Pseudomonas aeruginosa and P. maltophilia account for 80 percent of opportunistic infections by pseudomonads. Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns; the case fatality is 50 percent. Other infections caused by Pseudomonas species include endocarditis, pneumonia, and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin, and musculoskeletal system.

Structure, Classification, and Antigenic Types

Pseudomonas species are Gram-negative, aerobic bacilli measuring 0.5 to 0.8 μm by 1.5 to 3.0 μm. Motility is by a single polar flagellum. Species are distinguished by biochemical and DNA hybridization tests. Antisera to lipopolysaccharide and outer membrane proteins show cross-reactivity among serovars.

Pathogenesis

Neutropenia in cancer patients and others receiving immunosuppressive drugs contributes to infection. Pseudomonas aeruginosa has several virulence factors, but their roles in pathogenesis are unclear. An alginate is antiphagocytic, and most strains isolated produce toxin A, a diphtheria-toxin-like exotoxin. All strains have endotoxin, which is a major virulence factor in bacteremia and septic shock.

Host Defenses

Phagocytosis by polymorphonuclear leukocytes is important in resistance to Pseudomonas infections. Antibodies to somatic antigens and exotoxins also contribute to recovery. Humoral immunity is normally the primary immune mechanism against Pseudomonas infection but does not seem to resolve infection in cystic fibrosis patients despite high levels of circulating antibodies.
Pseudomonas species normally inhabit soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons. They often colonize hospital food, sinks, taps, mops, and respiratory equipment. Spread is from patient to patient via contact with fomites or by ingestion of contaminated food and water.

**Diagnosis**

Pseudomonas can be cultured on most general-purpose media and identified with biochemical media.

**Control**

The spread of Pseudomonas is best controlled by cleaning and disinfecting medical equipment. In burn patients, topical therapy of the burn with antimicrobial agents such as silver sulfadiazine, coupled with surgical debridement, has markedly reduced sepsis. Antibiotic susceptibility testing of clinical isolates is mandatory because of multiple antibiotic resistance; however, the combination of gentamicin and carbenicillin can be very effective in patients with acute P aeruginosa infections.

**Introduction**

The genus Pseudomonas contains more than 140 species, most of which are saprophytic. More than 25 species are associated with humans. Most pseudomonads known to cause disease in humans are associated with opportunistic infections. These include P aeruginosa, P fluorescens, P putida, P cepacia, P stutzeri, P maltophilia, and P putrefaciens. Only two species, P mallei and P pseudomallei, produce specific human diseases: glanders and melioidosis. Pseudomonas aeruginosa and P maltophilia account for approximately 80 percent of pseudomonads recovered from clinical specimens. Because of the frequency with which it is involved in human disease, P aeruginosa has received the most attention. It is a ubiquitous free-living bacterium and is found in most moist environments. Although it seldom causes disease in healthy individuals, it is a major threat to hospitalized patients, particularly those with serious underlying diseases such as cancer and burns. The high mortality associated with these infections is due to a combination of weakened host defenses, bacterial resistance to antibiotics, and the production of extracellular bacterial enzymes and toxins.
Clinical Manifestations

Figure 27-1. Diverse sites of infection by P aeruginosa. This opportunistic pathogen may infect virtually any tissue. Infection is facilitated by the presence of underlying disease (e.g., cancer, cystic fibrosis) or by a breakdown in nonspecific host defenses (as in burns). Pseudomonas aeruginosa causes various diseases (Fig. 27-1). Localized infection following surgery or burns commonly results in a generalized and frequently fatal bacteremia. Urinary tract infections following introduction of P aeruginosa on catheters or in irrigating solutions are not uncommon. Furthermore, most cystic fibrosis patients are chronically colonized with P aeruginosa. Interestingly, cystic fibrosis patients rarely have P aeruginosa bacteremia, probably because of high levels of circulating P aeruginosa antibodies. However, most cystic fibrosis patients ultimately die of localized P aeruginosa infections. Necrotizing P aeruginosa pneumonia may occur in other patients following the use of contaminated respirators. Pseudomonas aeruginosa can cause severe corneal infections following eye surgery or injury. It is found in pure culture, especially in children with middle ear infections. It occasionally causes meningitis following lumbar puncture and endocarditis following cardiac surgery. It has been associated with some diarrheal disease episodes. Since the first reported case of P aeruginosa infection in 1890, the organism has been increasingly associated with bacteremia and currently accounts for 15 percent of cases of Gram-negative bacteremia. The overall mortality associated with P aeruginosa bacteremia is about 50 percent. Some infections (e.g., eye and ear infections) remain localized; others, such as wound and burn infections and infections in leukemia and lymphoma patients, result in sepsis. The difference is most probably due to altered host defenses.

Pseudomonas maltophilia is the second most frequently isolated pseudomonad species in clinical laboratories. In nature, P maltophilia is found in water and in both raw and pasteurized milk. It has been associated with a variety of opportunistic infections in humans, including pneumonia, endocarditis, urinary tract infections, wound infections, septicemia, and meningitis. Pseudomonas cepacia, although primarily a plant pathogen (onion bulb rot), also is an opportunist. Most human infections caused by P cepacia are nosocomial and include endocarditis, necrotizing vasculitis, pneumonia, wound infections, and
urinary tract infections. Pseudomonas cepacia causes chronic lung infections in cystic fibrosis patients. These infections differ from those caused by P aeruginosa in that P cepacia has become systemic in a number of cystic fibrosis patients, whereas P aeruginosa infections remain confined to the lungs. Pseudomonas cepacia is highly resistant to aminoglycosides and other antibiotics, making it very difficult to control.

Unlike most pseudomonads, P mallei and P pseudomallei can cause disease in otherwise healthy individuals. Pseudomonas mallei is the agent of glanders, a disease primarily of equines. Humans generally become infected by inhalation or by direct contact through abraded skin. These infections are frequently fatal within 2 weeks of onset, although chronic infections also have been reported. Today, P mallei infections of equines are controlled and are rarely encountered in the western world. Similarly, melioidosis, an endemic glanders-like disease of animals and a human pulmonary infection caused by P pseudomallei, is rare in the western hemisphere. Melioidosis is still found in Southeast Asia, and travelers returning from that area are sometimes infected.

**Structure, Classification, and Antigenic Types**

Figure 27-2 Structure and pathogenic mechanisms of P aeruginosa The proposed role of other products is listed in Table 27-1. 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, and some strains have two or three flagella (Fig. 27-2). The flagella yield heat-labile antigens (H antigen). The significance of antibody directed against these antigens, aside from its value in serologic classification, is unknown. Clinical isolates usually have pili, which may be antiphagocytic and probably aids in bacterial attachment, thereby promoting colonization. The cell envelope of P aeruginosa, which is similar to that of other Gram-negative bacteria, consists of three layers: the inner or cytoplasmic membrane, the peptidoglycan layer, and the outer membrane. The outer membrane is composed of phospholipid, protein, and lipopolysaccharide (LPS). The LPS of P aeruginosa is less toxic than that of other Gram-negative rods. The LPS of most strains of P aeruginosa contains heptose, 2-keto-3-deoxyoctonic acid, and hydroxy fatty acids, in addition to side-chain and core polysaccharides. Recent evidence
suggested that the LPS of a large percentage of strains isolated from patients with cystic fibrosis may have little or no polysaccharide side chain (O antigen), and that this finding correlates with the polyagglutinability of these strains with typing sera. Studies of isolated outer membranes suggest strong conservation of many of the outer membrane proteins of P aeruginosa. Although numerous serologic types exist (based on evaluations of O-specific antigens), many of the outer membrane proteins from these strains are antigenically crossreactive.

Pseudomonas aeruginosa is a nonfermentative aerobe that derives its energy from oxidation rather than fermentation of carbohydrates. Although able to use more than 75 different organic compounds, it can grow on media supplying only acetate for carbon and ammonium sulfate for nitrogen. Furthermore, although an aerobe, it can grow anaerobically, using nitrate as an electron acceptor. This organism grows well at 25° C to 37° C, but can grow slowly or at least survive at higher and lower temperatures. Indeed, the ability to grow at 42° C distinguishes it from many other Pseudomonas species. In addition to its nutritional versatility, P aeruginosa resists high concentrations of salt, dyes, weak antiseptics, and many commonly used antibiotics. These properties help explain its ubiquitous nature and contribute to its preeminence as a cause of nosocomial infections.

**Pathogenesis**

<table>
<thead>
<tr>
<th>TABLE 27-1</th>
<th>Products of P aeruginosa Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
<td><strong>Incidence of Production (%)</strong></td>
</tr>
<tr>
<td>Endotoxin</td>
<td>100</td>
</tr>
<tr>
<td>Heat-stable hemolysin</td>
<td>65</td>
</tr>
<tr>
<td>Leukocidin</td>
<td>4</td>
</tr>
<tr>
<td>Phospholipase C</td>
<td>70</td>
</tr>
<tr>
<td>Pigments (pyocyanin and fluorescein)</td>
<td>60</td>
</tr>
<tr>
<td>Proteases (elastase and alkaline protease)</td>
<td>60</td>
</tr>
<tr>
<td>Toxin A</td>
<td>50</td>
</tr>
<tr>
<td>Exoenzyme B</td>
<td>90</td>
</tr>
</tbody>
</table>

Pseudomonas aeruginosa produces many factors that may contribute to its virulence. Table 27-1 lists some of them. Almost all strains of P aeruginosa are hemolytic on blood agar plates, and several different hemolysins have been described. A heat-stable hemolytic glycolipid consisting of two molecules each of L-rhamnose and L-β-hydroxydecanoic acid has been purified. Although this hemolytic glycolipid is not very toxic to animals (5 mg injected intraperitoneally is required to kill a mouse), it is toxic to alveolar macrophages. Furthermore, P aeruginosa strains isolated from respiratory tract infections produce more hemolysin than do environmental
Correlation of hemolysin production with infections of other sites has not been reported.

Several heat-labile protein hemolysins also have been described. One of these hemolysins may be identical to phospholipase C, which is produced by approximately 70 percent of all clinical strains of P aeruginosa. Phospholipase C, which hydrolyzes lecithin, is of unknown toxicity, and its role in P aeruginosa infections also remains unknown. Some strains of P aeruginosa produce a thermolabile protein (leukocidin), which lyses leukocytes from many species including humans but is nonhemolytic. This leukocidin (also called cytotoxin) damages lymphocytes and various tissue culture cells and is very toxic to mice (minimum lethal dose is 1 μg). Despite its toxicity, the role of leukocidin remains unknown.

Some strains of P aeruginosa produce large amounts of extracellular polysaccharide. These mucoid strains usually are isolated only from patients with cystic fibrosis. The role of these polysaccharides in the pathogenesis of P aeruginosa chronic lung infections is unknown, but they may impede phagocytosis and impair diffusion of antibiotics and thus facilitate colonization and persistence. Interestingly, mucoid strains are frequently deficient in production of elastase, toxin A, and flagella, and their LPS lacks long polysaccharide side chains.

Most strains of P aeruginosa also produce one or more pigments, the most common being pyocyanin (a phenazine pigment) and fluorescein. These pigments are nontoxic in animals. Pyocyanin, however, retards the growth of some other bacteria and thus may facilitate colonization by P aeruginosa. One or more of these pigments appear to function in iron acquisition by P aeruginosa. Additional work is needed to clarify the role of these pigments in P aeruginosa infections.

Approximately 90 percent of P aeruginosa strains produce extracellular protease. Three separate proteases have been purified that differ in pH optimum, isoelectric point, and substrate specificity. Although all are capable of digesting casein, one of them, protease II, also digests elastin. When injected into the skin of animals, purified P aeruginosa proteases induce formation of hemorrhagic lesions, which become necrotic within 24 hours. These proteases also cause rapid tissue destruction when injected into the cornea of animal eyes or into rabbit lungs; they also probably contribute to the tissue destruction that accompanies P aeruginosa eye or lung infections and may aid bacteria in tissue invasion. Their effects, however, appear to be localized, and they are not highly toxic to animals (LD50 - approximately 200 μg/mouse) (Table 27-1).
**Toxin A**

Toxin A, the most toxic known extracellular protein of *P. aeruginosa*, is produced by 90 percent of all strains. The median lethal dose of pure toxin A is about 0.2 μg/mouse. Its toxicity has been attributed to its ability to inhibit protein synthesis in susceptible cells. It achieves this by catalyzing the transfer of the ADP-ribosyl moiety of nicotinamide adenine dinucleotide (NAD) onto elongation factor 2 (EF-2) according to the following reaction:

\[
\text{Toxin A} \quad \text{NAD} + \text{EF-2} \rightarrow \text{ADP-ribosyl-EF-2} + \text{nicotinamide} + \text{H}^+.
\]

The resultant ADP-ribosyl-EF-2 complex is inactive in protein synthesis. This intracellular mechanism of action of toxin A is identical to that of diphtheria toxin fragment A (see Ch. 32). Also like diphtheria toxin, *Pseudomonas* toxin A is released by *P. aeruginosa* as a proenzyme. Toxin A is toxic to animals and cultured cells, but the proenzyme has little or no enzymatic activity. Table 27-2 shows the relationship between the various forms of toxin A and their enzymatic activity and mouse toxicity. Evidence suggesting that toxin A may be a major virulence factor of *P. aeruginosa* includes observations that toxin A-deficient mutants are less virulent in several animal models than their toxin A-producing parental strains, as well as the observation that most patients surviving *P. aeruginosa* sepsis have elevated levels of antitoxin A antibody or are infected with strains that produce little or no detectable toxin A in vitro. These studies need to be expanded before firm conclusions can be reached.

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Molecular Mass (Da)</th>
<th>Mouse LD₅₀ (μg)</th>
<th>Maximum ADP-ribosyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native toxin</td>
<td>70,000</td>
<td>0.2 μg</td>
<td>1.10</td>
</tr>
<tr>
<td>Reduced and denatured</td>
<td>70,000</td>
<td>&gt;500 μg</td>
<td>35</td>
</tr>
<tr>
<td>toxin A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragment A</td>
<td>27,000</td>
<td>&gt;500 μg</td>
<td>100</td>
</tr>
<tr>
<td>Fragment B</td>
<td>43,000</td>
<td>Not tested</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Toxin A was preincubated in 4 M urea plus 1 percent dithiothreitol for 15 minutes*
Exoenzyme S

A second ADP-ribosyltransferase, exoenzyme S, has been described. Exoenzyme S catalyzes the transfer of ADP-ribose onto a number of GTP-binding proteins, including the product of the proto-oncogene c-H-ras (p21C-H-ras); however, it does not modify elongation factor 2. Exoenzyme S is produced by about 90 percent of clinical isolates of P aeruginosa. Transposon-induced S-deficient mutants are less virulent in several animal models than is their S-producing parental strain; thus, exoenzyme S may be involved in the pathogenesis of some P aeruginosa infections.

Host Defenses

Although 85 percent of P aeruginosa isolates are resistant to serum alone, 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; however, once a P aeruginosa infection is established, other antibodies, such as antitoxin, may be important in preventing death. Although evidence suggests interaction between P aeruginosa and the cellular immune system, patients with diseases characterized by impaired cellular immune responses (e.g., Hodgkin’s disease) do not have an increased incidence of severe P aeruginosa infections. However, patients with diminished antibody responses caused by underlying disease or its associated therapy, have more serious P aeruginosa infections. This underscores the importance of the humoral response in controlling P aeruginosa infections. Cystic fibrosis is the exception. Most cystic fibrosis patients have high levels of circulating antibodies to many bacterial antigens, but are unable to clear P aeruginosa efficiently from their lungs.

Epidemiology

Pseudomonas aeruginosa commonly inhabits soil, water, and vegetation. It is found in 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, 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.

Several different typing systems are available for epidemiologic studies: serologic, phage, pyocin, and DNA fingerprinting. In the pyocin system, pyocins (bacteriocins or aeruginocins) produced by the test strain are assayed for bactericidal activity against a series of indicator strains. A number of different serologic typing systems are used. Some employ combinations of heat-stable and heat-labile antigens, whereas others use only heat-stable antigens. No system is universally accepted. Recently, DNA fingerprinting has identified probes that are useful in typing P aeruginosa strains.

**Diagnosis**

Diagnosis of P aeruginosa depends on its isolation and laboratory identification. It grows well on most laboratory media and commonly is isolated on blood agar plates 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 radiation helps in early identification of P aeruginosa colonies and also is useful in suggesting its presence in wounds. Other pseudomonads are identified by specific laboratory tests.

**Control**

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 mafenide or silver sulfadiazine, coupled with surgical debridement, has dramatically reduced 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, making susceptibility testing essential. The combination of gentamicin and carbenicillin is frequently used to treat severe Pseudomonas infections, especially in patients with leukopenia. Several types of vaccines are being tested, but none is currently available for general use.