18. Pseudomonas aeruginosa and non-fermenters

18.1. GENERAL FEATURES

Gram-negative, non-spore-forming, non-glucose fermenting and, except for Acinetobacter, motile bacteria. They are distributed widely in nature on organic substances and are capable of colonizing plants, animals and human. They have simple nutritional needs and are capable of quickly acquiring antibiotic resistance. This makes them common in hospital environments and, hence, likely to cause infections of hospitalized patients.

18.2. VIRULENCE FACTORS & PATHOGENESIS

Virulence factors are genetic, biochemical, or structural features that enable an organism to cause disease. **Virulence factors of *Pseudomonas aeruginosa* - the most significant pathogen compared to the other non-fermenter species.**

STRUCTURAL COMPONENTS: Pilli (fimbriae) and **nonpilus adhesins** bind to epithelial cells, similar to the pilli found in *N. gonorrhoeae.* **Capsule** made of the mucoid polysaccharide alginate inhibits antibiotic killing (e.g. aminoglycoside) and phagocytosis. **Lipopolysaccharide** has endotoxic activity.

TOXINS & ENZYMES: Pyocyanin inhibits ciliary function, increases release of cytokines, which mediate inflammation and causes tissue damage through production of toxic oxygen radicals (e.g. hydrogen peroxide). **Exotoxin A** inhibits protein synthesis, causes tissue damage (e.g. of the skin and cornea) and is immunosuppressive. **Exotoxin** S inhibits protein synthesis and is immunosuppressive. **Cytotoxin (leukocidin)** is cytotoxic to eukaryotic membranes. It disrupts leukocyte function. **Elastase** causes destruction of elastin-containing tissues (e.g. blood vessels, lung tissue, skin), collagen, immunoglobulins, and complement factors. **Phospholipase C** is a hemolysin. It causes tissue damage and stimulates an inflammatory response. **Alkaline phosphatase** causes destruction of tissues.

ANTIBIOTIC RESISTANCE. *P. aeruginosa* is resistant to many antibioitcs. It produces a number of different beta lactamases which renders resistance agains many beta lactam antibiotics, such as pencillins, cephalosporins and even carbapenems. Mutations in porin proteins provides another way for *P. aeruginosa* to be resistant to many antibiotics. These mutations change the structure of the pores so that antibiotics cannot pass through and reach their target sites within the bacteria.

18.3. INFECTIONS & EPIDEMIOLOGY

Any tissue or organ may be affected with ***P. aeruginosa*** **infections. Localized infections:** may occur in the eye (e.g. keratitis, endophtalmitis) and ear (e.g. necrotizing otitis externa). Skin infections may follow a trauma (wound sepsis). They can also be caused by use of invasive medical instruments or exposure to a contaminated environment such as a swimming pool. Urinary and respiratory tract infections are common in **hospitalized patients** after the use medical instruments such as catheters. **Gastrointestinal infections** range from mild diarrhea in children to necrotizing enterocolitis in neutropenic cancer patients. **Central nervous infections** – meningitis and brain abscesses usually associated with trauma or tumors. *P. aeruginosa* is a pathogen of chronic infection to patients with CF (as single infection or co-infection with *Burkholderia cepacia* complex) and to patients

chronic lung diseases. In patients with CF the strains adapt to the changing environment by producing a mucoid polysaccharide capsule (alginate) and biofilm. **Strains that produce biofilm are more resistant to antibiotics. Bacteremia, endocarditis, bone and joint infections** are a sequale of the spreading of localized infection to a systemic infection. **Other non-fermenters (e.g. *Acinetobacter baumannii, Stenotrophomonas maltophilia)*** are significant agents of hospital-acquired infections.

18.4. TREATMENT, PREVENTION & CONTROL

Pseudomonas aeruginosa infections. Treatment: Combined therapy of antipseudomonad penicillin (piperacillin, ticarcillin) in combination with an aminoglycoside (e.g. tobramycin) is useful. The empiric therapy should be confirmed by antibiotic susceptibility test as is the case of hospital-aquired infections caused by other non-fermenters. Prevention: hospital infection guidelines should be strictly followed to prevent or to cope with the infections.

18.5. LABORATORY DIAGNOSIS

a) Specimens: pus, sputum, urine, blood, and other clinical material depending upon the localization of the infection.

b) Microscopy: gram-negative rods usually in pairs.

c) Culture: because their simple nutritional need they grow well on basic culture media (nutrient agar). However in order to detect some specific properties, such as hemolysis, encriched media (blood agar) are used (fig. 1). To prevent competitive flora from appearing in the samples selective media are used.

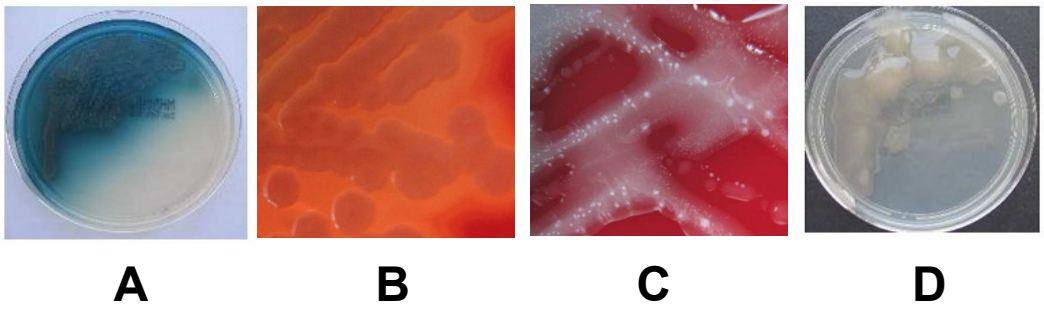


Fig. 1. Colonies of P. aeruginosa growing on nutrient (blue pigment pyocyanin)(A) and blood agar (hemolysis)(B), mucoid strains from sputum of CF patient growing on blood (C, white pigmented microcolonies in alginate could be seen, mucoid colonies contain only 10-20% bacterila cells and majority of the biomass is water into net of polysaccharide alginate) and nutrient agar (D).

***e) Phenotypical identification** is focused on detection of specific biological properties of the agent (microscopy, bacterial colonies, enzymes detection)(see also Chapter 6 – Identification).*

Screening tests (preliminary identification): detection of some phenotypic properties are demonstrated in fig. 2.

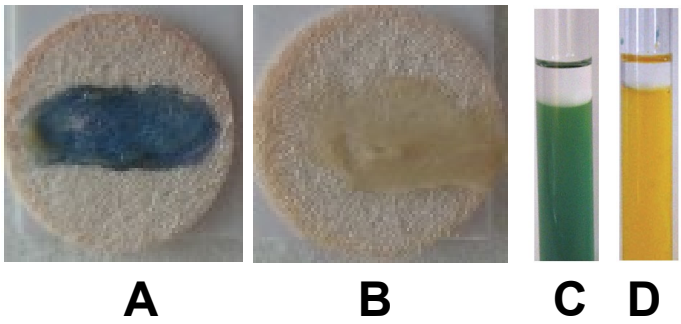


Fig. 2. Pseudomonas and other non-fermenters usually produce the enzyme oxidase (A – blue color indicates production of the enzyme) in contrast to enterobacteriae (B, oxidase negative reaction) which are also gram-negative bacteria and produce similar bacterial colonies on culture media. Pseudomonas and other non-fermenters never ferment glucose or other saccharides (C), in contrast to glucose fermentation in enterobacteria. This property is tested in semisolid medium containing glucose overlaid with mineral oil.

Biochemical identification. Multiple enzyme tests are performed to identify the bacteria. Positive and negative reactions are noted. The metabolic profiles of known species are compared with the strain being analysed. Key dichotomous method and numerical identification are used. (See also Chapter 6 – Identification).

f) Genotypical identification. There are several ways to identify a strain or species using genotype. For instance, comparing electrophoresed fragments allows visualisation of the restriction profile. Alternatively, homology of highly conserved regions, such as the 16S RNA gene, can assist identification of the species. (see also chapter 6 – Identification). (fig. 3).

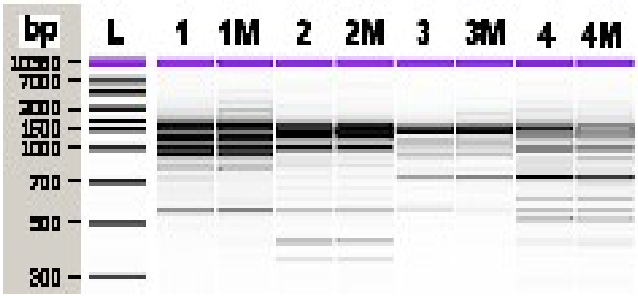
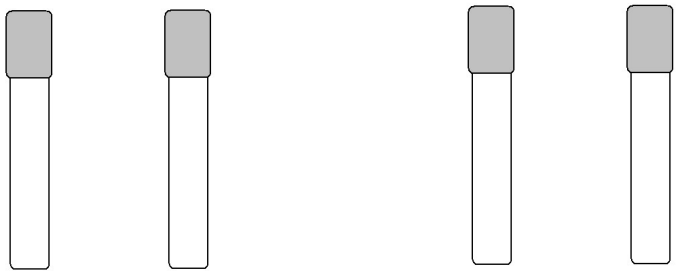


Fig. 3. Randomly Amplified Polymophic DNA (RAPD) analysis of four P. aeruginosa strains and their mucoid (M) counterparts isolated from CF patients revelaed that they are isogenic strains/clones. Infection of each patient was caused by a different clone and, hence, they are not clonally related.

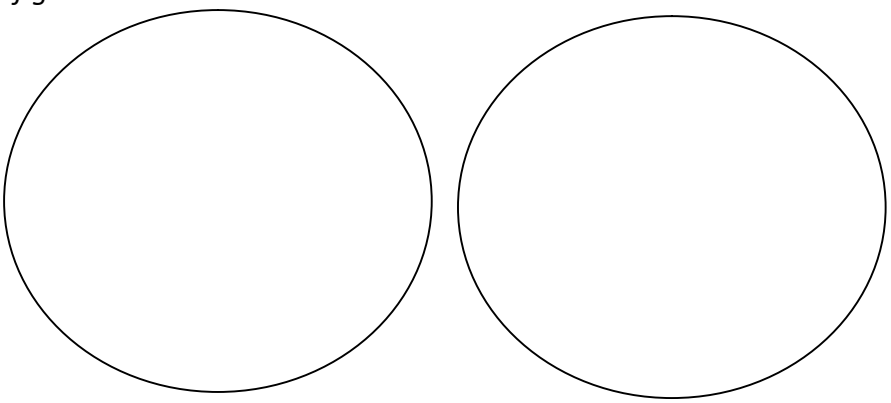
g) Susceptibility testing. Qualitative (disk diffusion method), quantitative methods or their combination (Etest) are used to test the susceptibility (see Table 5 – Antibiotic susceptibility testing).

18.5. PRACTICAL PART – PSEUDOMONAS AND NON-FERMENTERS

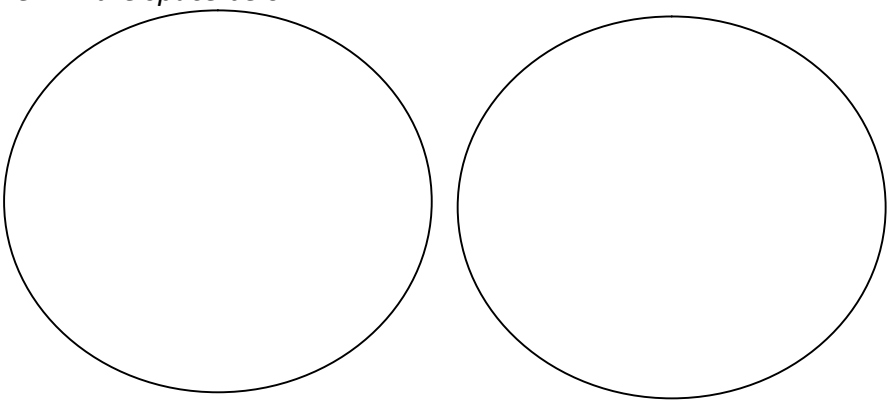
Excercise 1: READ AND INTERPRET OXIDATION/FERMENTATION (O/F) TEST. Read the results of the test after overnight cultivation of *P. aeruginosa* (or other non-fermenter) and compare with that of enterobacteria. Note the principle of the test below and draw the results below.



Excercise 2: *CULTURE OF P. AERUGINOSA ON GENERAL AND DIAGNOSTIC MEDIA. Draw them in the figures below.*



Excercise 3: *PIGMENT PRODUCTION. Specify the pigments characteristic for P. aeruginosa and draw them in the space below.*



Excercise 4: *INTERPRET THE RESULT OF BIOCHEMICAL IDENTIFICATION AND SUSCEPTIBILITY TESTING OF PSEUDOMONAS AERUGINOSA. Using producer recommendation interpret the result of biochemical identification of the analyzed strain. Using standardized criteria interpret the result of the disk diffusion method or determine the minimal inhibitory concentration of antibiotics tested.*

18.6. LAB QUIZ

1. Specify the virulence factors of *P. aeruginosa*.
2. Specify the infections caused by *P. aeruginosa* and the other non-fermenters.
3. Describe the principles of combined treatment of *P. aeruginosa* infections.

4. Specify the gold-standard methods to diagnose *P. aeruginosa* and other non-fermenters infections.
5. Specify the prevention and control of *P. aeruginosa* and other non-fermenters infections.