



28

PSEUDOMONAS

28.1 INTRODUCTION

Pseudomonas is a bacteria mostly saprophytic in nature, is found in soil, water and other moist environment. It has emerged as an important cause of Health Care Associated and Opportunistic Infections. Most of the clinical isolates of *Pseudomonas* are resistant to many antibiotics. *Pseudomonas aeruginosa* is also a pathogen of plants. *Pseudomonas* is a strict aerobe, motile Gram negative bacteria and belongs to the order Pseudomonadales, family- Pseudomonadaceae and Genus *Pseudomonas*. The family comprises of about eight groups and 191 species, the type species is *Pseudomonas aeruginosa*. Walter Migula vaguely described these Gram negative motile organisms in 1890 and named as *Pseudomonas*. Recently, it has been postulated that *Pseudomonas* may be the common nucleator of ice crystals in clouds, so the organism is important to the formation of snow and rain around the world.



OBJECTIVES

After reading this lesson, you will be able to:

- classify *Pseudomonas*;
- state the morphological characteristics of *Pseudomonas*;
- enumerate the biochemical characteristics;
- explain the mechanism of virulence, pathogenicity;
- describe how the biofilm is formed;
- enumerate the infections caused by *Pseudomonas*;
- culture and identify *Pseudomonas* from specimen;
- carry out antibiotic susceptibility and interpret the results.



Notes

28.2 HISTORY

As already explained that it was sometimes in 1890-1900 that this Gram negative bacteria was described. A scientist by the name of Migula gave the Genus name of Pseudomonas in 1894 to these bacteria. Pseudomonas is comprised of two Greek words-Pseudo meaning false and Monas meaning unit, so literally the term means “false unit”. However Pseudomonas is a real bacteria so we really do not know the basis of the nomenclature by Walter Migula. The bacteria now has many groups and species. Recently the Pseudomonas whole genome has been sequenced and the analysis of 16S rRNA has led to the exclusion of many groups and inclusion of many groups to the Genus pseudomonas.

28.3 CLASSIFICATION

The classification of Pseudomonas is given below:

- Class: Gamma Proteobacteria
- Order: Pseudomonadales
- Family: Pseudomonadaceae
- Genus: Pseudomonas
- Eight groups: *P. aeruginosa* ; *P. chlororaphis*; *P. fluorescens*; *P. pertucinogena*; *P. putida*; *P. stutzeri*; *P. syringae*; *P. incertae sedis*.
- The family has 191 valid species. These include bacteria which are saprophytic, free living, and human, animal and plant pathogens. The type species is *Pseudomonas aeruginosa*.

28.4 MORPHOLOGY

Pseudomonas is rod shaped, slender (0.5 to 0.8 μm by 1.5 to 3.0 μm) Gram negative organism, motile by polar flagella, sometimes more than two flagella may be present. Some strains of Pseudomonas particularly those isolated from cases of cystic fibrosis are very mucoid and have kind of pseudo capsule (glycocalyx) made of polysaccharides. The glycocalyx protects pseudomonas from host defense.

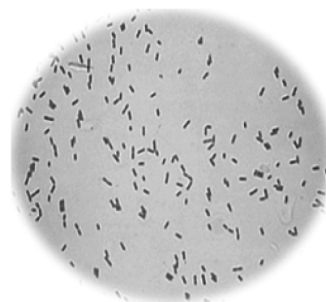


Fig. 28.1: Gram staining of pseudomonas: Gram negative, rod shaped bacteria seen

28.5 CULTURAL CHARACTERISTICS

Pseudomonas is a strict (obligate) aerobe, but sometimes it can grow anaerobically if nitrates (NO_3) act as respiratory electron acceptor) are present in the medium. *Pseudomonas* can grow at wide ranges of temperature; the optimum temperature is 37°C . It can grow on ordinary media like nutrient agar and grows almost on all the culture media used routinely in the bacteriology lab. *Pseudomonas* has been seen to grow in distilled water, also.



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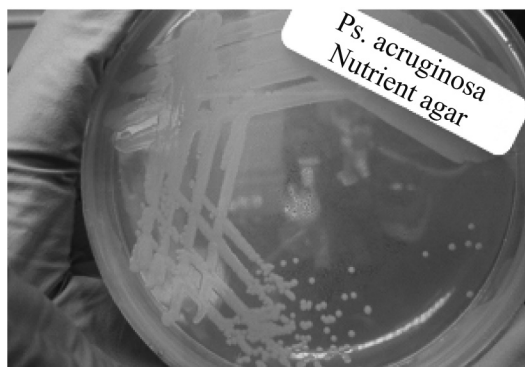


Fig. 28.2: *Pseudomonas aeruginosa* on nutrient agar showing greenish colouration due to production of Pyoverdine pigment.

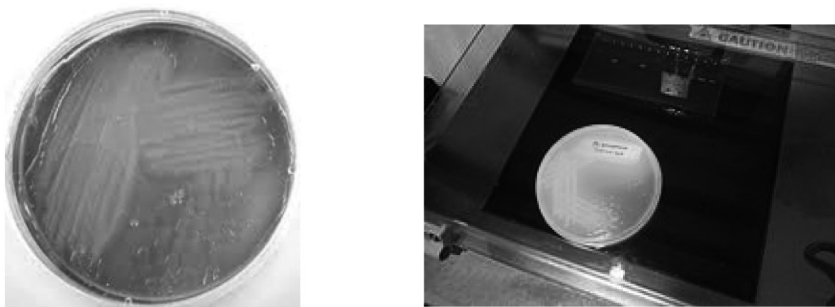


Fig. 28. 3: Growth of *P. aeruginosa* showing bluish green colouration due to production of Pyocyanin, produced by some strains of *Pseudomonas*

Pseudomonas produces large, opaque, flat colonies with irregular margins and distinctively fruity odour colonies. The colour of growth will depend upon the type of pigments (enumerated below) produced by the organism. The isolates from water and soil produce small round colonies. The isolates from clinical specimens like respiratory, urine, etc. may produce mucoid colonies. The bacteria which form mucoid colonies are more virulent compared to others.



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The pigments produced by Pseudomonas are:

- The fluorescent pigment pyoverdinin (greenish yellow)
- The blue pigment pyocyanin (bluish green)
- Pyorubin (red)
- Pyomelanin (brown)



INTEXT QUESTIONS 28.1

1. Pseudomonas is an
2. Pseudomonas is Gram bacteria.
3. Shape of Pseudomonas is
4. Pseudomonas grow on media.

28.6 BIOCHEMICAL CHARACTERISTICS

Pseudomonas has oxidative metabolism. Since organism is non fermentative the acid is not produced from peptone water sugars.

The important biochemical characteristics of Pseudomonas include:

- Oxidase test positive;
- Catalase test positive
- Nitrates are reduced to nitrites;
- Arginine dihydrolase test positive;
- Glucose is utilized oxidatively ⇒ Oxidative reaction in of media
- Indole, Methyl red (MR), Vogues Prauskar (VP) and H₂S production test are negative.

Commonest screening diagnostic biochemical test used in lab is the oxidase test.

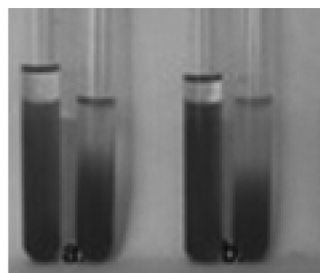


Fig. 28.4: Oxidative reaction of Pseudomonas in Hugh and Leifson's of media

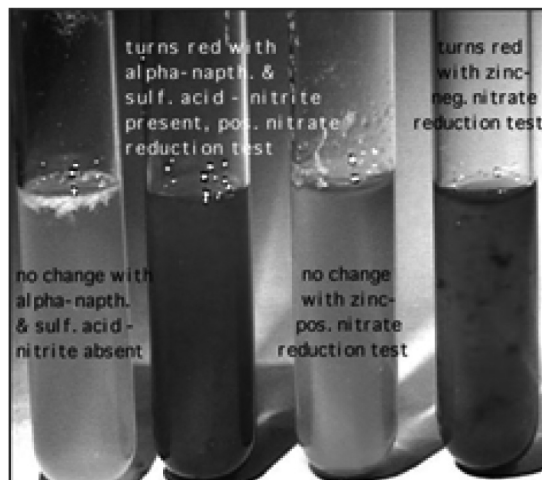


Fig. 28.5: Nitrate Test



Fig. 28.6: Arginine dihydrolase test

28.7 VIRULENCE AND PATHOGENICITY

Pseudomonas can infect any tissue, any organ system in an immune-compromised host. *Pseudomonas* usually cannot infect normal host. So, you see as compromised hosts are found in hospitals, *Pseudomonas* has emerged as a common cause of health care associated or nosocomial or hospital associated infections. In addition *Pseudomonas* produces many different organ system infections in the humans. These are described below.

P. aeruginosa produces exotoxin A which is a virulence factor. This exotoxin inactivates ADP ribosylate eukaryotic elongation factor 2 (EF 2) and thus interferes with the synthesis of protein resulting in death of the cell. *P. aeruginosa* also produces an exoenzyme “Exo U” which damages the cell membrane leading to lysis of the membrane and cell death.



Notes



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It is reported that low levels of phosphate in human intestines activate the symbiotic *Pseudomonas* to produce lethal toxins inside the intestinal tract which may severely damage or kill the host.

The most important risk factor for *Pseudomonas* infections is break down of host defense due to disease or other factors. *Pseudomonas* is both invasive and toxinogenic. Infection involves the following three steps:

- bacterial attachment and colonization;
- local invasion;
- disseminated systemic disease.

28.7.1 Bacterial attachment and Colonization

Pseudomonas infection may be endogenous (may be from intestines) or may be acquired from outside (exogenous). Individuals outside the hospital may be colonized with *Pseudomonas* at different sites (0-24%). The adhesins are the pili of *P. aeruginosa* with which bacteria adhere to specific galactose or mannose or sialic acid receptors on the mucosal epithelial cells of the upper respiratory tract and others. Production of protease enzyme by bacteria breaks down the fibronectin and exposes the pilus specific receptors on the epithelial cell surface. Tissue injury caused by viral infection and other phenomenon facilitates colonization by *Pseudomonas* (Opportunistic colonization).

Pseudomonas can also colonize by formation of biofilm which we will discuss later. *Pseudomonas* pili, mucoid polysaccharide, probably surface-bound exoenzyme S and possibly other cell surface adhesins help *Pseudomonas* to colonize.

28.7.2 Bacterial Invasion

P. aeruginosa produces enzymes and toxins that break down barrier to enter and damage host cells, resist phagocytosis and host immune defenses. The polysaccharide slime and sort of false capsule produced by *Pseudomonas* effectively protects cells from opsonization by antibodies, complement deposition, and phagocyte engulfment. Elastase and alkaline protease destroy the ground substance of the cornea and other supporting structures composed of fibrin and elastin resulting in invasion and injury. *Pseudomonas aeruginosa* produces three other soluble proteins involved in invasion: a cytotoxin (mw 25 kDa) and two hemolysins. The cytotoxin is a pore-forming protein. It was originally named leukocidin because of its effect on neutrophils, but it appears to be cytotoxic for most eucaryotic cells. Of the two hemolysins, one is a phospholipase and the other is a lecithinase. They appear to act synergistically

to break down lipids and lecithin. The cytotoxin and hemolysins contribute to invasion through their cytotoxic effects on neutrophils, lymphocytes and other eucaryotic cells. Pyocyanin the pigment, impairs the normal function of human nasal cilia and disrupts the respiratory epithelium. So, you see that *Pseudomonas* has a varied armamentarium consisting of pilli, enzymes, capsule and pigments which help it to establish in human tissues and produce harmful effects.

28.7.3 Bacterial dissemination:

Pseudomonas can invade the blood stream from initial site of infection and through blood is disseminated to different organs. The factors which help bacteria to invade as described above help to invade the organs, tissues wherever the bacteria reach. Bacterial endotoxin during septicemia, may cause fever, hypotension, and intravascular coagulation.

The virulence/pathogenicity armamentarium of *Pseudomonas* includes:

Adhesins

- Pili (N-methyl-phenylalanine pili)
- Polysaccharide capsule (glycocalyx)
- Slime

Invasins

- Elastase
- Alkaline protease
- Hemolysins (phospholipase and lecithinase)
- Cytotoxin (leukocidin)
- Pyocyanin

Toxins

- Exoenzyme S
- Exotoxin A
- Lipopolysaccharide (LPS)

Antiphagocytic elements

- False capsules, slime layer
- Lipo polysaccharide
- Biofilm formation





Notes

28.8 BIOFILM FORMATION

Like some other bacteria *Pseudomonas* forms biofilm which serves as a safe haven for the bacteria. Biofilm is formed on quorum sensing. When a critical number of bacteria are reached bacterial cells communicate with each other by forming some molecules. Quorum sensing results in expression of genes which help bacteria to adapt to the environment, multiply, release virulence factors and produce enzymes, exotoxins, slime, galactocalyx, etc. This is the biofilm, in which are embedded colonies of bacteria. These bacteria are protected from host defense and antibiotics. Bacteria from this biofilm can invade, enter blood stream and cause septicemia. Biofilms of *P aeruginosa* result in chronic opportunistic infections.



INTEXT QUESTIONS 28.2

1. Virulence factor of *Pseudomonas* is
2. *Pseudomonas* produce and which resists phagocytosis.
3. *Pseudomonas* invade by and
4. *Pseudomonas* colonies by the formation of

28.8 INFECTIONS AND DISEASES CAUSED BY PSEUDOMONAS

Pseudomonas aeruginosa is the most common cause of infection of burn injuries and otitis externa (infection of outer ear). As stated earlier, *Pseudomonas* can infect any tissue/organ system in the immunocompromised host. The disease produced will depend on the organ system infected. *Pseudomonas* colonizes medical devices, forms biofilms and causes chronic opportunistic infections. *Pseudomonas* can be present as commensal in healthy hosts and does not cause any disease.

The diseases caused by *Pseudomonas* include:

- **Respiratory infections:** Pneumonia in neutropenic cancer patients undergoing chemotherapy; diffuse broncho pneumonia, infection in cystic fibrosis patients;
- **Bacteremia and septicemia:** *Pseudomonas* causes hospital acquired Gram-negative bacteremias in immunocompromised patient and in severe burns.

Pseudomonas accounts for 25% of hospital acquired BSI caused by Gram negative bacilli.

- **Ear infections:** *Pseudomonas* usually causes otitis externa and "swimmer's ear".
- **Central nervous system infections:** *Pseudomonas* can invade meninges from near by structures like external ear /paranasal sinuses after invasive procedure or trauma to the head.
- **Urinary tract infection:** Usually causes hospital-acquired UTI related to urinary tract catheterization, instrumentation or surgery. *Pseudomonas aeruginosa* is the third leading cause(12%) of all hospital-acquired UTIs.
- **Endocarditis:** *Pseudomonas* bacteraemia may result in infection of damaged heart valves and prosthetic heart valves.
- **Bone and joint infections:** *Pseudomonas* bacteraemia may result in infection of bones and joints by direct inoculation. May cause chronic contiguous osteomyelitis from direct inoculation of bone. *Pseudomonas* also causes osteochondritis after puncture wounds of the foot.
- **Gastrointestinal infections:** Any part of the gastrointestinal system can be infected by *Pseudomonas* in immunocompromised host. The diseases produced include diarrhoea, gastroenteritis, perirectal infections. Sometimes *Pseudomonas* can produce necrotizing enterocolitis. It is an important cause of antibiotic associated diarrhea.
- **Skin and soft tissue infections, including wound infections, pyoderma and dermatitis:** Any part of skin and soft tissue compromised by trauma, burn injury, bad hygiene may be infected by *Pseudomonas*. The infections caused include folliculitis, acne vulgaris and abscesses.

**Notes**

28.9 DRUG RESISTANCE

Pseudomonas is heat sensitive is killed at 55° C in one hour. *Pseudomonas* is inherently resistant to many antibiotics, common disinfectants and can merrily grow in bottles of antiseptic solutions. The resistance to antibiotics is by different mechanisms like multi drug efflux pumps; antibiotic resistance chromosomal genes (*mexAB*, *mexXY*, etc.) and the low permeability of the bacterial false capsule in biofilms. *Pseudomonas* also acquires drug resistance by mutations which may be spontaneous and also drug induced. *Pseudomonas* is sensitive to aminoglycosides (amikacin and gentamicin); cephalosporins (ceftazidime and cefotaxime); fluoroquinolones (ciprofloxacin, pefloxacin) and penicillins like piperacillin, ticarcillin) and colistin. Localized infections can be treated with topical colistin, polymyxin B.



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28.10 LABORATORY DIGNOSIS OF INFECTIONS CAUSED BY PSEUDOMONAS

- Collect the appropriate sample. This will be according to the tissue/system affected. Specimen can be **pus, urine, blood, CSF, tissue**, etc.;
- Carry out the Gram staining- Gram negative bacilli and pus cells will be seen;
- Culture the specimen on Blood agar and MacConkey agar plates. Incubate overnight at 37° C;
- Examine the bacterial growth : Examine type of colonies, pigment production, odour and do oxidase test from MacConkey agar. Pale colonies on MaConkeyAgar, fruity odour, pigment (greenish, brownish) and oxidase positive test means the growth is probably Pseudomonas;
- Confirm by OF test and Arginine dihydrolase test
- Carry out the antibiotic susceptibility testing by Kirby Bauer disc diffusion method. Read the result-measure the inhibition zones and label as sensitive , resistant or intermediate sensitivity taking into account the zone size as has been explained to you in the chapter on antibiotic susceptibility testing.



INTEXT QUESTIONS 28.3

1. Pseudomonas causes and infection
2. Pseudomonas are sensitive and resistant to,
3. Pseudomonas are cultured on and
4. Pseudomonas are



WHAT YOU HAVE LEARNT

- Pseudomonas is a bacteria mostly saprophytic in nature, is found in soil, water and other moist environment. It is an important plant pathogen also. It has emerged as an important cause of Health Care Associated and Opportunistic Infections.
- Pseudomonas has eight groups: *P. aeruginosa* ; *P. chlororaphis*; *P. fluorescens*; *P. pertucinogena*; *P. putida*; *P. stutzeri*; *P. syringae*; *P. incertae sedis* and The family has 191 valid species.

Pseudomonas

- Pseudomonas is rod shaped, slender (0.5 to 0.8 μm by 1.5 to 3.0 μm) Gram negative organism, motile by polar flagella.
- Pseudomonas is a strict (obligate) aerobe, can grow at wide ranges of temperature; the optimum temperature is 37° C. It can grow on ordinary media like nutrient agar and others and even indistilled water.
- *Pseudomonas aeruginosa* on nutrient agar showing greenish yellow colouration due to production of Pyoverdin pigment.
- The pigments produced by Pseudomonas are:
 - The fluorescent pigment pyoverdin (greenish yellow)
 - The blue pigment pyocyanin (bluish green)
 - Pyorubin (red)
- Pseudomonas has oxidative metabolism.
- Since organism is non fermentative the acid is not produced from peptone water sugars.
- The important biochemical characteristics include: Oxidase test positive and Catalase test positive, no fermentation of sugars in peptone water.
- Pseudomonas can infect any tissue, any organ system in an immune-compromised host. Pseudomonas usually cannot infect normal host.
- Pseudomonas has emerged as a common cause of health care associated or nosocomial or hospital associated infections.
- The most important risk factor for Pseudomonas infections is break down of host defense due to disease or other factors. Pseudomonas is both invasive and toxinogenic.
- Pseudomonas forms biofilm which serves as a safe haven for the bacteria.
- Pseudomonas causes a large number of infections affecting almost any part of body in immunocompromised host. These include: Respiratory infections, Bacteremia and septicemia, hospital acquired Gram-negative infection, otitis externa and "swimmer's ear", Central nervous system infections, hospital-acquired UTI related to urinary tract catheterization, instrumentation or surgery, Endocarditis, Bone and joint infections, Pseudomonas also causes skin and soft tissue infections, including wound infections, and is an important cause of infection in burns patients.
- To detect and identify Pseudomonas from specimen in the lab: Collect the appropriate sample; make smear and do Gram staining; culture the specimen on Blood agar and MacConkey agar plates; examine the bacterial growth; and do oxidase test from MacConkey agar.
- Pale colonies on Mac Agar, fruity odour, pigment (greenish, brownish) and oxidase positive test means the growth is Pseudomonas; carry out the antibiotic susceptibility testing.

MODULE

Microbiology



Notes



Notes

**TERMINAL QUESTIONS**

1. Briefly classify *Pseudomonas*.
2. What are the important morphological, cultural and biochemical characteristics of *Pseudomonas aeruginosa*.
3. Enumerate the important enzymes and toxins produced by *Pseudomonas* which help the bacteria to evade the host defense and produce disease.
4. Briefly describe how biofilm is formed and how it helps *Pseudomonas*?
5. Enumerate the infections and diseases caused by *Pseudomonas*.
6. Is *Pseudomonas* a sensitive organism? If not give reasons for resistance.
7. Briefly describe the steps for identification of *Pseudomonas* from pus collected from burn case.

**ANSWERS TO INTEXT QUESTIONS****28.1**

1. Aerobe
2. Negative
3. Rod
4. Nutrient

28.2

1. Exotoxin
2. Enzyme, toxin
3. Cytotoxin, hemolysins
4. Biofilm

28.3

1. Septicemia, Opportunistic
2. Heat, antibiotic, disinfectants
3. Blood agar, MacConkey agar
4. Aerobes