, fish and meat. A hint to detect possible contamination of *C. botulinum* within canned products is bulging or swelling of the can lid, due to production of gas. The toxin is resistant to gastric acid, thus it can enter the lymph and blood vessels through the intestine acting on the nervous

system, causing a progressive **flaccid** paralysis. Botulism has also been linked to intravenous or intradermic drug users. Worth mentioning is the possible use of the botulinic toxin as a biological weapon.

The onset of symptoms is marked by double vision (diplopia), drooping of eyelids, slurred speech (dysarthria), altered voice (dysphonia) difficulty swallowing (dysphagia), dry mouth and muscle weakness (four D's). If it is left untreated, the descending flaccid paralysis leads to death by paralysis of the respiratory muscles. Treatment has to be initiated as soon as possible and is achieved by administration of **botulinum antitoxin**.

Bacteriological Diagnosis

Collection and Identification

Specimens appropriate for collection include blood for serology, vomit, faeces and the suspected food. The bacteriological diagnosis is performed in reference laboratories.

Identification is based on:

- Gram-stain: Gram positive bacilli + **spores**
- Anaerobic growth
- Neurotoxin detection: immunoassay, molecular techniques

Essential to remember:

- Anaerobic
- Gram-positive bacillus with a subterminal spore
- Neurotoxin secretion: botulinic toxin that causes botulism (foodborne disease)