Risk of Cross-Colonization and Infection by *Pseudomonas aeruginosa* in a Holiday Camp for Cystic Fibrosis Patients

JACOMINA A. A. HOOGKAMP-KORSTANJE,1* JACQUES F. G. M. MEIS,1 JOHAN KISSING,1 JOHANNES VAN DER LAAG,2 AND WILLEM J. G. MELCHERS1

Department of Medical Microbiology, University Hospital Nijmegen, Nijmegen,1 and Wilhelmina Childrens Hospital, Utrecht,2 The Netherlands

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The risk of cross-colonization and subsequent infection by *Pseudomonas aeruginosa* in holiday camps for cystic fibrosis patients was studied in 91 children by culturing sputum at their arrival, at their departure, 2 months later, and at regular intervals thereafter. The isolated strains were subjected to serotyping, phage typing, pyocin typing, and genotyping by random amplified polymorphic DNA fingerprinting-PCR. It was concluded from random amplified polymorphic DNA fingerprinting-PCR typing that the *Pseudomonas* flora was not constant in most children. Some children harbored one genotype, whereas some harbored two or more different genotypes simultaneously. Most culture-positive children easily acquired a strain of another genotype which replaced the former one or coexisted with the original one. The incidence of sputum conversion was 7.7% in previously negative children; the incidence of permanent colonization and infection was 1.9%. This risk was comparable with that observed in the community. We conclude that the risk of cross-infection is trivial compared with the obvious joy and social benefit derived from a holiday camp.

Chronic lung infection with *Pseudomonas aeruginosa* is primarily responsible for the pulmonary deterioration and reduced life expectancy in patients with cystic fibrosis (CF). The means by which this organism is acquired and transmitted are not well understood. Although Hoiby and Rosendal (6) concluded that cross-infection occurred quite frequently, Grotheus et al. (5) found little evidence of cross-infection.

We also tried to provide a conclusive answer after prospectively studying patients with CF who had taken part in three summer holiday camps. These camps are equipped with medical staff and have facilities for appropriate treatment. The contact between patients is naturally very close, and therefore, there is a real risk of cross-colonization. We investigated the epidemiology of *P. aeruginosa* in holiday camps for CF patients in 1980 and could not exclude the possibility of cross-colonization using the typing techniques available at that time because those typing techniques had a rather low discriminatory power (7). With the recent developments in genotyping techniques it became possible to reexamine the strains and attempt to provide a definitive conclusion. We opted for PCR-based fingerprinting (random amplified polymorphic DNA fingerprinting-PCR [RAPD-PCR]) since it has already proven its value for the genotypic characterization of many different medically important microorganisms, including *Pseudomonas* strains (17, 18).

**MATERIALS AND METHODS**

**Study population.** Forty-eight boys and 43 girls (ages, between 6 and 16 years; mean age, 11.8 years) in three groups attended a holiday camp in The Netherlands for 10 days during 1976. The holiday camp was situated on a farm in a largely rural area on the coast of the North Sea. The children slept in eight-bed rooms, in which they also had physiotherapeutic and inhalation treatments at regular intervals. They shared a common dining room, and no contact restrictions were placed on them.

Sputum specimens were collected at arrival, at departure, 2 months later, and at regular intervals thereafter. Sputum samples were obtained after physiotherapy to ensure that the sample had originated from the deeper airways. A total of 79 children could be followed completely and evaluated during the follow-up, four patients died within 2 months after attending the holiday camp, and eight patients lived too far from the study center to be evaluated in the follow-up. Microbiologically, after mixing an equal volume of sputum and 10% *Sputasol* (0.1% dithiothreitol; Oxoid, Haarlem, The Netherlands) samples were centrifuged at 10,000 × g for 15 min and were then cultured quantitatively by the technique of Monroe et al. (10) on sheep blood agar, saponin blood agar, MacConkey agar, and Sabouraud glucose agar. Plates were incubated aerobically for 24 to 48 h at 37°C and were examined daily.

At entry 41 children were infected with one bacterial species of *Staphylococcus aureus*, *Haemophilus influenzae*, or *P. aeruginosa*, 40 children were infected with mixed bacteria, and from 10 children no significant pathogen was isolated. The distribution of organisms cultured is summarized in Table 1. Twelve children had significant numbers of *Candida albicans* in their sputa, and one had *Aspergillus fumigatus*. At the end of the camp, 28 children (30.7%) had acquired one or more microorganisms in significant numbers.

All *P. aeruginosa* strains from colonies distinmillar in macroscopic appearance were processed separately. A total of 78 macroscopically different *P. aeruginosa* strains were isolated from 30 children at entry. 15 children harbored one strain, 20 harbored 2 strains, 5 harbored 3 strains, and 2 harbored 4 strains. Four children lost their strain during the camps, four previously negative children became colonized, and the other children kept their *Pseudomonas* flora. One of four colonized children was still positive after 2 months.

The 91 strains isolated from 18 children during one of the three camps were further characterized. All strains were also freeze-dried and kept in ampoules.

**Typing methods.** Serotyping was performed by agglutination with specific polyclonal anti-O antisera (15), phage typing was performed with a routine set of 20 bacteriophages as described by Asheshov (2), and pyocin typing was performed by active typing with eight indicator strains (4).

Susceptibility patterns were determined by agar diffusion by standard methods (11). The antibiotics tested were ampicillin, tetracycline, kanamycin, amikacin, gentamicin, tobramycin, polymyxin B, carbencillin, and piperacillin. Each antibiogram pattern has been assigned a code.

For genotyping, *P. aeruginosa* genomic DNA was isolated as follows. A single colony was resuspended in 250 μl of STET buffer (233 mM sucrose, 50 mM Tris-HCl [pH 8.0], 20 mM EDTA, 0.5% Triton X-100) and 0.5 mg of lysozyme (Sigma, St. Louis, Mo.). The solution was incubated at room temperature for 5 min, heated at 100°C for 1 min, and put on ice for 2 min. Subsequently, 0.1 μg of proteinase K (Boehringer, Mannheim, Germany) and sodium dodecyl sulfate were added to a final concentration of 0.5%, and the solution was incubated for 2 h at 55°C. DNA was extracted with phenol-chloroform and then by ethanol precipitation. The DNA was resuspended in 100 μl of distilled water, 20 μg of RNAase was added, and the mixture was incubated for 30 min at 37°C. The DNA concentration was determined by using a GeneQuant RNA/DNA Calculator (Pharmacia LKB, Cambridge, United Kingdom).

PCR-based fingerprint analysis was performed with a number of arbitrary primers (RAPD-PCR) and two enterobacterial repetitive intergenic consensus

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* Corresponding author. Mailing address: Department of Medical Microbiology, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Phone: 31-80-614356. Fax: 31-80-540216.
TABLE 1. Children positive for the microorganisms indicated at entry into the camp, children who acquired one or more organisms during the camp after 2 weeks, and children in whom persistence was recorded after 2 months of follow-up

<table>
<thead>
<tr>
<th>Organism</th>
<th>At entry</th>
<th>Acquisition during camp</th>
<th>Persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>42</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>28</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>39</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Members of family</td>
<td>9</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>13</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>91 (91)</td>
<td>28 (91)</td>
<td>5 (79)</td>
</tr>
</tbody>
</table>

Persistence

prions (17). The arbitrary primer D8635 (5’-GAGCGGCCCAGAGGAGGCA-CAACG-3’) gave the best resolution and had the highest discriminatory power. Amplification of 50 ng of bacterial DNA was performed in a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl2, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM (each) deoxyribonucleotide triphosphate, 0.2 U of SuperTaq DNA polymerase (Sphero-Q, Leiden, The Netherlands), and 50 pmol of arbitrary primer D8635. Four cycles of 94°C for 5 min, 40°C for 5 min, and 72°C for 5 min were performed by low-stringency amplification; this was followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min (high-stringency amplification) and a final incubation at 72°C for 10 min (1). Amplified DNA (20 μl) was separated by gel electrophoresis in 1.5% agarose and was visualized by ethidium bromide staining. Gels were photographed and interpreted visually by three different observers. Each different genotype was coded with a letter. Very closely related genotypes in which only subband differences were found were coded by the same letters, with one letter containing an additive (for example, b versus b'). Seven unrelated clinical isolates of P. aeruginosa strains and ATCC 35364 and ATCC 35354 were included as controls.

RESULTS

A total of 91 strains, including 67 mucoid isolates from children in the second of three camps, were extensively studied. They were isolated from 18 children; 14 children were positive at entry (31 strains), 17 children were positive at the end of the camp (40 strains), and 13 children were positive at follow-up (20 strains). Three positive children (patients D, E, and F) died within 2 months after the camp; one child (patient O, the brother of patient P) did not come for follow-up cultures.

Forty-three strains (47%), 28 mucoid and 15 nonmucoid, were serologically nontypeable, whereas the other 48 strains belonged to 15 different O serotypes. Strains dissimilar in macroscopic appearance were both of different serotypes and of identical serotypes. Most patients harbored more than one serotype simultaneously and the isolates demonstrated a large variation in time, suggesting a quick turnover. No particular cluster or any similarity between strains isolated at the start, at the end, or after the follow-up could be observed.

Twenty-two unique pyocin typing patterns were found among 88 strains; 3 strains could not be typed. One to three different patterns could be found in one sputum sample. For strains from some patients, strains dissimilar in macroscopic appearance had different pyocin patterns, but for those from other patients they had identical patterns. There was no correlation with the serotype. The pyocin types were not conclusive to determine whether cross-colonization had occurred. Eighty-one strains could be phage typed, but the reaction was often weak. Almost each strain had its own phage pattern; there was no correlation with any serotype or pyocin type.

Mucoid and nonmucoid strains from the same patient with the same serotype and pyocin type had different phage patterns.

Three main susceptibility patterns could be distinguished: pattern 3 (susceptible to tetracycline, kanamycin, gentamicin, tobramycin, and carbenicillin; 12 strains), pattern 10 (susceptible to gentamicin, tobramycin, and carbenicillin; 50 strains), and pattern 11 (susceptible to gentamicin and tobramycin; 29 strains). Most children harbored strains with various susceptibility patterns. There was no relationship with particular serotypes, pyocin types, or phage types.

Genotyping demonstrated that strains dissimilar in macroscopic appearance and of different serotypes, pyocin types, and phage types could be of the same, unique genotype. The genotypes found are listed in Table 2 and presented in Fig. 1. All strains were processed in one experiment at the same time under the same conditions. A repeat experiment gave no other information. At the start 14 children were infected with strains of 10 different genotypes; type b was found in 3, children, types g and p were found in 2 children each (those infected with type p were siblings), and 2 children harbored two different genotypes simultaneously in the same sample (patients A and I).

At the end of the camp, the genotypes of the strains infecting four children had changed or they acquired a strain of another genotype (A; C; Cb'; Dg, Lk) and four previously negative children had picked up a Pseudomonas strain (G; i, M0, Nb',...
Qg). Strains of genotypes b' and g were acquired by cross-colonization in the camp. The strain of genotype b' originated from patient D and colonized patients C and N and possibly patient K. The strain of genotype g was originally found in samples from patients E and F and colonized patient D. None of these strains persisted at follow-up, although this possibility could not be excluded for patient D, who died before the next sampling time. The origins of the strains of genotypes i, k, o, and q colonizing patients G, M, and Q could not be cleared. The strain of genotype o persisted at follow-up, while those of the other three genotypes disappeared. At follow-up 4 of 13 children were infected with strains with genotypes different from those of the strains with which they were infected 2 months earlier, and one child (patient R), who was not known to be infected with P. aeruginosa earlier, was colonized with a strain of genotype 1 that was possibly acquired from the community.

**DISCUSSION**

The prevalence of P. aeruginosa in our study population entering the holiday camp was what we expected of patients with CF at that age, with 42% of the patients being chronically colonized with P. aeruginosa (3). The poor prognosis of CF is especially associated with an early onset of Pseudomonas lung infection. It is not quite clear which factors are involved in colonization and subsequent infection, but cross-infection from other CF patients should be avoided if possible. The frequency of cross-infection has been the subject of several studies, but the results that have been reported are contradictory. Hoiby and Rosendal (6) found one epidemiological type of Pseudomonas predominant in their patients and concluded that cross-infection during hospitalization was the most likely explanation. Other investigators, however, found little evidence of cross-infections between patients, even when the patients were treated in the same clinic (5). The contradictory results of these studies may reflect the difficulty in typing P. aeruginosa.

Our observations of CF patients in the holiday camp showed that the acquisition of microorganisms from each other was rather common under these circumstances, because 31% of the children the organisms in their sputa change. Persistent colonization was estimated to be only 5.4% for bacteria and 1.9% for P. aeruginosa.

In 1980 we reported that cross-colonization in general could not be excluded (7). The results of serotyping and phage typing were not helpful in allowing us to draw a definite conclusion and the serotypes and phage types were therefore not further evaluated.

The methods used for typing P. aeruginosa showed that strains varying in colony morphology, being mucoid or not of a given serotype, pyocin type, or phage type, could nevertheless be of an unique genotype. Like other investigators (12, 13) we found that molecular characterization is the most helpful tool for typing CF strains. We used RAPD-PCR instead of restriction fragment length polymorphism typing because RAPD-PCR has a higher discriminatory power and ready has proven its value in epidemiological studies medically important microorganisms including P. aeruginosa (17, 18). Some of the children in our study showed a colonization with rather constant Pseudomonas flora, with strains different phenotypes but of one genotype over a long period of time. This is in agreement with the findings of Thomassen et al. (16) and Horrevorts et al. (8). However, we also found patients who were colonized with more than one genotype simultaneously, confirming the findings of Hoiby and Rosendal (We also found that within a very short period of time the majority of the children easily acquired strains of other genotypes).
types which sometimes replaced their original strain or which coexisted with the original one. This suggests that the composition of the flora is not constant as a rule, as stated by Horrevorts et al. (8), but may fluctuate considerably. Prolonged intimate family contact clearly led to a brother and sister carrying the same unique genotype (patients O and P), as has been reported by others (5). The risk of becoming colonized with *P. aeruginosa* was estimated to be 7.7% for previously negative children. This figure is comparable to that for the acquisition of *Pseudomonas cepacia* in other summer camps (14). During follow-up it appeared that the incidence of permanent colonization and infection was approximately 1.9%, which is not different from the risk of becoming colonized and infected in the community. We also observed that one child became infected during the follow-up period, suggesting that exposure is not the only determinant for becoming colonized and infected. We therefore conclude that the risk of cross-colonization by participating in a summer holiday camp is trivial compared with the obvious social and psychological benefits.

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REFERENCES