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# MOLECULAR EPIDEMIOLOGY OF *PSEUDOMONAS AERUGINOSA*

**David P. Speert**

*Research Centre, Vancouver, B.C., V5Z 4H4, Canada*

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## 1. ABSTRACT

*Pseudomonas aeruginosa* is a serious opportunistic pathogen in certain compromised hosts, such as those with cystic fibrosis, thermal burns and cancer. It also causes less severe noninvasive disease, such as otitis externa and hot tub folliculitis, in normal hosts. *P. aeruginosa* is phenotypically very unstable, particularly in patients with chronic infection. Phenotypic typing techniques are useful for understanding the epidemiology of acute infections, but they are limited by their discriminatory power and by their inability to group isolates that are phenotypically unrelated but genetically homologous. Molecular typing techniques, developed over the past decade, are highly discriminatory and are useful for typing strains from patients with chronic infection where the bacterial phenotype is unstable; this is particularly true in cystic fibrosis, where patients often are infected with the same strain for several decades, but the bacteria undergo phenotypic alteration. Molecular typing techniques, which have proven useful in typing *P. aeruginosa* for epidemiological purposes, include pulsed field gel electrophoresis, restriction fragment length polymorphic DNA analysis, random amplified polymorphic DNA analysis, repetitive extrapalindromic PCR analysis, and multilocus sequence typing. These methods are generally only available in specialized laboratories, but they should be used when data from phenotypic typing analysis are ambiguous or when phenotypic methods are unreliable, such as in cystic fibrosis.

## 2. INTRODUCTION

*Pseudomonas aeruginosa* is a Gram-negative bacterial pathogen of tremendous importance because of its capacity to infect certain compromised hosts. It is the leading cause of respiratory tract infections in patients with cystic fibrosis (CF) (1) and is a prevalent pathogen in individuals who are neutropenic as a result of cancer chemotherapy (2, 3) and in patients suffering from thermal burns (4, 5). In addition to its capacity to cause devastating invasive disease, *P. aeruginosa* also causes less serious, noninvasive infections in immunocompetent individuals; examples include otitis externa (swimmer's ear) (6), hot foot syndrome (7) and hot tub folliculitis(8).

A universally acceptable explanation for the success of *P. aeruginosa* as an opportunistic pathogen has not been forthcoming, but it appears to be capable of causing disease for several reasons as follows: (i) it has several putative virulence determinants which allow it to breach anatomic barriers (9); (ii) after having invaded, the bacteria can elaborate additional potential virulence determinants; (iii) it is resistant to a wide range of antimicrobial agents and disinfectants, allowing it to survive under conditions averse to other bacterial species (10); and (iv) it is hydrophilic and has to capacity to reside in a wide range of ecological niches (11). This last characteristic puts *P. aeruginosa* at a particular advantage over other opportunistic pathogens as it often finds itself "in the right place at the right time" allowing it to take advantage of compromised immunity and cause disease in vulnerable hosts.

*P. aeruginosa* can be recovered from a wide range of environmental sources, but it is particularly prevalent in moist places (11). The organism is highly versatile, and can adapt to diverse environmental niches. It is this versatility that equips it so well for infection in compromised hosts. For instance, the bacterial phenotypic changes that are seen during chronic infection in patients with CF may reflect similar morphological adaptations that have evolved for survival under conditions of nutrient limitation in aquatic environments (12).

Natural environments from which *P. aeruginosa* can regularly be recovered include (but are not limited to) the following: seawater near sewage outfalls, polluted rivers, domestic drinking water, sink drains (particularly those in hospitals), toilets, showers and hot tub recirculation tubing. Non-aqueous environmental sources include soil – particularly in the rhizosphere (the area immediately proximate to plant roots and tubers) – and from a wide range of raw vegetables (11).

Whereas *P. aeruginosa* can readily be recovered from the natural environment, it is rarely found colonizing healthy humans. Indeed "colonization resistance" is a characteristic of the normal human gastrointestinal tract (13); colonization of the gut can usually be achieved only after the GI flora has been perturbed by administration of antibiotics, such as ampicillin and orally nonabsorbable aminoglycosides (14). Similarly, the bacteria are not found under normal conditions in the respiratory tract. Patients at risk for lower respiratory tract infection with *P. aeruginosa* include those receiving mechanical ventilation and those with CF; in both cases the adhesion of *P. aeruginosa* to respiratory tract epithelium appears to be enhanced (15, 16).

Antibiotic resistance is a feature common to all *P. aeruginosa* strains (10). Resistance is both intrinsic and inducible. The former is due to relative impermeability of the outer membrane, and the latter may emerge during therapy. Development of resistance during infection is a particular problem in patients with CF who often require prolonged and repeated courses of antibiotic therapy. Induction of resistance during therapy renders the antibiotic susceptibility profile relatively unreliable as an epidemiological tool (17).

Because of the burden of infection of this important pathogen and its apparent ability to cause nosocomial infections, substantial attention has been addressed to its epidemiology both within and outside hospitals. Any epidemiological studies are only as good as the typing methods employed. The purpose of this review is to discuss the various typing methods which have been used to evaluate the epidemiology of *P. aeruginosa* and to focus on newer methods which use genetic technology.

## 3. INFECTIONS CAUSED BY *PSEUDOMONAS AERUGINOSA*

### 3.1. Cystic fibrosis

CF is an inherited defect, effecting predominantly Caucasians. Patients have a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator, a chloride channel; as a result, respiratory secretions are abnormal and the endobronchial space is highly susceptible to infection with *P. aeruginosa*. By the time CF patients reach adulthood, approximately 80% are chronically infected with *P. aeruginosa*. Most patients remain infected with the same strain of *P. aeruginosa* (18), although transient infection has been described. During the course of infection, the bacteria undergo a phenotypic evolution from rough to smooth lipopolysaccharide (LPS) (19), nonmucoid to mucoid colonial morphology (20), motile to nonmotile (21), highly toxigenic to less toxigenic (22), relatively antibiotic susceptible to highly resistant, and serum resistant to serum sensitive (19). This phenotypic plasticity on the same genetic background renders typing by phenotypic means very misleading.

### 3.2. Sepsis in compromised hosts

Normal hosts are highly resistant to invasive infection with *P. aeruginosa*, indicating an important role for intact host defense mechanisms. Polymorphonuclear leukocytes play an essential role in defense against these bacteria as demonstrated by the high risk of Pseudomonas septicemia in patients with depressed neutrophil counts (23). Patients with malignancies undergoing cancer chemotherapy are at particularly high risk for septicemia with *P. aeruginosa* (3, 24). Strains of *P. aeruginosa* recovered from neutropenic hosts (in contrast to those

from patients with CF) usually have a smooth LPS and are nonmucoid. Typing methods based upon phenotypic characteristics appear to be useful in this patient population, but newer genetic techniques provide a higher degree of discriminatory power (25).

### 3.3. Burn wound infections

Patients with burns are at extraordinary risk of acquiring *P. aeruginosa* infection of the thermal wound with subsequent septicemia and death (5). Whereas defects in systemic immunity have been documented in burned individuals, the primary host defense defect appears to be loss of the integrity of the skin. Since *P. aeruginosa* is a hydrophilic organism with the capacity to survive in numerous aqueous environments (including water and liquid disinfectants), it can easily be introduced into skin wounds in patients recovering from thermal burns. Its high level intrinsic resistance to antimicrobial agents enhances its selection in the hospital environment where heavy antibiotic pressures exist. For all these reasons, *P. aeruginosa* is a scourge of hospital burn units and substantial efforts are taken to prevent its emergence.

### 3.4. Superficial infections

Whereas serious, life threatening, invasive infections with *P. aeruginosa* occur in compromised hosts, superficial infections are regularly documented in individuals with normal host defenses. As a hydrophilic organism, it preys on hosts exposed to certain sources of fresh water. *P. aeruginosa* can survive at temperatures up to $42^0$C, putting users of hot tubs at risk (8). Indeed hot tub folliculitis is usually caused by *P. aeruginosa*. Otitis externa (swimmer's ear) is a problem in children who spend a great deal of time in fresh water swimming pools, and the predominant etiologic agent is *P. aeruginosa*. Hot foot syndrome is another superficial infection with *P. aeruginosa* to which swimmers are susceptible (7).

## 4. PHENOTYPIC TYPING METHODS

Methods for typing *P. aeruginosa* based on phenotypic (physical) features have been available for many years and have been successfully used to investigate numerous outbreaks of infection. However, the phenotypic plasticity of *P. aeruginosa* has rendered such methods impractical in situations where patients are infected for prolonged periods of time, such as in CF; under such situations, the organisms have ample opportunity to adapt to their mammalian environment and change their phenotype without altering their genetic background. Genotypic methods are therefore preferable to phenotypic ones for typing isolates from patients with chronic infection.

### 4.1. Lipopolysaccharide (LPS) serotyping

LPS serotyping has been a mainstay of epidemiological investigation of *P. aeruginosa* for several decades (26, 27). This method is based upon identification of differences in the O-polysaccharide of LPS with up to 17 different antisera. These antisera are usually polyclonal antibodies from animals such as rabbits. The antisera are able to agglutinate *P. aeruginosa* in a type-dependent fashion, but the system relies upon a well-developed O-polysaccharide moiety in the bacterial LPS. This method fails when the strains have a rough O-polysaccharide, as is typical of isolates from patients with CF (19). Isolates from patients with CF are often "polyagglutinable" as they can be agglutinated by multiple sera, each of which recognizes common features of the exposed core of the LPS molecule. Furthermore, the results of serotyping CF isolates can be very misleading, as apparent clonality may simply reflect the greater capacity of one of the multiple antisera to most avidly recognize the core region of rough LPS; this results in apparent clustering of isolates of one clone, when no such cluster exists. LPS serotyping is also of limited discriminatory power, as there are no more than 17 potential types which can be identified (27). Despite these weaknesses, LPS serotyping has been employed with great success to answer a wide range of questions about the epidemiology of *P. aeruginosa*. It is a simple method and one which can be used by any diagnostic microbiology or infection control service.

### 4.2. Other phenotypic typing methods

A wide array of methods have been used to type *P. aeruginosa* for epidemiological investigations. Each method has unique characteristics, strengths and weaknesses, but all suffer from their inability to circumvent the challenge presented by the phenotypic plasticity of *P. aeruginosa*. Phenotypic methods which have been reported for typing *P. aeruginosa* include phage, pyocin and antimicrobial susceptibility typing. In a large multicentered typing study, these different methods were compared for their capacity to reproducibly type 200 different strains of *P. aeruginosa* from a wide range of clinical and environmental sources (25). For strains other than those from patients with CF, LPS serotyping using commercial reagents provided the most reproducible and discriminatory results. For CF strains, genetic typing methods were required.

## 5. GENOTYPIC TYPING METHODS.

Molecular typing techniques embody most attributes one would seek for epidemiological investigations as follows: reproducibility, sufficient discriminatory power to differentiate among strains not epidemiologically related, and the ability to characterize as similar isolates from the same source. The discriminatory power must be high enough to differentiate among unrelated strains, but not so great as to define isolates of common lineage (but minor genetic variation) as unrelated. Further attributes of the ideal typing system include ease of use, low cost and unambiguous interpretation of derived data. Many different methods are available for molecular typing of bacteria, based upon analysis of the entire genome or portions thereof. Whereas, multiple methods have been used, discussion will be limited to the five methods that have been widely used for typing *P. aeruginosa*.

### 5.1. Restriction Fragment Length Polymorphism (RFLP)

This was the first method to be widely used for typing strains of *P. aeruginosa* from patients with CF (28).

Genomic DNA is extracted from the bacterium of interest, digested with one of several restriction enzymes, and the DNA fragments are then separated by electrophoresis. A radiolabelled probe, directed to a specific portion of the bacterial genome, is then added, and a hybridization reaction is carried out. The most discriminatory probes are those which react with a hypervariable portion of the bacterial genome. In the case of *P. aeruginosa*, the most informative data have been derived from studies in which a probe "upstream" from the gene for exotoxin A (*exoA*) was used (28). This method with the *exoA* probe has proven to be very useful in characterizing the molecular epidemiology of *P. aeruginosa*, but it has been largely supplanted by pulsed field gel electrophoresis and random amplified polymorphic DNA analysis.

**5.2.Pulsed Field Gel Electrophoresis (PFGE)**

This method evaluates genetic polymorphisms within the entire bacterial genome by "macrorestriction." Genomic DNA is extracted and then digested with rare-cutting restriction enzymes which break the DNA into large fragments (29). These fragments are then separated using a highly specialized electrophoresis apparatus to allow the large fragments to migrate through the gel. The DNA fragments are then stained and the pattern examined by eye or by computer-assisted methods. This method has proven to be very effective for typing *P. aeruginosa*, providing a high degree of discrimination among strains. Whereas it is a very powerful epidemiological tool, the method suffers from labor-intensity and the need for using sophisticated electrophoresis equipment which is only available in specialized laboratories.

**5.3. PCR-based methods**

**5.3.1. *Random Amplified Polymorphic DNA (RAPD) analysis***

This polymerase chain reaction (PCR)-based method is a simplified means by which one can gain a "snapshot" of the entire bacterial genome (18). A short PCR primer (usually about 10 bases) is used to amplify random sections of the bacterial genome. The amplified DNA segments are then separated by electrophoresis, stained and analyzed by eye or computer as for PFGE. Further discrimination among isolates is possible by using different PCR primers and/or digesting the PCR amplification products with restriction enzymes. This method is simpler and less labor-intensive than PFGE. However it suffers from lack of reproducibility in some hands. It is most reproducible if performed repetitively under identical conditions with the same equipment and the same operator, and if it is run on a regular rather than a sporadic basis.

**5.3.2. *Repetitive Extrapalindromic PCR (REP-PCR)***

This is another PCR-based fingerprinting method which is analogous to RAPD except that the short primers used to amplify the fingerprint are targeted at known DNA sequences which occur at multiple positions in the bacterial genome. The sequences are known as repetitive extrapalindromic DNA and the fingerprinting method has become known as REP-PCR (30). The method is now available commercially (Bacterial Bar Codes Inc; http://www.bacterialbarcodes.com/) and may offer some advantages to diagnostic laboratories since it is readily available, standardized and highly reproducible.

**5.4. Multilocus Sequence Typing (MLST)**

The most precise differences among bacterial isolates are determined by directly sequencing the genomic DNA. Sequencing of the entire genome is impractical; therefore a method has been developed to sequence the PCR amplified segment of a highly variable region (31). This method is very discriminatory among strains but requires technical sophistication which is beyond the capability of most diagnostic laboratories. MLST schemes for *P. aeruginosa* are being investigated by several laboratories, but working systems are currently not available.

## 6. EPIDEMIOLOGY OF *P. AERUGINOSA* IN PATIENTS WITH CYSTIC FIBROSIS

Patients with CF harbor very high densities of *P. aeruginosa* in their sputa, and once infected, they often retain the same bacterial strain for life (18). Since CF patients cough frequently and are often in close contact with one another, the likelihood of patient-to-patient transmission would logically be very high. The actual risk of transmission of *P. aeruginosa* among patients with CF has been very difficult to gauge in the past because of the imprecision of phenotypic typing methods. However, with the advent of the genotypic methods described above, there has been substantial interest lately in determining the risk of spread among patients within or outside hospitals. Determination of patient-to-patient spread of a chronic infectious agent, in which the date of acquisition cannot be determined reliably, presents great epidemiological challenges. Identification of a clonal cluster does not necessarily imply patient-to-patient spread, but could instead result from exposure to a common source. Furthermore, the logistics and cost of genotypic analysis of a large collection of isolates from any given CF center is beyond the means of routine microbiology and infection control units. Therefore, surveys conducted to determine if nosocomial or other spread has occurred in a particular center has been dependent upon the expertise of local investigators with particular interest in the epidemiology of *P. aeruginosa* in CF.

**6.1. Evidence for patient to patient spread**

As described above, *P. aeruginosa* has remarkable phenotypic plasticity. Therefore, recognition of a potential cluster of a single clone within a CF center might only be suggested by an unusual phenotypic trait, such as a rare pigmentation or a novel antimicrobial susceptibility profile. Indeed clusters of *P. aeruginosa* in different CF centers have recently been suggested by such phenotypes and clonality substantiated by genotypic methods. The first such clearly documented "epidemic" in a CF center was reported from Liverpool, United Kingdom by Cheng and colleagues (32). The epidemic was considered after the appearance of a high number of *P. aeruginosa* isolates resistant to ceftazidime and other beta-lactam antibiotics among the patients in the CF clinic population. Clonality

was confirmed by pulsed field gel electrophoresis and by assessment of flagellin gene polymorphisms by amplification of the whole gene and restriction enzyme digestion. A subsequent epidemic was also reported from the United Kingdom (33). Again this epidemic was suggested by the emergence of a phenotypically unusual lineage with a discrete antimicrobial susceptibility pattern. Confirmation of clonality was obtained by genetic fingerprinting. These two epidemics and another one from Sydney Australia (D. Armstrong and K. Grimwood, personal communication) illustrate the potential for patient-to-patient spread of *P. aeruginosa* in CF. However, it seems most likely that the majority of infections with *P. aeruginosa* in CF are acquired from widely disseminated environmental sources.

**6.2. Evidence against patient to patient spread**

There is a dearth of reports of lack of transmission of *P. aeruginosa*; this may be due to a number of factors, but is probably a result of simple lack of reporting of "negative" studies or – more likely – due to lack of looking. Documentation of the epidemiology of *P. aeruginosa* in any CF center depends upon active surveillance using genetic typing techniques. These techniques are expensive and only provided by highly specialized laboratories. Unless a concern has been raised about the spread of *P. aeruginosa* among patients with CF within a specific clinic, it is unlikely if genetic epidemiological typing would be performed.

Studies in Vancouver have been conducted since 1981 in an effort to determine the means by which *P. aeruginosa* is acquired by patients with CF. The first study was performed in a summer camp in 1981 (34), and failed to demonstrate transmission of any strains of *P. aeruginosa* from one patient to another. Indeed there were no documented new acquisitions of the organism during the camp period. That study was performed prior to the general availability of genetic typing techniques.

A subsequent study was performed in an effort to determine if *P. aeruginosa* could be spread from one CF patient to another as a result of sharing a hospital room during therapy for a pulmonary exacerbation (35). Transient apparent cross-infection was identified in three of seven hospital roommates studied, by comparing the isolate from the "donor" and the "recipient" by LPS serotyping. In none of those situations was cross-infection durable. Furthermore, when the isolates were later evaluated by RAPD and by PFGE, it was found that each pair was composed of different strains, and therefore did not support the earlier suggestion of even transient cross-infection (unpublished observation).

To determine if there has been inapparent cross-infection in any of the Vancouver CF clinics, we conducted a study of all patients from whom *P. aeruginosa* had been recovered between 1981 and 1999 (36). Multiple isolates from 174 patients were analyzed by RAPD and PFGE. There was no evidence of cross-infection except in ten of 12 sibships and in one pair of unrelated patients. Similarly, lack of apparent patient-to-patient spread has been reported from surveys in Colorado (28) using RFLP analysis and from Brazil (37) using PFGE.

**6.3. Rational infection control practices based upon local epidemiology**

In order to ascertain the potential risk of patient-to-patient spread of *P. aeruginosa* in CF clinics in which there is no evidence of the emergence of a unique phenotypic clone, active surveillance is required. Such surveillance is expensive, labor-intensive and therefore impractical in most centers. Indeed active surveillance using genetic fingerprinting techniques is only carried in centers where the epidemiology of *P. aeruginosa* in CF is of particular research interest. The risk of spread of *P. aeruginosa* is likely variable among centers. Differences could be due to heterogeneity of strain types infecting patients in those centers or to the nature of the standard of care and the nature of the facility within which the care is delivered. Further studies are clearly needed to define strategies to prevent the potential spread of *P. aeruginosa* from one CF patient to another. Until the risk of patient-to-patient spread has been defined and the means by which the bacterium is acquired by patients with CF is determined, infection control strategies should be consistent with local experience.

**7. EPIDEMIOLOGY OF *P. AERUGINOSA* IN OTHER MEDICAL CONDITIONS**

**7.1.Burn Wound Infections**

The skin provides a highly effective barrier against infection with environmental organisms. This truth is clearly supported by the risk of invasive infection with *P. aeruginosa* in patients who have sustained thermal burns (5). Such patients are at risk for infection with a number of different bacterial pathogens, but the most problematic in many burn wound treatment centers is *P. aeruginosa*. The likelihood of infection and the severity of infectious disease in burned patients is directly proportional to the extent of the burn. Topical antibiotic therapy has reduced but not eliminated the risk of infection (5), and *P. aeruginosa* remains problematic because of its high intrinsic resistance to a wide range of antimicrobial agents.

A clearer understanding of the epidemiology of *P. aeruginosa* infection in burn units has been facilitated by the advent of molecular typing techniques. Clonal clusters have alerted the responsible physicians to an epidemic and investigation of possible sources undertaken. Molecular methods which have been used include PFGE (38) and RAPD (39). Using molecular techniques (39) and phenotypic methods (40), epidemic spread of *P. aeruginosa* was documented in a burn unit and traced to moist environments such as hydrotherapy tanks or irrigation tubing (41). If *P. aeruginosa* is recovered from cutaneous lesions or from normally sterile sources (such as blood) of multiple patients in a burn unit, an epidemic should be considered. Isolates from each of the patients should be evaluated for clonality by a genetic fingerprinting technique, such as PFGE or RAPD. If a clonal cluster is identified, the source should be identified immediately and strategies for decontamination instituted.

### 7.2.Ventilator-associated pneumonia

*P. aeruginosa* is a very unusual commensal organism in the upper respiratory tract, but it can be commonly recovered from individuals who are receiving intensive care, particularly if they are dependent upon mechanical ventilation. Respiratory tract infection appears to be facilitated by adhesion of bacteria to respiratory tract epithelial cells; *P. aeruginosa* is relatively resistant to adhesion to such cells because of the presence of fibronectin on their surface (42). During hospitalization in intensive care, patients apparently lose fibronectin from the surface of their respiratory tract epithelial cells, and adhesion of *P. aeruginosa* is facilitated (15). It is quite possible that this alteration in the local environment in the upper respiratory tract sets in motion a chain of events which results in pneumonia. The presence of an endotracheal tube interferes with the normal capacity of tracheal epithelial cells to evacuate inhaled bacteria. The presence of *P. aeruginosa* in the upper airway coupled with compromised tracheal toilet, may conspire to facilitate the development of ventilator associated pneumonia (VAP). Conditions which predispose to VAP include duration of time in intensive care, mechanical ventilation and prior use of antibiotics (43).

*P. aeruginosa* is one of the leading causes of VAP, and the prognosis is poor (44-46). The origin and potential for patient-to-patient spread of *P. aeruginosa* in intensive care units has been investigated using molecular typing techniques. Most studies have demonstrated multiple clonal types of *P. aeruginosa* and argue against patient-to-patient spread as a common cause for acquisition. However, patients in an intensive care unit have been found to share a specific strain, suggesting patient-to-patient spread (47). One would anticipate that such spread would result from the shared use of contaminated respiratory care equipment or on the hands of hospital care givers. After initial colonization, the most likely source of lower respiratory tract disease appears to be auto-infection. The bacteria usually first appear in the respiratory or gastrointestinal tract (47), either by exogenous introduction or by selection during antibiotic therapy. “Contamination” and infection of the lower respiratory tract may subsequently occur because of failure of the normal mucociliary elevator. Molecular methods for investigating the epidemiology of *P. aeruginosa* in intensive care units and as the cause of VAP include PFGE and RAPD (47-51). Most studies indicate that patient-to-patient spread is the exception rather than the rule and that endogenous infection is usually responsible.

### 7.3.Cancer and neutropenia

Neutrophils play a critical role in defense of the normal host against infection with *P. aeruginosa*; this fact is dramatically illustrated by the vulnerability of neutropenic hosts to invasive Pseudomonas infection (23). Patients with cancer who are receiving oncolytic chemotherapy are at particular risk for invasive infection with *P. aeruginosa*, and molecular epidemiology has been employed to determine the route of acquisition and infection. Animal models have also been developed to gain a clearer understanding of the pathogenesis of infection in these vulnerable hosts. It appears that the usual route of infection is endogenous from a gastrointestinal source (52). As discussed above, the normal host is resistant to colonization with *P. aeruginosa*, and very few healthy individuals carry the organism as part of their normal GI flora (13). However, therapy with antibiotics can perturb the normal gut flora, diminish the state of “colonization resistance” and allow *P. aeruginosa* to gain a foothold (13). Once *P. aeruginosa* has colonized the intestinal tract, it is poised to cause bacteremia and sepsis after breaching the epithelial barrier. Certain microbial products, such as those secreted by the type III system are actively exported to enhance epithelial cell transmigration and bloodstream invasion (53). Neutropenic hosts are severely compromised in their capacity to deal with the bacteria after they have invaded the bloodstream, and fatal disease frequently ensues (2).

Prospective studies have demonstrated that *P. aeruginosa* infection in neutropenic hosts usually emerges from an endogenous source in the gastrointestinal tract. A study reported in 1985, demonstrated that most leukemic patients who acquire *P. aeruginosa* bacteremia, carry the same strain in their gastrointestinal tract prior to development of invasive disease (52). The likelihood of developing Gram-negative bacteremia was correlated with neutropenia. One would expect that these data would be confirmed if molecular epidemiological techniques were applied for comparing intestinal and bloodstream isolates of *P. aeruginosa* from the same patient.

## 8. CONCLUSIONS

Molecular tools have aided in the understanding of the epidemiology of *P. aeruginosa* infections. Phenotypic methods have been of limited value in situations where *P. aeruginosa* undergoes physical change during chronic infection; this is particularly true in patients with CF who often are infected with the same strain of *P. aeruginosa* for decades. Molecular methods are highly discriminatory, permitting differentiation among many strains which might be considered to be identical by methods such as LPS serotyping in which no more than 17 types can be identified. Whereas molecular methods are very useful in investigating the epidemiology of *P. aeruginosa*, they are labor-intensive, available only in specialized laboratories and susceptible to lack of reproducibility. Nonetheless, methods such as PFGE are now considered the “gold standard” for investigation of possible *P. aeruginosa* epidemics, particularly among patients with CF.

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## 10. REFERENCES

1. Govan J.R. & V. Deretic: Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* 60, 539-574 (1996)

2. Maschmeyer G. & I. Braveny: Review of the incidence and prognosis of *Pseudomonas aeruginosa* infections in cancer patients in the 1990s. *Eur J Clin Microbiol Infect Dis* 19, 915-925 (2000)
3. Rolston K.V. & G.P. Bodey: *Pseudomonas aeruginosa* infection in cancer patients. *Cancer Invest* 10, 43-59 (1992)
4. McManus A.T, A.D.J. Mason, W.F. McManus & B.A.J. Pruitt: Twenty-five year review of *Pseudomonas aeruginosa* bacteremia in a burn center. *Eur J Clin Microbiol* 4, 219-223 (1985)
5. Pruitt B.A. Jr., A.T. McManus, S.H. Kim & C.W. Goodwin: Burn wound infections: current status. *World J Surg* 22, 135-145 (1998)
6. Reid T.M. & I.A. Porter: An outbreak of otitis externa in competitive swimmers due to *Pseudomonas aeruginosa*. *J Hyg (Lond)* 86, 357-362 (1981)
7. Fiorillo L, M. Zucker, D. Sawyer & A.N. Lin: The Pseudomonas hot-foot syndrome. *N Engl J Med* 345, 335-338 (2001)
8. Gregory D.W. & W. Schaffner: Pseudomonas infections associated with hot tubs and other environments. *Infect Dis Clin North Am* 1, 635-648 (1987)
9. Lyczak J.B, C.L. Cannon & G.B. Pier: Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes Infect* 2, 1051-1060 (2000)
10. Hancock R.E. & D.P. Speert: Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and impact on treatment. *Drug Resist Updat* 3, 247-255 (2000)
11. Botzenhart K. & G. Doring: Ecology and epidemiology of *Pseudomonas aeruginosa*, in *Pseudomonas aeruginosa as an opportunistic pathogen*, edited by Campa M, M. Bendinelli & H. Friedman: New York, Plenum Press, 1-18, (1993)
12. Speert D.P, S.W. Farmer, M.E. Campbell, J.M. Musser, R.K. Selander & S. Kuo: Conversion of *Pseudomonas aeruginosa* to the phenotype characteristic of strains from patients with cystic fibrosis. *J Clin Microbiol* 28, 188-194 (1990)
13. Gorbach S.L, M. Barza, M. Giuliano & N.V. Jacobus: Colonization resistance of the human intestinal microflora: testing the hypothesis in normal volunteers. *Eur J Clin Microbiol Infect Dis* 7, 98-102 (1988)
14. Hentges D.J, A.J. Stein, S.W. Casey, J.U. Que: Protective role of intestinal flora against infection with *Pseudomonas aeruginosa* in mice: influence of antibiotics on colonization resistance. *Infect Immun* 47, 118-122 (1985)
15. Johanson W.G. Jr., D.E. Woods & T. Chaudhuri: Association of respiratory tract colonization with adherence of gram-negative bacilli to epithelial cells. *J Infect Dis* 139, 667-673 (1979)
16. Woods D.E, J.A. Bass, W.G. Johanson Jr. & D.C. Straus: Role of adherence in the pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. *Infect Immun* 30, 694-699 (1980)
17. Thomassen M.J, C.A. Demko, B. Boxerbaum, R.C. Stern & P.J. Kuchenbrod: Multiple isolates of *Pseudomonas aeruginosa* with differing antimicrobial susceptibility patterns from patients with cystic fibrosis. *J Infect Dis* 140, 873-880 (1979)
18. Mahenthiralingam E, M.E. Campbell, J. Foster, J.S. Lam & D.P. Speert: Random amplified polymorphic DNA typing of *Pseudomonas aeruginosa* isolates recovered from patients with cystic fibrosis. *J Clin Microbiol* 34, 1129-1135 (1996)
19. Hancock R.E, L.M. Mutharia, L. Chan, R.P. Darveau, D.P. Speert & G.B. Pier: *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis: a class of serum-sensitive, nontypable strains deficient in lipopolysaccharide O side chains. *Infect Immun* 42, 170-177 (1983)
20. Doggett R.G: Incidence of mucoid *Pseudomonas aeruginosa* from clinical sources. *Appl Microbiol* 18, 936-937 (1969)
21. Mahenthiralingam E, M.E. Campbell & D.P. Speert: Nonmotility and phagocytic resistance of *Pseudomonas aeruginosa* isolates from chronically colonized patients with cystic fibrosis. *Infect Immun* 62, 596-605 (1994)
22. Gallant C.V, T.L. Raivio, J.C. Olson, D.E. Woods & D.G. Storey: *Pseudomonas aeruginosa* cystic fibrosis clinical isolates produce exotoxin A with altered ADP-ribosyltransferase activity and cytotoxicity. *Microbiology* 146 ( Pt 8), 1891-1899 (2000)
23. Speert D.P.: *Pseudomonas aeruginosa* - phagocytic cell interaction, in *Pseudomonas aeruginosa as an opportunistic pathogen*, edited by Campa M, M. Bendinelli & H. Friedman. New York, Plenum Press, 1993, 163-182
24. Bodey G.P.: *Pseudomonas aeruginosa* infections in cancer patients: have they gone away? *Curr Opin Infect Dis* 14, 403-407 (2001)
25. Speert D.P. & The International *Pseudomonas aeruginosa* Typing Study Group: A multicenter comparison of methods for typing strains of *Pseudomonas aeruginosa* predominantly from patients with cystic fibrosis. *J Infect Dis* 169, 134-142 (1994)
26. Homma J.Y, K.S. Kim, H. Yamada, M. Ito & H. Shionoya: Serological typing of *Pseudomonas aeruginosa* and its cross-infection. *Jpn J Exp Med* 40, 347-359 (1970)
27. Fisher M.W, H.B. Devlin & F.J. Gnabasik: New immunotype schema for *Pseudomonas aeruginosa* based on protective antigens. *J Bacteriol* 98, 835-836 (1969)
28. Ogle J.W, J.M. Janda, D.E. Woods & M.L. Vasil: Characterization and use of a DNA probe as an epidemiological marker for *Pseudomonas aeruginosa*. *J Infect Dis* 155, 119-126 (1987)
29. Tenover F.C, R.D. Arbeit, R.V. Goering, P.A. Mickelsen, B.E. Murray, D.H. Persing & B. Swaminathan: Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33, 2233-2239 (1995)
30. Versalovic J, T. Koeuth & J.R. Lupski:: Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 19, 6823-6831 (1991)
31. Spratt B.G.: Multilocus sequence typing: molecular typing of bacterial pathogens in an era of rapid DNA sequencing and the internet. *Curr Opin Microbiol* 2, 312-316 (1999)
32. Cheng K, R.L. Smyth, J.R.W. Govan, C. Doherty, C. Winstanley, N. Denning, D.P. Heaf, H. van Saene & C.A. Hart: Spread of B-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. *Lancet* 348, 639-642 (1996)
33. Jones A.M, W.E. Webb, J.R. Govan, C.A. Hart & M.J. Walshaw: *Pseudomonas aeruginosa* cross-infection in cystic fibrosis. *Lancet* 359, 527-528 (2002)

34. Speert D.P, D. Lawton & S. Damm: Communicability of *Pseudomonas aeruginosa* in a cystic fibrosis summer camp. *J Pediatr* 101, 227-228 (1982)
35. Speert D.P. & M.E. Campbell: Hospital epidemiology of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *J Hosp Infect* 9, 11-21 (1987)
36. Speert D.P, M. Campbell, D.A. Henry, R. Milner, F. Taha, A. Gravelle, A.G.F. Davidson, L. T. Wong, & E. Mahenthiralingam: Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis in British Columbia, Canada. *Am J Respir Crit Care Med* (in press), (2002)
37. Silbert S, A.L. Barth & H.S. Sader: Heterogeneity of *Pseudomonas aeruginosa* in Brazilian cystic fibrosis patients. *J Clin Microbiol* 39, 3976-3981, (2001)
38. Douglas M.W, K. Mulholland. V. Denyer & T. Gottlieb: Multi-drug resistant *Pseudomonas aeruginosa* outbreak in a burns unit--an infection control study. *Burns* 27, 131-135 (2001)
39. De Vos D, A. Lim Jr., J.P. Pirnay, L. Duinslaeger, H. Revets, A. Vanderkelen, R. Hamers & P. Cornelis: Analysis of epidemic *Pseudomonas aeruginosa* isolates by isoelectric focusing of pyoverdine and RAPD-PCR: modern tools for an integrated anti-nosocomial infection strategy in burn wound centres. *Burns* 23, 379-386 (1997)
40. Richard P, R. Le Floch, C. Chamoux, M. Pannier, E. Espaze & H. Richet: *Pseudomonas aeruginosa* outbreak in a burn unit: role of antimicrobials in the emergence of multiply resistant strains. *J Infect Dis* 170, 377-383 (1994)
41. Kolmos H.J, B. Thuesen, S.V. Nielsen, M. Lohmann, K. Kristoffersen & V.T. Rosdahl:Outbreak of infection in a burns unit due to *Pseudomonas aeruginosa* originating from contaminated tubing used for irrigation of patients. *J Hosp Infect* 24, 11-21 (1993)
42. Woods D.E, D.C. Straus, W.G. Johanson & J.A. Bass: Role of fibronectin in the prevention of adherence of *Pseudomonas aeruginosa* to buccal cells. *J Infect Dis* 143, 784-790 (1981)
43. Bauer T.T, R. Ferrer, J. Angrill, G. Schultze-Werninghaus & A. Torres: Ventilator-associated pneumonia: incidence, risk factors, and microbiology. *Semin Respir Infect* 15, 272-279 (2000)
44. Chastre J. & J.L. Trouillet: Problem pathogens (*Pseudomonas aeruginosa* and Acinetobacter). *Semin Respir Infect* 15, 287-298 (2000)
45. Rello J. D, F. Mariscal, F. March, P. Jubert , F. Sanchez, J. Valles & P. Coll: Recurrent *Pseudomonas aeruginosa* pneumonia in ventilated patients: relapse or reinfection? *Am J Respir Crit Care Med* 157, 912-916 (1998)
46. Rello J. & A. Torres: Microbial causes of ventilator-associated pneumonia. *Semin Respir Infect* 11, 24-31 (1996)
47. Bonten M.J, D.C. Bergmans, H. Speijer & E.E. Stobberingh: Characteristics of polyclonal endemicity of *Pseudomonas aeruginosa* colonization in intensive care units. Implications for infection control. *Am J Respir Crit Care Med* 160, 1212-1219 (1999)
48. Bergmans D.C, M. J. Bonten, E.E. Stobberingh, F.H. van Tiel, S. van der Geest, P.W. de Leeuw & C.A. Gaillard: Colonization with *Pseudomonas aeruginosa* in patients developing ventilator-associated pneumonia. *Infect Control Hosp Epidemiol* 19, 853-855 (1998)
49. Bergmans D.C, M.J. Bonten, F.H. van Tiel, C.A. Gaillard, S. van der Geest, R.M. Wilting, P.W. de Leeuw & E.E. Stobberingh: Cross-colonisation with *Pseudomonas aeruginosa* of patients in an intensive care unit. *Thorax* 53, 1053-1058 (1998)
50. Berthelot P, F. Grattard, P. Mahul, J. Pain, R. Jospe, C. Venet, A. Carricajo, G. Aubert, A. Ros, A. Dumont, F. Lucht, F. Zeni, C. Auboyer, J.C. Bertrand & B. Pozzetto: Prospective study of nosocomial colonization and infection due to *Pseudomonas aeruginosa* in mechanically ventilated patients. *Intensive Care Med* 27, 503-512 (2001)
51. Ruimy R, E. Genauzeau, C. Barnabe, A. Beaulieu, M. Tibayrenc & A. Andremont: Genetic diversity of *Pseudomonas aeruginosa* strains isolated from ventilated patients with nosocomial pneumonia, cancer patients with bacteremia, and environmental water. *Infect Immun* 69, 584-588 (2001)
52. Tancrede C.H. & A.O. Andremont: Bacterial translocation and gram-negative bacteremia in patients with hematological malignancies. *J Infect Dis* 152, 99-103 (1985)
53. Hirakata Y, B.B. Finlay, D.A. Simpson, S. Kohno, S. Kamihira & D.P. Speert: Penetration of clinical isolates of *Pseudomonas aeruginosa* through MDCK epithelial cell monolayers. *J Infect Dis* 181, 765-769 (2000)

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**Send correspondence to:** David P. Speert, M.D., Room 377, Research Centre, 950 West 28th Avenue, Vancouver, B.C., V5Z 4H4, Canada, Tel: 604-875-2438, Fax: 604-875-2226, E-mail: dspeert@cw.bc.ca