Transmission of *Pseudomonas aeruginosa* in children with cystic fibrosis attending summer camps in The Netherlands

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Abstract

**Background:** Cross-infection of *Pseudomonas aeruginosa* has been reported to occur at holiday camps for children with Cystic Fibrosis (CF) with varying frequency. The study aimed to establish the degree of transmission resulting in subsequent infection of *P. aeruginosa* among CF children (*n*=80) attending holiday camps in The Netherlands.

**Methods:** The study was performed in the summer of 2001 in four camps organised simultaneously at different locations. Sputum was collected on day 1 of the holiday, and three and six months later. Different morphotypes of *P. aeruginosa* from sputum were genotyped by AFLP™ analysis. Criteria were defined for the degree of evidence of transmission.

**Results:** There were 18 cases possible, 2 cases of probable transmission and 1 case of highly probable transmission. Two predominant types of *P. aeruginosa* were found (types 18 and 23). Type 18 was already prevalent on day 1 mostly in younger children and was involved in eleven cases of transmission; type 23 was involved in six cases of transmission among older children.

**Conclusions:** There was a considerable risk of transmission of *P. aeruginosa* during holiday camps for CF children in The Netherlands. Two genotypes of *P. aeruginosa* appeared to be easily transmissible, one of which seemed common in the Dutch CF population.

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1. Introduction

*Pseudomonas aeruginosa* is a notorious pathogen in Cystic Fibrosis (CF) patients. The acquisition of *P. aeruginosa* and subsequent development of chronic infection leads to increased morbidity and earlier mortality in CF patients as compared to those who have remained *P. aeruginosa* free [1]. Therefore, it is important to protect patients against infection with *P. aeruginosa*. Bacteria belonging to *Burkholderia cepacia* complex, organisms with a transmission mode similar to that of *P. aeruginosa* [2,3], have been shown to be transmitted among CF children in holiday camps [4]. As a consequence, children with strains belonging to the *B. cepacia* complex can no longer join holiday camps or other social activities with CF patients. It has become a policy in many countries to stop organising holiday camps of CF patients in order to prevent *P. aeruginosa* acquisition. For the latter organism, however, it has not been shown unambiguously that there is a high risk of acquisition in these camps. One retrospective analysis of a study performed in a summer camp in 1976 in The Netherlands showed a rate of sputum conversion for *P. aeruginosa* of 7.7% among previously *P. aeruginosa* negative children, but only 1.9% remained colonised after two months of follow-up [5]. The authors, therefore, saw no reason to advise CF children to refrain from...
attending a holiday camp with other CF patients. In a retrospective investigation in Denmark [6], transmission was found in all children (n=5) who did not carry *P. aeruginosa* prior to attending the holiday camp. The advice was to segregate the CF children into groups according to their *P. aeruginosa* status during social activities such as these camps. From a study by Hunfeld et al. [7] it was concluded that the risk of cross-colonisation during a summer camp probably is not much higher than the reported incidence of acquisition of the pathogen from the environment. The risk of *P. aeruginosa* spreading in camps was considered minor in comparison to the medical, social and psychological benefits of such camps for CF patients. Another study to determine the transmission of *P. aeruginosa* among CF patients during three consecutive winter camps in a health camp could not demonstrate cross-infection of *P. aeruginosa* [8].

The conflicting outcomes of these studies [5–8] on the risk of transmission of *P. aeruginosa* during holiday camps challenged us to gather more evidence before recommendations on the continuation of CF camps in The Netherlands could be given. The aim of the present prospective study was to establish the degree of transmission resulting in subsequent infection of *P. aeruginosa* among children with CF attending holiday camps in The Netherlands.

2. Methods

2.1. Patients and camps

The study was performed in the summer of 2001 in four summer holiday camps organised simultaneously at different locations in The Netherlands. The period of stay in each camp was eight days. The camps ranged according to the age category of the participants: camp 1 had 16 participants age 6–11 years, camp 2 had 19 participants age 11–13, camp 3 had 24 participants age 13–15 and camp 4 had 21 participants age 15–19 years.

All children had a ‘non-cepacia’ declaration from their medical attendant, which implies that bacteria of the *B. cepacia* complex had never been cultured from the child’s sputum and that within the last six months at least one sputum culture was done. All participants received written information about the purpose and the consequences of the investigation. It was agreed that patients nor their medical attendants would be informed about the individual culture results, unless a strain belonging to the *B. cepacia* complex or a methicillin resistant *Staphylococcus aureus* (MRSA) would be isolated. Consent was obtained from the participants. The study was approved by the ethics committee of HagaZiekenhuis (former Ziekenhuis Leyenburg).

2.2. Bacteriology

Sputum was collected on day 1 of the holiday (T1), after three months (T2), and after six months (T3). These sampling points were chosen as practical for establishing transmission of *P. aeruginosa* resulting in chronic infection. Patients were asked to produce sputum spontaneously by coughing, while a pharyngeal swab was taken if no sputum could be produced. In 8 times (7 children) no culture could be obtained due to absence at the sampling times. No environmental sampling was done in and around the camps. All sputum samples and swabs were processed for isolation and identification of *P. aeruginosa* in one laboratory. Species identification of *P. aeruginosa* was performed on the basis of a positive oxidase test, pigment production, hydrolysis of acetamide, resistance to the combination of C-390 and phenantroline. In case of doubt, additional identification with the API 20-NE system (bioMérieux, Marcy l’Etoile, France) was performed. Different morphotypes of *P. aeruginosa* from the sputum sample were isolated and stored in glycerol broth at minus 80 °C for typing at a later date. All samples were also investigated for the presence of *B. cepacia* complex and MRSA as their presence would result in isolation measures.

2.3. AFLP analysis

Genotypic relatedness of strains was assessed by AFLP™, a high resolution genomic fingerprinting method [9]. Briefly, purified DNA was digested using EcoRI and *Mse*I, while ligation of EcoRI and *Mse*I adapters to the restriction fragments was performed simultaneously. PCR was done with a Cy5-labelled EcoRI primer and a *Mse*I+C primer (C, selective nucleotide). The ALF express system (Amersham Biosciences, Roosendaal, The Netherlands) was used for fragment separation. Isolates were grouped for their similarity of fragments of 50–500 bp by cluster analysis with the BioNumerics software release 2.5 (Applied Maths, Sint-Martens-Latem, Belgium). Pearson product moment coefficient (r) was used as a measure of similarity, and the unweighted pair group average linkage method (UPGMA) for grouping. The optimisation setting was 0.5%, and the final area for cluster analysis was 7.0–95.2% of the profile.

2.4. Pulsed field gel electrophoresis (PFGE)

Macrorestriction analysis by PFGE was performed as described previously [10] with the following modifications [11]: a Gram-positive bacteria lysis step (using lysis buffer containing 500 µg lysozyme ml⁻¹) was included prior to the addition of Gram-negative lysis buffer. DNA was digested with 30 U SpeI (Helena BioSciences Europe, Tyne & Wear, UK) in the accompanying Y′/Tango buffer with BSA. Fragments were separated with the CHEF DRII apparatus (Bio-Rad, Hemel Hempstead, UK) in a 1.2% agarose gel. Initial and final switching times were 1 and 50 s respectively. Running conditions were: temperature 12 °C, angle of 120°, and run time 30 h at 6 V/cm. Following staining with ethidium bromide, gels were photographed and profiles were analysed visually and by cluster analysis with BioNumerics.
software using the band based Dice coefficient as a similarity measure and UPGMA for grouping.

2.5. Definitions of transmission

Transmission was defined as the finding of a *P. aeruginosa* AFLP type that was not present in the sputum of the patient at entry of the camp (T1), but was present at a later time (T2 and/or T3), and that this specific type of *P. aeruginosa* was present in another patient at T1 in this camp.

If at T1 from a patient no *P. aeruginosa* was cultured, the history of *P. aeruginosa* infection of this patient was sought. Culture results of sputum or throat in the two years preceding the camp were inquired at the medical centre the patient had visited. For this purpose a minimum of three cultures was considered acceptable.

Criteria were defined for the degree of evidence of transmission. Transmission was considered “highly probable”, if a patient had a negative history of *P. aeruginosa* prior to the camp period, and if this patient was (*P. aeruginosa*) negative at T1, but (*P. aeruginosa*) positive at T2, and positive or negative at T3 for a given strain type circulating in the camp. Transmission was considered “probable” in case of a negative history of *P. aeruginosa*, a negative culture at T1 and T2 but positive at T3 for a type circulating in the camp. Transmission was assumed “possible” if a patient had a positive history of *P. aeruginosa* (of an unknown strain type), was negative at T1 but positive at T2 and/or T3 with a type of *P. aeruginosa* found among the camp attendants. All other combinations were considered as “no transmission”.

3. Results

3.1. Patients and camps

The study comprised of 80 children with CF in the age range of 6–19 years, including a few sets of siblings, attending one of four summer camps. These children were treated in 24 different hospitals in The Netherlands. Seventy-one out of the 80 children were positive for *P. aeruginosa* at one or more of the sampling moments (T1–T3). Twenty-three patients were negative at T1, but further enquiries showed that 17 of them had a positive sputum or throat culture in the two years prior to the camp. Of the 215 sputum cultures 162 were positive for *P. aeruginosa* (75%). In total 17 throat cultures were taken from which 13 were positive for *P. aeruginosa* (76%). The general sensitivity of culture to establish infection with *P. aeruginosa* in these groups, taking their history of prior *P. aeruginosa* infection into account, was 77, 78 and 78% respectively for T1, T2 and T3.

From each of the 71 children, the isolates were selected per sample in the clinical laboratory on the basis of their unique colony morphology and considered possibly to represent different strains. Thus, 255 isolates including different morphotypes from individual patients were left for genotyping by AFLP analysis. Concordance of AFLP results with PFGE analysis was sought for a subset of isolates. No patients harboured strains of *B. cepacia* complex, and one patient had a positive sputum culture for MRSA at T2.

3.2. Genotyping: validation and results

AFLP analysis was first validated for its ability to type *P. aeruginosa* strains by assessing the marker stability – i.e., the stability of AFLP profiles of strains – the discriminatory capacity and the reproducibility of the method [12]. Marker stability was estimated by comparing profiles of sequential isolates from 14 patients each. The discriminatory capacity of the method was tested on a set of 18 epidemiologically unrelated isolates. Cluster analysis of the resulting profiles depicted in a dendrogram showed that, at a cluster cutoff level of 88%, multiple isolates of each patient clustered together, while unrelated strains were ungrouped (data not shown). The reproducibility of the method was assessed by comparing independently processed samples of one control strain (ATCC 27853) the profiles of which grouped at 84.54±3.41%. Based on the noted marker stability and the reproducibility of the method, the 85% level was considered the level to delineate strains. First, this level was used to exclude multiple isolates of the same strain in one patient sample, resulting in 197 isolates from 71 patients. Next, at the same cluster cutoff level AFLP analysis of these 197 isolates was done. Thus, a total of 24 well separated clusters (AFLP types), designated 1–24 were distinguished (Fig. 1). Predominant AFLP types were type 18 in 47 patients, type 23 in seven patients, type 13 in six patients and type 1 in five patients.

To verify the validity of the AFLP profiling, a subset of ten isolates, including seven of AFLP type 18 and three additional isolates of AFLP types 13, 15 and 19 were analysed by PFGE. The analysis also included two epidemic CF strains circulating in Liverpool and Manchester (United Kingdom). The grouping of the PFGE profiles of the organisms as depicted in a dendogram, the actual PFGE profiles, the AFLP designation and origin are shown in Fig. 2. The figure shows clearly the consistency of PFGE banding profiles for the isolates allocated to AFLP type 18; these clustered at 85% and had no more than three band differences. The other profiles including those from the epidemic UK strains were clearly distinct. Further analysis revealed that the AFLP types 13, 15, 18 and 19 were also distinct from two epidemic strains clone C from Hanover (G) [13], the epidemic strain Mdl from Birmingham (UK) [Scott] and from isolates from CF patients in the Czech Republic (data not shown).

3.3. Distribution of different strains among patients and camps

In camp 1, with the youngest children, only five different types (types 4, 8, 16, 18, 22) were distinguished at T1, of which type 18 was the most predominant. In one child in this
Fig. 1. Dendrogram, clustering AFLP types of *P. aeruginosa* isolates found in this study with the number of isolates and the number of patients involved for this type.
group an additional, unique type (type 11) was found at T2 and T3. In camp 2, five types (types 10, 14, 18, 19, 24) were found at T1, while one additional type (type 8) was found in one patient at T2. In camp 3, nine types (types 1, 2, 3, 8, 13, 15, 16, 18, 21) were found at T1, and two additional types (types 10, 20 in two children) at T2/T3. In camp 4, eight types (types 1, 5, 6, 12, 13, 17, 18, 23) were found at T1, while at T2/T3 in these patients two additional types (types 7, 9) were encountered.

The occurrence of type 18 at T1, in particular in camps 1–3, was strikingly high with a distribution in camps 1–4 in 8, 9, 16 and 3 children respectively, while other types were relatively rare.

3.4. Transmission

There were 18 cases of possible, 2 cases of probable transmission and there was 1 case of highly probable transmission (Table 1). All cases of transmission in camps 1 and 2 were confined to type 18; types 3 and 21 played a role in camp 3. In camp 4 with the oldest CF patients, type 18 played a minor role as compared to types 13 and 23. At T1 one child in camp 4 was the only carrier of a P. aeruginosa of type 23; at T2 however six other children were also infected with this type (categorised as possible transmissions). In these camps 10 sets of siblings were included (n = 21). Seven of them had identical types of P. aeruginosa in their sputum during the whole period. Three sets of siblings had different types during this period of six months. We did not see transmission between these three sets of siblings.

4. Discussion

In 21 of the 80 children, who attended one of the four separate camps, infection of a new P. aeruginosa type, that was present at the beginning of these summer camps, was established. As one can never be sure of the exact moment of transmission, the patterns of culture/typing results over time were categorised into evidence of possible, probable and highly probable transmission. No typing data of the P. aeruginosa strains cultured in the period before these camps took place were available. As a consequence of this lack of information the transmissions in this particular group were graded not higher than “possible”, as it could not be excluded, that the children were already carrying this strain type before joining the camp.

Another weakness is the sensitivity of culture (78%) to detect infection with P. aeruginosa. This means that some infected children were missed at T1 and when their cultures were positive at T2 or T3 for a camp-related type, this was categorised as a transmission. But using the grading system we categorised this as a “possible” transmission. This sensitivity is confined to this group of clinically stable children aged 6 to 19 years.

On the other hand, only seven of the 80 children had a unique P. aeruginosa type at T2 or T3, that was not present
in the camp at T1. This supports the argument that transmissions of *P. aeruginosa* have occurred in the period that the camps were attended.

Three of the six children, with no prior history of *P. aeruginosa* infection, had a camp-related *P. aeruginosa* type three or six months after the camp. This percentage of 50% may be less than the findings of Ojeniyi [6], but is definitely more than the incidence of permanent colonisation and infection of *P. aeruginosa* of 1.9% after 2 months of follow-up as seen in a previous study in The Netherlands [5]. The transmissions in this group were graded as probable or highly probable due to their negative history of *P. aeruginosa* infection up to two years preceding this camp.

An unexpected finding was the dominant presence of one type of *P. aeruginosa* (type 18), particular in the younger children. The high prevalence of type 18 at the time of the camp within the groups 1, 2 and 3 may have contributed that most transmissions have occurred with this specific type. Frequent previous participations in these holiday camps could not be related to the high prevalence of type 18. The 36 children, who had a type 18 at T1, had participated in these camps a 192 times cumulatively (mean 5.3 times). The 33 children, who were not infected with type 18 at T1 and thereafter, had participated in these camps a 184 times cumulatively (mean 5.6 times).

Linking types to the CF centres attended by the infected children showed that nearly all centres (20/24) had one or more children in their care infected with type 18, emphasizing its wide occurrence in the Netherlands CF patient population. Remarkable is that in camp 4, which was the camp with the oldest children, only 3 of the 21 children were infected with type 18 at T1. This suggests that type 18 is not universally present within the total CF population or the environment, and therefore supports the argument that the observed transmissions have taken place in the camps.

In camp 4 possible transmission of *P. aeruginosa* type 23 appeared to have occurred from one single child to six others. Seemingly types 18 and 23 had the capacity to spread among the CF children and, consequently, might be called epidemic strains. Future studies are required to assess the prevalence of these strains in the different centres and whether this ability to spread epidemically is also linked with an increase in virulence, as was described for an epidemic strain from Liverpool, UK [14].

The prevalence of *P. aeruginosa* in our study population was surprisingly high at the start: only six children attending these holiday camps had no history of infection with *P. aeruginosa*. An explanation could be that parents keep their non-infected children away from joining other CF children in summer camps; or children infected with *P. aeruginosa* are more handicapped by their disease so that they prefer a summer camp with specific medical support.

The living and social conditions in camps for children or adults with CF vary and may partly explain the differences of outcome of the other studies [5–8] on transmission of *P. aeruginosa*. The children of these four Dutch camps were from the same age group, with many adolescents, most being already familiar with each other and a holiday camp is very much considered as a social paradise for these individuals: no segregation policy was in place.

The organisers were knowledgeable that in some other countries these holiday camps had been ended because of the risk of transmission of *P. aeruginosa*. One prior study in summer camps in The Netherlands [5] did not demonstrate a risk of transmission of *P. aeruginosa* and in that study no predominant strains were detected. The organisers and their medical advisory board were therefore reluctant to follow the guidelines of other countries to end these popular camps. The results of this study have made the Dutch CF community aware, that epidemic strains may have emerged and that participating in these summer camps, without some form of segregation, does carry a risk of acquiring *P. aeruginosa*.

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