# CHAPTER III

## LITERATURE REVIEW

## I *Pseudomonas aeruginosa*

### General characteristics

*P. aeruginosa* is motile with polar flagella and rod-shaped organism, measuring about 0.6x2 μm. It is gram-negative and occurs as single cell, in pairs, and occasionally in short chains. It is an obiligate aerobe that grows readily on many types of culture media. Some strains producing a sweet or grape-like odor and hemolyze blood. Most strain produce characteristic blue green pigment, pyocyanin, fluorescein (pyoverdin the dark red pigment pyorubin or black pigment pyomelanin (11). *P. aeruginosa* can produce multiple colony types, on a giving the impression of a culture of mixed species of bacteria. These different colony types may also have different biochemical and enzymetic activities and even antimicrobial susceptibility patterns. From patients with cystic fibrosis, colonies of *P. aeruginosa* are very mucoid thus due to overproduction of alginate, and exopolysaccharide. *P. aeruginosa* grows well at 37-42°C. The organism can be differentiate from the other *Pseudomonas* species by the ability to grow at 42°C. It is oxidase-positive and does not ferment carbohydrates, but many strains oxidize glucose. Identification is usually based on colonial morphology, oxidase test, presence of characteristic pigments, and growth at 42°C. Differentiation of *P. aeruginosa* from other pseudomonads requires testing with a large battery of test.

### Natural Habitats

*P. aeruginosa* is environmental organisms found in water and soil. It is distributed worldwide. Because of their abilities to survive in aqueous environments, these organisms have become particularly problematic in the hospital environment. *P. aeruginosa* has been found in a variety of aqueous solutions, including disinfectants, ointments, soaps, irrigation fluids, eyedrops, and dialysis fluids, and in dialysis equipment. It is frequently found in the aerators and traps of sinks, whirlpool, hydrotherapy baths, respiratory therapy equipment, and showerheads. *P. aeruginosa* is also found on the surfaces of many types of raw fruits and vegetables. Therefore, profoundly immunosuppressed individuals are not allowed these foods, because ingestion of the pseudomonads with subsequent colonization and translocation may lead to bacterimia. Inconclusive, almost any site in the hospital may harbor the organism, and can be some of infection. In addition to its nosocomial sources, *P. aeruginosa* may be found in swimming pools, hot tubs, contact lens solutions, cosmetics, illicit injectable drugs, and the innersoles of sneakers.

*P. aeruginosa* is uncommonly isolated from healthy individuals. Colonization of the organism in the respiratory tract is common in hospitalized individuals, especially who are intubated. The likelihood of colonization increases the longer the patient is hospitalized. And colonized in the gastrointestinal tract can be occurred in one who ingested *P. aeruginosa* contaminated food (17).

**Pathogenesis**

*P. aeruginosa* seldom caused infection in healthy individuals, but prone to be the major cause of infection in the hospitalized patients particularly the patients with underlying disease such as cystic fibrosis or cancer. *P. aeruginosa* is pathogenic only when introduced into areas devoid of normal defenses for instance disrupted mucous catheter membranes and skin direct tissue damage, retained urinary catheters. The bacterium adheres and colonized on the mucous membrane or skin, then invades, and

can produces systemic disease. These processes promoted by structural factors such as pili, enzymes, and toxins. Lipopolysaccharide endotoxin, plays a direct role in causing fever, shock, oliguria, leukocytosis or leukopenia, disseminated intravascular coagulation, and adult respiratory distress syndrome.

P. *aeruginosa* has a number of virulence factors, including structural components, exotoxin, endotoxin, and enzymes (Table 1). P. *aeruginosa* virulence is multifactoral.

## Virulence factor :

### 1. Pili

The polar pili of P. *aeruginosa* are long linear filaments with a mean diameter of 5.2 nm and an average length of 2500 nm (18) and are composed of a single protein termed pilin that generally has a size of ~15kDa (19). Pilin is initially synthesized as larger precursor that is proteolytically modified in the membrane and then reversibly assembled into a functional pilus in the outer membrane (20).

Pili is used by P. *aeruginosa* as adhesins to mediate attachment to both human buccal epithelial cell (BEC) and human tracheal epithelial cell (TEC) (21). Thus a number of systems have been used to examine P. *aeruginosa* adherence, and a clear role for pili as adhesins has been established. Equilibrium analysis of P. *aeruginosa* binding to BEC in the presence of purified pili indicates that pili inhibit the adherence of whole cells by a competitive inhibition mechanism and thus bind to the same receptors as do whole bacteria. Schmidt et al (22) found considerable homology between linear section of the pilin associated proteins that function as the adhesin for the E. *coli* digalactoside-binding pilin. Further, they found that synthetic peptides of these sequences induced the synthesis of protective antibody that prevented colonization and infection in an animal model.

Variability in the sequence of *P. aeruginosa* pilin accounts for significant variation in pilus affinity for respiratory epithelial cells, and may allow for a degree of variation in the structure required for pilus receptor activity. The affinity and the number of receptors present on a BEC cell surface varies substantially from strain to strain. A given strain's virulence may thus directly depend upon the pilin produced.

### 2. Polysaccharide

A polysaccharide matrix or glycocalyx is secreted by *P. aeruginosa* as an aid to colonization. This glycocalyx is believed to provide a protected microenvironment, inside which the invading organism forms microcolonies and grows. Recent evidence has shown that the glycoprotein component of thes matrix traps secreted proteases and contains metalloprotease activity (21). These metalloproteases degrade collageneous and non-collageneous components of the intact basement membranes to oligopeptides and other low molecular weight products. The extracellular release of proteases thus contributes to the virulence of the invading organism.

### 3. Capsule

Another virulence factor is surface polysaccharide. It protects the organism from phagocytosis. Polysaccharide capsules are responsible for the mucoid colonies seen in cultures from patients with cystic fibrosis. This layer can also anchor the bacteria to cell surfaces, particularly in patients with cystic fibrosis or other chronic respiratory diseases who are predisposed to colonization with mucoid strains of *P. aeruginosa*. The lipopolysaccharide, which exists in multiple immunotypes, is responsible for many of the endotoxic properties of the organism. There are more

than one lipopolyssaccharide immunotype and piocin(bacteriocin) susceptibility pattern.

The mucoid colony type of *P. aeruginosa* is associated with presence of alginic acid, and extracellular polysaccharide composed of L-guluronic and D-mannuronic acid.(23) The alginic acid or mucoid substance of Hoiby(24) following synthesis and excretion remains loosely associated with the *P. aeruginosa* cell envelope in what has been termed "slime layer" or perhaps what more appropriately may be termed a peripheral capsule.(25)

The presence of an alginic acid peripheral capsule appears to decrease susceptibility of the organism to antibiotics and to surface-active agents such as deoxycholate.(26) The peripheral capsule also inhibits phagocytosis of *P. aeruginosa* by macrophages *in vitro* (27) and appears to enhance the survival of the organism in high oxygen tension environments, possibly by the scavenging of free radicals.(28)

The expression of the peripheral capsule or mucoid phenotype is highly regulated, with expression dependent upon several regulatory loci that are separated from the structural genes for the synthesis and export of alginate.(29) It is now evident that even nonmucoid *P. aeruginosa* strains have some cell surface-localized alginate present in the form of a partial microcapsule.(30) The microalginate capsule is considerably smaller in dimension that the capsule found on mucoid strains, which appear to have lost the ability to regulate the synthesis of alginate and thus the size of their capsule.

Elucidation of the role of the *P. aeruginosa* capsule has been complicate by the realization that mucoid strains and their alginate varies considerably.(31) Alginate has been shown to vary tremendously in the ratio of guluronic to mannuronic acid and in degree of acetylation.(32) Structural variation in alginates from various strains is also reflected immunologically, several discrete alginate epitopes have been identified. (33) In addition, various lectins (rat lung, human fetal lung and muman placental) also bind to specific structural forms of *P. aeruginosa* alginates.(34) Several authors have

suggested that alginate may be involved in the adhesion of the pathogen to epithelial surface.

### 4. Exotoxin A

Exotoxin A is the most toxic product of *P. aeruginosa* and, with rare exceptions, almost every *P. aeruginosa* strains produces exotoxin A. Exotoxin is lethal for vertebrates with an experimentally determined $LD_{50}$ for mice in the range of 2.5 µg/kg. This is approximately 10,000 times the toxicity associated with *Pseudomonas* lipopolysaccharide (21). Exotoxin A is also lethal for rats rabbits, dogs, and rhesus monkeys, although clinical aspects vary among species. In mice, death occurs within 48 hr following toxin infection and appears to be associated with severe liver pathology (35). Early hepatocellular necrosis was observed consistently, indicating that liver toxicity appears to be a major factor in the pathology associated with exotoxin A (36). This has become an important consideration in studies involving the use of exotoxin A or active fragments in the construction of immunotoxin conjugates or chimeric proteins for use indirect cytotoxicity applications. In addition to in vivo studies, several in vitro studies have shown that exotoxin A is cytotoxic for a variety of cultured cells (37)

Several studies, taken together, indicate that exotoxin A contributes to the pathogenesis associated with *P. aeruginosa* infections. The fact that exotoxin A is a potent inhibitor of eucaryotic protein synthesis is a clear indication of its pathogenic potential. It has been established that exotoxin A is lethal when administered to various species. Clinical experience indicates that exotoxin A is produced in human infection and those patients recovering from Pseudomonas infections exhibit significant anti-exotoxin A titers. Analysis of clinical infections indicated that increased chance of survival was associated with high anti-exotoxin A titers (38).

## 5. Exoenzyme S

This extracellular toxin is produced by one third of the clinical isolates of *P. aeruginosa* and can inhibit protein synthesis in the host cells. Exo-S is reported to catalyze the transfer of ADP-ribose from NAD to a number of eucaryotic proteins. Nicas and Iglewski report two forms of exo-S; a larger 53-kD form that is enzymatically inactive, and a 49-kD form that has ADP-ribosyltransferase activity.(39) Exo-S purification from *P. aeruginosa* has been described by Woods et al.(40) in a report that indicated exo-S has an estimated molecular weight of 105,000 (by gel filtration analysis) and appears to consist of a complex of smaller components as revealed by SDS-PAGE analysis. A physical description of exo-S remains unclear. The cloning of the structural gene, and therefore the determination of the gene sequence for exo-S, have not been reported.

Several studies have attempted to determine whether exo-S plays a role in pathogenesis. The most convincing studies have employed a genetic approach using transposon insertional mutagenesis to create mutant strains that have lost the ability to produce exo-S. The virulence of these mutant strains was compared with the wild-type strain in both the chronic lung infection model (41) and the burned mouse model.(42) In both model systems evidence indicates that mutants deficient in exo-S are less virulent than the wile-type strain.

## 6. Elastase

Elastase was named because of its ability to degrade elastin. Several studies show that elastase is clearly involved in the pathogenicity associated with *Pseudomonas* infections.(43) The precise role elastase plays in the pathogenic process depends upon the type of infection, but it is clear that this proteolytic enzyme is capable of contributing to the destruction of host tissue or defenses in numerous ways.

Because approximately 85% of *P. aeruginosa* strains have been shown to posses elastolytic activity, the probability is great that elastase will be involved in pathogenesis.(44)

The toxic and biological properties of purified elastase have been clearly demonstrated. The $LD_{50}$ for mice varies between 60 and 400 µg, depending upon the route of inoculation, indicating that elastase is considerably less toxic than Exotoxin A. (45) Of particular note is the ability of purified elastase to degrade a number of biologically important molecules that include various complement components, the immunoglobulins IgG, IgA and secretory IgA, as well as fibrin and human collagens. (46) Elastase has also been reported to inactivate the human alpha-1-proteinase inhibitor and recent evidence suggests that elastase contributes to the inactivation of human gamma interferon. In addition, studies using purified elastase have demonstrated that elastase damages pulmonary and corneal tissue and has direct effect of epithelial junctions.(47)

**Protease**

*Pseudomonas* isolated from a variety of environmental sources and from the tissues of infected patients secrete the protease, which are probably instrumental in initiating and controlling the tissue invasion and necrosis characteristic of *Pseudomonas* infections.

Whereas there is no clearly defined role for proteases in the growth of *Pseudomonas* in the natural environment, there is a wealth of direct and indirect evidence for their role in tissue invasiveness, injury, and necrosis in the host. Protease synthesis is associated with organisms isolated from acute lung infections.(48) Similarly, corneal infections, and experimental alveolar necrosis and hemorrhage, which closely resemble the pathology observed in patients with *P. aeruginosa*

pneumonia, are also associated with protease production. In addition, purified proteases injected intradermally have hemorrhagic and necrotic effects in skin. (49)

Two observations in particular indicate that proteases are released extracellularly *in vivo*. First, the proteases have been detected in the sputum of cystic fibrosis(CF) patients, many of whom suffer from chronic *Pseudomonas* infections of the respiratory tract.(50) Second, antibodies to the proteases are often detected in patents colonized by *Pseudomonas*.(51) The proteases themselves are not cytotoxic, but degrade many extracellular matrix components that are constituents of connective tissues, including elastin, collagen types III and IV.(52)

### 8. Phospholipases

In recent years an increasing number of reports have concerned the production and secretion of phospholipase enzymes by *P. aeruginosa*. A 78-kD protein with phospholipase and hemolytic activity was described by Kurioka and liu.(53) Designated phospholipase C (PLC), this secreted protein was described as a hemolytic toxin that might be associated with the virulence of *P. aeruginosa*. Liu observed that the production of the phospholipase C hemolysin was phosphate-regulated and proposed that phospholipase C and alkaline phosphatase acted together as a phosphate scavenging system. Phospholipase C has the ability to degrade phospholipids commonly found in the membranes of eucaryotic cells but not present in membranes of procaryotic cells. The process of phospholipid degradation also produces diacylglycerol, which can result indirectly in toxic side effects in animals.(54)

Many current investigations regarding phospholipase activity in *Pseudomonas* have used a genetic approach. For example, a series of recent papers have described the cloning and expression of the PLC structural gene (*plc*S) in both *E. coli* and *P. aeruginosa* host strains. The construction of a mutant strain with a deletion of the entire *plc*S gene led to the discovery of a second, nonhemolytic phospholipase protein,

PLC-N (55). The structural gene that encodes for the non-hemolytic phospholipase (plcN) has been cloned and sequenced, revealing remarkable sequence homology between the plcS and plcN gene products (56). The non-hemolytic PLC-N is 73.5 kD, whereas the hemolytic PLC-H has a molecular weight of 78.4 kD (57). Sequence comparison analysis indicates two domains or regions with high homology: one at the amino terminus (70%) and a middle region that contains 81% homology.

The role of the phospholipases in the virulence of Pseudomonas has yet to be defined. Initial studies indicated that purified PLC-H caused numerous pathologic consequences, including death, when injected into mice (58) and can cause the aggregation of human platelets. Another study has shown that purified PLC-H induces a series of inflammatory responses in mice.(59) An earlier work using PLC-containing culture supernatants from Pseudomonas strains isolated from infected patients provided evidence that PLC caused the release of inflammatory mediators from rat peritoneal mast cells and human granulocytes.(60) Additional evidence in support of the importance of these factors in pathogenicity comes from an analysis of mutant strains in which the plcSR operon genes have been insertionally inactivated or deleted.(61) These mutant strains were tested in the burned mouse model to determine the effect of the loss of the hemolytic phospholipase(PLC-G) or the plcR gene products on the virulence of the organism. When grown under phosphate limiting conditions, the mutant strains exhibited a 200 to 10,000-fold reduction in virulence, in comparison with the PAO1 wild-type strain; however, under high phosphate conditions, only a modest difference (10-fold) was seen, perhaps because phosphate regulates the production of PLC-H. Of potential significance in these studies is the observation that the plcR mutant strain is more hemolytic, but less virulent than the wild-type PAO1 strain, which implies that the plcR gene products may have some role in pathogenesis. However, at the present time the role of the plcR genes remains unresolved.

Table 1 Virulence Factors Associated with *Pseudomonas*

| Virulence factors | Biological effects |
|---|---|
| Pili | Adherence to respiratory epithelium |
| Polysaccharide capsule | Adherence to tracheal epithelium; antiphagocytic |
| Exotoxin A | Inhibition of protein synthesis |
| Exoenzyme S | Inhibition of protein synthesis |
| Elastase | Vascular tissue damage; inhibition of neutrophil function |
| Alkaline protease | Tissue damage; anticomplementary; inactivation of IgG; inhibition of neutrophil function |
| Phospholipase C | Tissue damage |

**Clinical Significance**

*P. aeruginosa* is the most important human pathogen in the genera *Pseudomonas* and *Burkholderia* with respect to both the numbers and types of infections caused and their associated morbidity and mortality. It successfully combines adaptability to a variety of moist environments with a collection of potent virulence factors. The spectrum of disease caused by this agent ranges from superficial skin infections to fulminant sepsis.

The relative frequency of infections caused by *P. aeruginosa* has increased dramatically over the last 30 years. Two main factors appear to account, in large part, for this change: (1) advances in medical treatments and technologies that have resulted in the prolonged survival of immunologically impaired hosts; and (2) the increased prevalence of gram-negative bacillary nosocomial infections as a consequence of

widespread antibiotic use.(62) Infections with *P. aeruginosa* continue to be associated with a higher mortality than other gram-negative bacillary infections. *P. aeruginosa* has been associated with a variety of clinical syndromes and can involve nearly every organ system.

### a. Bacteremia

Bacteremia due to *P. aeruginosa* is almost exclusively a nosocomial infection and usually occurs in patients with severe disruptions in their immunocompetence. Predispositions include granulocytopenia, especially in those who become colonized with *Pseudomonas*, hematologic malignancies, previous antibiotic or corticosteroid therapy, prematurity, congenital disease, organ transplants, traumatic injury, or the presence of a serious underlying disease.(63)

Between the first clinical description of bacteremia in 1890 and the first extensive literature review on the subject in 1947, 91 cases had been reported.(64) Since that time, *P. aeruginosa* has accounted for 7-18% of all episodes of gram-negative septicemia and has become an important cause of septicemia in neutropenic patients.(65) The fatality rate from *Pseudomonas* sepsis, ranging from 38% to96%, exceeds that from all other causes of gram-negative sepsis. *P. aeruginosa* is also involved in polymicrobial bacteremias in susceptible hosts.(66)

### b. Endocarditis

Prior to the last two decades, infective endocarditis caused by *P. aeruginosa* was extremely rare. It accounted for less than 0.25% of all cases seen at The New York Hospital in the 30-year period preceding 1973.(67) Almost two-thirds of those cases of endocarditis had evidence of underlying heart disease. Since that time, there has been a marked increase in the prevalence of *P. aeruginosa* endocarditis

The major predisposition to gram-negative bacillary endocarditis is the illcit parenteral use of drugs, usually heroin, although recent reports have emphasized the role of other illicit drugs.(68) Some of these agents, such as pentazocine and tripelennamine, may confer a selective survival advantage to particularly virulent serotypes of *P. aeruginosa*.(69) In 348 cases of gram-negative endocarditis reported in 1980, 32% occurred in intravenous drug users and 10% occurred in patients with prosthetic valves, the other major risk factor for this disease. In that study *P. aeruginosa* accounted for 58% of all addict-associated gram-negative endocarditis, although a marked regional variation in etiologies was noted.(70)

The disease in parenteral drug users usually occurs on native valves, and it has been hypothesized that the valves of these individuals are subject to injury secondary to the foreign materials mixed with the narcotics, or previous bouts of endocarditis. The injury results in fibrotic changes on the valves, creating a potential nidus of infection.(71) This may explain why more than 25% of these cases had no prior history of cardiac abnormalities. The source of the organism has been hypothesized to be tap water or other environmental sources contaminating drug paraphernalia. *P. aeruginosa* may be readily cultured from syringes and other equipment used by addicts.(72)

### c. Respiratory infections

In the early clinical reviews, *P. aeruginosa* was noted to uncommonly cause pneumonia. Recent data, however, have shown this organism to be an important cause of nosocomial pneumonia, accounting for approximately 15% of these cases, largely in intensive care unit settings. It is usually acquired via endogenous aspiration of organisms from a colonized oropharynx. The susceptibility to colonization is inversely related to the basic health of an individual. *P. aeruginosa* is a rare cause of community acquired pneumonia.(73)

This infection occurs almost exclusively in patients with compromised local or systemic host defenses. Disorders that impair local defenses in the pulmonary system and predispose to *P. aeruginosa* pneumonia include cystic fibrosis, tracheostomy, tracheal intubation, or the use of contaminated respiratory inhalation equipment.(74) Serious underlying diseases such as heart failure and emphysema also appear to predispose patents to this infection. Malignancies, especially of the hematologic variety, appear to predispose the host to the bacteremic form of *Pseudomonas* pneumonia. The mortality rate from the bacteremic form of pneumonia is approximately 80%,(75) higher than that of primary, nonbacteremic pneumonia.

### d. Urinary Tract Infections

Primary infection of the urinary tract with *P. aeruginosa* is almost always either a nosocomial or iatrogenic event. Occasionally the genitourinary tract will be infected secondarily from a hematogenous source,(63) but more often will serve as a source of bacteremia.

Whereas *Pseudomonas* accounts for less than 1% of the isolates in populations with recurrent urinary tract infections, it causes approximately 11% of nosocomial genitourinary tract infections.(76) The most important predispositions appear to be the presence of urinary catheters and other foreign bodies; genitourinary tract manipulation; anatomic abnormalities of the urinary tract; and spinal cord injury.(77)

### e. Wound Infections

Because of its ubiquitous presence in nature, particularly in moist environments, *P. aeruginosa* is a common isolate from wounds. This organism was noted to account for 25% of isolates in the wound infections of Israeli soldiers after the Yom Kippur War, and 67% of the wound isolates in soldiers in Vietnam.(78)

Pseudomonas was most often isolated after multiple days of hospitalization, reflecting its status as a nosocomial pathogen.(79)  It should be noted that the presence of *P. aeruginosa* in a wound may only indicate colonization in the absence of clinical infection may lead to secondary bacteremia.

*P. aeruginosa* is also a major cause of burn wound sepsis and a significant cause of morbidity and mortality in these patients. *Pseudomonas* tends to colonize the burn site, and these patients are immunocompromised due to the destruction of their mechanical barrier defense and the variety of immunologic defects related to serious thermal injury.(80)

### g. Cystic Fibrosis

Cystic fibrosis(CF) is the most common lethal genetic disease in white populations, affecting approximately 1 in 2000 to 1 in 4000 live births in Europe and North America.(81)  Fifty-three years after it was first described, the gene responsible for the generalized metabolic disorder was identified on chromosome 7.(82)  It is expressed in a variety of tissues that are affected in CF patients, such as the lungs, pancreas, liver, sweat glands, and nasal epithelia. The gene encodes a membrane protein that most probably regulates ion transport and/or is an ion channel itself, and was therefore named "Cystic Fibrosis Transmembrane Conductance Regulator" (CFTR).  The major mutation in CFTR, present in about 70% of the CF mutant chromosomes, is the deletion of a single amino acid residue at position 508.  The frequency of $\Delta$F508 varies considerably depending on the geographic location; so far, more than 60 mutations in CFTR differing from $\Delta$F508 have been reported from CF patients.(83)

Bacterial colonization and infection of the CF respiratory tract occurs soon after birth.  Almost invariably, *Staphylococcus aureus* is the first detectable pathogen. Anaerobes, fungi, and a number of gram-positive and gram-negative bacteria may also

be found, but *P. aerruginosa* is the dominant pathogen.(84) Difficult to treat and never eradicated, *P. aeruginosa* remains in the airways of CF patients until their premature death. It is the bacterial lung infection, most notably with *P. aeruginosa*, that causes progressive pulmonary disease and therefore plays a central role in CF, because it is the direct cause of death in the vast majority of these patients.(85) Nevertheless, a large heterogeneity exists among CF patients with respect to the severity of the disease, pancreas or liver involvement, and the onset and development of *P. aeruginosa* lung infection, even in patients homozygous for $\Delta$F508.(86)

Major advances in prolonging the lives of CF patients have been made possible by the development of pancreatic enzyme replacement therapy to treat the more or less impaired pancreas function and by improved antibiotic treatment regimes to reduce the number of *P. aeruginosa* organisms in the airways and, as a consequence, to reduce airway obstruction and inflammation.(87) Thus, a median survival of 25-30 years is now common in countries where these drugs are available and are used to treat CF patients, whereas life expectancy is still below 5 years in countries without such treatment.

**Antimicrobial Susceptibility**

Community-acquired isolates of *P. aeruginosa* are usually susceptible to the antipseudomonal penicillins (ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, and mezlocillin), the aminoglycosides (gentamicin, tobramycin, and amikacin), ciprofloxacin, cefoperazone, ceftazidime, and imipenem. Susceptibility is less predictable for ticarcillin, carbenicillin, other broad-spectrum cephalosporins (ceftriaxone and cefotaxime), fluoroquinolones other than ciprofloxactin, and the monobactam aztreonam. The organism is uniformly resistant to antistaphylococcal penicillins, ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, tetracyclines, macrolides, rifampin, cholramphinicol trimethoprim-sulfamethoxazole, first- and

second- generation cephalosporins, and oral broad-spectrum cephalosporins (cefixime and cefpodoxime).

Nosocomially acquired *P. aeruginosa* isolates tend to be more resistant than community-acquired strains to antimicrobial agents, frequently displaying resistance to multiple calsses of antimicrobial agents. This multidrug resistance is of particular concern in chronically infected CF patients. Development of resistance during monotherapy with either cell wall-active agents (antipseudomonal penicillin, cefoperazone, and ceftazidime) or ciprofloxacin occurs frequently and is usually due to mutation.

Erika D, et al reported that P. aeruginosa was the most common species from ceftazidime-resistant gram-negative bacilli (CAZ-RGN) from patients in two surgical intensive care units (SICU) during a nonoutbreak period (88).

**Typing Method of *P. aeruginosa***

**Classification of Typing Methods**

A convenient way of classifying typing systems is to divide them into phenotvpic techniques, i.e., those that detect characteristics expressed by the mecroorganisms, and genotypic techniques, i.e., those that involve direct DNA-based analyses of chromosomal or extrachromosomal genetic elements.

**Phenotypic Techniques**

Typing methods that assess phenotypic differences are inherently limited by the capacity of microorganisms to alter the expression of the underlying genes.(89) Such changes may occur unpredictably or in response to various environmental stimuli.(90) In addition, point mutations representing a single nucleotide in the entire

chromosome can result in the abnormal regulation or function of the gene responsible for a particular phenotype. Thus, isolates that represent the same strain and are gene tically indistinguishable(or almost so) can vary in the phenotype detected. Some phenotypic approaches, such as serotyping and bacteriophage typing, require specific reagents for detecting individual types. Since the available materials may not be appropriate for all strains, a relatively large fraction of strains may give a null phenotype and consequently be nontypeable.

### 1. Biotyping

Biotyping makes use of the pattern of metabolic activities expressed by an isolate and may include specific biochemical reactions, colonial morphology, and environmental tolerances (e.g., the ability to grow on certain media or at extremes of pH or temperature). In general, however, biotyping has only limited ability to differentiate among strains within a species, and consequently, the technique had relatively poor discriminatory power.

### 2. Antimicrobial Susceptibility Testing

Clinical microbiology laboratories routinely test most bacterial isolates for susceptibility to a panel of antimicrobial agents. Both manual and automated methods are widely available, rigorously quality controlled, typically easily performed, and relatively inexpensive. These data are readily available to clinicians and infection control practitioners. The identification of a new or unusual pattern of antibiotic resistance among isolates cultured from multiple patients is often the first indication of an outbreak. Antibiotic susceptibility testing has relatively limited utility not only because phenotypes vary but also because antibiotic resistance is under extraordinary selective pressure in contemporary hospitals. There are multiple genetic mechanisms

by which a given strain can become abruptly resistant to a particular antibiotic. These include spontaneous point mutations and the acquisition of specific resistance genes via plasmids and transposons from other strains or even other species. Since a single plasmid or compound transposon can carry multiple resistance determinants, resistance to multiple antimicrobial agents may be acquired simultaneously. Since a single plasmid or compound transposon can carry multiple resistance determinants, resistance to multiple antimicrobial agents may be acquired simultaneously. On the otherhand, in the absence of specific selective pressure, such elements may be lost (91). As a consequence of these various genetic mechanisms, different strains may develop similar resistance patterns and, conversely, the susceptibility patterns of sequential clinical isolates representing the same strain may differ for one or more antibiotic (92).

Eventhough the antibiotic susceptibility patterns appeared more discriminative than the genotypic methods, antibiotic susceptibility testing has relatively limited utility in epidemiological studies because antibiotic resistance is affected by extraordinary selective pressure in contemporary hospitals. (93)

### 3. Serotyping

Serologic typing (serotyping) is based on the long-standing observation that microorganisms of the same species can differ in the antigenic determinants expressed on the cell surface. Many different surface structures, including lipopolysaccharides, capsular polysaccharides, membrane proteins, and extracellular organelles (e.g., flagella and fimbriae) exhibit such antigenic variation.

The available rapid, reliable serologic assays for detecting antigen-antibody reactions use a wide variety of methods, including direct agglutination, latex coagglutination, enzyme labelling, and fluorescence labelling. If high-quality commercial reagents are not available, the preparation of specific typing antibodies is a difficult process and is generally restricted to reference and research laboratories.

Serotyping often exhibits poor discriminatory power, even for bacterial species with large numbers of antigenic variants, because many strains may represent only a few serotypes or may be nontypeable.

## 4. Bacteriophage and Bacteriocin Typing

Among species for which numerous lytic bacteriophages (i.e., viruses capable of infecting and lysing bacterial cells) have been identified, strains can be characterized by their patterns of resistance or susceptibility to a standard set of phages. Because of the need to maintain stocks of biologically active phages and control strains, phage typing is available only at reference laboratories. Even for experienced workers, the procedure is technically very demanding and suffers from considerable experimental as well as biologic variability. Since many strains are nontypeable with the standard phages, additional, experimental phages often need to be considered.

In bacteriocin typing, and isolate is assessed for susceptibility to a set of bactericidal peptides produced by selected strains (94). Although the method has been useful for certain pathogens, such as *Pseudomonas aeruginosa* (95), bacteriocin typing has limitations similar to those described for phage typing.

Table 2 Characteristics of phenotypic typing systems

| Typing system | Proportion of strains typeable | Reproducibility | Discriminatory power | Ease of interpretation | Ease of performance |
|---|---|---|---|---|---|
| Bityping | All | Poor | Poor | Excellent | Excellent |
| Antimicrobial susceptibility | All | Fair | Poor | Excellent | Excellent |
| Serotyping | Most | Good | Fair | Good | Fair |
| Bacteriophage typing | Most | Fair | Fair | Fair | Poor |

## Genotypic Techniques

Because problems with typeability, reproducibility, or discriminatory power have been associated with many phenotypic techniques, numerous systems with use of DNA-based methods have been developed. Initially these techniques were used only in a few research laboratories, but they are rapidly becoming more widely available for use in clinical practice. In addition to the technical intricacies of these methods, the substantial complexities involved in effectively interpreting the results are becoming increasingly apparent.

### 1. Restriction Endonuclease Analysis of Chromosomal DNA (REA)

A restriction endonuclease enzymatically cuts ("digests") DNA at a specific ("restricted") nucleotide recognition sequence. Both the recognition sequence of the enzyme and the composition of the DNA influence the number and sizes of the restriction fragments generated by digesting a given piece of DNA. Thus, an enzyme whose recognition sequence is composed of only guanine (G) and cytosine (C) will cut DNA with a low G+C content less frequently and consequently will generate fewer and larger restriction fragments than an enzyme recognizing sequences of only adenine and thymine. An enzyme that recognizes a sequence of 6 bp (a "6-bp cutter") typically has more recognition sites than an 8-bp cutter, will digest DNA more frequently, and consequently will generate more and smaller restriction fragments.

In conventional REA, bacterial DNA is digested with endonucleases that have relatively frequent restriction sites, thereby generating hundreds of fragments ranging from ~0.5 to 50 kb in length. Such fragments can be separated by size by using constant-field agarose gel electrophoresis, and the pattern can be detected by staining the gel with ethidium bromide and examining it under UV light. Different strains of

the same bacterial species have different REA profiles because of variations in their DNA sequences that alter the number and distribution of restriction sites. (96)

The major limitation of REA is the difficulty of comparing the complex profiles, which consist of hundreds of bands that may be unresolved and overlapping. In addition, restriction fragments derived from plasmids may confound the patterns, whose DNA can readily contaminate genomic-DNA preparations. Thus, isolates that differ only in their plasmid contents may be considered different strains.

## 2. Southern Blot Analysis of RFLPs

REA patterns are complex because the ethidium bromide stain detects all of the hundreds of fragments generated by the restriction enzymes used. In contrast, Southern blot analyses detect only the particular restriction fragments associated with specific chromosomal loci. Southern blots, named after the investigator who first described the technique(97), are prepared by digesting bacterial DNA, separation the restriction fragments by agarose gel electrophoresis, and then transferring("blotting") the fragments onto a nitrocellulose or nylon membrane. The fragment(s) containing specific sequences (loci) is then detected by using a labelled piece of homologous DNA as a probe. Under the appropriate conditions, the probe binds ("hybridizes") by complementary base pair matching only to those fragments containing identical or nearly identical nucleotide sequences. Variations in the number and sizes of the fragments detected are referred to as restriction fragment length polymorphisms (RFLPs) and reflect variations in both the number of loci that are homologous to the probe and the location of restriction sites within or flanking those loci. All strains carrying loci homologous to the probe are typeable, and the results are, in general, highly reproducible. The discriminatory power of an analysis is directly related to the frequency with which the fragments detected vary in number and/or in size. Thus, the choice of probes is a critical consideration.

Ribotyping refers to a Southern blot analysis in which strains are characterized for the RFLPs associated with the ribosomal operon(s). Operons are clusters of genes that share related functions and are often coordinately regulated; the ribosomal operons comprise nucleotide sequences coding for 16SrRNA, 25SrRNA, and one or more tRNAs. Ribosomal sequences are highly conserved, and probes prepared from isolated *E. coli* rRNA or a cloned ribosomal operon(rrn) (98) hybridize to the chromosomal ribosomal operons of a wide range of bacterial species. All bacteria carry these operons and are therefore typeable. In general, ribotypes are stable and reproducible, with isolates from an outbreak typically having the same ribotype.(99)

For organisms with multiple (five to seven) ribosomal operons, such as *E. coli* and *Klebsiella*, *Haemophilus*, and *Staphylococcus* spp., ribotype patterns commonly have 10 to 15 bands and moderate discriminatory power. Nevertheless, epidemiologically unrelated isolates not infrequently demonstrate the same pattern, limiting the utility of the method. For example, ~20% of 188 bloodstream isolates of *E. coli* from each of three geographically dispersed sites (Massachusetts, California, and Kenya) represented the same ribotype(14), although another genotypic technique (i.e., pulsed-field gel electrophoresis[PFGE];) generally resolved each isolate as a distinct strain. Isolates of this ribotype typically expressed adhesin and hemolysin virulence factor; as discussed above, virulent genotypes are frequently over represented among collections of bacterial pathogens. For bacterial species with only a single ribosomal operon, such as mycobacteria, ribotyping typically detects only one or two bands and has limited utility for epidemiological studies.(100)

## 3. Typing Systems Applying the PCR

The PCR is being increasingly applied to the detection of infectious agents. The essential feature of PCR is the ability to rapidly and exponentially replicate ("amplify") a particular DNA sequence (the "template"). The basic procedure

involves several distinct components. (i) The template should represent a relatively small fragment of DNA, typically 0.5 to 2.0 kb, because larger target sequences are difficult to amplify efficiently. Only minute quantities of the template need be present; theoretically, even a single copy is detectable, (ii) Two small oligonucleotides ("primers"), corresponding to sequences at opposite ends of the template, define the sites at which replication is initiated. The primers should be long enough to define those sites uniquely; statistical calculations indicate that 18 to 20 bp is typically sufficient. A cycle of replication involves denaturing the double-standed DNA template, binding the primers to each strand of the template, and then synthesizing ("polymerizing") the complementary strand. (iii) A rapid, self-contained "chain reaction" is achieved by using thermostable DNA polymerases and programmable thermocyclers. An entire procedure, consisting of 20 to 30 cycles, can be conducted in a small, closed container (e.g., an microcentrifuge tube) and within a few hours will generate sufficient product ("amplicon") to be visualized and sized directly in an agarose or polyacrylamide gel.(11)

PCR can be readily performed with commercially available reagents and thermocycles. Since contamination with even a single copy of target DNA can produce a false-positive result, many experienced workers recommend having two physically separate work areas, one for preparing the samples and the other for performing the reactions.

## 4. PFGE of Chromosomal DNA

As noted above, a major limitation of REA with enzymes that have relatively frequent recognition sites is the difficulty of analyzing the resulting patterns composed of large numbers of overlapping, poorly resolved restriction fragments. If the bacterial genome is digested with enzymes that have relatively few restriction sites, then considerably fewer but much larger restriction fragments are generated. Until

relatively recently, the characterization of large DNA fragments was limited by two major factors. First DNA fragments of $\geq$ 25 kb are separated poorly or not at all by conventional agarose gel electrophoresis;; second DNA prepared in solution is spontaneously sheared into random fragments typically of $\leq$ 100 kb. PFGE, developed by Schwartz and Cantor in 1984 (101), is a variation of agarose gel electrophoresis in which the orientation of the electric field across the gel is changed periodically ("pulsed") rather than being kept constant as in the conventional agarose gel electrophoresis used for the REA and Southern blot studies described above. For technical reason (102), this critical modification enables even megabasesize DNA fragments to be effectively separated by size. Suitable unsheared DNA is obtained by embedding intact organisms in agarose plugs ("inserts") and then enzymatically lysing the cell wall and digesting the cellular proteins. The isolated genomes are then digested *in situ* with restriction enzymes that have few recognition sites (103). PFGE analysis of such digests provides a chromosomal restriction profile typically composed of 5 to 20 distinct, well-resolved fragments ranging from approximately 10 to 800 kb. All bacterial isolates are theoretically typeable by PFGE, and the results are highly reproducible. The relative simplicity of the restriction profiles greatly facilitates the analysis and comparison of multiple isolates.

For several common bacterial pathogens, such as *E. coli, enterococci, staphylococci, P. aeruginosa*, and *M. avium* (14), PFGE analysis has proven to be highly discriminatory and comparable or superior to other available techniques.

Total DNA profiles obtained by PFGE (CHEF) method gave excellent typability, as all strains yielded a DNA profile and presented very good stability. Reproducibility was very good in triplicate experiments, and comparison of different gels was facilitated by including a reference strain. Lastly the discriminatory power appeared good (104). Boukadida E. et al used the ribotyping, randomly amplified polymorphic DNA analysis, and pulsed-field gel electrophoresis in the epidemiologic evaluation of *P. aeruginosa* isolated from eight children and two *P. aeruginosa* O:12

environmental isolates from a hematology ward. Randomly amplified polymorphic DNA analysis and pulsed-field gel electrophoresis had been shown to be able to discriminate isolates that were indistinguishable by biochemical typing, O-serotyping, or ribotyping. In such study, random PCR was able to differentiate 2 strains among the ten *P. aeruginosa* O:12 related isolates while PFGE was able to differentiate 3 strains among the same group (105). However, PFGE has two notable limitations. First, because of the need for all buffers and enzymes to be diffused into the agarose insert, the preparation of suitable DNA involves several extended incubations and takes from 2 to 4 days. This effort is partially offset by the fact that DNA in agarose is stable for years at 4°C and can easily be released into solution for use in other protocols. Second, PFGE requires relatively expensive, specialized equipment.

Table 3   Characteristic of genotypic typing systems

| Typing system | Proportion of strains typeable | Reproducibility | Discriminatory power | Ease of Interpretation | Ease of performance |
|---|---|---|---|---|---|
| REA | All | Good | Good | Poor | Excellent |
| Ribotyping | All | Excellent | Fair | Good | Good |
| PCR restriction digest | All | Excellent | Good | Excellent | Good |
| PFGE | All | Excellent | Excellent | Excellent | Good |

## II. Epidemiological

### Incidence of Infections

Because *P. aeruginosa* causes infections only in compromised patients, most of the recorded infections occur in hospitals. However, due to the frequent isolation of *P. aeruginosa* from sewage and other reservoirs from outside of the hospital, patients with CF, for example, may also acquire the pathogen outside the hospital. Data from the National Nosocomial Infections Surveillance System (NNIS) instituted by the U.S. Centers for Disease Control in Atlanta reveal that *P. aeruginosa* caused 11.4% of nosocomial infections of all sites, 12.7% of urinary tract infections, 16.9% of lower respiratory infections, and 8.9% of surgical wound infections.(106) Similar results were obtained in an epidemiological studies carried out from 1980 to 1984 in the North Carolina Memorial Hospital.(107) From 1984 to 1988 the NNIS also reported that *P. aeruginosa* rose in rank from the fourth to the first as the most frequent pathogen of nosocomial septicemia.(108) Responsible for this trend is increased drug resistance to various antibiotics including aminoglycosieds, cephalosporins, and quinolones. It has also been shown that the pathogens most frequently isolated from nosocomial infections in ICUs (n = 3617 infections, October 1986 to May 1988) were *P. aeruginosa* (13.4%) (9). This organism had been reported as the most common organism causing bacteremia in a U.S. burn center between 1953 and 1983, representing 10.3%.(109) A significant reduction in the frequency of *P. aeruginosa* bacteremia was achieved when patients were isolated in the following years in a new burn unit, reflecting the beneficial application of improved hygiene. Isolation of hospitalized CF patients also led to a decrease in the number of *P. aeruginosa* pulmonary infections.(110) Both the less frequent isolation of *P. aeruginosa* in new hospital settings as well as improved aseptic techmiques may explain the signifecant drop in the percentage of intubated patients colonized with *P. aeruginosa* (14% to

6.5%; p <0.001) in the first year after and intensive therapy unit moved into a new building .(21) However, no such trend was noticed in a similar study done elsewhere. (111) Furthermore, nosocomial cross-colonization has not been regarded as an important mode of dissemination of *P. aeruginosa* strains.

**Routes of Transmission**

The elucidation of bacterial transmission routes in general and especially within the hospital is a difficult task, because we must distinguish among contacts between patients, between patients and healthy carriers (*e.g.*, hospital personnel), and between patients and environmental sources. A reliable and highly discriminatory typing method is crucial to any investigation of transmission routes.

**a. Transmission via Hospital Personnel and Hospital Equipment**

In the study done by Wolz, C. et al (112). *P. aeruginosa* strains from 46 CF patients were typed, four episodes of cross-infection were detected. Whether cross-infection was due to a direct patient-to-patient contact or whether transmission occurred via contaminated environmental reservoirs was not investigated in this study. However, the researcher suggested that the environment could possibly be the source of the organism because the majority of the newly acquired *P. aeruginosa* strains were not derived from the other infected CF patients. To investigate the environment-patient transmission route of *P. aeruginosa*, the organisms from patients who had minimal or no contact at all with each other, such as patients with acute leukemia receiving cytostatic therapy, paraplegic patients, and intubated patients were isolated and typed. Of the 119 patients with leukemia, 18 were found to be colonized or infected during a 2 year study period. Twelve different *P. aeruginosa* genotypes were detected. Groups of up to three patients were colonized by identical genotypes,(113)

suggesting nosocomial transmission. In another epidemiological study of *P. aeruginosa* in oncology patients by PFGE, 12% of the patients were colonized at admission and 10% acquired *P. aeruginosa*.(114) A considerable incidence of nosocomial infection was also found in hospitalized paraplegic patients by PFGE. Up to four patients were infected by single *P. aeruginosa* genotype that could also be isolated from the environment of the ward. *P. aeruginosa* strains were present in 51% of all accessible water reservoirs of such wards. The result showed that the identical *P. aeruginosa* genotypes were isolated from a male paraplegic patient and from the toilet of the female personnel strongly suggested the transmission by hospital personnel.(115)

Hand washing continues to be the most effective and simple infection control to stop the transmission of pathogens. Most endemic infections are transmitted by the hands of healthcare workers, and outbreaks of nosocomial infections have also bee related to transmission by the hands of hospital employees. At the University of Iowa Hospital, *P. aeruginosa* was isolated three times more frequently from the surgical intensive care unit (SICU) than had been reported previously, a statistically significant increase (p<0.05). *P. aeruginosa* was isolated from nine patients in the SICU, seven patients were infected and two colonized (116). Hand cultures of 60 healthcare workers in the SICU as well as 129 stool and throat cultures from SICU patients were performed. Only one hand culture was positive for the same *P. aeruginosa* outbreak strain. It belonged to a nurse providing direct care for these three patients. In this report, transmission of *P. aeruginosa* by the hands of a healthcare worker was strongly supported by such evidence. It also showed the growing need and interest in the use of molecular epidemiologic typing systems, together with good clinical and epidemiololgic investigations, as an effective combined approach to infection control research (117).

During the six months from January-June 1994, 10 cases of severe and 11 of less severe pulmonary infection caused by *P. aeruginosa* were diagnosed in patients

with chronic obstructive airways disease at University Hospital Gent, Belgium. *P. aeruginosa* was isolated from four of the 22 nebulizers tested. Three types were identified and the distribution of types in patients with severe infection was as follows (one patient had a multiple infection). Type I was isolated from two nebulizers and from sputa , and/or blood and/or bronchial protected specimen brush samples or bronchial large fluid from four patients. Type II came from the sputa of three patients and a third nebulizer; and type III from sputa and/or blood of four further patients and another nebulizer. The data provided evidence for the relation between *P. aeruginosa* as a cause of infection and the contamination of the nebulizers. When nebulizer mouthpiece were changed every 24 h and sterilized between patients, no more contamination occurred, and the outbreak ceased (118).

Jumaa P. and Chattopadhyay B. (13) reported that the organism isolated from an outbreak of gentamicin, ciprofloxacin-resistant *P. aeruginosa* in an intensive care unit was found to be associated with contaminated quivers, used to store suction tubing between use on ventilated patients. Once the quivers were disinfected and changed between patients daily, the outbreak stopped. Suction of ventilated patients may be important source of contamination of the respiratory tract with nosocomial pathogens.

Kerr J. R. et al. (119) indicated that from July to September 1993 in the intensive care unit of the Royal Victoria Hospital there were 10 cases of pneumonia associated with sputum culture of *P. aeruginosa*. The isolates had an identical RAPD typing profile. The same strain of *P. aeruginosa* was recovered from the sink plug-hole in two rooms, and the tap handles and ventilator tubing in a third room. Recommendations to medical and nursing staff included secretion isolation precautions, terminal disinfection after patient discharge, use of disposable vinyl gloves by hospital staff for all body substance contacts, through handwashing with 4% chlorhexidine gluconate before and after dealing with all patient contacts, and prompt, appropriate antibiotic treatment for P. aeruginosa pneumonia.

In order to identify the possible reservoirs and routes of cross-infection with *P. aruginosa* during a six week period in autumn 1992 from patients, their visiting parents, staff and the inanimate environment of the Danish Cystic Fibrosis (CF) Center and for a control ward with common pediatric disease, the study was performed by Zembrzuska-sadkowska E. et al (120) who indicated that 310 strains of *P. aeruginosa* were isolated from 240 CF patients and only six strains from 1000 swabs from environment were positive *P. aeruginosa* . These six environmental strains and 20 *P. aeruginosa* strains from CF patients with identical serotype and phage type were examined with pulsed-field gel electrophoresis. None of the patients harbored strains similar to the environmental strains indicating the present isolation procedure and hygienic precautions were effective in this CF center, and prevented contamination of the environment with *P. aeruginosa*

This notion was further substantiated in a longitudinal investigation of intubated patients receiving mechanical ventilation in an intensive therapy unit in the same hospital. In at least eight of the 15 immobile patients colonized or infected with *P. aeruginosa*, the respective *P. aeruginosa* genotype was hospital-acquired and most probably transmitted by hospital personnel, because the strains were isolated from the hospital environment or from other patients who were already colonized. Furthermore, such a transmission route from the sinks to hands was also found in the mixed infectious disease ward of a children's hospital,(121) where *P. aeruginosa* was sampled longitudinally from patients, hospital personnel and the hospital environment. In total, 46% of the personnel had *P. aeruginosa*-positive hand cultures. Colonization of personnel hands occurred after the personnel had entered the hospital; hand cultures were negative in all samples taken at entrance. This suggested that *P. aeruginosa* colonization of hospital personnel and subsequently of patients derived at least in part from the widespread contamination of sanitary installations and other hospital equipment where the organism multiplied and persisted for long periods of time. This

theory is also substantiated by various studies on *P. aeruginosa* strain transmission in hospitals which have been performed during the past 20 years. (122)


## b. Aerosol Transmission from Sink Drains


Because the transmission of sink drain organisms to patients is difficult to understand at first glance, the role of waste-traps as reservoirs from which infection may start in hospitals has been questioned in the past. Nevertheless, backsplash and aerosol production has been shown to occur during handwashing and toilet flushing and *P. aeruginosa* was detected on agar plates placed up to 10 feet away from a washbasin while the faucet was activated for three 10-sec intervals.(122) When a toilet was contaminated with *E. coli*, and agar plates were exposed throughout the room, flushing of the toilet resulted in detectable bacteria in a limited area around the toilet within the first 2 hr and for up to 6 hr in a more random distribution of *E. coli* in the room.(123) When hand washing was performed in a *P. aeruginosa* contaminated washbasin without soap, 2400 colony forming units(CFU) of PAO1 were grown on the filter membrane after the hands were dried and immersed in a sterile plastic bag containing 100 ml of physiological saline. Hand washing with soap yielded 1200 PAO1 CFU per 100 ml saline.(121) Thus, normal handwashing without appropriate disinfection afterwards may lead to contamination with microorganisms persisting in the sink drains rather than to decontamination. If patients with a high risk of acquiring *P. aeruginosa* or other bacteria (i.e., patients with CF) washed their hands or brush their teeth in contaminated washbasins, colonization and infection might occur. Close contact with contaminated reservoirs seems to be important for the contraction of pathogens via aerosols, because survival of *P. aeruginosa* in aerosols is relatively short and comparable to the survival times of other gram-negative rods. Microbial hazard is highest the first time water is run or when toilets are flushed in the morning, when planktonic *P. aeruginosa* organisms have multiplied considerably from the wall-

attached glycocalyx. Therefore, hygienic measures to decontaminate sinks and toilets are recommended, as well as improved hygienic measures for hand disinfection.

### c. Endogenous Infection Routes

The question is difficult to answer whether *P. aeruginosa* infection in hospitals originate from contaminated environmental reservoirs or are a consequence of endogenous bacterial carriage prior to admission to a special unit of the hospital. Both possibilities have to be considered. Patients precolonized at the time of admission to intensive care units seem to be responsible for a considerable percentage of subsequent *P. aeruginosa* infections. Intestinal carriage of *P. aeruginosa*, which is enhanced after antibiotic therapy, may lead to oropharyngeal colonization via retrograde migration of the organisms, especially in patients treated with antacids. In a recent study in an intensive therapy unit, *P. aeruginosa* colonization of the gastrointestinal tract preceded colonization of the oropharynx with identical genotypes in two colonized cases. An external transmission route involving hospital personnel cannot be ruled out.(21) Another possible infection route for *P. aeruginosa*, especially in neutropenic patients, is translocation from the gastrointestinal tract to the bloodstream. Eighty-one percent of 16 such patients with bacteremia caused by *P. aeruginosa* were intestinal carriers of the same strain.