

College of Technology & Health Sciences 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  $0.6 \times 2 \mu m$ . 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 37–42°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: Gramnegative 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 <u>selective for Pseudomonas</u> 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.

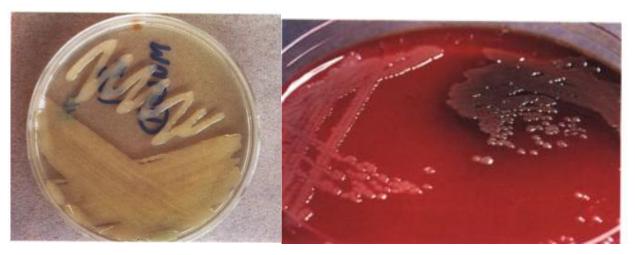


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In **Centrimide** agar: *Pseudomonas aeruginosa* colonies (greenish-blue in color) are medium sized and characterized by an irregular growth

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 Indolenegative 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
P. aeruginosa	+	-	+	-	+
P. pseudomallei	+	+	+	-	+

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 coccal 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.