Pseudomonas aeruginosa

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Gram stain of Pseudomonas aeruginosa cells

Pseudomonas

Pseudomonas aeruginosa is the epitome of an opportunist pathogen of humans. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect, if the tissue defenses are compromised in some manner.

Pseudomonas aeruginosa is a Gram-negative, aerobic rod, belonging to the bacterial family Pseudomonadaceae. The family includes Xanthomonas, which together with Pseudomonas, comprise the informal group of bacteria known as Pseudomonads. These bacteria are common inhabitants of soil and water. They occur regularly on the surfaces of plants and occasionally on the surfaces of animals. The pseudomonads are better known to microbiologists as pathogens of plants rather than animals, but three Pseudomonas species are pathogens of humans. Pseudomonas aeruginosa is an opportunistic pathogen that causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a variety of systemic infections, particularly in patients with severe burns, and in cancer and AIDS patients who are immunosuppressed. Pseudomonas aeruginosa is occasionally a pathogen of plants, as well. Pseudomonas mallei causes a disease in horses known as glanders. It is a true parasite, since it is unable to survive in nature in the absence of its host. The
primary focus of infection is the lungs. The disease can be transmitted to humans from the horse. *Pseudomonas pseudomallei* is the agent of *melioidosis*, a highly fatal tropical disease of humans and other mammals. It is also an opportunistic pathogen contracted through the contamination of wounds with mud or soil.

The typical *Pseudomonas* bacterium in nature might be found in a **biofilm**, attached to some surface or substrate, or in a **planktonic form**, as a single cell actively motile my means of polar flagella. *Pseudomonas* is one of the most vigorous, fast-swimming bacteria seen in hay infusions and pond water samples. *Pseudomonas aeruginosa* is motile by means of a single polar flagellum. *P. aeruginosa* can live in a sessile biofilm form, or it can live in a planktonic form, as a free-swimming cell.

**Pseudomonas aeruginosa**

*Pseudomonas aeruginosa* is not particularly distinctive as a pseudomonad, but there are a few characteristics that are noteworthy and relate to its pathogenesis. The organism can be isolated from soil and water, particularly in enrichments for denitrifying bacteria. Although the bacterium is respiratory and never fermentative, it will grow in the absence of O₂ if NO₃ is available as a respiratory electron acceptor. *P. aeruginosa* possesses the metabolic versatility for which pseudomonads are so renowned. Organic growth factors are not required, and it can use more than thirty organic compounds for growth. *Pseudomonas aeruginosa* is often observed growing in "distilled water" which is evidence of its minimal nutritional requirements. Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees. Its tolerance to a wide variety of physical conditions, including temperature, contributes to its ecological success as an opportunistic pathogen. *Pseudomonas aeruginosa* does, however, show a predilection for growth in moist environments, a reflection of its natural existence in soil and water.

*P. aeruginosa* isolates may produce three **colony types**. Natural isolates from soil or water typically produce a small, **rough** colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large, **smooth**, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a **mucoid** appearance, which is attributed to the production of **alginate slime**. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.

*P. aeruginosa* produces two types of soluble pigments, **pyocyanin** and (fluorescent) **pyoverdin**. The latter is produced abundantly in media of low-iron content, and could function in iron metabolism in the bacterium. Pyocyanin (from "pyocyaneus") refers to "blue pus" which is a characteristic of suppurative infections caused by *Pseudomonas aeruginosa*.

*Pseudomonas aeruginosa* is notorious for its **resistance to antibiotics** and is, therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane LPS. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations antibiotics. Since its natural habitat is the soil, living in association with the bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally-occuring antibiotics. Moreover, *Pseudomonas* maintains
antibiotic resistance plasmids, both R-factors and RTFs, and it is able to transfer these genes by means of the bacterial processes of transduction and conjugation. Only a few antibiotics are effective against *Pseudomonas*, including fluoroquinolones, gentamicin and imipenem, and even these antibiotics are not effective against all strains. The futility of treating *Pseudomonas* infections with antibiotics is most dramatically illustrated in cystic fibrosis patients, virtually all of whom eventually become infected with a strain that is so resistant that it cannot be treated.

*Pseudomonas aeruginosa* colonies on agar

*Pseudomonas aeruginosa* can usually be isolated from soil and water, as well as the surfaces of plants and animals. It is found throughout the world, wherever these habitats occur, so it is quite a "cosmopolitan" bacterium. It is sometimes present as part of the normal flora of humans, although the prevalence of colonization of healthy individuals outside the hospital is relatively low (estimates range from 0 to 24 percent depending on the anatomical locale). Although colonization usually precedes infections by *Pseudomonas aeruginosa*, the exact source and mode of transmission of the pathogen are often unclear because of its ubiquitous presence in the environment.

*Pseudomonas aeruginosa* is primarily a nosocomial pathogen. According to the CDC, the overall incidence of *P. aeruginosa* infections in US hospitals averages about 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly-isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections.

**Pathogenesis**

For an opportunistic pathogen such as *Pseudomonas aeruginosa*, the disease process begins with some alteration or circumvention of normal host defenses. The pathogenesis of *Pseudomonas* infections is multifactorial, as suggested by the number and wide array
of virulence determinants possessed by the bacterium. Multiple and diverse
determinants of virulence are expected in the wide range of diseases caused by
*Pseudomonas aeruginosa* such as *Pseudomonas septicemia*, *urinary tract infections*,
*Pseudomonas pneumonia* and *chronic lung infections*, *endocarditis*, *dermatitis*, and
*osteochondritis*.

Most *Pseudomonas* infections are both invasive and toxinogenic. The ultimate
*Pseudomonas* infection may be seen as composed of three distinct stages: (1) bacterial
attachment and colonization; (2) local invasion; (3) disseminated systemic disease.
However, the disease process may stop at any stage. Particular bacterial determinants of
virulence mediate each of these stages and are ultimately responsible for the
characteristic syndromes that accompany the disease.

Colony

The fimbriae of *Pseudomonas* will adhere to the epithelial cells of the upper respiratory
tract and, by inference, to other epithelial cells as well. These adhesins appear to bind to
specific galactose or mannose or sialic acid receptors on epithelial cells. Colonization of
the respiratory tract by *Pseudomonas* requires **fimbrial adherence** and may be aided by
production of a protease enzyme that degrades fibronectin in order to expose the
underlying fimbrial receptors on the epithelial cell surface. Tissue injury may also play
a role in colonization of the respiratory tract since *P. aeruginosa* will adhere to tracheal
epithelial cells of mice infected with Influenza virus but not to normal tracheal
epithelium. This has been called **opportunistic adherence**, and it may be an important
step in *Pseudomonas* keratitis and urinary tract infections, as well as infections of the
respiratory tract.

The receptor on tracheal epithelial cells for *Pseudomonas* pili is probably sialic acid (N-
acetylneuraminic acid). Mucoid strains, which produce an a **exopolysaccharide**
(alginate) have an additional or alternative adhesin which attaches to the
tracheobronchial mucin (N-acetylglucosamine). Besides pili and the mucoid
polysaccharide, there are possibly two other cell surface adhesins utilized by
*Pseudomonas* to colonize the respiratory epithelium or mucin. In addition, it is likely
that surface-bound **exoenzyme S** could serve as an adhesin for glycolipids on
respiratory cells.

The mucoid exopolysaccharide produced by *P. aeruginosa* is repeating polymer of
mannuronic and glucuronic acid referred to as **alginate**. Alginate slime forms the matrix
of the *Pseudomonas biofilm* which anchors the cells to their environment and, in
medical situations, protects the bacteria from the host defenses such as lymphocytes,
phagocytes, the ciliary action of the respiratory tract, antibodies and complement.
Biofilm mucoid strains of *P. aeruginosa* are also less susceptible to antibiotics than
their planktonic counterparts. Mucoid strains of *P. aeruginosa* are most often isolated
from patients with cystic fibrosis and they are usually found in post mortem lung tissues
from such individuals.

Invasion

The ability of *Pseudomonas aeruginosa* to invade tissues depends upon its resistance to
phagocytosis and the host immune defenses, and the extracellular enzymes and toxins
that break down physical barriers and otherwise contribute to bacterial invasion. As mentioned above, the bacterial capsule or slime layer effectively protects cells from opsonization by antibodies, complement deposition, and phagocyte engulfment.

Two extracellular proteases have been associated with virulence that exert their activity at the invasive stage: elastase and alkaline protease. Elastase has several activities that relate to virulence. The enzyme cleaves collagen, IgG, IgA, and complement. It also lyses fibronectin to expose receptors for bacterial attachment on the mucosa of the lung. Elastase disrupts the respiratory epithelium and interferes with ciliary function. Alkaline protease interferes with fibrin formation and will lyse fibrin. Together, elastase and alkaline protease destroy the ground substance of the cornea and other supporting structures composed of fibrin and elastin. Elastase and alkaline protease together are also reported to cause the inactivation of gamma Interferon (IFN) and Tumor Necrosis Factor (TNF).

P. aeruginosa produces three other soluble proteins involved in invasion: a cytotoxin (mw 25,000) 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 eukaryotic 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 eukaryotic cells.

The Pseudomonas pigments are probably determinants of virulence for the pathogen. The blue pigment, pyocyanin, impairs the normal function of human nasal cilia, disrupts the respiratory epithelium and exerts a proinflammatory effect on phagocytes. A derivative of pyocyanin, pyochelin, is a siderophore that is produced under low-iron conditions to sequester iron from the environment for growth of the pathogen. No role in virulence is known for the fluorescent pigment, pyoverdin.

Dissemination

Blood stream invasion and dissemination of Pseudomonas from local sites of infection is probably mediated by the same cell-associated and extracellular products responsible for the localized disease, although it is not entirely clear how the bacterium produces systemic illness. P. aeruginosa is resistant to phagocytosis and the serum bactericidal response due to its mucoid capsule and possibly LPS. The proteases inactivate complement, cleave IgG antibodies and inactivate IFN, TNF, and probably other cytokines. The Lipid A moiety of Pseudomonas LPS (endotoxin) mediates the usual pathologic aspects of Gram-negative septicemia, e.g. fever, hypotension, intravascular coagulation, etc. It is also reasonable to assume that Pseudomonas Exotoxin A exerts some pathologic activity during the dissemination stage.

Toxinogenesis

P. aeruginosa produces two extracellular protein toxins, Exoenzyme S and Exotoxin A. Exoenzyme S is probably an exotoxin. It has the characteristic subunit structure of the A-component of a bacterial toxin, and it has ADP-ribosylating activity (for a variety of eukaryotic proteins) characteristic of exotoxins. Exoenzyme S is produced by bacteria growing in burned tissue and may be detected in the blood before the bacteria
are. It has been suggested that exoenzyme S may act to impair the function of phagocytic cells in the bloodstream and internal organs to prepare for invasion by \textit{P. aeruginosa}.

\textbf{Exotoxin A} has exactly the same mechanism of action as the \textit{diphtheria toxin}, it causes the ADP ribosylation of eukaryotic elongation factor 2. It is partially-identical to diphtheria toxin, but it is antigenically-distinct. It utilizes a different receptor on host cells but otherwise it enters cells in the same manner as the diphtheria toxin and it has the exact enzymatic mechanism. The production of Exotoxin A in is regulated by exogenous iron, but the details of the regulatory process are distinctly different in \textit{C. diphtheriae} and \textit{P. aeruginosa}.

Exotoxin A appears to mediate both local and systemic disease processes caused by \textit{Pseudomonas aeruginosa}. It has necrotizing activity at the site of bacterial colonization and is thereby thought to contribute to the colonization process. Toxinogenic strains cause a more virulent form of pneumonia than nontoxinogenic strains. In terms of its systemic role in virulence, purified Exotoxin A is highly lethal for animals including primates. Indirect evidence involving the role of exotoxin A in disease is seen in the increased chance of survival in patients with \textit{Pseudomonas} septicemia that is correlated with the titer of anti-exotoxin A antibodies in the serum.

Table 1 (below) is a summary of the virulence determinants of \textit{Pseudomonas aeruginosa}. Table 2 is a brief description of the diseases caused by \textit{Pseudomonas aeruginosa}.

\begin{table}[h]
\centering
\begin{tabular}{|l|}
\hline
\textbf{Adhesins} \\
\quad fimbriae (N-methyl-phenylalanine pili) \\
\quad polysaccharide capsule (glycocalyx) \\
\quad alginate slime (biofilm) \\
\hline
\textbf{Invasins} \\
\quad elastase \\
\quad alkaline protease \\
\quad hemolysins (phospholipase and lecithinase) \\
\quad cytotoxin (leukocidin) \\
\quad siderophores and siderophore uptake systems \\
\quad pyocyanin diffusible pigment \\
\hline
\textbf{Motility/chemotaxis} \\
\quad flagella \\
\hline
\textbf{Toxins} \\
\quad Exoenzyme S \\
\quad Exotoxin A \\
\quad Lipopolysaccharide \\
\hline
\textbf{Antiphagocytic surface properties} \\
\quad capsules, slime layers \\
\quad LPS \\
\hline
\textbf{Defense against serum bactericidal reaction} \\
\hline
\end{tabular}
\caption{Summary of the Virulence Determinants of Pathogenic \textit{Pseudomonas aeruginosa}}
\end{table}
slime layers, capsules
LPS
protease enzymes

**Defense against immune responses**
capsules, slime layers
protease enzymes

**Genetic attributes**
genetic exchange by transduction and conjugation
inherent (natural) drug resistance
R factors and drug resistance plasmids

**Ecologic criteria**
adaptability to minimal nutritional requirements
metabolic diversity
widespread occurrence in a variety of habitats

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**Table 2. Disease caused by *Pseudomonas aeruginosa***

**Endocarditis.** *Pseudomonas aeruginosa* infects heart valves of IV drug users and prosthetic heart valves. The organism establishes itself on the endocardium by direct invasion from the blood stream.

**Respiratory infections.** Respiratory infections caused by *Pseudomonas aeruginosa* occur almost exclusively in individuals with a compromised lower respiratory tract or a compromised systemic defense mechanism. Primary pneumonia occurs in patients with chronic lung disease and congestive heart failure. Bacteremic pneumonia commonly occurs in neutropenic cancer patients undergoing chemotherapy. Lower respiratory tract colonization of cystic fibrosis patients by mucoid strains of *Pseudomonas aeruginosa* is common and difficult, if not impossible, to treat.

**Bacteremia.** *Pseudomonas aeruginosa* causes bacteremia primarily in immunocompromised patients. Predisposing conditions include hematologic malignancies, immunodeficiency relating to AIDS, neutropenia, diabetes mellitus, and severe burns. Most *Pseudomonas* bacteremia is acquired in hospitals and nursing homes. *Pseudomonas* accounts for about 25 percent of all hospital acquired Gram-negative bacteremias.

**Central Nervous System infections.** *Pseudomonas aeruginosa* causes meningitis and brain abscesses. The organism invades the CNS from a contiguous structure such as the inner ear or paranasal sinus, or is inoculated directly by means of head trauma, surgery or invasive diagnostic procedures, or spreads from a distant site of infection such as the urinary tract.

**Ear infections including external otitis.** *Pseudomonas aeruginosa* is the predominant bacterial pathogen in some cases of external otitis including "swimmer's ear". The bacterium is infrequently found in the normal ear, but often inhabits the external auditory canal in association with injury, maceration, inflammation, or simply wet and humid conditions.
**Eye infections.** *Pseudomonas aeruginosa* can cause devastating infections in the human eye. It is one of the most common causes of bacterial keratitis, and has been isolated as the etiologic agent of neonatal ophthalmia. *Pseudomonas* can colonize the ocular epithelium by means of a fimbrial attachment to sialic acid receptors. If the defenses of the environment are compromised in any way the bacterium can proliferate rapidly and, through the production of enzymes such as elastase, alkaline protease and exotoxin A, cause a rapidly destructive infection that can lead to loss of the entire eye.

**Bone and joint infections.** *Pseudomonas* infections of bones and joints result from direct inoculation of the bacteria or the hematogenous spread of the bacteria from other primary sites of infection. Blood-borne infections are most often seen in IV drug users, and in conjunction with urinary tract or pelvic infections. *Pseudomonas aeruginosa* has a particular tropism for fibrocartilagenous joints of the axial skeleton. *Pseudomonas aeruginosa* causes chronic contiguous osteomyelitis, usually resulting from direct inoculation of bone, and is the most common pathogen implicated in osteochondritis after puncture wounds of the foot.

**Urinary tract infections.** Urinary tract infections (UTI) caused by *Pseudomonas aeruginosa* are usually hospital-acquired and related to urinary tract catheterization, instrumentation or surgery. *Pseudomonas aeruginosa* is the third leading cause of hospital-acquired UTIs, accounting for about 12 percent of all infections of this type. The bacterium appears to be among the most adherent of common urinary pathogens to the bladder uroepithelium. As in the case of *E. coli* urinary tract infection can occur via an ascending or descending route. In addition, *Pseudomonas* can invade the bloodstream from the urinary tract, and this is the source of nearly 40 percent of *Pseudomonas* bacteremias.

**Gastrointestinal infections.** *Pseudomonas aeruginosa* can produce disease in any part of the gastrointestinal tract from the oropharynx to the rectum. As in other forms of *Pseudomonas* disease, those involving the GI tract occur primarily in immunocompromised individuals. The organism has been implicated in perirectal infections, pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis. The GI tract is also an important portal of entry in *Pseudomonas* septicemia.

**Skin and soft tissue infections, including wound infections, pyoderma and dermatitis.** *Pseudomonas aeruginosa* can cause a variety of skin infections, both localized and diffuse. The common predisposing factors are breakdown of the integument which may result from burns, trauma or dermatitis; high moisture conditions such as those found in the ear of swimmers and the toe webs of athletes and combat troops, in the perineal region and under diapers of infants, and on the skin of whirlpool and hot tub users; neutropenia; and AIDS. *Pseudomonas* has also been implicated in folliculitis and unmanageable forms of acne vulgaris.