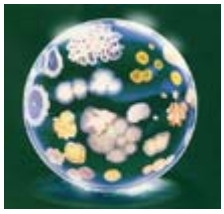
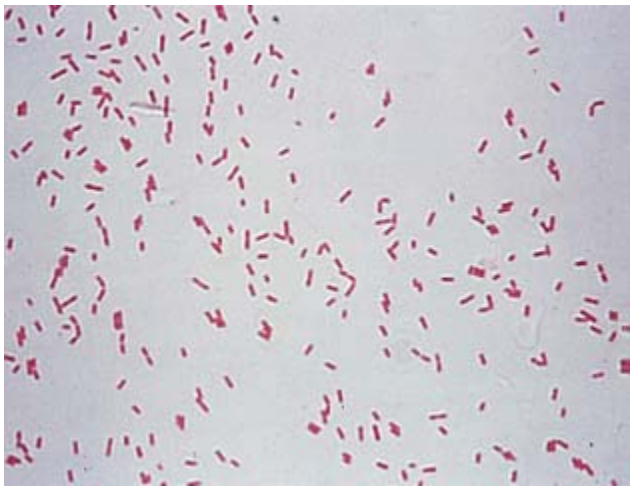


Todar's Online Textbook of Bacteriology

Pseudomonas aeruginosa



Gram stain of *Pseudomonas aeruginosa* cells

Pseudomonas aeruginosa is the epitome of an opportunistic 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 other genera, which, together with certain other organisms, constitute the bacteria informally known as **pseudomonads**. These bacteria are common inhabitants of soil and water. They occur regularly on the surfaces of plants and occassionally on the surfaces of animals. The pseudomonads are well known to plant microbiologists because they are one of the few groups of bacteria that are true pathogens of plants. In fact, *Pseudomonas aeruginosa* is occasionally a pathogen of plants. But *Pseudomonas aeruginosa* and two former *Pseudomonas* species (now reclassified as *Burkholderia*) are pathogens of humans. A general treatment of the pseudomonads is presented in The Genus *Pseudomonas* . This chapter deals specifically with *Pseudomonas aeruginosa* as a pathogen of humans.

Pseudomonas aeruginosa is an **opportunistic pathogen,** meaning that it exploits some break in the host defenses to initiate an infection. It causes **urinary tract infections**, **respiratory system infections**, **dermatitis**, **soft tissue infections**, **bacteremia, bone and joint infections, gastrointestinal infections** and a variety of **systemic infections**, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. *Pseudomonas aeruginosa* infection is a serious problem in patients

hospitalized with cancer, cystic fibrosis, and burns. The case fatality rate in these patients is 50 percent.

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.

Characteristics

Pseudomonas aeruginosa is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. Almost all strains are motile by means of a single polar flagellum.

The bacterium is ubiquitous in soil and water, and on surfaces in contact with soil or water. Its metabolism is respiratory and never fermentative, but it will grow in the absence of O_2 if NO_3 is available as a respiratory electron acceptor.

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 unicellular organism, actively swimming by means of its flagellum. *Pseudomonas* is one of the most vigorous, fast-swimming bacteria seen in hay infusions and pond water samples.

In its natural habitat *Pseudomonas aeruginosa* is not particularly distinctive as a pseudomonad, but it does have a combination of physiological traits that are noteworthy and may relate to its pathogenesis.

--*Pseudomonas aeruginosa* has very simple nutritional requirements. It is often observed "growing in distilled water" which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of *Pseudomonas aeruginosa* consists of acetate for carbon and ammonium sulfate for nitrogen.

--*P. aeruginosa* possesses the metabolic versatility for which pseudomonads are so renowned. Organic growth factors are not required, and it can use more than seventy-five organic compounds for growth.

--Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees.

--It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.

--*Pseudomonas aeruginosa* has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water.

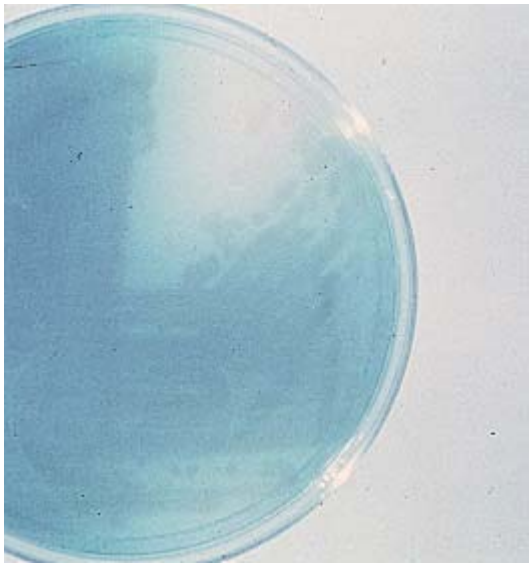
These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominance as a nosocomial pathogen.

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.

***Pseudomonas aeruginosa* colonies on agar**

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



The soluble blue pigment pyocyanin is produced by many, but not all, strains of *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 permeabiliity 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 my 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.

Diagnosis

Diagnosis of *P. aeruginosa* infection depends upon isolation and laboratory identification of the bacterium . It grows well on most laboratory media and commonly is isolated on blood agar or eosin-methylthionine blue agar. It is identified on the basis of its Gram morphology, inability to ferment lactose, a positive oxidase reaction, its fruity odor, and its ability to grow at 42° C . Fluorescence under ultraviolet light is helpful in early identification of *P. aeruginosa* colonies. Fluorescence is also used to suggest the presence of *P. aeruginosa* in wounds.

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, which include **septicemia**, **urinary tract infections**, **pneumonia, 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.

Colonization

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. 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).

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 **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. Also, 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 a 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, it 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 production of extracellular enzymes and toxins that break down physical barriers and damage host cells, as well as resistance to phagocytosis and the host immune defenses. 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 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 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.

One *Pseudomonas* pigment is probably a determinant 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 pigments.

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 assumed

that *Pseudomonas***Exotoxin A** exerts some pathologic activity during the dissemination stage (see below).

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 *P. aeruginosa*.

Exotoxin A has exactly the same mechanism of action as the **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 *C. diphtheriae* and *P. aeruginosa*.

Exotoxin A appears to mediate both local and systemic disease processes caused by *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 *Pseudomonas* septicemia that is correlated with the titer of anti-exotoxin A antibodies in the serum. Also, tox^- mutants show a reduced virulence in some models.

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

Table 1. Summary of the Virulence Determinants of Pathogenic *Pseudomonas aeruginosa*

Adhesins

fimbriae (N-methyl-phenylalanine pili)
polysaccharide capsule (glycocalyx)
alginate slime (biofilm)

Invasins

elastase
alkaline protease
hemolysins (phospholipase and lecithinase)
cytotoxin (leukocidin)
siderophores and siderophore uptake systems
pyocyanin diffusible pigment

Motility/chemotaxis

flagella

Toxins

Exoenzyme S
Exotoxin A
Lipopolysaccharide

Antiphagocytic surface properties

capsules, slime layers
LPS

Defense against serum bactericidal reaction

slime layers, capsules
LPS
protease enzymes

Defense against immune responses

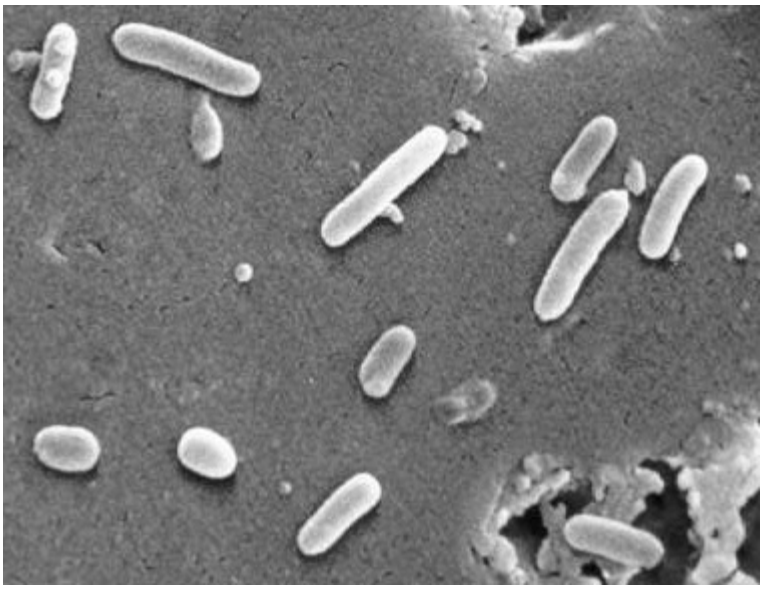
capsules, slime layers
protease enzymes

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



***Pseudomonas aeruginosa* Scanning electron micrograph. CDC**

Table 2. Diseases 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 and Septicemia. *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. Individuals with AIDS are easily infected. *Pseudomonas* has also been implicated in folliculitis and unmanageable forms of acne vulgaris.

Host Defenses

Most strains of *P. aeruginosa* are resistant to killing in serum alone, but the addition of polymorphonuclear leukocytes results in bacterial killing. Killing is most efficient in the presence of type-specific opsonizing antibodies, directed primarily at the antigenic determinants of LPS. This suggests that phagocytosis is an important defense and that opsonizing antibody is the principal functioning antibody in protecting from *P. aeruginosa* infections.

Once *P. aeruginosa* infection is established, other antibodies, such as antitoxin, may be important in controlling disease. The observation that patients with diminished antibody responses (caused by underlying disease or associated therapy) have more frequent and more serious *P. aeruginosa* infections underscores the importance of antibody-mediated immunity in controlling infections. Cystic fibrosis is the exception. Most cystic fibrosis patients have high levels of circulating antibodies to bacterial antigens, but are unable to clear *P. aeruginosa* efficiently from their lungs. Cell-mediated immunity does not seem to play a major role in resistance or defense against *Pseudomonas* infections.

Epidemiology and Control of *P. aeruginosa* Infections

Pseudomonas aeruginosa is a common inhabitant of soil, water, and vegetation. It is found on the skin of some healthy persons and has been isolated from the throat (5 percent) and stool (3 percent) of nonhospitalized patients. The gastrointestinal carriage rates increase in hospitalized patients to 20 percent within 72 hours of admission.

Within the hospital, *P. aeruginosa* finds numerous reservoirs: disinfectants, respiratory equipment, food, sinks, taps, and mops. Furthermore, it is constantly reintroduced into the hospital environment on fruits, plants, vegetables, as well by visitors and patients transferred from other facilities. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated reservoirs, and by the ingestion of contaminated foods and water.

The spread of *P. aeruginosa* can best be controlled by observing proper isolation procedures, aseptic technique, and careful cleaning and monitoring of respirators, catheters, and other instruments. Topical therapy of burn wounds with antibacterial agents such as silver sulfadiazine, coupled with surgical debridement, dramatically reduces the incidence of *P. aeruginosa* sepsis in burn patients.

Pseudomonas aeruginosa is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed. The combination of gentamicin and carbenicillin is frequently used to treat severe *Pseudomonas* infections. Several types of vaccines are being tested, but none is currently available for general use.

Return to Todar's Online Textbook of Bacteriology
