

Assist. Prof. Dr. Ameer Mezher Hadi

Lecture. 4

Second Stage

Evaluation Lecturer



Al-Mustaqbal University

College of Health and Medical Techniques

Department of Medical Laboratory
Technique

Pseudomonas and Acinitobacter

Pseudomonads

Pseudomonads The pseudomonads are **Gram-negative, motile, aerobic rods, some of which produce water-soluble pigments.** The pseudomonads occur **widely in soil, water, plants, and animals.** *P. aeruginosa* is frequently present in small numbers in the normal **intestinal flora and on the skin of humans,** and is the major pathogen of the group. Other pseudomonads infrequently cause disease.

In the general population *P. aeruginosa* is carried by very few people but this can rise **to over 30% after a stay in hospital.** The invasive potential of this organism means that it causes disease in a wide range of **hospital patients.** It is a particular problem to the **neutropenic patient** where it can cause fulminant **septicaemia and death.**

Patients undergoing **artificial ventilation** for **extended periods** in intensive therapy units may become colonized with *P. aeruginosa* and **secondary lower respiratory tract infection may follow.**

Extensive burns become colonized and **septicaemia** develops in a proportion of patients. **Multidose optical solutions** can be contaminated by *P. aeruginosa* which, when used, can produce a rapidly progressive corneal infection which ends in **ocular perforation.** *Pseudomonas aeruginosa* is an important pathogen for patients with **cystic fibrosis** where colonization with this organism is inevitable. Skin infection may arise in healthy subjects exposed to high infective doses such as **deep sea divers** and **users of contaminated hydrotherapy pools and Jacuzzi.**

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Pseudomonas aeruginosa distribution

It is widely distributed in nature and is commonly present in moist environments in hospitals. It can colonize normal humans; in whom it is a saprophyte. It causes disease in humans with abnormal host defenses, especially in individuals with neutropenia.

Classification: There are more than 100 species in the genus *Pseudomonas*. There are two primary pathogens, *P. pseudomallei* and *P. mallei*.

Morphology and Identification:

A. Typical Organisms *P. aeruginosa* is **motile** (except ***P. mallei***) and **rod shaped**, measuring about $0.6 \times 2 \mu\text{m}$. It is **Gram-negative** and occurs as **single** bacteria, in **pairs**, and occasionally in **short chains**.

B. Culture *P. aeruginosa* is an **obligate aerobe** that grows readily on many types of culture media, sometimes producing a **sweet** or **grape-like** or **corn taco-like odor**. Some strains **hemolyze** blood. *P. aeruginosa* forms **smooth round colonies** with a **fluorescent greenish color** **pyoverdin** which gives a greenish color to the agar. It often produces the **non-fluorescent bluish pigment** **pyocyanin**, which **diffuses** into the agar. Other *Pseudomonas* species do not produce **pyocyanin**, Some strains produce the **dark red pigment** **pyorubin** or the **black pigment** **pyomelanin**.

C. Growth Characteristics *P. aeruginosa* grows well at $37\text{--}42^\circ\text{C}$; its growth at 42°C helps differentiate it from other *Pseudomonas* species that produce fluorescent pigments. It is oxidase positive. It does not ferment carbohydrates, but many strains oxidize glucose.

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Antigenic Structure and Toxins:

- Pili: Adhere to epithelial cells
- Exopolysaccharide: Anti-phagocytic property/ inhibit pulmonary clearance.
- Lipopolysaccharide: Endotoxic effect Enzymes
- Elastases: Digests protein (elastin, collagen, IgG)
- Proteases
- Hemolysins
- Phospholipases C (heat labile): Degrade cytoplasmic membrane components

Exotoxin A: Cytotoxic by blocking protein synthesis.

Endotoxin: like that of other gram-negative bacteria, causes the symptoms of sepsis and septic shock.

Pathogenesis: *Pseudomonas aeruginosa* is primarily an opportunistic pathogen that causes infections in hospitalized patients (e.g., those extensive burns), with in whom the skin host defenses are destroyed; in those with chronic respiratory disease (e.g., cystic fibrosis), in whom the normal clearance mechanisms are impaired; in those who are immunosuppressed;

- Urinary tract infection- chronic, complicated Urinary tract infection and associated with indwelling catheter.
- Wound infection of burn sites, pressure sores and ulcers.
- Septicaemia- “Ecthyma gangrenosum” skin lesion (haemorrhagic skin necrosis) •
- Otitis externa- Malignant external ear infection in poorly treated diabetic patients.
- Pneumonia- Infection of the lung in patients with cystic fibrosis.

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- Eye infection- Secondary to trauma or surgery.

Laboratory diagnosis:

Isolation Bacteria of the genus *Pseudomonas* grow readily on simple media such as **nutrient** or **blood** agar, and will also grow on the less inhibitory selective media such as **MacConkey**.

Specimen: pus, urine, sputum, blood, eye swabs, surface swabs Smear: Gram-negative rods. *Pseudomonas pseudomallei* is usually isolated from sputum, blood or pus from abscesses.

Culture: Obligate aerobe, grows readily on all routine media over wide range of temperature (5-42 °C). Bluish-green pigmented large colonies with characteristic “fruity” odor on culture media.

Media can be made **selective for Pseudomonas** by the incorporation of one or more of the **antibiotics** or **disinfectants** to which it is naturally resistant such as **irgasin**, **cetrimide** or **nalidixic acid**.

Colonies of *P. aeruginosa* are morphologically diverse and dwarf, rough, mucoid, rugose, coliform-like colonies and the more commonly encountered large convex, flat, oval colonies are described.

A culture of *P. aeruginosa* has a characteristic musty odour. The colonies of *P. pseudomallei* and *P. mallei* are slower to appear and are typically wrinkled with a **faint pinkish colour** developing after about five days.

P. aeruginosa are lactose and fructose oxidation, arginine dehydrolase, gelatinase and lysine decarboxylase.

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In **Centrimide** agar: *Pseudomonas aeruginosa* colonies (greenish-blue in color) are medium sized and characterized by an irregular growth

In blood agar: Colonies of *Pseudomonas aeruginosa* surrounded by a wide zone of beta-hemolysis. Cultivation 48 hours in an aerobic atmosphere, 37°C.



***Pseudomonas aeruginosa* may produce the characteristic blue-green pigment or none at all**

Biochemical reactions: Oxidase positive Catalase-positive Citrate-positive Indole-negative Produce acid from carbohydrate by oxidation, not by fermentation.

Examples of biochemical tests used in the identification of *Pseudomonas* spp.

| Species | Oxidase | Lactose | ADH | ODC | Gelatin |
|------------------------|---------|---------|-----|-----|---------|
| <i>P. aeruginosa</i> | + | - | + | - | + |
| <i>P. pseudomallei</i> | + | + | + | - | + |

ADH: Arginine dehydrolase, ODC: Ornithine decarboxylase

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Acinetobacter

Acinetobacter species are aerobic, Gram-negative bacteria that are widely distributed in soil and water and can occasionally be cultured from skin, mucous membranes, secretions, and the hospital environment. *A baumannii* is the species most commonly isolated. *Acinetobacter lwoffii* and other species are isolated occasionally.

A. Morphology and Identification: Acinetobacters are usually coccobacillary or coccial in appearance; they resemble neisseriae on smears, because diplococcal forms predominate in body fluids and on solid media. Rod-shaped forms also occur, and occasionally the bacteria appear to be Gram-positive.

B. Culture: *Acinetobacter* grows well on most types of media used to culture specimens from patients. *Acinetobacter* recovered from patients with meningitis, bacteremia, female genital, sputum, skin, pleural fluid, and urine, usually.