Transmission and prevalence of *Burkholderia cepacia* in Welsh cystic fibrosis patients

L. MILLAR-JONES*, H. C. RYLEY†, A. PAULL† AND M. C. GOODCHILD*

*Cystic Fibrosis Unit, Department of Child Health, University Hospital of Wales, Cardiff, U.K.
†Department of Medical Microbiology, University of Wales College of Medicine, Cardiff, U.K.

From 1987 to 1994, 16 of 162 cystic fibrosis (CF) patients attending CF clinics at three different hospitals in South Wales, U.K. were found to have respiratory secretions colonized with *Burkholderia cepacia* (*B. cepacia*). Bacteriological typing by polymerase chain reaction (PCR) ribotyping demonstrated seven strains of *B. cepacia* among these 16 CF patients. This typing confirmed that cross-infection was the mechanism of colonization in six of the nine patients who were colonized at the paediatric CF clinic at the University Hospital of Wales in Cardiff, and in three of the six patients who were colonized at the adult CF clinic at Llandough Hospital in Cardiff (cross-infection rate nine of 16 patients or 56%).

A search was made for a nosocomial source, with screening of wards and clinics. Swabs from fomites produced four positive cultures for *B. cepacia*. Two isolates had the same PCR ribotype as that of the previous CF room occupant.

To establish prevalence of *B. cepacia* among CF children living throughout Wales, respiratory secretions were cultured from 151 of 186 CF children (age <16 years). This failed to demonstrate *B. cepacia* colonization other than in the CF patients already identified.

**Introduction**

*Burkholderia cepacia* (*B. cepacia*), formerly *Pseudomonas cepacia*, has become an important pathogen among cystic fibrosis (CF) patients in recent years (1), and its incidence has been rising (2,3). The effects of *B. cepacia* can be different from those produced by *Pseudomonas aeruginosa* (1,4,5); it is of concern that rapid deterioration and death has been described in a few CF patients (1,4–7) within a short time of colonization. This fulminant course has been associated with necrotizing pneumonia and septicemia (8,9).

The mode of transmission of *B. cepacia* remains controversial. Evidence has been increasing for person-to-person transmission associated with hospital attendance (10,11) and social contact (12–14). Nosocomial acquisition of *B. cepacia* has also been suggested (15,16), and acquisition from the environment outside the hospital (10).

Since 1987, within Cardiff, there has been a gradual increase in the number of CF patients colonized with *B. cepacia*, both in the paediatric clinic at the University Hospital of Wales and the adult clinic at Llandough Hospital, which are on opposite sides of the city.

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Concern over the rising incidence of *B. cepacia* colonization within the two clinics in Cardiff prompted the authors to investigate first, the suspected chain of cross-infection by identifying times of contact between patients and typing the isolates of *B. cepacia*. Initial typing involved bacteriocin production and sensitivity (Govan JRW, Edinburgh 17,18) and has been reported previously (11); more recent typing has been done by the polymerase chain reaction (PCR) 'Ribotyping' method of Kostman et al. (19) modified by Ryley et al. (20). Second, in order to establish the prevalence of *B. cepacia* among paediatric CF patients living in Wales, respiratory secretions were screened from as many patients as possible. Third, since many of the patients appeared to become colonized at the time of hospital admission or shortly afterwards, search for a nonnosocomial source was also commenced.

**Patients and Methods**

**PATIENTS**

Sixty-nine CF children (mean age ± 1 SD 9.4 ± 3.8 years, 33 female) were studied at the paediatric CF clinic at the University Hospital of Wales. An additional 82 CF patients (mean age ± 1 SD 6.7 ± 3.8 years, 39 female) who attended other clinics in Wales were studied from March 1992 to April 1993 by culture of respiratory secretions. Eighty adult CF patients (mean age ± 1 SD 23.3 ± 4.7 years, 43 female) were studied at Llandough Hospital.
RESPIRATORY SECRETION COLLECTION

During the 14-month period from March 1992 until April 1993, children with CF attending the University Hospital of Wales had respiratory secretions collected at outpatient visits (usually every 3 months), also on admission to the ward and weekly for inpatients. Respiratory secretions were similarly collected from adult CF outpatients and inpatients attending Llandough Hospital.

During this same time period, respiratory cultures were obtained from 151 of the estimated 186 CF patients in Wales (21), who were less than 16 years. Respiratory secretions from CF patients outside Cardiff were taken by an experienced member of the Cardiff CF team at the time of peripheral clinic visits. They were placed either into sterile universal containers or obtained as cough swabs (produced by asking or stimulating the patient to cough onto a swab positioned in the pharynx) and placed in Stuart medium. To avoid the problems of variation in isolation rates for B. cepacia from different laboratories, all specimens were brought back to the University Hospital of Wales for culture and identification. For specimens obtained at a considerable distance from Cardiff, there was an inevitable delay in getting specimens to the laboratory (maximum transport time, 3 days).

The authors had demonstrated previously that it was possible to isolate B. cepacia from cough swabs and sputum samples kept for up to 4 days, either at room temperature or at 4°C. Patients outside Cardiff usually had two respiratory secretions tested in this manner over the 14 months.

BACTERIOLOGICAL CULTURE AND TYPING

Secretions were inoculated onto B. cepacia selective media containing ticarcillin and polymyxin B (Selectatab Agar, Mast Laboratories, Merseyside, U.K.), and incubated aerobically for 48 h at 37°C. Suspect colonies were identified using standard bacteriological techniques including the API 20 NF identification system (Bio Merieux Ltd, Hampshire, U.K.).

Typing of the organism was carried out using a modification of the PCR 'Ribotyping' method of Kostman et al. (19) as described by Ryley et al. (20). Essentially, PCR amplification was done on the intergenic space between the 16S and 23S rRNA genes on extracted chromosomal DNA with the same primers and under similar conditions to those described by Kostman et al. (19). Prior to electrophoretic analysis, the PCR products were digested with a restriction endonuclease, TaqI, resulting in more complex final electrophoretic patterns which gave greater discrimination between types. Pattern differentiation was based on the number of bands seen and the relative position and intensity of staining of each band within the electrophoretic pattern.

ENVIRONMENTAL SCREENING

From March 1992 over a 12-month period, five single occupancy cubicles of known B. cepacia positive patients were swabbed in eight designated areas (bed frame, mattress, locker, window sill, blind, sink, door handle and floor) on the following occasions: after discharge (within 30 min of the patient vacating the cubicle) before and after cleaning the cubicle and, where possible, before admission. Immediately following discharge, the atmosphere in the cubicle was tested for 1 min using a slit sampler (30 l min⁻¹).

Two University Hospital of Wales outpatient clinic rooms (used for respiratory function testing and consultations) were investigated similarly with swabs and a slit sampler, on three separate clinic days set aside for patients colonized with B. cepacia. Swabs were taken from the plug holes in the sinks, from the finger probe on the pulse oximeter, from inside the vitalograph flow head and from the grid at the mouth-piece end of the peak flow meter. Timing of samples was prior to the clinic visit and immediately after each patient had left the room. At the end of the clinic, the vitalograph flow-head was dismantled and swabs taken from the filters inside. All swabs were moistened with polymyxin broth and inoculated immediately into B. cepacia enrichment medium. Incubations, subculture and identification were the same as for the respiratory secretions. Cultures were obtained, onto B. cepacia selective medium, from 193 swabs and 36 slit sampler plates.

Results

PAEDIATRIC PATIENTS

Since 1987, nine CF children (mean age at colonization +1 so 11.7 ± 2.7 years, five female) attending the paediatric clinic at University Hospital of Wales become colonized with B. cepacia (Patients A–J, Fig. 1, Table 1). Three of the patients died (A–C). PCR typing, prior colonization with Pseudomonas aeruginosa or Pseudomonas species, age at acquisition of B. cepacia, mean duration of B. cepacia colonization and age at death are shown in Table 1.

The first patient (A) was identified after a 4-week visit to a Canadian holiday camp for people with CF. Burkholderia cepacia had never been isolated from this patient's sputum prior to this holiday, despite testing at least 3 monthly over the previous 2 years. On the basis of typing, it was concluded that a chain of cross-infection derived from this index case was responsible for six subsequent cases (Patients B–F and J) (Fig. 1). Apart from the index case and the siblings, none of the patients had had known contact with any other CF patients outside the hospital environment. After recognition of the first four cases in 1990, management changes were instituted to segregate CF patients into single occupancy cubicles while on the ward. Children slept and performed physiotherapy in the cubicles, but socialized in the school room and playroom. Separate clinics were established for B. cepacia colonized and non-colonized patients.

Following the failure to contain cross-infection (with recognition of Patient F), further management changes followed in June 1992 with all paediatric CF patients being
Concurrent admission resulting in cross-infection

PCR type 1
PCR type 2
PCR type 3
PCR type 4
PCR type 5
PCR type 6

Holiday in Canada


![Diagram showing transmission of Burkholderia cepacia during hospital admission and PCR ribotyping results.](image)

Fig. 1. Transmission of Burkholderia cepacia during hospital admission in cystic fibrosis (CF) patients. The paediatric CF patients are A–H and J; the adult patients L–P and R. PCR, polymerase chain reaction.

partially barrier-nursed. This involved all CF patients being nursed in single occupancy cubicles; no socializing was allowed in any part of the hospital and social contact outside the hospital was discouraged. Each patient had his own examination equipment within the cubicle, and strict hand washing was performed before and after patients were examined. Due to the lack of cubicles with bathroom facilities, patients were allowed to use the general ward facilities. Despite these efforts to separate patients, Patient J became colonized in April 1994 after admission to hospital at the same time as Patients C and E.

PCR ribotyping demonstrated three distinct banding patterns with variation in number, position and intensity of individual bands. Patients A–F and J all carried PCR type 1, in keeping with the presumed spread of colonization by cross-infection between these patients. Patients G and H, however, who had had no known contact with other CF patients colonized with B. cepacia, carried ribotypes 2 and 3, different from the foregoing and different from each other.

No patient was colonized more than one type of B. cepacia and no changes of type were noted. Once colonized with B. cepacia, all patients, except Patient H, were chronically colonized with this organism. Patient H was treated with a 2-week course of intravenous antibiotics at the time of first isolation of B. cepacia; following this, the authors were unable to re-isolate the bacterium from three sputum cultures over 4 months. However, the culture became positive again at 5 months, when the PCR ribotype was the same as before treatment.

None of the 82 patients outside Cardiff was colonized with B. cepacia, and fewer cultured positive for P. aerugi-
nosa (Table 2). As a group, the CF population outside Cardiff was significantly younger than those attending the Cardiff clinic (P<0.001). Eight months after the close of the study, a paediatric patient (Patient K) attending Singleton Hospital in Swansea was identified by the local paediatrician to be colonized with B. cepacia in December 1993. This patient carried yet another PCR ribotype, type 7 (Table 1).

ADULT PATIENTS

Since February 1992, six adult patients (mean age at colonization ± 1 SD 19.1 ± 1.9 years, five female) attending the CF clinic at Llandough Hospital were found to have become colonized with B. cepacia (Patients L–R, Fig. 1, Table 1). Two of these patients died (N and R). PCR typing, other microbiological details and age at death are shown in Table 1.

In Llandough hospital, CF patients were not barrier nursed in cubicles; however, patients colonized with B. cepacia were segregated onto a different ward from those not colonized with B. cepacia. Despite being discouraged, some patients socialized in other parts of the hospital.

Bacteriological ribotyping by PCR showed that the isolates from the paediatric and adult clinics were different, and identified three distinct electrophoretic patterns among the six patients attending the adult clinic (Table 1). Four of these patients (L, M, P and R) who had known contact within hospital shared the same type of B. cepacia (PCR ribotype 4). Patient N who had had several admissions with Patient M and was known to be a close friend, was
Table 1. Details of patients colonized with *Burkholderia cepacia* and polymerase chain reaction typing of isolates

<table>
<thead>
<tr>
<th>Patient/sex</th>
<th>PCR typing of <em>Burkholderia cepacia</em> (1-7)</th>
<th>Prior isolation of <em>Pseudomonas aeruginosa</em> (±)</th>
<th>Age at acquisition of <em>Burkholderia cepacia</em> (years)</th>
<th>Duration of <em>Burkholderia cepacia</em> colonization (years)</th>
<th>Age at death (years)</th>
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<tr>
<td>Paediatric</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/female</td>
<td>1</td>
<td>+</td>
<td>12.1</td>
<td>3.6</td>
<td>15.7</td>
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<tr>
<td>B/female</td>
<td>1</td>
<td>+</td>
<td>9.1</td>
<td>3.2</td>
<td>12.3</td>
</tr>
<tr>
<td>C/male</td>
<td>1</td>
<td>+</td>
<td>11.7</td>
<td>4.6</td>
<td>16.3</td>
</tr>
<tr>
<td>D/female</td>
<td>1</td>
<td>+</td>
<td>9.5</td>
<td>4.9</td>
<td>—</td>
</tr>
<tr>
<td>E/male</td>
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<td>+</td>
<td>8.5</td>
<td>4.8</td>
<td>—</td>
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<tr>
<td>F/male</td>
<td>1</td>
<td>+</td>
<td>9.3</td>
<td>2.9</td>
<td>—</td>
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<tr>
<td>G/male</td>
<td>2</td>
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<td>15.4</td>
<td>2.7</td>
<td>—</td>
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<td>3</td>
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<td>14.3</td>
<td>2.4</td>
<td>—</td>
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<td>J/female</td>
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<td>15.0</td>
<td>0.9</td>
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<tr>
<td>K/male</td>
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<td>—</td>
<td>5.4</td>
<td>1.3</td>
<td>—</td>
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<td>Adult</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L/female</td>
<td>4</td>
<td>+</td>
<td>19.8</td>
<td>3.1</td>
<td>—</td>
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<tr>
<td>M/female</td>
<td>4</td>
<td>+</td>
<td>18.0</td>
<td>2.9</td>
<td>—</td>
</tr>
<tr>
<td>N/female</td>
<td>5</td>
<td>+</td>
<td>22.4</td>
<td>0.6</td>
<td>23.0</td>
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<tr>
<td>O/male</td>
<td>6</td>
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<tr>
<td>P/female</td>
<td>4</td>
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<td>21.2</td>
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<tr>
<td>R/female</td>
<td>4</td>
<td>+</td>
<td>23.6</td>
<td>0.8</td>
<td>24.4</td>
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<tr>
<td>Age (years)</td>
<td>Mean ± 1 SD</td>
<td></td>
<td>14.8 ± 5.7</td>
<td>2.7 ± 1.4</td>
<td>18.3 ± 5.1</td>
</tr>
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</table>

Table 2. Prevalence of *Burkholderia cepacia* colonization of paediatric cystic fibrosis (CF) patients in Wales for the period March 1992–April 1993

<table>
<thead>
<tr>
<th>Cardiff paediatric clinic</th>
<th>Clinics outside Cardiff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of CF patients</td>
<td>69</td>
</tr>
<tr>
<td>Mean age ± 1 SD (years)</td>
<td>9.4 ± 3.8</td>
</tr>
<tr>
<td>Number of respiratory secretions tested per patient median (range)</td>
<td>8 (1–20)</td>
</tr>
<tr>
<td>Number of patients colonized with:</td>
<td></td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em></td>
<td>6 (9%)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>39 (57%)</td>
</tr>
</tbody>
</table>

colonized with a different type of *B. cepacia* (PCR ribotype 5). Patient O, who had no known contact with other *B. cepacia* patients, had the third type of *B. cepacia* found in the adult clinic (PCR ribotype 6).

Environmental Screening

Only four positive isolates were found: from a ward cubicle floor, from a slit sampler used in a clinic room and from each of two sinks in separate clinic rooms. The isolates from the cubicle floor and the slit sampler were both PCR ribotype 1, the same as that of Patient C who had recently occupied the cubicle and clinic room. Samples form the sinks were of a different PCR ribotype (not recognized among the patients), here termed an 'environmental strain'. Testing of lung function equipment in the paediatric clinic failed to culture *B. cepacia*.

Discussion

*Burkholderia cepacia* has caused widespread concern among the CF community for patients and carers alike. Confusion over the mode of transmission has caused some of this anxiety. Over recent years, there has been increasing evidence to support person-to-person transmission of *B. cepacia* between CF patients, both within the hospital environment (3,4,11,14,20,22,23) and by social contact outside the hospital (3,12–14). Conversely, other studies have not supported person-to-person spread within the CF population (5,24–26), suggesting that epidemic spread of *B. cepacia* is not inevitable as not all strains have epidemic potential (26). The risk of transmission may be strain dependent, and a subgroup of *B. cepacia* strains has been responsible for clustering of cases in particular centres (3). In the present study, acquired cross-infection with *B. cepacia* appears to have caused colonization in nine out of 16 cases (56%). Six of these patients (all with similar ribotype) attended University Hospital of Wales and three patients (all with a similar ribotype but different from the paediatric cases) attended Llandough Hospital. Transmission within the home environment appears to have
been responsible for two of the cases attending University Hospital of Wales (one in each of two families) who were siblings of known cases.

Nosocomial acquisition of *B. cepacia* by surgical patients via cleaning fluids has been well documented (27,28). Evidence for environmental acquisition has been less certain, although contamination has occurred (1,3,10,16,24,29). The authors' search for a nosocomial source at University Hospital of Wales produced few positive results despite repeat testing; only four positive isolates were obtained, two of which were of the same ribotype as the previous room occupant, suggesting that nosocomial acquisition may be possible. The authors did not obtain positive cultures from lung function equipment, like others (1), but these have been recorded (1,3,16).

Controversy surrounds the management of patients colonized with *B. cepacia*. Many centres have introduced segregation policies and actively discouraged social contact such as attendance at CF meetings and CF holiday camps (3,30–32). Such themes have been taken up by the U.K. CF Trust which has issued guidelines on restriction of social and hospital contact (33), and by the U.S. CF Foundation which has ceased to advocate or sponsor summer camps for *B. cepacia* (34). All this imposes a considerable burden on patients and staff alike, and the long-term psychological effects are unknown. Segregation of colonized from non-colonized patients can create 'microbiological outcasts' as patients are removed from their usual hospital CF clinic or ward, away from friends and familiar hospital staff (32). In addition, non-colonized patients become increasingly anxious about getting *B. cepacia* (32,35).

In areas where strict segregation has been used, the incidence of *B. cepacia* colonization of CF patients has remained low or has decreased (31,36). Segregation policies for patients colonized with *P. aeruginosa* have been operating at the Copenhagen CF clinic since 1981 (36); similar practices for *B. cepacia* are thought to have contributed to the remaining low incidence of this bacterium at this clinic (36,37). At University Hospital of Wales, Cardiff, a segregation policy for *B. cepacia* has been in place since 1992, following which no further cross-infection occurred for 2 years, until Patient J became colonized in 1994. Despite counselling, this teenage patient socialized with known *B. cepacia* CF patients who were on the ward at the same time. At Llandough Hospital, Cardiff, colonization of two further CF patients with *B. cepacia* followed the recognition of the earlier two patients, even although efforts were made to separate colonized and non-colonized patients on different wards.

Five patients in this study (31%) acquired *B. cepacia* having had no known contact with other *B. cepacia* patients. These five patients carried individual ribotypes, different from each other and different from the two chains of presumed cross-infection recognized at University Hospital of Wales and Llandough Hospital. Segregation could not have prevented colonization of these patients with these particular organisms.

As in other reports (23,30), the majority of patients in this study were colonized with either *Pseudomonas* species or *P. aeruginosa*, prior to *B. cepacia*, and there may have been a tendency for females to do worse (1,7): 10 of the 16 patients with *B. cepacia* were female (among a population which was 51% female) as were four of the five deaths. Among the 10 paediatric patients colonized, mean age of acquisition of *B. cepacia* (11.0 years ± 3.2) was comparable with other reports involving paediatric clinics (1,23,38).

Investigation of 151 of 186 paediatric CF patients throughout Wales showed that the prevalence of *B. cepacia* in April 1993 was 4%. At that time, all these six patients attended the Cardiff paediatric CF clinic; four were of a similar ribotype and colonized by cross-infection. The other two were of two further, differing ribotypes and the source of their infection is unknown. For these patients, it is possible that their older age may have rendered them more liable to colonization with *B. cepacia*. Since isolation of *B. cepacia* from cough swabs kept for up to 4 days was consistently possible, the authors are confident that the delay of up to 3 days for some swabs reaching the laboratory would not have affected the detection of this organism from patients attending clinics outside Cardiff. However, it is possible that testing of fewer respiratory secretions from outside Cardiff may have influenced the number of positive findings.

Person-to-person transmission of *B. cepacia* between CF patients has been the main cause of the rise in incidence of colonization of CF patients with this organism in Cardiff. Segregation of patients seems to have reduced this transmission, but sporadic acquisition of *B. cepacia* has been recognized, which would have occurred despite segregation.

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### References


