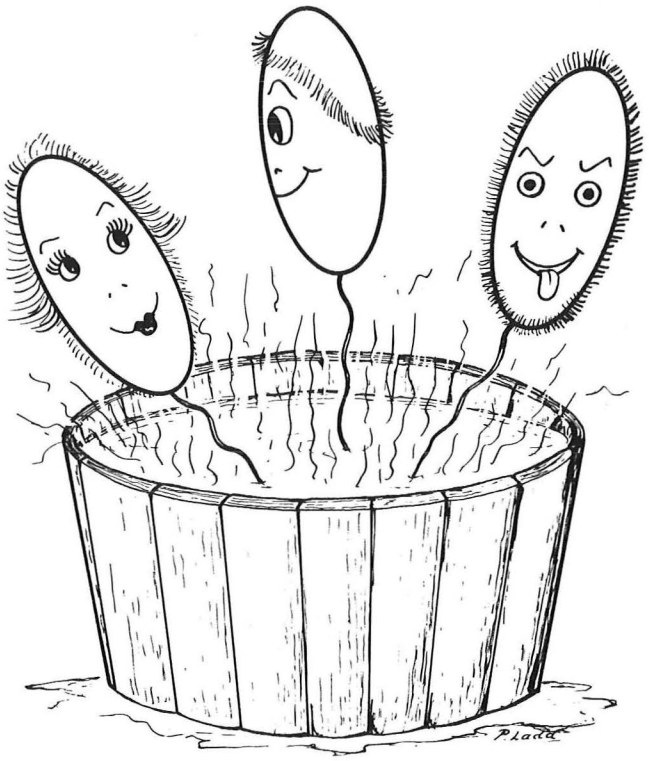
Infectious

THE SLIPPERY SAGA OF *PSEUDOMONAS AERUGINOSA*



Medical Grand Rounds

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THE SLIPPERY SAGA OF *PSEUDOMONAS AERUGINOSA*

THE ORGANISM

DISEASES IN "NORMAL" HOSTS:

- External Otitis
- Dermatitis
- Puncture-wound Osteomyelitis
- Endocarditis and Osteomyelitis in Intravenous Drug Abusers
- Noscomial Infections

DISEASES IN "ABNORMAL" HOSTS

- MOTILE MODE -- INVASION WITH LOCAL SPREAD:
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- MOTILE MODE -- INVASION WITH SYSTEMIC SPREAD:
  - Bacteremia, Pneumonia, and Burn Wound Sepsis

CELL STRUCTURE; PATHOGENETIC MECHANISMS -- MOTILE MODE

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DIAGNOSIS

THERAPY

- Antimicrobial Chemotherapy
- Immunotherapy
- Therapy for Specific Pseudomonal Diseases

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THE ORGANISM

P. aeruginosa is the type species of the genus *Pseudomonas*, which consists of gram-negative, rod-shaped bacteria that possess polar flagella and carry out oxidative but not fermentative metabolism. In other words, the cells are *motile aerobes*. Features that distinguish *P. aeruginosa* from some of the other pseudomonads are indicated in the following table (1,2).

Species name	Flagella	Growth at 41°C	Fluorescent pigment	Gelatin Hydrolysis
P. aeruginosa	single polar	yes	yes	yes
P. alcaligenes	"	yes	no	
P. putida	polar tufts	no	yes	no
P. fluorescens	"	no	yes	yes
P. cepacia	"	usually	no	usually

P. aeruginosa strains are characteristically oxidase-positive; they hemolyze blood, oxidize gluconate, and utilize mannitol and acetamide. They do not produce gas from carbohydrates. Most strains produce a greenish-blue color in growth media as the result of the production of a blue phenazine pigment, pyocyanin.

Several schemes have been developed for serotyping isolates of *P. aeruginosa*. A detailed discussion of these and other typing methods is available in the paper by Brokopp & Farmer (3). The two most commonly used serotyping schemes are those of Fisher and Habs (the latter is similar to the International Antigenic Typing Scheme [IATS]). They are based on the O-antigenic variation in the cell wall lipopolysaccharides.

IATS type	Fisher type
1	4
2	3
3	
4	
5	7
6	1
7	
8	6
9	
10	5
11	2
12-17	

The normal habitat of *P. aeruginosa* includes surface water and soil. The organism probably enters the environment from animal wastes, including those of man. Although *P. aeruginosa* is found in 10-15% of fecal samples

from healthy American adults, the number of organisms per gram of stool is usually quite low (and much less than the number of coliforms).

Much of the clinical importance of P. aeruginosa derives from its ability to prosper in a variety aqueous environments. This is best appreciated by considering some of the diseases that P. aeruginosa produces in individuals who have no specific immunologic or anatomic defect.

DISEASES IN "NORMAL" HOSTS

EXTERNAL OTITIS

Otitis externa (inflammation of any portion of the skin of the external auditory canal) has many causes. The pathogenesis of diffuse bacterial external otitis is thought to involve

1. Maceration of epithelial cells in the ear canal from prolonged exposure to moisture
2. Plugging of sweat and sebaceous gland ducts
3. Invasion by exogenous organisms through breaches in a damaged epithelium
4. Moisture absorption by the stratum corneum at levels of high humidity (and high temperature)
5. An absence of cerumen or the presence of an alkaline secretion.

The process usually begins with a preinflammatory stage, in which the lining of the ear canal loses its normal protective cover (e.g., following cerumen removal, or because of prolonged water exposure). At the same time, overactivity of the apocrine glands may produce a feeling of fullness in the ear, which is relieved by scratching and damaging the unprotected epithelial surface. The surface keratin may absorb moisture, causing edema of the stratum corneum, and if the ducts are blocked, keratin debris accumulates in the orifices of the apopilosebaceous units, with the result that there is a deficiency of ear wax. This gives momentum to the itch-scratch cycle. With the surface barriers to microbial invasion lowered, normally nonpathogenic endogenous organisms are able to penetrate and thrive in the orifices of the sebaceous glands, setting up a low-grade inflammatory process. Exogenous invaders, primarily Gram-negative organisms, can also invade, usually producing a more severe, secondary inflammation (4).

Contact with water is recognized as a major component of this pathogenetic scheme. Several reports indicate that S. epidermidis and diphtheroids are the most commonly isolated organisms in individuals with normal ear canals. P. aeruginosa is only rarely isolated (usually less than 1-2% of normal individuals). In contrast, P. aeruginosa has been found in the cultures of approximately 50-60% of individuals with external otitis; this frequency appears to increase if one samples patients with severe otitis externa. Other gram-negative bacteria (Proteus, Klebsiella, E. coli) also play a role in external otitis, accounting for up to 20% of the isolates in patients with disease duration exceeding 16 days. One study found that swimmers are 5 times more likely to have otitis externa than are normal

individuals; the ear cultures contained P. aeruginosa in 78% of diseased swimmers, compared with 33% of non-swimmers with external otitis (5, 6). Divers are at higher risk of otitis externa (usually due to P. aeruginosa) than swimmers. Both fresh water and chlorinated pool water have been incriminated in P. aeruginosa external otitis (7).

DERMATITIS

P. aeruginosa may cause several skin infections, including toe web intertrigo, the green-nail syndrome, burn and surgical wound infections, and suprainfections in patients with acne vulgaris. Recently, much attention has been given to pseudomonas folliculitis, an interesting rash disease that occurs only in patients who bathe in contaminated water. Although most recent outbreaks have afflicted users of hot tubs or heated whirlpools, others have occurred in persons using indoor swimming pools and water slides (8, 10-14).

The rash is not unique in its appearance, and physicians seeing it for the first time have confused it with insect bites, scabies, contact dermatitis, bromoderma, staphylococcal folliculitis, and even varicella or herpes zoster (8).

Helpful clues to the diagnosis are the following:

1. History of bathing in a hot tub or swimming pool. The incubation period to onset of rash is usually less than 72 hours. The disease has a high attack rate: others similarly exposed may also develop a rash.

   A typical epidemic curve was reported by Gustafson et. al. from an outbreak of rash and otitis externa in persons who used a health spa swimming pool in Tennessee (8):

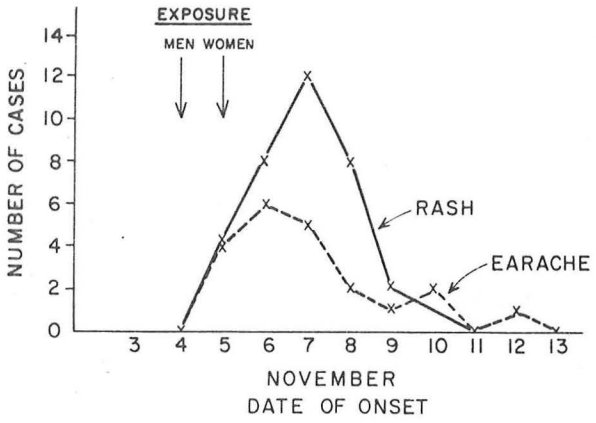


Figure 1. Number of cases of rash and earache and dates of onset in members of a health spa (Tennessee, 1980).

2. The lesions progress from a follicular pruritic papule to a red papulopustule within 24-48 hours. Lesions in many different stages are usually present simultaneously. The lesions heal spontaneously in 2-5 days, leaving fine desquamation and red-brown macules. Scarring is unusual.
3. The lesions have a characteristic distribution, illustrated in the diagrams in Figure 2 (from reference 8).

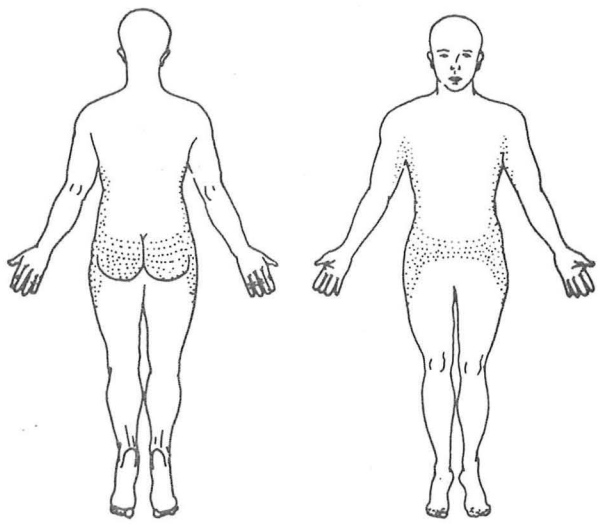


Figure 2. Distribution of pseudomonas folliculitis. The rash can occur on any hair-bearing skin surface but is characteristically concentrated in the areas shown.

Note that the palms, soles, and mucous membranes are spared; in hot tub victims, the face is also spared (no head immersion). It is hypothesized that the skin of the buttocks, hips, and axillae is subjected to more moisture and friction than skin elsewhere--perhaps explaining the predilection for infection of the hair follicles in these areas.

4. Involvement of other organs. Mastitis occurs in both males and females, involving some 6 to 20% of patients. Otitis externa may occur when head immersion has taken place (more common in swimming pool outbreaks).

Gustafson and colleagues (8) have suggested that the folliculitis and the other P. aeruginosa skin infections show an interesting spatial relationship to apocrine sweat glands. The folliculitis tends to be most dense in areas where apocrine sweat glands are located, including the axillary, pubic, and circumanal skin. Moreover, the ceruminous glands of the ear, the mammary glands, and the glands of Montgomery are considered to be modified apocrine sweat glands. Their "unitary" hypothesis suggests that P. aeruginosa may, for unknown reasons, be able to flourish in the apocrine glands.

Factors usually invoked to explain the occurrence of this disease include: (1) the persistence of low numbers of P. aeruginosa in chlorinated

water, and the rapid multiplication of the organisms when the free chlorine (HOCl) level drops below 0.5 mg/L or the water pH is greater than 7.8, (2) the agitation and high temperature of hot tubs, which increase the evaporation of chlorine from the water, (3) crowding, particularly in hot tubs, with increased concentration of nitrogenous wastes in the water, favoring bacterial growth (and decreasing the efficacy of chlorination, since when NH_3 combines with chlorine, the product monochloramine is ineffective). It is typical for outbreaks to occur after prolonged, heavy use of hot tub water.

The hydrolysis of chlorine occurs according to the reaction

$$Cl_2 + HOH \rightleftharpoons HOCl + H^+ + Cl^-$$

Hypochlorous acid (HOCl), the bactericidal agent, is a weak acid that dissociates:

$$HOCl \rightleftharpoons H^+ + OCl^-$$

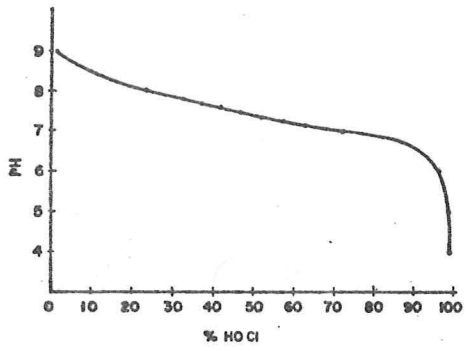
The methods for measuring "available" chlorine do not distinguish between HOCl and OCl^-.

The important effect of water pH on freel chlorine (HOCl) levels is illustrated in the following table and figure from Black (15):

Table —Amount of *free available* chlorine required to provide 0.40 ppm *free chlorine at pool pH*

pH of pool water	Percentage of free available chlorine present as HOCl	Conversion factor	*Free available* chlorine required to provide 0.4 ppm *free* chlorine
7.2	62.3	1.60	0.64
7.3	57.2	1.77	0.70
7.4	52.1	1.94	0.77
7.5	47.0	2.14	0.85
7.6	41.9	2.37	0.95
7.7	36.8	2.70	1.09
7.8	31.7	3.14	1.26

Figure 3—Effect of pH on per cent HOCl for any free available chlorine residual



The U.S. Public Health Service guidelines (39) for swimming pool and hot tub water safety thus stress control of pH as well as the free available chlorine level.

PUNCTURE-WOUND OSTEOMYELITIS

Reported series of patients from many centers indicate that P. aeruginosa is the most common etiologic agent of puncture-wound associated osteomyelitis of the small bones of the foot. Although most of the reported patients have been children, a recent series from Houston suggests that the same association may hold true in adults (17-19).

Following the initial puncture and an acute inflammatory stage, the disease usually takes a subacute course, so that most patients come to medical attention 1 to 4 or more weeks later. The presenting symptoms (pain, swelling, occasionally a discharge) are usually not accompanied by systemic toxicity but, because of the long interval between introduction of the organisms and examination, X-rays typically show signs of osteomyelitis. The ESR is often elevated but there is no leukocytosis. In the Parkland-Childrens Medical Center experience, 5 of 7 children with puncture-associated osteomyelitis of the foot over the period 1959-1981 had P. aeruginosa grown in pure culture from bone biopsy specimens; in the other 2 children no organism was recovered (data kindly provided by Dr. Mary Ann Jackson). These data are in agreement with the reported experience, in which $\geq$ 90% of puncture-associated osteomyelitis is caused by P. aeruginosa.

The pathogenesis of this disease is uncertain. Perhaps the most obvious explanation would be that P. aeruginosa gets into the wound when the child soaks his foot in contaminated water after the puncture. On the other hand, there is interesting recent evidence that the organisms may be introduced at the time of the puncture. Workers in Philadelphia have reported that they isolated P. aeruginosa from the sneakers of 5 of 6 children with P. aeruginosa osteomyelitis of the foot, as well as from 6 of 66 discarded sneakers from children who did not have osteomyelitis (21). No positive cultures were obtained from 30 new tennis shoes from a single manufacturer. Interestingly, the positive cultures were obtained from sneaker punch biopsy specimens that sampled the inner layers of rubber in the soles; cultures of the inside heel surfaces or bottoms of the shoes were negative. These workers hypothesize that breaks in the lining of the sole allow contaminated water to penetrate into the layers of rubber, where it persists. A nail or other object, passing through the sole, could then pick up P. aeruginosa and carry it into the soft tissue/bone of the foot.

It is not possible to test this hypothesis by reviewing the published cases, since the case reports usually do not indicate whether or not the patient was wearing shoes at the time of the puncture.

ENDOCARDITIS AND OSTEOMYELITIS IN INTRAVENOUS DRUG ABUSERS

P. aeruginosa is a well-established cause of osteomyelitis, septic arthritis, and endocarditis in individuals who inject drugs intravenously. Osteomyelitis or septic arthritis usually involves vertebral bodies (mainly lumbar), the sacroiliac joint, or sternoclavicular joint; the patients (in contrast to those with systemic Staphylococcus aureus disease) are often not toxic (low grade fever, normal WBC count, moderately elevated ESR), and the diagnosis is often suggested by localized pain and a positive bone scan (23). P. aeruginosa endocarditis may involve the right (75%) or left (25%) sides of the heart. Tricuspid valve endocarditis is usually a subacute disease that features cough, sputum production, pleuritic chest pain, pulmonary infiltrates, and pleural effusions. Systemic emboli are unusual. Left-sided P. aeruginosa endocarditis is a more acute disease that is characterized by heart failure and large systemic emboli. Splenic abscesses are common. Peripheral signs of infective endocarditis (Osler nodes, etc.) or ecthyma gangrenosum are rare (24).

One of the striking features of these diseases is the fact that they occur almost exclusively in addicts who live in Detroit or Chicago. (The only patient seen at Parkland with P. aeruginosa endocarditis in the last 3 years was a young man who had recently arrived in Dallas after spending several days enroute from Detroit, where he had injected drugs IV). The explanation for this geographical localization is uncertain. On the other hand, there is some evidence that the organism is introduced into the blood from contaminated syringes: in a study from Chicago, 4 of 4 syringe cultures from patients with P. aeruginosa endocarditis grew P. aeruginosa of the same serotype as the blood isolate, whereas syringe cultures from 3 addicts with S. aureus endocarditis were negative (25). P. aeruginosa has not been recovered from heroin and is not a usual colonizer of the skin. These observations support the notion that P. aeruginosa may be injected along with the drug that is drawn up into a contaminated syringe. The most likely source of P. aeruginosa would then be the water that is used to "clean" the syringe or dilute the drug.

NOSOCOMIAL INFECTIONS

P. aeruginosa has been implicated as the etiologic agent in a variety of nosocomial epidemics. Although the organism is able to thrive in sites such as hospital sinks, ice machines, and the like, from which it may be carried to patients via ice or unwashed hands, many of these outbreaks have involved a contaminated detergent or "disinfectant" preparation. A representative selection is presented below:

P. aeruginosa contamination of	Associated with	Reference
Hexachlorophene used to disinfect cystoscope	Urinary tract infection	Strand (27)
Prepodyne^{R} (poloxamer-iodine) used to prep indwelling peritoneal catheters	Peritonitis	Parrott (28)
Povidone-iodine used to prep skin for drawing blood cultures	Pseudobacteremia	Craven (29)
Inhalation therapy equipment (nebulizer)	Nosocomial pneumonia	Pierce (30)
Chlorhexidine used for surgical prep	Postoperative wound infection	Anyiwo (31)
Hydrotherapy (Hubbard) tank	Wound infections	McGuckin (32)

In addition to the common-source outbreaks listed above, P. aeruginosa also has been associated with cross-infection (patient to patient) outbreaks, most commonly involving the respiratory tract and affecting patients who are intubated or have tracheostomies. These outbreaks usually have occurred when in-use respirator tubing was not changed regularly or when aseptic respiratory care technique was not practiced (33).

SUMMARY. The obvious common thread that unites the above diseases is the presence of P. aeruginosa in water. This microorganism is indeed extremely versatile. P. aeruginosa is able to multiply in distilled water, using traces of dissolved CO_2 as its carbon source. It also produces esterases that enable it to hydrolyze nonionic surfactants such as those used in cosmetics, ointments, steroid creams, hand lotions, and the like. Remarkably, the organisms can also grow on many petroleum products, and it is not surprising that the first patented organism of any kind was a P. aeruginosa strain that is able to digest spilled oil. Finally, as is indicated by the nosocomial outbreaks traced to contaminated disinfectants, P. aeruginosa is able to adapt to grow in a wide variety of "disinfectant" compounds, including quaternary ammonium compounds, iodophors, and even phenolic disinfectants.

It is of some interest that one of the 16 serotypes of P. aeruginosa, serotype 011, has figured prominently in several of these water-associated diseases. Although this serotype accounts for a small portion (~15%) of isolates from patients with non-epidemic nosocomial P. aeruginosa disease, two-thirds of 23 single-strain nosocomial outbreaks were associated with this serotype (33). Moreover, serotype 011 has accounted for the majority of the outbreaks of P. aeruginosa folliculitis and for at least one series of patients with endocarditis related to contaminated syringes. The reason(s) for this association are uncertain and deserve investigation.

PREVENTION. It is important to note that the usual methods for quantitating bacteria in water supplies are intended to detect coliforms (this name has been used since 1901 to describe organisms now classified in the genera Escherichia, Enterobacter, and Klebsiella - operationally they are lactose-fermenting, gas-forming, aerobic and facultatively anaerobic, gram-negative rods that do not form spores.) P. aeruginosa may be present in water that does or does not contain coliforms. Moreover, although most authorities agree that the presence of P. aeruginosa in water supplies should be taken seriously, there are currently no standards or guidelines such as those that exist for declaring coliform-contaminated water unsafe for drinking or swimming (2).

On the other hand, it is known that hyperchlorination of water is able to eliminate P. aeruginosa, and effective disinfection of hot tube or swimming pool water should follow the guidelines provided in a recent CDC document (39a). Overcrowding and prolonged use of this water should be avoided. Apparently effective guidelines also exist for disinfection/sterilization of various mechanical devices in common use (Appendix).

Guidelines for care of respiratory therapy equipment (largely developed by Drs. Pierce, Sanford, Johanson, and their colleagues at Parkland in the early 1970's) included initial sterilization with ethylene oxide and in-use disinfection with 0.25% acetic acid. Use of disposable equipment has largely removed the need for these measures today.

DISEASES IN "ABNORMAL" HOSTS

P. aeruginosa can cause disease in many organ systems. This discussion will focus on a few diseases in which P. aeruginosa plays a distinctive or

important role. The discussion of these diseases will highlight important pathogenetic factors and emphasize existing and potential modes of therapy.

It is conceptually useful to consider these diseases in terms of two general pathogenetic mechanisms (modified slightly from Costerton (79)):

(1) P. aeruginosa in the motile mode invade via a disruption in a normal anatomical barrier and cause local or systemic disease;

(2) P. aeruginosa in the stationary (microcolony) mode colonize and persist, avoiding host defense mechanisms, producing disease locally.

MOTILE MODE -- INVASION WITH LOCAL SPREAD

Malignant (Invasive) External Otitis (IEO)

In 1959, Meltzer and Kelemen described a fatal case of Pseudomonas osteomyelitis of the temporal bone, mandible, and zygoma in an elderly diabetic man. Chandler (1968) described 13 cases of a similar disease and, because of its high mortality, named it "Malignant Otitis Externa." This is an infection that begins in the external ear canal, spreads to the adjacent soft tissues at the base of the skull and sometime results in multiple cranial neuropathies and osteomyelitis of the temporal bone. The condition occurs almost always in elderly diabetic patients (45), and it is caused almost always by P. aeruginosa. The few children who have developed this disease have been anemic, malnourished, or severely ill (43).

In one large recent review (42), the average age of reported patients was 68.5 years, with a range of 7 months to 91 years. Diabetes was present in 89% of the patients; the average duration of diabetes prior to the onset of IEO was 16.4 years. Physical examination showed discharge from the external ear canal in 96% of patients and granulation tissue (in the posterior- inferior canal wall or at the junction of the bony and cartilaginous canal) in 94%. Most patients with IEO present because of pain or drainage from the ear; symptoms of cranial neuropathy have been present in less than half of the patients in the recent series. The facial nerve has been involved most commonly; the IX, X, XI, and XII nerves also may be affected, while rarely IV or VI nerve palsies may be seen.

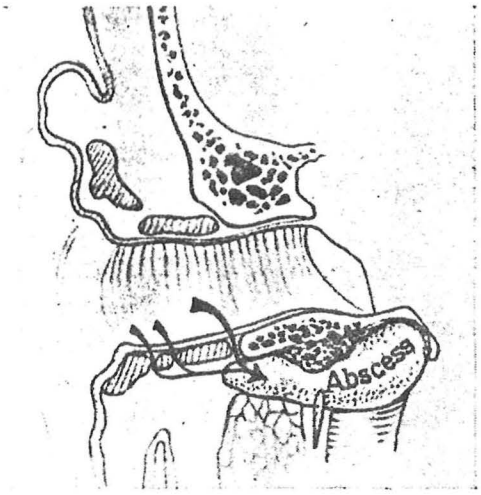


Fig. 4 Diagrammatic illustration of the external auditory canal with arrows demonstrating spread of infection via the fissures of Santorini into the subtemporal space. Note subtemporal abscess.

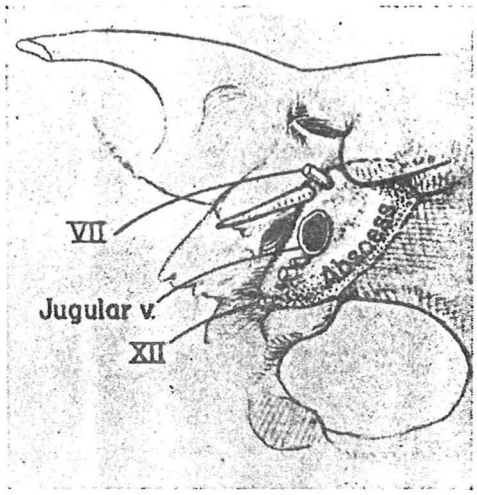


Fig. 5 Oblique basal view of subtemporal abscess.

The floor of the external auditory canal at the bony-cartilaginous junction appears to be particulary vulnerable to trauma, often presenting with pathologic perforation. The fissures of Santorini through the cartilage provide natural conduits to the subcutaneous tissues beneath the ear, including the parotoid and temporomandibular joint. Inflammation may involve the facial nerve (1) as it emerges from the stylomastoid foramen (probably most common) or (2) as it passes through the temporal bone (coursing behind the tympanic membrane then through the facialis canal). As the soft tissue and bone about the jugular bulb become involved, paralysis of cranial nerves IX, X, and XI can occur, producing further disability associated with pharyngeal and laryngeal dysfunction, and lateral venous sinus thrombosis can lead to intracranial involvement. In some series, there has usually been evidence of osteomyelitis of the skull when facial nerves other than VII have been involved. The process is indolent, progressing slowly over weeks and usually unaccompanied by systemic symptoms such as fever or weight loss. Bacteremia has not been described. Meyerhoff and his co-workers have emphasized the distinction between IEO and pseudomonal mastoiditis, which usually occurs in association with otitis media (44).

Since IEO is such a distinctive clinical syndrome, the diagnosis has usually been established by demonstrating the presence of P. aeruginosa in the external canal discharge or biopsy tissue of a patient who fits the typical clinical description. Biopsy of the local subcutaneous tissue, while not often performed, is reasonable to establish a definitive microbiological diagnosis (P. aeruginosa is found in the canals of some 60% of individuals who have only acute external otitis without invasion) and to exclude the possibility that carcinoma is present. Polytomography of the base of the skull is indicated, since patients with osteomyelitis may require longer antibiotic therapy or surgical intervention (49). The role of CT in the management of IEO is not well established (47).

	EXTERNAL OTITIS	INVASIVE EXTERNAL OTITIS
Age of patient	all ages	usually over 60
Underlying disease	none	diabetes mellitus
Physical examination		
Erythema, edema or canal	yes	yes
Pain	variable	yes
Granulation tissue at bony-cartilaginous margin	no	yes (90%)
Soft tissue swelling beneath or around ear	unusual	common
Cranial nerve palsy	no	common
Fever, systemic symptoms	no	no
Culture of ear discharge	P. aeruginosa (60%) S. aureus (20%) others (20%)	P. aeruginosa (95+%)
Therapy	Cortisporin otic drops	- tobramycin or gentamicin plus carbenicillin or ticarcillin +/- surgical debridement

The response to therapy may be predicted from the presenting findings: patients with no cranial neuropathies do well (90-100% survival), while patients who have neuropathies at the onset of therapy do very poorly (40-50% survival).

The close association of IEO with diabetes mellitus is poorly explained. Unlike rhinocerebral mucormycosis, another infectious disease that is seen almost exclusively in diabetics, patients with IEO do not often have ketoacidosis. It seems most likely at present that diabetic microangiopathy, present in the skin and soft tissue of the external ear canal, results in poor local tissue perfusion and creates an environment favorable for local invasion by P. aeruginosa. Presumably the bacterial factors described later in the handout contribute to the invasiveness of this organism in this setting, although the pathogenesis of this disease has not been studied.

MOTILE MODE -- INVASION WITH SYSTEMIC SPREAD

Bacteremia, Pneumonia, and Burn Wound Sepsis

Much of P. aeruginosa's bad reputation derives from its ability to cause lethal bacteremia in compromised hosts, particularly in neutropenic patients. Although P. aeruginosa is found less frequently than E. coli and Klebsiella pneumoniae in most series of bacteremic neutropenic patients, the poor prognosis of patients with pseudomonas bacteremia and the difficulty with

which this organism is treated account for its notoriety. Both pneumonia and bacteremia due to P. aeruginosa are usually (>80%) hospital-acquired. Colonization of the stool (GI tract) frequently precedes systemic disease with this organism; in one study, approximately 50% of the neutropenic patients who became colonized with P. aeruginosa developed pseudomonas bacteremia. The organisms are thought to cross microulcerations in the GI tract--a transgression that is facilitated by the absence of the usual neutrophilic "policemen."

As noted above, P. aeruginosa does not figure prominently in the normal human gut flora. It is also difficult to colonize the GI tract of normal individuals with P. aeruginosa, probably due to the "colonization resistance" provided by normal gut anaerobes. The administration of broad-spectrum antibiotics and/or antacids greatly increases an individual's susceptibility to gut colonization with this organism (and others). It is thus likely that most neutropenic patients who become colonized with P. aeruginosa acquire the organism in the hospital, and recent attention has focused on simple measures that might reduce this risk. It appears that a common source of P. aeruginosa is food, particularly salad-makings; studies have found approximately 5,000 P. aeruginosa per 80 gm sample of tomato (an average helping), and other fresh vegetables have been similarly criticized (50, 51). Elimination of salads from the diet of neutropenic patients would seem reasonable; one might also wonder about other commonly consumed items such as ice (hospital ice machines are often pseudomonal culture vats).

Pneumonia due to P. aeruginosa usually occurs in hospitalized patients who are receiving mechanical ventilation (the P. aeruginosa is usually introduced from some source in the inanimate environment or by cross-infection from another patient--it is not part of the endogenous upper respiratory flora), who are neutropenic, or who develop pneumonia as a complication of Pseudomonas bacteremia. Community-acquired P. aeruginosa pneumonia is very unusual (53).

P. aeruginosa rapidly colonizes burn wounds, so that by five days post-burn as many as 60% of burned patients may have wound infection with this organism (55, 56). It is thought that most patients acquire the organisms from their environment (stool carriage precedes burn colonization in a minority of patients) yet precise definition of the environmental source is not usually possible. Occasional outbreaks of P. aeruginosa disease in burn units have been linked to sources such as defective, contaminated mattress covers (57). Invasive burn wound sepsis (defined as a wound biopsy culture with greater than 10^5 organisms/gram, with histopathology showing bacterial invasion beyond the burn injury) increases in frequency as burn size increases, rarely occurring in burns of less than 30% of the total body surface. Patients with large burns may have many abnormalities in host defense, including decreased immunoglobulin levels and impairment of a number of normal neutrophil functions; these doubtless contribute to the susceptibility of burned patients to invasive disease. Important distant complications of P. aeruginosa burn wound sepsis include ecthyma gangrenosum and metastatic (hematogenous) pneumonia.

A variety of skin lesions has been found in patients with P. aeruginosa bacteremia. The most common and best characterized of these is ecthyma gangrenosum, first described in Vienna in 1897 (58). This lesion, which

appears in only 3-5% of patients with P. aeruginosa bacteremia, commonly occurs on the buttocks, genitalia, or abdomen. Typically the lesion(s) progress over a few hours through a characteristic pattern: edema, erythema, hemorrhagic bullae, and frank necrosis (59). The edematous and erythematous phases may merge, central hemorrhage causes the center of the lesion to enlarge and become bullous, and finally the blister collapses, leaving a central zone of hemorrhagic necrosis. Classically, in the final stage the central lesion is surrounded by a halo of uninvolved skin, and finally by a narrow violaceous ring. Microscopically, the lesion is distinctive: bacteria are present outside and in the walls of the veins, but there is no intimal involvement and there is practically no infiltration with neutrophils. This lesion is caused by very few other bacteria; Aeromonas hydrophila, a much less common cause of bacteremia than P. aeruginosa, is the major one.

The pathology of ecthyma gangrenosum and pseudomonas pneumonia is similar and their pathogenesis may be the same: the bacteria invade by moving through capillaries (dermal capillaries in the skin, alveolar capillaries in the lung), spread through perivascular tissue spaces to ("centripetally") involve larger vessels, particularly veins; and cause necrosis of tissue cells and hemorrhage as vessel walls are weakened. Inflammatory cells, when present, are predominantly lymphocytes or monocytes; neutrophils are scarce although pycnotic neutrophilic nuclei may be found. This schema suggests that P. aeruginosa must have the ability to penetrate capillary walls, seek out and damage nearby blood vessels, and ward off or destroy the host's neutrophilic defense. The pathology of burn wound sepsis is similar, although the local inflammatory response is more exuberant than that described above; there is the same tendency for bacteria to cuff and destroy vessels, producing infarction and hemorrhage.

Patients with P. aeruginosa bacteremia have also had other kinds of skin lesions (61):

1. subcutaneous abscesses, deep nodules without fluctuation, cellulitis with necrosis and hemorrhage (62); these may be the subdermal presentation of the same process that causes ecthyma in the dermis-epidermis. Microscopically there is again hemorrhage and a striking absence of neutrophilic infiltration.
2. gangrene of extremities or genitalia (64).
3. "erysipelaslike" lesions (63).
4. rose spots, like those of typhoid fever, in "Shanghai fever," a typhoid-like form of P. aeruginosa septicemia that was reported from Shanghai (66).

SUMMARY. The diseases discussed in this section share certain features: (1) they occur in patients with drastically altered barriers to infection--whether a damaged GI mucosa, an altered oropharyngeal epithelium, or an extensive burn; (2) their severity, and the risk of systemic (bloodstream) spread, is usually related to the status of the host's neutrophils (whether deficient in number or dysfunctional)--and upon the presence of immunoglobulin opsonins; (3) the offending P. aeruginosa are usually acquired from an exogenous source (environmental, food or water, personnel to patient spread); and (4) the histopathology of these diseases is similar, and distinctive from that found with most other gram-negative bacterial pathogens: vascular involvement, hemorrhage, and a relative paucity of

neutrophils occur in all of these diseases when P. aeruginosa is the etiologic agent. Although it is arguable that the extent of invasion is determined by the status of the host defense, and not by specific microbial factors, the bacterial cells that cause these diseases are motile and they possess certain attributes that doubtless contribute to their invasiveness. Some of these properties are discussed in the following section.

CELL STRUCTURE; PATHOGENETIC MECHANISMS -- MOTILE MODE

Much experimental work has attempted to define specific bacterial factors that contribute to the virulence of P. aeruginosa. A variety of approaches has been used:

In clinical studies:

1. Demonstration that isolates of P. aeruginosa from environmental or other sites lack a "factor" that is present in isolates from clinically important sites (e.g., blood).
2. Association of outcome from serious infection with levels of pre-existing serum antibody to specific bacterial "factors."

These studies suffer from the fact that many host conditions (underlying disease, age, antibiotic therapy, supportive care, etc.) may influence the outcome of P. aeruginosa disease in man; these therefore may confound interpretation of the contribution of single variables such as antibody levels.

In animal studies:

1. Demonstration that mutants lacking a certain factor (F^-) lack virulence in an animal model of infection, and that virulence is regained (restored) in revertants to wild type (F^+); demonstration that introduction of the gene(s) coding for the specific factor confers virulence upon a F^-, non-virulent strain.
2. Demonstration of the toxicity or biological activity of a specific highly purified bacterial product in animals.
3. Protection of animals from experimental infection by the administration of antibodies that are specific for the factor of interest, or by active immunization using the purified factor as a vaccine.

These studies suffer from the fact that animal models do not entirely mimic human disease. Several models have been used most extensively:

a) bacteremia in granulocytopenic animals (rabbits, rats, mice)
b) burn wound sepsis in mice
c) bronchopneumonia in guinea pigs and rats.

No single factor has been shown by all of the above methods to be essential for virulence in P. aeruginosa. A summary of the available evidence that supports a role for various bacterial factors is provided below; more detailed review of the pathogenetic mechanisms of this organism is available in the references cited.

PILI (FIMBRIAE) Like many gram-negative bacteria, P. aeruginosa have short hair-like surface projections that appear to play a role in the adherence of the organism to mucosal surfaces. The best evidence that P. aeruginosa pili enhance mucosal adherence comes from the studies of Johansen and his colleagues on the adherence of bacteria to human buccal epithelial cells (67). They found that P. aeruginosa adhere poorly to epithelial cells from normal individuals but, in contrast, stick quite readily to epithelial cells from patients with serious illness. The ability of P. aeruginosa to adhere to epithelial cells correlated directly with the levels of protease present in the subject's saliva and indirectly with the amount of fibronectin on the epithelial cell surface. Purified P. aeruginosa pili inhibited the adherence of P. aeruginosa to epithelial cells (67):

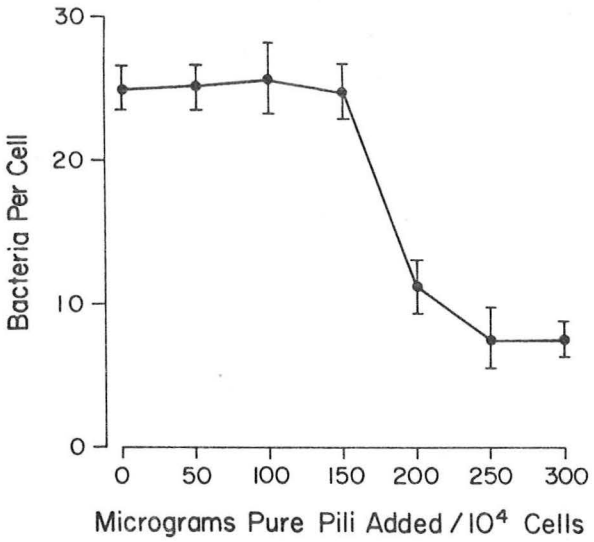


Figure 6. Dose-response curve reflecting the ability of purified pili to block the adherence of intact *Pseudomonas aeruginosa*. Buccal epithelial cells were exposed to increasing amounts (0–250 μg/ml) of purified pili for 1 hr at 37 C before being tested for intact *P. aeruginosa* adherence.

LIPOPOLYSACCHARIDE (LPS; endotoxin). All P. aeruginosa possess cell wall LPS, yet a role for LPS in virulence has not been established. In fact, the LPS of P. aeruginosa is relatively non-toxic when compared with LPS from enteric bacteria such as E. coli. Antibodies to LPS have been shown to protect animals from death due to experimental P. aeruginosa sepsis and have been correlated with survival in human patients with P. aeruginosa bacteremia, suggesting that LPS antigens provide a site for the initiation of successful opsonization by antibody.

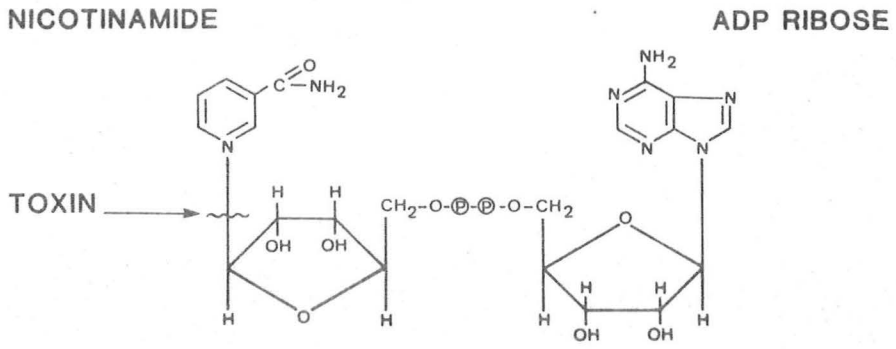
LPS antigens also provide the basis for the available methods for serotyping P. aeruginosa.

FLAGELLA. P. aeruginosa possess a single polar flagellum. The presence of this organelle appears to be important for tissue invasion: non-motile mutants have been shown to have decreased virulence in an animal model of burn wound sepsis (84). Active immunization with purified P. aeruginosa flagellae afforded protection in the same model.

EXOTOXIN A. Although the existence of a potent exotoxin was suspected by early students of P. aeruginosa, isolation of the "lethal" toxin was not achieved until the 1960's. Subsequent study has shown that the toxin, named "exotoxin A" by Liu, is a protein that has a molecular weight of approximately 66,000. It has a mouse LD_{50} of 60-80 nanograms (the mouse LD_{50} for P. aeruginosa LPS is at least 10,000-fold greater). Injection of purified exotoxin A into animals induces leukopenia, a finding commonly observed in P. aeruginosa sepsis, in addition to shock, liver injury, and death (69, 71, 72).

The molecular mechanism of action of exotoxin A is known. Like diphtheria toxin, exotoxin A is an enzyme that catalyzes the transfer of ADP-ribose from NAD to a specific site on elongation factor 2 (EF2), a critical component of the protein synthetic machinery. Protein synthesis is thus paralyzed.

$$\text{NAD} + \text{EF2} \xrightarrow{\text{exotoxin A}} \text{ADPR-EF2} + \text{nicotinamide} + H^+$$



Although exotoxin A and diphtheria toxin share this mechanism of action, they are structurally dissimilar, apparently utilize different cell-surface receptors (85), and their enzymatic activities cannot be cross-neutralized with their respective antisera.

A role for exotoxin A in the pathogenesis of P. aeruginosa disease is suggested by numerous observations:

1. Exotoxin A is produced by almost all (85-90%) P. aeruginosa isolates from specimens indicative of serious human disease.

2. Patients and animals with P. aeruginosa disease develop antibodies to exotoxin A.

3. The presence of serum antibodies to exotoxin A early in the course of P. aeruginosa bacteremia has been correlated with the probability of survival (133a).

4. In a mouse model of eye (corneal) infection, a toxin-producing strain was more virulent (destructive) than non-toxin-producing mutants. (In contrast, toxin-producing P. aeruginosa were less virulent than tox mutants in a model of pneumonia in guinea pigs.)

5. Mice with burn wound infection with toxin-producing P. aeruginosa manifest decreased functional EF2 levels in tissue; enzymatically active exotoxin A has been found in the burn wounds and serum of these animals. Passive immunization with antibodies against exotoxin A prevented the metabolic changes and enhanced survival. In contrast, mice that were infected with non-toxin-producing strains showed no alteration in protein synthesis and were not benefited by antitoxin.

On the other hand, there is evidence that non-toxin-producing strains retain normal virulence in experimental lung infection, at least in the guinea pig model (82); studies in a rat pneumonia model have been more consistent with a pathogenetic role for exotoxin A (71).

It seems likely that exotoxin A production enhances the virulence of P. aeruginosa. Excretion of the toxin at certain local sites may, by inducing tissue damage, foster local invasion, while release of exotoxin A into the circulation may produce some of the systemic effects observed in P. aeruginosa bacteremia.

PROTEASES. P. aeruginosa make and release a variety of extracellular enzymes, particularly proteases. Of these, alkaline protease and elastase have received most study. The available evidence, derived largely from the work of Wretlind (68), suggests that the proteases, which are inhibited by the serum protein α_2-macroglobulin, may contribute to the pathogenicity of P. aeruginosa at local sites (e.g., eye infections, burn wounds) but not to the lethality of bloodstream infections. Wretlind calls elastase a "virulence-enhancing factor in certain types of infections." In more recent experiments, Blackwood et. al. found evidence that elastase contributes to the virulence of P. aeruginosa in the lung (experimental guinea pig pneumonia) (82). Purified protease and elastase, when injected into animals, produce hemorrhagic-necrotic skin lesions somewhat similar to those seen with Pseudomonas sepsis (72). Recent studies also suggest that protease and elastase inhibit neutrophil chemotaxis in vitro (86). Danish workers have found high levels of antibodies to alkaline protease and elastase in the serum of patients with CF, indicating that these enzymes are produced by P. aeruginosa in vivo in man.

PHOSPHOLIPASE C. Extracellular phospholipase C may damage cell membranes (causing hemolysis, for example), and a bacterial glycolipid is thought to contribute to this process (73). A definitive role for this enzyme in pathogenesis is not established.

SUMMARY. Adherence of P. aeruginosa to human mucosal surfaces is probably mediated by pili. In the upper respiratory tract, adherence is promoted by a deficiency in cell-surface fibronectin, which appears to occur when there is excess salivary protease (as in acutely ill hospitalized patients). Local invasion is probably enhanced by the excretion of exotoxin A (best shown in burn wounds and ocular infections) and bacterial proteases (best shown for pneumonia); flagellar motility is probably essential for invasion to occur. Bloodstream invasion is poorly understood but occurs most readily in granulocytopenic animals or in animals whose host defenses (e.g., neutrophil function) have been altered by overwhelming burn injury, suggesting that the bacterial virulence mechanisms are not normally sufficient to overcome host

defenses (opsonization, phagocytosis) that prevent bacteremia. Exotoxin A probably produces some of the systemic manifestations of sepsis (neutropenia, liver injury, shock).

STATIONARY (MICROCOLONY) MODE

Lower respiratory tract infection in patients with cystic fibrosis

Cystic fibrosis is an autosomal recessive disease of generalized dysfunction of the exocrine secretory glands. Mucus-producing glands throughout the body, including those in the lung, produce abnormal secretions that tend to precipitate in the duct lumina and obstruct the flow of secretions. Pulmonary manifestations often begin early in life, with bronchiolitis, bronchitis, and eventually bronchiectasis. Colonization of the lower respiratory tract with P. aeruginosa appears to be almost inevitable in the patient with cystic fibrosis who survives beyond 10 years of age (87). Indeed, this organism (along with S. aureus) plays a major role in the pulmonary disease that often leads to the death of patients with CF. Children with CF usually become colonized with rough (nonmucoid) strains of P. aeruginosa early in life, and subsequently (usually by the 'teens) the strains become mucoid (i.e., excrete an exopolysaccharide that makes the colonies appear shiny, irregular, and "runny" on agar plates) and the severity of the lung disease increases (87). The histopathology is that of a (usually mild) bronchopneumonia superimposed upon chronic changes of bronchiectasis, peribronchial fibrosis, and granulation tissue formation. The organisms, surrounded by exopolysaccharide, may themselves contribute to the plugging of small airways, but parenchymal invasion is not striking and bloodstream invasion is exceedingly uncommon.

Why do children with CF become colonized with P. aeruginosa? In an another intriguing study, Woods and his colleagues (88) found that P. aeruginosa adhered to buccal epithelial cells from patients with CF much more commonly than they did to cells from normal control subjects. Nonmucoid cells adhered in much higher numbers than did mucoid cells, and increased adherence was associated with decreased amounts of fibronectin on the CF epithelial cell surfaces and with increased levels of proteases in CF saliva. The authors suggested that their findings may explain the observation that nonmucoid organisms are the initial colonizers in CF patients. Because of their excess salivary protease levels, which presumably produce the deficiency in epithelial cell fibronectin (which coats the cell surface and apparently prevents gram-negative bacterial adherence), these children become colonized more easily with P. aeruginosa than do normal children. They acquire nonmucoid strains because these are the strains that they encounter; mucoid isolates are rarely obtained from environmental sources (water, etc.). The observations of Woods et. al. might also be used to explain the finding that P. aeruginosa do not seem to be transmitted between CF patients, even in the environment of a summer camp for children with CF (91-93). Presumably in these older children the isolates are largely mucoid, and these cells would not have the adherence advantage of nonmucoid cells.

A discussion of mucoid P. aeruginosa will be preceded by a brief overview of the exopolysaccharides produced by this organism.

EXOPOLYSACCHARIDES. This is a confusing area, largely because there is disagreement in the literature on the composition and importance of at least three different exopolysaccharides.

Slime. P. aeruginosa cells may secrete compounds that form a loose capsular slime around the cells. A clue to the complex nature of this material is its current descriptive name: glycolipoprotein (GLP). The precise chemical composition of GLP is not known. When injected into mice, GLP causes leukopenia and death (LD_{50} = 30 ug/g body weight). Antibodies to GLP apparently opsonize P. aeruginosa, promoting phagocytosis, and protect mice from experimental challenge with live organisms. Purified GLP is said to produce neutropenia in mice (74).

High molecular weight polysaccharides (HMWPS). Pier and his associates (77) have isolated high molecular weight polysaccharides from the supernatants of broth cultures of P. aeruginosa. These compounds contain antigen(s) that, unlike slime polysaccharides, cross-react with the O antigen of the cell wall LPS, but the HMWPS lack the toxicity of LPS and their sugar composition differs quantitatively from that of LPS. The HMWPS appear to be immunogenic in experimental animals and man, and the antibodies raised by HMWPS vaccination appear to function normally as opsonins. There is a distinct HMWPS for each LPS type (7 or more, depending on the system used).

Alginates (mucoid strain polysaccharides). These are acetylated polymers of mannuronic and guluronic acids (75); cells that secrete them produce mucoid colonies on agar plates. The alginates may form a gel in the presence of calcium and may surround the cells in a "microcolony mode" (Costerton) that is relatively sheltered from host defense mechanisms (79); the negatively charged polysaccharide may also (at least theoretically) trap positively charged antibiotics such as aminoglycosides (76). Govan found that mucoid cells were cleared less quickly from the tracheobronchial tree than non-mucoid cells in an animal model of experimental infection (78).

The extracellular alginate seems to be the most important exopolysaccharide for the pathogenesis of bronchial infection. Alginate synthesis and excretion is thought to be controlled by at least 2 chromosomal genes, and strains of P. aeruginosa can be forced to switch back and forth between rough and mucoid cell types by various manipulations in vitro (78). None of these manipulations has been evaluated in vivo. Similarly, the components of CF sputum that create a favorable environment for P. aeruginosa persistence are not known (90); one recent study suggests that sputum iron levels might be a reasonable subject for investigation (89), and there is good evidence that antibiotic therapy (particularly with carbenicillin) may encourage the selection of mucoid cells (78). Patients with other chronic lung diseases may also harbor mucoid P. aeruginosa in their sputum, indicating that the CF milieu, per se, is not required for the persistence of mucoid strains.

Other bacterial factors may contribute to the microcolony mode. Most mucoid strains appear to be non-flagellated (80) and thus non-motile, a fact that may help explain the relatively non-invasive nature of mucoid P. aeruginosa bronchopulmonary infection. A recent study also suggests that the LPS of these strains may contribute. The serum sensitivity (susceptibility to killing by normal serum) of gram-negative bacteria is generally related to

the length of the polysaccharide component of the LPS; long and short polysaccharides are usually found in the LPS of resistant and sensitive strains, respectively. Hancock and co-workers (98) have recently reported that many of the P. aeruginosa isolates from sputum of CF patients had short LPS polysaccharides and were serum sensitive. They suggest that the presence of such isolates may help explain the apparently low rate of blood invasion found in CF patients; a larger and more complete study is required.

It is interesting that patients with CF may harbor more than one strain of P. aeruginosa, that most who have mucoid isolates also have nonmucoid strains in the same specimen, and that different isolates from the same sputum sample may have strikingly different antimicrobial susceptibilities (78). In part, these differences may possibly relate to the tendency of organisms to stop producing alginate when cultured in vitro; it is also easy to imagine that the presence or absence of the alginate layer might influence the diffusion of antibiotics to their site of action (99).

One recent study found that 22% of young CF patients (less than 10 years of age) had low serum levels of immunoglobulins G and/or A, while older CF patients had either normal or high immunoglobulin levels (100). Although none of several parameters of immune function was abnormal in these patients, the patients with hypogammaglobulinemia had significantly less severe lung disease than did age-matched patients with normal or elevated IgG levels. Interestingly, the hypogammaglobulinemic children also had a significantly lower rate of colonization with P. aeruginosa. These observations are consistent with a schema in which P. aeruginosa colonization promotes both antibody formation and deterioration in lung function. Other studies have found that CF patients make antibodies to a variety of P. aeruginosa antigens; the mean time interval between colonization and the development of antibodies to pseudomonas elastase and protease was 11 and 15 months, respectively (97). Antibody titers tend to increase with repeated exacerbations of P. aeruginosa infection. Examination of CF sputum for the presence of these pseudomonal enzymes found that the enzymes were present only when antibodies to them were absent (96). These studies suggest that the antibodies in sputum may be able to neutralize the extracellular antigens produced by the bacteria; since these enzymes may contribute to the tissue damage caused by the organisms in the "motile" mode, perhaps these observations help explain the relative lack of tissue invasiveness seen with P. aeruginosa colonization of the lower airways in CF.

SUMMARY. Adherence of non-mucoid P. aeruginosa to epithelial cells that have low amounts of surface fibronectin occurs early in the life of most CF patients, leading to respiratory tract colonization with these organisms. At some subsequent point, for unclear reasons, the organisms may "switch" to a mucoid phenotype in which they produce large amounts of alginate. The alginate may sequester the bacteria from antibody, phagocytic cells, and even certain antibiotics. Although the bacteria may produce extracellular enzymes such as protease and elastase, these are probably neutralized by host antibody and thus do not promote bacterial invasion. Similarly, the bacteria become non-flagellated (non-motile) and, due to a lack of LPS polysaccharide, they may be quite susceptible to killing by normal serum. Taken together, these properties tend to make the organisms rather non-invasive, and the disease that they cause in the bronchopulmonary tract of CF patients is more a consequence of mechanical obstruction to airways than it is an invasive infection in the usual sense.

PREVENTION: ACTIVE IMMUNIZATION

Studies in animals have shown that immunization with whole-cell or cell-wall (LPS) P. aeruginosa vaccines can prevent death from experimental pneumonia or sepsis, and several immunization trials using LPS-based vaccines have been conducted in man. Two preparations have been used for most of these studies, a heptavalent vaccine prepared by Parke-Davis using the Fisher immunotypes (see page 2), and a hexadecavalent vaccine (PEV-01) prepared by Burroughs Wellcome. As indicated in the following table, taken from a recent review by Bodey (101), these vaccines have been somewhat successful:

Vaccine	Population immunized	No. of patients		Mortality/sepsis		Ref.
		vaccine	control	vaccine	control	
Heptavalent	Cancer pts	176	185	7.3/7.8	16.7/10.2	102
Heptavalent	Burn pts	96	75	3.1/8	14.1/18	103
PEV-01	Burn pts	18	20	0/...	15/...	104
PEV-01	Burn pts	51	56	0/0	18.7/12.5	105

Most of these trials involved randomization of patients to vaccine or control groups; the above results reflect instances of mortality and sepsis due to P. aeruginosa. No comparisons of the heptavalent and PEV-01 vaccines have been reported. Hyperimmune anti-P. aeruginosa serum has also not been tested for prophylaxis, although it may be a very useful adjunct to therapy (see below).

Adverse side-effects have been frequently observed in recipients of these vaccines, particularly the heptavalent vaccine. This toxicity is thought to reflect the presence of LPS in the vaccines, and workers have tried to find other suitable immunogens. One of these, the HMWPS (Pier, see page 19) is antigenically cross-reactive with LPS but lacks lipid A, the toxic moiety. Experiments in animals and human volunteers indicate that type-specific antibodies can be raised using HMWPS as immunogen (112). Unfortunately, a vaccine using HMWPS as antigen would have to be polyvalent. Clinical protection trials are anticipated.

Japanese workers have pioneered in studies on OEP ("original endotoxin protein"), an antigen common to most serotypes of P. aeruginosa. This material is non-toxic, and a recent report indicated that a multivalent vaccine (that included OEP, elastase, and protease) was effective in preventing epidemic P. aeruginosa pneumonia in minks (106). Human studies with this vaccine have not been reported.

There has been widespread interest in exotoxin A "toxoids," stimulated by the studies cited above that implicate exotoxin A in the pathogenesis of invasive P. aeruginosa diseases. Although a variety of methods for producing toxoids exists, and an interesting cross-reactive (but non-toxic) mutant toxin has been purified by Cryz and co-workers (107), none of these preparations has been extensively evaluated. The available evidence suggests that such toxoids, given alone, may not provide complete immunity; the induction of opsonic antibody may be more important for protection than the induction of antitoxic immunity (109). Interestingly, one study found that formalinized toxoid, given with adjuvant (108), could produce 100% survival in a mouse model of burn wound sepsis provided that one dose of gentamicin was also given (111).

Vaccines using P. aeruginosa flagellae or enzymes would also theoretically make attractive vaccine candidates, based on the animal studies cited above. No human studies using these immunogens have been reported.

Vaccination of CF patients with PEV-01 had no demonstrable effect upon the course of their disease, in keeping with the observations that such patients often have high titers of anti-pseudomonal antibody and yet are unable to eradicate colonization. More recently, workers have suggested that vaccination of children with cystic fibrosis, if performed early in life, might prevent the acquisition of P. aeruginosa. This approach would seem reasonable, particularly in view of the arguments advanced above regarding the possible role of P. aeruginosa in the causation of both hyperglobulinemia and deterioration in lung function in these patients. On the other hand, it is not possible to be sure that hyperglobulinemia itself does not contribute to the pathogenesis of the pulmonary disease (100); immunization might only make things worse. Although LPS-based vaccines would seem the obvious choice for investigating this problem (the HMWPS seem most attractive), a number of other possible vaccine candidates might be considered: (1) P. aeruginosa pili, hoping to produce oral antibody that might prevent the attachment of P. aeruginosa to the upper respiratory epithelium (2) GLP might be used, since antibodies to this substance appear to be opsonic. Another approach might involve investigation of the genetic and enzymatic basis for the "switch" from non-mucoid to mucoid phenotype, with the goal of developing either a chemical or immunologic approach to preventing this switch. An analogy may be provided by Streptococcus mutans, which produces an extracellular glucan that is thought to allow adherence of this organism to dental enamel, where it initiates caries. The streptococcal enzymes (glucosyl transferases) produce the glucon extracellularly. Vaccines using purified enzymes as immunogens have led to the appearance of oral antibody that successfully inhibited dextran production by S. mutans and prevented caries in animals (112a). It is conceivable that a similar approach might work to prevent alginate synthesis by P. aeruginosa in patients with cystic fibrosis.

DIAGNOSIS

Wound appearance. Although the association of P. aeruginosa wound infections with blue-green pus has been noted for over 100 years, this appearance is not very specific for this pathogen. Greenish discoloration often occurs in wounds infected by other pathogens and probably reflects simply the presence of (disintegrated) neutrophils. A more diagnostically useful characteristic of pseudomonas-infected wounds may be the presence of a fluoroscein pigment that can be detected by ultraviolet light (Woods lamp). The presence of fluoroscein has suggested the diagnosis of P. aeruginosa ocular infection (113) and tricuspid valve infection (114); the specificity of this finding is not known.

Culture, gram stain. P. aeruginosa is a strict aerobe. It will not grow in an anaerobic environment. This property distinguishes P. aeruginosa from most of the other common gram-negative bacilli that are grown from blood, since these organisms (E. coli, Klebsiella, Proteus, etc.) are facultative anaerobes and usually grow in both the aerobic and anaerobic blood culture bottles. A laboratory report indicating that a patient has gram-negative rods growing only in the aerobic blood culture bottles should alert one to

the possibility that the organism could be P. aeruginosa. (Other gram-negative bacilli that are strict aerobes: brucella, other pseudomonads, Acinetobacter).

The gram-stain appearance of P. aeruginosa is usually different from that of the enteric gram-negative rods. P. aeruginosa tend to be smaller in diameter than, say, E. coli. This difference is not sufficiently reliable to allow definitive identification, but it may be useful when one is designing anticipatory therapy based on a gram stain of infected material.

Antigen detection. Two solid-phase ELISA techniques have been developed for the detection of P. aeruginosa antigen (LPS) in blood and urine (116, 117). A similar method exists for detecting exotoxin A (116), and a radio-immunoassay has been developed for P. aeruginosa elastase. Clinically useful applications of these methods have not been described, however.

THERAPY

Antimicrobial chemotherapy

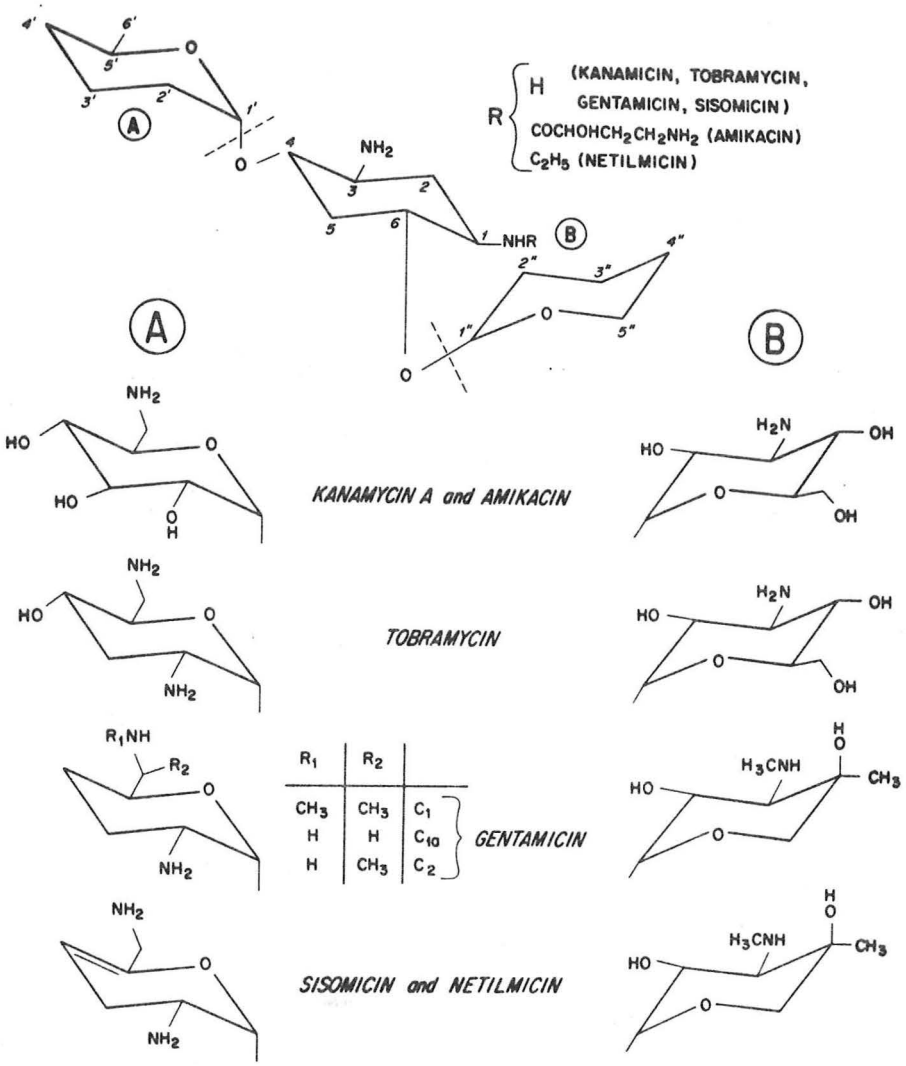
The following comments pertain specifically to antimicrobial therapy for P. aeruginosa. Generalizations should not be applied to antimicrobial therapy for other gram-negative bacilli, although often regimens that are effective for sensitive P. aeruginosa will also be effective for other sensitive organisms. This is particularly important with regard to combination therapy, where evidence for efficacy is better for P. aeruginosa than for most other pathogens.

Aminoglycosides. These drugs continue to form the cornerstone of antibiotic therapy for P. aeruginosa. Although several new aminoglycosides have been introduced in recent years, none appears to have a definite advantage over the "standard" anti-pseudomonal aminoglycosides, gentamicin (note spelling), tobramycin, and amikacin. The following comments are largely derived from an excellent review by Eliopoulos and Moellering (118).

Aminoglycosides are really aminoglycosidic aminocyclitols; they have a six-membered aminocyclitol ring to which one more amino-containing sugars are attached.

The mechanism of action of the aminoglycosides is complex and poorly understood, even after decades of serious study. The drugs must penetrate intracellularly, a process that includes both energy-independent (binding) and energy-dependent (uptake) steps. Once inside the cell they bind to ribosomes, thereby producing an inhibition of protein synthesis (probably by interfering with the initiation of translation) and the synthesis of nonsense proteins (misreading). Bacterial resistance to the drugs can involve a block in one or more of these steps: (1) drug uptake requires energy, and transport does not occur in the absence of oxygen (thus the inactivity of the aminoglycosides towards anaerobes, or in anaerobic conditions), (2) a variety of enzymes, present in the periplasmic space (between the cytoplasmic and outer membranes), may inactivate the drugs as they cross this space, thereby inhibiting transport, ribosomal binding, and/or ribosomal action of the

drugs, (3) the ribosomal target site may be altered, so that the drug does not bind or does not have its usual action.



Most clinically significant resistance involves plasmid-encoded enzymes that acetylate (a NH_2 group), adenylate (a OH group), or phosphorylate (a OH group) the aminoglycosides. Different aminoglycosides are susceptible to different enzymes, and different plasmids may contain one or more of the enzymes. Gentamicin and tobramycin, for example, are usually susceptible to several of the same inactivating enzymes (e.g., the major adenylytransferase [ANT(2'')]) whereas amikacin is different--it is susceptible to only one of the known gram-negative bacterial enzymes, a 6' acetyltransferase (AAC[6']). This property of amikacin, as well as the apparently low prevalence of the AAC (6')-producing plasmid in clinical isolates, may account for the observation that amikacin resistance has not increased in hospitals which have used this antibiotic extensively for a period of years (119). This experience contrasts, in most instances, with the emergence of resistance to gentamicin and tobramycin under comparable circumstances of use. Longer periods of observation may be necessary to reach secure conclusions about this point, however (120).

In recent years, isolates of P. aeruginosa at PMH have been gratifyingly sensitive to the commonly used aminoglycosides. Isolates of P. aeruginosa usually are somewhat more sensitive in vitro to tobramycin than to gentamicin, and even tobramycin and gentamicin-resistant isolates are usually sensitive to amikacin. These statements are supported by recent data from the PMH microbiology laboratory (provided by Dr. Paul Southern) (121):

Susceptibility of Pseudomonas aeruginosa to Aminoglycosides

Agent	No. Tested	MIC Range (ug/ml)	MIC_{50} (ug/ml)	MIC_{90} (ug/ml)	Per Cent Susceptible[1]
Tobramycin	515	<0.25->16	0.5	2	95
Gentamicin	350	<0.25->16	2	16	60
Amikacin[2]	425	<2 ->64	8	16	96

[1] Susceptible to clinically relevant serum levels.

[2] Amikacin only tested against strains having Tobramycin or Gentamicin MIC $\geq$ 8 ug/ml.

As summarized by Eliopoulos and Moellering (118), "There is no evidence that any aminoglycoside is more effective clinically than others to which the pathogen is equally susceptible in vitro". Other factors, such as toxicity and cost, must thus influence the choice of aminoglycoside in a given clinical situation. The ototoxicity of the aminoglycosides is apparently quite similar--10-14% when evaluated using audiometry. There is evidence (albeit controversial) that tobramycin is less nephrotoxic than gentamicin, and it would seem reasonable to favor tobramycin for long-term therapy. On the other hand, tobramycin is more expensive than gentamicin, and amikacin is even more expensive.

Recommendations: use tobramycin rather than gentamicin (1) when the patient has tissue infection with a P. aeruginosa isolate that is more sensitive in vitro to tobramycin, (2) when long-term aminoglycoside therapy is anticipated (e.g., endocarditis), (3) for anticipatory therapy, when there is a high risk of P. aeruginosa sepsis (febrile neutropenic patient, burn wound sepsis, pneumonia). Use gentamicin rather than tobramycin for anticipatory therapy in patients who are unlikely to have P. aeruginosa infection, or in whom a period of suboptimal therapy is unlikely to alter the outcome (e.g., UTI, abdominal sepsis). In the U.S., gentamicin-resistant isolates of P. aeruginosa are exceedingly unusual outside of hospitals. Use amikacin for gentamicin- and tobramycin-resistant isolates, for anticipatory therapy in patients in whom GM-, TM-resistant gram-negative isolates are suspected (e.g., neutropenic patients who have been hospitalized for several days or treated with TM or GM), and in patients with renal failure who might benefit from ticarcillin-aminoglycoside combination therapy (see below) --

unlike gentamicin and tobramycin, amikacin does not appear to undergo inactivation by antipseudomonal penicillins in vivo or in vitro.

Dr. Craig Brater and his colleagues have devised a computer program that can assist the physician who wants to decide an initial aminoglycoside dose; the program can be used via telephone contact with the computer operator (688-2209). The same group also provides a computer-based algorithm for adjusting aminoglycoside dosage using blood levels. Although it is arguable that all patients who receive aminoglycosides should have blood levels determined, another approach would be to limit these determinations to patients most likely to benefit from careful dosing: the elderly and other patients with impaired renal function; burn patients; patients in whom high levels of drug are desirable (such as those with P. aeruginosa endocarditis); patients who are hypotensive or receiving medications (such as NSAID) that may alter renal function; obese patients.

Who should have audiometry performed before and during therapy? Current recommendations (118) include patients who will require long courses of therapy, those who develop symptoms suggestive of ototoxicity (unfortunately, high frequency hearing loss occurs first, and this is unlikely to be clinically detected), patients who have received "substantial quantities" of aminoglycosides in the past, those with renal impairment, and patients in whom even minimal hearing or balance dysfunction would be a major disability. Most of the patients who have developed severe hearing impairment while receiving aminoglycosides at PMH have been dialysis patients; unless indicated by clinical circumstances or measured levels, such patients should probably not receive post-dialysis does of aminoglycoside more than 3 or 4 times a week, regardless of the number of dialyses performed.

Antipseudomonal penicillins. These drugs fall into two chemical categories, the carboxypenicillins (carbenicillin, ticarcillin) and the newer acylureido penicillins (azlocillin, mezlocillin, piperacillin).

Although initial reports suggested that carbenicillin alone might be effective therapy for P. aeruginosa disease, much subsequent experience indicates that this drug (as well as the other drugs in this class) should usually be used in combination with an aminoglycoside (101). Combination therapy may be more effective than monotherapy (see below) and may also prevent the emergence of resistant strains. Ticarcillin and carbenicillin have essentially the same antimicrobial spectrum, but because ticarcillin is more potent and can be given in lower dosage (with lower sodium load and, presumably, with lower risk of platelet dysfunction), it has generally replaced carbenicillin in most hospitals.

The newer antipseudomonal penicillins were reviewed in a recent Grand Rounds (Dr. James Smith, October 20, 1983) (122) and will not be discussed in detail here. As is shown in the table on the following page (again, data were kindly provided by Dr. Paul Southern from the PMH laboratory), azlocillin and piperacillin are more active than mezlocillin toward our isolates (121).

The role that these new, expensive drugs will play in the therapy of P. aeruginosa and other pathogens is uncertain. Azlocillin has been widely used in Europe and it appears to be at least as effective as ticarcillin (when

used in combination with an aminoglycoside) for serious infection. Piperacillin is probably a poorer drug than azlocillin, although good data to solidify this conclusion are not available. The side-effects of these drugs are probably similar--though serum-sickness-like symptoms were reported in one study of cystic fibrosis patients who received piperacillin or azlocillin, and similar symptoms were not observed in patients who received ticarcillin (142). Workers in Detroit tried to use piperacillin plus an aminoglycoside for treating patients with P. aeruginosa endocarditis but found that ticarcillin plus an aminoglycoside was superior (24). At the moment it seems reasonable to use the older drugs (e.g., ticarcillin), reserving the newer agents for ticarcillin-resistant isolates or other special circumstances.

Susceptibility of Pseudomonas aeruginosa to Newer Beta-Lactam Antimicrobial Agents

Agent	No. Tested[1]	MIC Range (ug/ml)	MIC_{50} (ug/ml)	MIC_{90} (ug/ml)	Per Cent Susceptible
Ticarcillin	237	16 - NI	64	256	82
Azlocillin	525	1 - 512	32	128	92
Mezlocillin	420	2 - 512	64	256	80
Piperacillin	746	1 - 512	16	256	87
Moxalactam	1320	2 - 128	32	64	51
Cefotaxime	771	1 - 64	32	64	58
Cefoperazone	985	1 - 64	8	64	89
Cefsulodin	426	1 - 128	4	32	93
Ceftazidime	305	2 - 64	8	64	88
Azthreonam	621	1 - 64	4	64	60
N-formimidoyl-thienamycin	478	0.5 - 64	2	8	98

[1] Total number of isolates tested = 1422. All isolates not tested against each agent.

[2] Susceptible to clinically relevant serum levels.

Combination chemotherapy. Combination therapy with two drugs (antipseudomonal penicillin and aminoglycoside) has a basis in (1) in vitro evidence for synergism (greater than additive effect) using two drugs, (2) experimental studies performed in animals, and (3) several clinical trials. In most of the animal models reviewed by Young (123), the same therapeutic effect could be achieved with large doses of single drugs and smaller doses of drugs used in combination. Equally convincing studies have found no difference in the efficacy of single drugs (particularly if used in high

dosage) and two drug combination therapy. In these experimental animal models, combination therapy has usually been superior to a single drug for the treatment of P. aeruginosa sepsis, while it has been harder to show a benefit of two drugs for tissue infections such as pneumonia (125) and osteomyelitis (126). Pennington has argued that these differences might relate to the poor (and variable) tissue penetration of the drugs, so that their actual tissue concentrations do not resemble those achieved in vitro. However, one recent study found no synergy between ticarcillin and tobramycin in a model of P. aeruginosa sepsis, in contrast to the earlier studies using similar design (128). The explanation for these discrepancies is uncertain.

Both retrospective and prospective studies, largely performed in cancer centers, have suggested strongly that combination therapy can be superior to single drug therapy for treating gram-negative rod sepsis. Unfortunately, this conclusion is generally valid only if the patient's isolate is susceptible to both drugs in vitro. Moreover, Young is probably correct in stating that "no single study has been convincing in establishing the superiority of combination therapy for a specific gram-negative bacillary infection," including P. aeruginosa sepsis (123). The practice of using two drugs (usually ticarcillin plus tobramycin) as anticipatory therapy in the febrile neutropenic patient is based on the desirability of using at least one effective agent and the added potential benefit of synergistic therapy should the patient's isolate be sensitive to both. Several useful reviews of combination antimicrobial chemotherapy have been published (123, 129-131).

Additional evidence that supports the use of aminoglycoside-antipseudomonal penicillin combinations for serious P. aeruginosa disease comes from uncontrolled studies of the treatment of endocarditis. Reyes, et. al. examining isolates from 30 patients with P. aeruginosa endocarditis, found that combinations of carbenicillin with gentamicin or tobramycin were synergistic against 25 strains. Medical therapy failed for all five patients whose isolates were not killed synergistically, and for 12 of 25 of those with isolates susceptible to synergy. High dose therapy was neccessary for success: 8 mg/kg/day gentamicin or tobramycin, aiming for peak blood levels of 12-20 ug/ml, combined with carbenicillin (400 mg/kg/day, or usually 30 gm/day) or ticarcillin (24 gm/day). Interestingly, an attempt to substitute piperacillin for ticarcillin, although supported by in vitro susceptibility data, had to be abandoned when this regimen failed to clear P. aeruginosa bacteremia in several patients (23).

In conclusion, aminoglycosides remain the backbone of therapy for serious P. aeruginosa disease. Although it is not possible to reach firm conclusions about the role of adding an antipseudomonal penicillin such as ticarcillin in every situation, the available evidence suggests that two drug (aminoglycoside-antipseudomonal penicillin) therapy probably benefits patients with P. aeruginosa bacteremia and endocarditis, and (much less convincingly) patients with tissue infections such as osteomyelitis and pneumonia. Clinical benefit from two drug therapy is more likely if the patient's drug is synergistically killed in vitro by the two drugs.

Newer cephalosporins. As also reviewed by Dr. Smith (122), there is a bewildering number of new cephalosporins, some of which may provide effective therapy for P. aeruginosa. Ceftazidime, cefsulodin, and thienamycin seem particularly promising. Ongoing studies should establish the place of these

drugs in everyday therapy; at the present time it would seem best to use the standard regimens discussed above.

Immunotherapy. Much evidence supports the effectiveness of passive immunotherapy for P. aeruginosa bacteremia and burn wound sepsis.

1. The likelihood that one will survive an episode of P. aeruginosa bacteremia correlates directly with the serum level of antibody to P. aeruginosa LPS and/or exotoxin A that is present at the onset of sepsis. Pollack has recently reported that the rate of survival from P. aeruginosa bacteremia is also higher if patients have high titers of antibody to core endotoxin glycolipid (E. coli J5) (133).

2. Studies in experimental animals suggest that antibodies to P. aeruginosa LPS can prevent extension of burn wound infection beyond the skin (i.e., bacteremia) and can improve the survival of neutropenic animals with P. aeruginosa sepsis (134). Interestingly, in these studies antibodies to exotoxin A and elastase had no appreciable beneficial effect.

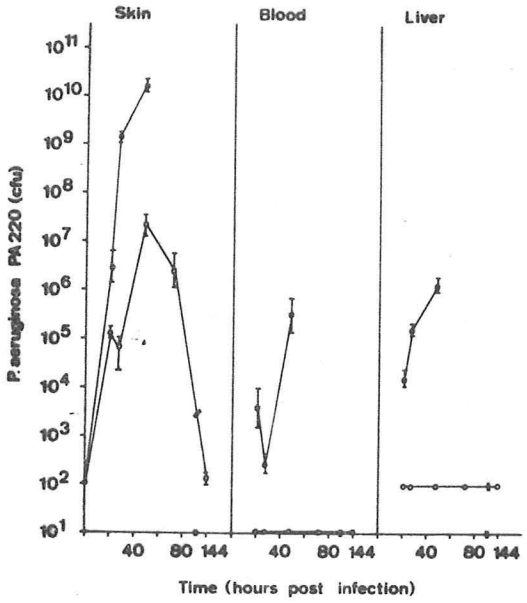


Figure 8. Quantitation of P. aeruginosa PA220 in the skin (at the site of infection), blood, and liver ± standard error of the mean at various times after challenge with 50 P. aeruginosa PA220 organisms (time 0). Each mouse received 0.2 ml of either normal rabbit IgG (●) or anti-LPS IgG (o) 24 h before challenge.

In a dog model of P. aeruginosa sepsis, other workers found that anti-P. aeruginosa IgG greatly improved the benefit provided by transfused granulocytes (135).

3. IgG antibodies present in "routine" gamma globulin preparations and in P. aeruginosa hyperimmune globulin can effectively opsonize P. aeruginosa for phagocytosis (136, 137).

4. In burn centers, hyperimmune globulin has been reported to improve the outcome of patients with P. aeruginosa burn wound sepsis (56).

5. Antiserum to E. coli J5 (endotoxin core) improved the outcome of patients with septic shock (138); approximately 20% of the patients in both treatment and control groups had P. aeruginosa bacteremia. Unfortunately, the results of therapy for each offending bacterium were not presented.

Trials to study the efficacy of passive immunotherapy for P. aeruginosa sepsis should be performed soon. It seems likely that this approach will prove useful.

THERAPY FOR SPECIFIC DISEASES

External otitis. Topical measures suffice. Cortisporin otic, local cleansing.

Folliculitis. The rash resolves without therapy in 3-5 days. Corticosteroid applications have usually made the rash worse.

Puncture-wound osteomyelitis. Most authorities recommend at least 3 weeks of parenteral therapy using an antipseudomonal penicillin plus an aminoglycoside. Some suggest omitting the aminoglycoside after two weeks of therapy. Surgical debridement may be beneficial in some cases.

Endocarditis. High dose aminoglycoside (6-8 mg/kg/day) and ticarcillin (20-24 g/day) for 6 weeks. Left-sided endocarditis often requires valve replacement; some would operate as soon as the diagnosis is made (26). Serum-cidal levels do not correlate well with the outcome (24).

UTI. An aminoglycoside alone should suffice; the course should last 7-10 days. Most would treat P. aeruginosa pyelonephritis for at least 2 weeks.

Bacteremia, burn wound sepsis:

Neutropenic or burn patient: ticarcillin-aminoglycoside therapy for 10-14 days, depending upon the patient's response. Adjunctive immunotherapy may be beneficial.
Non-neutropenic patient (e.g., bacteremic pyelonephritis): aminoglycoside +/- ticarcillin, for 10-14 days.

Pneumonia. Most experts would use combination therapy, although benefit from adding the antipseudomonal penicillin is not proven. Some (Klastersky) advocate administering antibiotic via the endotracheal tube, but there is little enthusiasm generally for this approach. The role of immunotherapy in the treatment of pneumonia is unclear.

Cystic fibrosis with respiratory exacerbation (139-145). Antibiotic therapy probably helps reduce the number of P. aeruginosa organisms that colonize these patients, but eradication (cure) of infection is not possible. Most experts use two drug combinations for 7-10 days to treat respiratory exacerbations. Studies of various combinations have not shown any one regimen to be impressively superior to the others. Some have advocated administering antibiotics via aerosol as out-patient therapy for patients with frequent hospitalization for pulmonary exacerbations; this is

controversial at present. Most authorities emphasize the beneficial role of good bronchopulmonary toilet in CF patients.

As noted earlier in the handout, P. aeruginosa isolates with quite different antimicrobial sensitivities may be grown from the same specimen of CF sputum, making interpretation of bug-drug interactions quite difficult.

APPENDIX

Recommendations for Disinfection and Sterilization

	Disinfection				Sterilization (Will enter tissue or vascular system)	
	Will not come in contact with skin or tissue		Will come in contact with skin or mucous membrane			
Object	Procedure	Minutes	Procedure	Minutes	Procedure	Hours
Smooth, hard-surfaced objects	A	≧10	A	≧30	C	18
	D	≧10	C	≧30	K	mfr. rec.
	E	≧10	F	≧30	L	12
	G	≧10	H	≧30	M	10
	I	≧10	J[a]	≧30	P	mfr. rec.
			L	≧30		
			M	≧30		
			N	≧30		
Rubber tubing and catheters			F	≧30	K	mfr. rec.
			H	≧30	P	mfr. rec.
			M	≧30		
			N	≧30		
Polyethylene tubing and catheters[b, c, d]			A	≧30	C	18
			F	≧30	K	mfr. rec.
			H	≧30	L	12
			M	≧30	M	10
			N	≧30	P	mfr. rec.
Lensed instruments			L	≧30	K	mfr. rec.
			M	≧30	L	12
					M	12
Thermometers (oral & rectal)[e]			B	≧30	C	18
			M	≧30	K	mfr. rec.
					L	12
					M	10
Hinged instruments					K	mfr. rec.
					L	12
					M	10
					P	mfr. rec.

Key:

A Ethyl or isopropyl alcohol (70%–90%)
B Ethyl alcohol (70%–90%)
C Formaldehyde (8%)-alcohol (70%) solution
D Quaternary ammonium germicidal detergent solution (2% aqueous solution of concentrate)
E Iodophor germicidal detergent (100 p.p.m. available iodine)
F Iodophor (500 p.p.m. available iodine)
G Phenolic germicidal detergent solution (1% aqueous solution of concentrate)
H Phenolic solutions (3% aqueous solution of concentrate)
I Sodium hypochlorite (100 p.p.m. available chlorine)
J Sodium hypochlorite (1000 p.p.m. available chlorine)
K Ethylene oxide gas (for time, see manufacturer's recommendations)
L Aqueous formaline (40% formaldehyde)
M Glutaraldehyde (2% aqueous solution)
N Wet pasteurization at 75°C after detergent cleaning
P Heat sterilization (see manufacturer's recommendations)

Notes:

[a] Not recommended for metal instruments.
[b] Tubing must be completely filled for disinfection.
[c] Instruments or catheters that enter tissue or the vascular system should be sterilized.
[d] Thermostability should be investigated when indicated.
[e] Thermometers must be thoroughly wiped, preferably with soap and water, before disinfection or sterilization. Alcohol-iodine solutions will remove markings on poor-grade thermometers. Do not mix rectal and oral thermometers at any stage of handling or processing.

Modified from U.S. Department of Health, Education, and Welfare. *Isolation Techniques for Use in Hospitals* (2d ed.). DHEW Publication No. (CDC) 76–8314. Atlanta: Center for Disease Control, 1975.

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