Lack of Evidence of Transmission of *Pseudomonas aeruginosa* among Cystic Fibrosis Patients Attending Health Camps at the Dead Sea, Israel

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Abstract

Background: Transmission of *Pseudomonas aeruginosa* among cystic fibrosis patients attending health camps has been reported previously.

Objectives: To determine the transmission of *P. aeruginosa* among CF patients during three winter camps in the Dead Sea region in southern Israel.

Methods: Three consecutive CF patient groups were studied, each of which spent 3 weeks at the camp. The patients were segragated prior to camp attendance: patients who were not colonized with *P. aeruginosa* constituted the first group, and colonized patients made up the two additional groups. Sputum cultures were obtained upon arrival, at mid-camp and on the last day. Environmental cultures were also obtained. Patients were separated during social activities and were requested to avoid social mingling. Isolates were analyzed by antibiotic susceptibility profile and by pulsed field gel electrophoresis.

Results: Ninety isolates from 19 patients produced 28 different fingerprint patterns by PFGE. Isolates from two siblings and two patients from the same clinic displayed the same fingerprint pattern. These patients were already colonized with these organisms upon arrival. Two couples were formed during the camp, but PFGE showed no transmission of organisms. All other patients' isolates displayed unique fingerprint patterns and were distinguishable from those of other attendees, and none of the *P. aeruginosa*-negative patients acquired *P. aeruginosa* during camp attendance. Environmental cultures were negative for *P. aeruginosa*.

Conclusions: We were unable to demonstrate cross-infection of *P. aeruginosa* among CF patients participating in health camps at the Dead Sea who were meticulously segregated.

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Cystic fibrosis is the most common potentially fatal autosomal recessive disease in Europe and North America, afflicting approximately 1 in 2,000 live births among Caucasians [1]. Recurrent lung infections leading to respiratory failure and death is the major concern among CF patients. *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, and *Staphylococcus aureus* are the most common pulmonary pathogens in those patients [2]. These organisms are resistant to a wide variety of antimicrobial agents and thus pose a serious therapeutic challenge [3]. This bacterium (i.e., *Pseudomonas aeruginosa)* can spread from one CF patient to another through close contact and shared facilities, such as respiratory equipment [4]. However, the transmissibility of these bacteria may vary depending on several factors, such as the presence of other CF patients in the family, policy of the CF center, biological characteristics of the colonizing strains, patient population characteristics, and environment [3–8].

Health camps are well established as an important treatment modality to improve the well-being of CF patients worldwide. Transmission of respiratory bacteria among CF patients attending health camps has been demonstrated in various studies by epidemiologic molecular methods [9,10–13].

The Dead Sea region has been established as a healthcare facility for patients with chronic respiratory illnesses [14,15]. During the winter months, patients from European countries visit the Dead Sea to benefit from its mild weather and health activities such as spa, physiotherapy and others. The CF patients studied here spent 3 weeks at the Dead Sea during the winter 1999–2000. The goals of this study were to determine the epidemiology and potential transmission rate of *P. aeruginosa* among these patients.

Patients and Methods

Health camps for CF patients from two Western European countries (henceforth referred to as Country A and Country B) have been conducted at the Dead Sea for several years. Duration of the camp is 3 weeks. According to the program guidelines, patients were segregated by their physician in their countries of origin according to their colonization status with *P. aeruginosa* and *B. cepacia*. In addition, sputum cultures were taken upon arrival, at mid-camp and at the end of the stay. The patients were notified that the data regarding their sputum cultures would be used for study purposes as well. The local Ethics Committee approved the use of the data obtained during the camp for future studies.

During the winter of November 1999 to March 2000, three consecutive CF patient groups participated in the program; each came 24 hours after the previous group had left, without overlapping between groups. The first group included patients whose sputum cultures were negative for *P. aeruginosa* or *B. cepacia* in their country of origin. The other two groups included patients reported to be colonized with *P. aeruginosa* in their country of origin. No patient was reported as being colonized with *B. cepacia* in his/her country of origin. All participants stayed at the same hotel on the Dead Sea shore. Patients were separated during health activities, such as physical therapy (which was done in the patients' own

CF = cystic fibrosis
PFGE = pulsed field gel electrophoresis

rooms) or spa. Camp attendees did not share health equipment such as inhalers and/or nebulizers.

As part of the program policy, patients were requested to avoid sharing social activities with other CF patients within the camp. Organized social activities were limited and took place in open-air areas (e.g., hiking). Each group stayed in different rooms from the previous group to avoid any possibility of transmission of organisms due to room sharing. Each patient had a separate room with its own washroom facilities. Patients ate in the same dining room. The hotel has several elevators and patients used them simultaneously.

Twenty environmental culture swabs were obtained from swimming pools, the Dead Sea water, the dining room, sinks, and spa equipment shared by the patients. Most patients were on various antibiotic treatments, either oral or inhaled, or both.

Microbiology

Culture media and identification of *P. aeruginosa* and *B. cepacia* were performed according to standard bacteriologic methods. All *P. aeruginosa* isolates displaying different colony morphology appearance were tested for antibiotic susceptibility to the following drugs: ceftriaxone, gentamicin, ciprofloxacin, ceftazidime, amoxicillin/clavulanate, mezlocillin, piperacillin, amikacin, tobramycin, imipenem, and piperacillin/tazobactam.

Genotypic analysis

P. aeruginosa isolates were analyzed by pulsed field gel electrophoresis. The restriction enzyme *Spe*I was used. PFGE was carried out with a contour-clumped homogenous electric field apparatus (CHEF- DR III, BioRad, Hercules, CA, USA). The CF patients' isolates were compared according to their PFGE fingerprint patterns. Further comparison was made taking into consideration the patients' background (country and city of origin). The DNA fragment patterns generated by PFGE were interpreted according to the recent consensus criteria suggested by Tenover et al. [16].

Results

Between November 1999 and March 2000, 31 CF patients – 16 females and 15 males – attended three health camps. Twenty-one patients came from Country A and 10 from Country B. Participants from the first group were all from Country A; the median age was 8 years (range 4–29 years). The second group included 7 patients from Country A and 6 from Country B; the median age was 28 years (range 5–37). The third group included 4 patients from Country A and 4 from Country B; the median age was 17 years (range 13–40). Only one patient refused to participate in the study.

Sputum culture was positive in 24 (77.4%) of the 31 patients: 22 had *P. aeruginosa* and 2 had *S. aureus*. The following organisms were recovered: 105 *P. aeruginosa* isolates (one in group 1, 69 in group 2, and 35 in group 3) and 14 *S. aureus* isolates. No methicillin-resistant isolate was recovered. No *B. cepacia* isolates were recovered. All cultures obtained from the environment were negative for these bacteria.

P. aeruginosa isolates were susceptible to the following antibiotics: piperacillin/tazobactam 89%, ciprofloxacin 85%, ceftazidime 82%, piperacillin 78%, tobramycin 74%, imipenem 70%, amikacin 55% and gentamicin 36%.

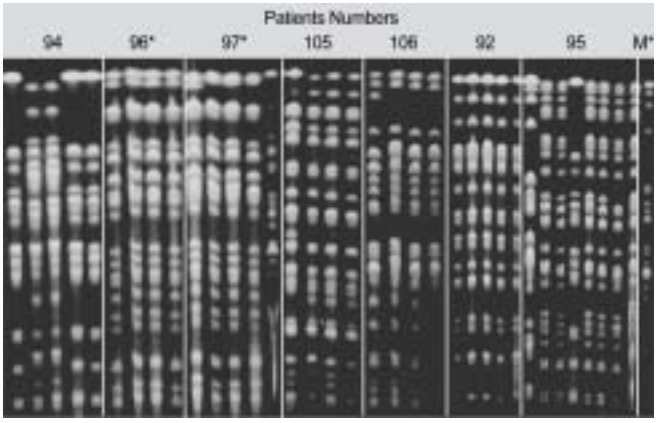

Figure 1. Pulsed field gel electrophoresis fingerprint patterns of *Pseudomonas aeruginosa* isolated from patients originating from Country B.
M = molecular weight. * Patients 96 and 97 were from the same clinic.

Ninety-eight of the 105 *P. aeruginosa* isolates (93%) were analyzed by PFGE. Twenty-four distinctive PFGE fingerprint patterns were noted among isolates. In addition, in some patients there was more than one PFGE fingerprint pattern. No predominant clone was found among *P. aeruginosa* isolates.

Consecutive isolates from the same patient showed identical PFGE patterns over the 3 week period. Furthermore, isolates from different patients were not identical with the exception of two occurrences. In the first, two siblings, aged 13 and 16 years old, carried an identical strain of *P. aeruginosa*, which was detected upon their arrival at the health camp; in another group, two patients who were treated by the same physician, in the country of origin, at the same CF center, displayed the same PFGE fingerprint pattern that did not change for the entire period of the stay in the health camp [Figure 1; patients 96 and 97]. No new acquisition of *P. aeruginosa* strains or cross-transmission between patients was found within each group or between groups according to PFGE fingerprint analysis [17].

Comparison of the groups using PFGE analysis with the phenotypic analysis showed no correlation, and isolates with identical PFGE fingerprint pattern had different colony morphology and/or different antibiotic susceptibility patterns. Isolates from the two siblings were phenotypically different: in four isolates the colony description was mucoid green, one was tetracycline-resistant, two were sensitive and one was resistant to tetracycline and gentamicin. In these patients four other colony morphologies were described: dark green, gray-green, small colonies, and dry colonies. Each different colony morphology expressed different antibiotic susceptibility patterns (i.e., some were resistant to pipracillin/tazobactam and some to gentamicin). All of these isolates were genotypically identical by PFGE.

Discussion

The Dead Sea is characterized by environmental factors such as dry air, a magnesium-enriched ecological niche and high barometric

pressure. The dry weather and descent to low altitude improve arterial oxygenation, exercise performance and sleep oximetry, and consequently, the quality of life in patients with hypoxemia and advanced lung disease is improved [14]. Some authors also suggest another potential mechanism for the improvement of lung function tests in patients with chronic lung diseases that may be related to the absorption of magnesium through the skin or by inhalation passing through the lungs, due to its involvement in anti-inflammatory and vasodilatory processes [17].

It is well established that discordance between CF patients regarding their colonization status in health camps can cause the spread of *P. aeruginosa* from colonized to non-colonized CF patients [13,18]. Acquisition of an additional *P. aeruginosa* strain among colonized CF patients in the same clinic may occur after 5.4 to 13.5 months [19]. Segregation of CF patients according to their *P. aeruginosa* and *B. cepacia* colonization status is common practice in many CF centers [20].

In this study we successfully used the practice of segregating patients according to their *P. aeruginosa* colonization status before they arrived at the health camp. Thus, only patients colonized with *P. aeruginosa* participated in the same health camp and were not allowed to participate in non-*P. aeruginosa*-colonized patient camps. In addition, the practice of obtaining serial sputum cultures at the start, middle and end of the stay enabled us to follow and document potential acquisition and transmission of *P. aeruginosa* strains among patients for the whole camp duration. All participants remained colonized with their original strains that were identified upon arrival and at the end of the camp. Furthermore, no person-to-person transmission was detected among patients within groups and between groups. We speculate that these results were due to several factors. First, the meticulous segregation policy among patients prior to attendance in the camps, which was suggested previously by others and followed carefully, ensured the homogeneity of patients according to their colonization status, which led to the reduction in transmission [21]. Second, the non-sharing of equipment and facilities may also have contributed to this reduction. Third, the program policy of minimum social contacts, which was followed successfully by the participants and program directors, may also have impacted the final results. Fourth, the duration of the camp may have been too short to allow for transmission among patients who were initially colonized, as shown in patients from CF clinics [19].

No transmission of *P. aeruginosa* strains was documented among patients at mid- or end of camp. Identical strains were isolated in cultures obtained from two siblings and from two patients treated at the same CF center for many years. These findings possibly demonstrate that prolonged exposure is needed for patients already colonized to acquire new strains, and perhaps the relatively short exposure period of the patients during the health camp is not long enough to allow transmission of *P. aeruginosa* strains [19,21]. It is also possible that competition among strains for the same biologic niche makes it difficult for new strains to be acquired.

Comparison of *P. aeruginosa* isolates according to their PFGE fingerprint patterns revealed heterogeneity among isolates from different patients. Previous studies showed that genetic heterogeneity among *P. aeruginosa* isolates recovered from CF patients is common [13], whereas epidemics in this setting are characterized by isolation of organisms with identical genotypes [22]. Phenotypic analysis showed no correlation between isolates with identical PFGE fingerprint pattern that had different colony morphology and antibiotic susceptibility patterns (among the isolates the anti-pseudomonal antibiotic susceptibility rate was above 75%). In some instances, isolates had different genotypes but showed an identical PFGE pattern. Establishing identity or differences among *P. aeruginosa* isolates on the basis of colony morphology or antibiogram may lead to false conclusions, and genotypic methods should be used in order to detect transmission of organisms from person to person.

It is well recognized that CF patients may harbor more than one *P. aeruginosa* strain. In our study group, six patients had more than one strain, both initially and throughout the entire camp period.

Our study had two limitations: it involved a limited number of patients and the follow-up was only 3 weeks. Large-scale studies should be carried out to support our results. However, it has been strongly recommended that all CF camps and overnight education retreats be discontinued [23], precluding the performance of similar studies in the near future.

In summary, we demonstrated that health camps conducted for a short period at the Dead Sea for CF patients previously segregated according to their colonization status do not contribute to patient-to-patient transmission of *P. aeruginosa*.

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I married him for better or for worse, but not for lunch

Hazel Weiss, after her husband retired as general manager of the New York Yankees in 1960

Capsule

Fixing spinal injury in fish

The tragedies of spinal cord damage in humans would be much alleviated if spinal cord neurons could regenerate, but human neurons show great reluctance to do so. Restricting factors seem to be both intrinsic to the damaged neuron and extrinsic to the surrounding central nervous system (CNS) tissue. In zebrafish, the CNS seems to have less of the general repressive nature, but Mauthner neurons still cannot regenerate reliably. Bhatt et al. found that application of cAMP (adenosine 3', 5'-monophosphate) to the cell body of the Mauthner neuron in zebrafish induced its regeneration and restored Mauthner neuron-dependent behavioral responses.

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Capsule

Source of T cells

Although the thymus is known as the major source of T cells, many lines of evidence point to other organs, such as the liver and intestine, as significant alternative sites of T cell development. In particular, intraepithelial lymphocytes, the abundant T cells that reside between the epithelial cells of the intestinal mucosa, are thought to derive largely from specific developmental islands within the mucosa. Using a fate-mapping approach, Eberl and Littman provide direct evidence that intraepithelial lymphocytes are, instead, the direct descendants of thymocytes. Extra-thymic development is thus unlikely to contribute significantly to the T cell pool.

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