

Original Article

Burkholderia cenocepacia ST-250 in cystic fibrosis patients in Switzerland: Genomic investigation of transmission routes

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ABSTRACT

This report describes the characterization of *Burkholderia cenocepacia* isolates belonging to sequence type (ST)-250, detected in eight patients with cystic fibrosis (CF) in Switzerland. We retrospectively analyzed 18 isolates of *B. cenocepacia* ST-250 isolated between 2003 and 2015 by whole-genome sequencing and evaluated clinical and epidemiological data. Single nucleotide polymorphism analysis of the *B. cenocepacia* ST-250 lineage showed that the isolates from all patients cluster tightly, suggesting that this cluster has a recent common ancestor. Epidemiological investigations showed that six out of eight patients acquired *B. cenocepacia* ST-250 in the years 2001–2006, where participation in CF summer camps was common. Two patients were siblings. Genomic relatedness of the *B. cenocepacia* ST-250 isolates supported transmission by close contact, however, a common source or nosocomial routes cannot be excluded. With respect to the fatal outcome in six patients, our study shows the importance of infection control measurements in CF patients with *B. cenocepacia*.

1. Introduction

Bacteria of the *Burkholderia cepacia* complex (Bcc) are opportunistic pathogens but are known to cause increased morbidity and mortality among persons with cystic fibrosis (CF). The species most frequently isolated in CF patients are the closely related bacteria *Burkholderia multivorans* and *Burkholderia cenocepacia* [1]. *B. cenocepacia* has the higher morbidity and mortality in CF patients and displays a genetic diversity with occurrence of epidemic strains [2,3]. Patient-to-patient transmission of highly pathogenic epidemic strains of *B. cenocepacia* has been described [2–4]. Early recognition of chronic infection by pathogenic *B. cenocepacia* strains are crucial for patient management and infection control measurements. Currently the most detailed and

extensive global epidemiology data for Bcc bacteria has been obtained by multilocus sequence typing (MLST) [5]. The availability of novel methods such as whole-genome sequencing (WGS) allows relatedness to be examined using much more data than MLST. The use of epidemiological investigations in conjunction with WGS analysis of bacterial isolates is a high resolution way to understand and prevent transmission of bacteria in addition to implementing infection control precautions in vulnerable populations.

In a retrospective analysis of clinical Bcc isolates in Switzerland, we detected 18 isolates of *B. cenocepacia* sequence type (ST) 250 by whole-genome analysis from eight CF patients. The aim of our study was to characterize the *B. cenocepacia* ST-250 isolates and to describe clinical details of the corresponding patients. We uniquely describe whole-

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genome sequences of *B. cenocepacia* ST-250 and elucidate the relatedness of the isolates in the context of possible patient-to-patient transmission.

2. Methods

2.1. Patients and clinical data

Clinical data of the eight CF patients was retrospectively retrieved from the archived medical charts. The patients attended different CF centers in the German-speaking part of Switzerland (University Hospital of Zurich, University Hospital of Berne, Center Quartier Bleu de Berne, Cantonal Hospital Lucerne, Children's Hospital of Eastern Switzerland, St. Gallen).

Medical charts were reviewed and measurements of forced expiratory volume in 1s (FEV1), shown as a percentage of the predicted value, were used to describe the lung function, where available.

2.2. Bacterial isolates

The 18 bacterial isolates were isolated from clinical respiratory samples of eight CF patients between 2003 and 2015 and were routinely identified using conventional biochemical methods or matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonik, Bremen, Germany), 16S rRNA gene analysis and *recA* gene analysis [6,7]. The isolates were frozen in skimmed milk (Difco Laboratories, Detroit, USA) at -80 °C at the Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland. Bacterial isolates were thawed and incubated on Columbia 5 % sheep blood agar (bioMérieux, Marcy l'Etoile, France) at 37 °C. After subculturing twice, isolates were analyzed for WGS and antimicrobial susceptibility testing.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disk diffusion method on Mueller-Hinton agar (Becton Dickinson, Franklin Lakes, NJ) and data were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines M100, 32th edition [8]. The following antibiotics were tested: meropenem, ceftazidime, trimethoprim-sulfamethoxazole, levofloxacin, and minocycline (i2a, Montpellier, France). *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality control strains.

2.4. Whole-genome sequencing analysis

DNA was extracted from isolates using the UltraClean Microbial Kit (QIAGEN, Germany) according to the manufacturer's instructions. Whole-genome sequencing was performed on extracted DNA from each isolate by Illumina MiSeq (Illumina®, San Diego, CA, USA), paired-end 150nt after QIAseq FX library preparation to a mean read depth of over 23x. All data was submitted to the ENA under project number PRJEB65335 under the accession numbers given in Table S1. Raw data were processed and trimmed by trimmomatic version 0.39 [9] and assembled into contigs using Unicycler v0.4.8 [10]. We determined the multi-locus sequence types from PubMLST (<http://pubmlst.org/bcc/>) [11]. We performed single nucleotide polymorphism (SNP) analysis in CLC Genomics Workbench v22.0.2 using the unicycler assembly of isolate BCC021, as one of the best assemblies, as a reference. Variants were called with 10x minimum coverage, 10 minimum count and 70 % minimum frequency. Gubbins v.3.2.1 [12] was used to detect recombinations in the resulting 7.5 Mb alignment, using default parameters and five iterations. BactDating v1.1.1 [13] was run on the recombinant corrected phylogeny using the arc model. The program was run three times using 1 million to 5 million MCMC iterations, giving highly similar results: the presented results are from 5 million iterations.

2.5. Ethical approval

This study was approved by the Cantonal Ethic committee of the Canton of Zurich, Switzerland (BASEC-Nr. 2023-00561). The study was carried out as per approved guidelines.

3. Results

3.1. Case descriptions and clinical parameters

The eight CF patients identified became chronically infected with *B. cenocepacia* between 1997 and 2006 (Table 1). Patient 1 acquired *B. cenocepacia* in 2003; the patient had a stable outcome (FEV1 78 %) with moderate infect exacerbations and survived. Patient 2 acquired Bcc in 1997. Lung function worsened in 2008 and the patient died in 2009 possibly due to cepacia syndrome. Patient 3 acquired *B. cenocepacia* in 2006. The patient developed a CF-related hepatopathy; in 2021 the modulator therapy (Trikafta) was installed, and the patient survived. Chronic infection with *B. cenocepacia* is still documented. Patient 4 acquired *B. cenocepacia* in 2003, and in 2014 he developed a severe infect exacerbation caused by *B. cenocepacia*. *B. cenocepacia* was present in blood cultures and the patient died 2014 possibly due to cepacia syndrome. Patient 5 acquired *B. cenocepacia* in 2005 and was hospitalized multiple times due to the *B. cenocepacia* infection. The patient died 2010. Patient 6 acquired *B. cenocepacia* in 2003 and developed chronic bronchitis in 2008. He was treated with ceftazidime. Lung function worsened 2011 (FEV1 26 %), the patient was hospitalized due to *B. cenocepacia* infection exacerbation. In 2012, the patient had a lung transplantation and died in 2013 due to septic shock. Patient 7 (sibling of patient 6) was first infected by *B. cenocepacia* in 2001; in 2003 the lung function worsened (FEV1 25 %). During 2004-2011, the patient had multiple hospitalizations due to *B. cenocepacia* infection. The patient received a bilateral lung transplantation in 2005, re-transplantation in 2006 and died in 2011. Patient 8 first was diagnosed with *B. cenocepacia* in 1998. In 2009 the lung function worsened (FEV1 32 %), in 2012 a lung transplantation was undertaken but *B. cenocepacia* was still detectable; the patient died in 2012. From 2009 on, he was treated with ceftazidime, in 2012 he received minocycline.

3.2. Isolates and antibiotic resistance profiles

B. cenocepacia isolates (n = 18) from eight patients were obtained (Table 2). Five patients had multiple isolates collected over up to nine years (Table 2). Isolates exhibited high levels of resistance towards meropenem (15/18, 83.3 %), minocycline (14/18, 77.8 %), trimethoprim-sulfamethoxazole (17/18, 94.4 %) and ceftazidime (12/18, 66.7 %) (Table 2). Isolates derived from the same patients over time displayed a progressive increase in resistance towards different antibiotics. Patient 6 developed resistance towards ceftazidime and patient 8 against ceftazidime and minocycline.

Table 1
Patient characteristics and clinical outcomes.

Patient	Year of acquisition	Age at first + culture	Sex	Pancreatic insufficiency	CF-related diabetes	outcome
1	2003	19	M	Yes	No	Alive
2	1997	9	M	Yes	No	Death
3	2006	20	M	Yes	No	Alive
4	2003	24	M	Yes	No	Death
5	2005	29	F	Yes	Yes	Death
6	2003	15	M	Yes	No	Death
7	2001	16	F	Yes	Yes	Death
8	1998	12	M	Yes	Yes	Death

CF, cystic fibrosis.

Table 2
Antibiotic resistance profile of 18 *B. cenocepacia* ST-250 isolates from eight CF patients.

Patient	Isolate	Culture date	Ceftazidime	Meropenem	Minocycline	Trimethoprim-sulfamethoxazole
1	BCC048	2004	R	R	R	R
2	BCC055	2006	S	S	R	S
3	BCC018	2006	R	R	R	R
3	BCC019	2015	R	R	R	R
4	BCC046	2003	I	R	R	R
5	BCC032	2007	S	R	R	R
5	BCC121	2007	R	R	R	R
6	BCC022	2004	S	R	R	R
6	BCC023	2012	R	R	R	R
6	BCC071	2012	R	R	R	R
7	BCC020	2003	R	R	R	R
7	BCC056	2004	R	R	S	R
7	BCC021	2005	R	I	I	R
8	BCC054	2004	S	R	I	R
8	BCC030	2007	I	S	R	R
8	BCC070	2012	R	R	I	R
8	BCC069	2012	R	R	R	R
8	BCC031	2012	R	R	R	R

Interpreted according to CLSI [8].

3.3. Genomic characterization of *B. cenocepacia* ST-250 isolates

High quality draft genomes were obtained from 18 *B. cenocepacia* isolates from eight CF patients (Table S1). All isolates were found to belong to *B. cenocepacia* ST-250. Phylogenetic analysis showed a moderate SNP diversity over the time period, suggesting a recent common ancestor (Fig. 1). Clustering of isolates from patients is apparent, and within-patient genomic variation was observed through sampling over up to nine years, and up to 14 years post infection. The genomic analysis supported both potential common source or patient-to-patient transmission occurring to cause the spread of the ST-250 among the CF cohort (Fig. 1). The isolates of patients 6 and 7 share a common ancestor in the phylogeny, suggesting transmission between siblings.

A dated phylogeny suggests a most likely root date, inferring the common ancestor, of 1985.43 [95 %CI:1975.76-1991.91] (Fig. 2) and estimating a mutation rate of 8.28 SNPs per genome per year [95 %CI:

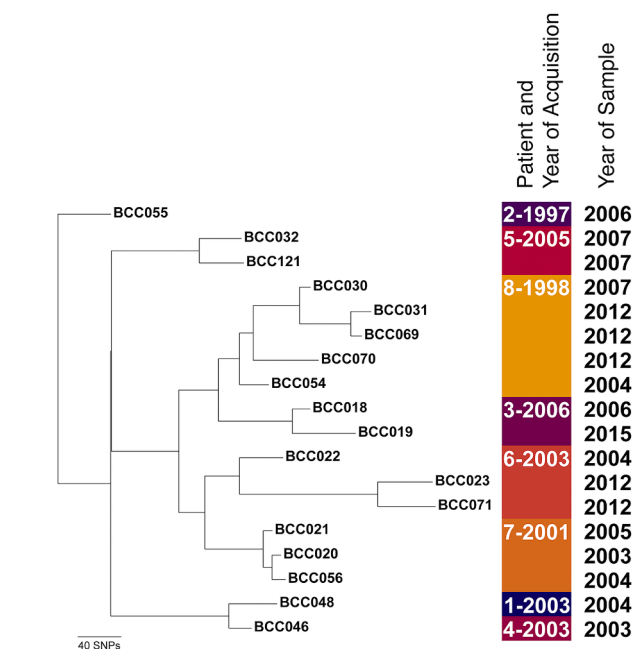


Fig. 1. Recombination-corrected phylogeny of *B. cenocepacia* ST-250 isolates. Genomes cluster by patient, displaying diversity within patients over time. Scale bar shows number of SNPs. The tree was rooted based on a *B. cenocepacia* ST-208 outgroup.

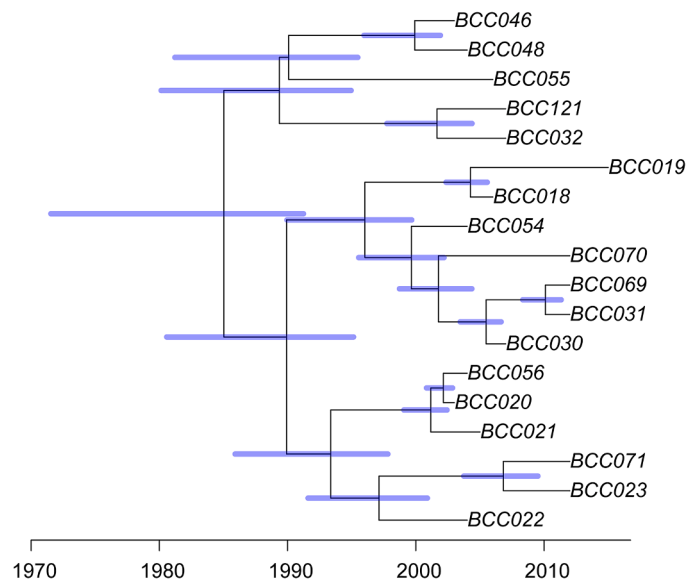


Fig. 2. Dated phylogeny of the outbreak. The blue bars show 95 %CI of each node against the time scale.

5.26-11.5].

4. Discussion

Within the species *B. cenocepacia*, epidemic lineages with a higher potential to transmission and pathogenic potential are known, e.g., the epidemic lineage E12 which spread intercontinentally [3]. This is the first report describing genomic data of *B. cenocepacia* ST-250, according to literature and PubMLST. Detailed information regarding this specific strain is scarce. Lupo *et al.* described four isolates of *B. cenocepacia* ST-250, which were isolated between 1998 and 2007 in CF patients in Switzerland [14]. Due to the overlapping timeframe, these could have been part of the same outbreak as the isolates described in our study. In the PubMLST database, an additional six isolates of *B. cenocepacia* ST-250 are deposited: five originating from CF patients in the USA (2000), Canada (2015) and Switzerland (2007); and one industrial isolate (<http://pubmlst.org/bcc/>). *B. cenocepacia* ST-250 belongs to the former taxonomic subgroup of *B. cenocepacia* IIIA, which comprises multiple epidemic clones [3]. To date, *B. cenocepacia* ST-250 has not been described as an epidemic strain, but considering its potential

transmissibility and pathogenicity as we describe herein an infection with this ST must be treated with caution.

Spread of epidemic *B. cenocepacia* strains are caused by multiple potential routes. Common sources resulting from contaminated industrial products [15], nosocomial patient-to-patient transmission during hospitalization [4] or patient-to-patient transmission by direct contact account for transmission of *Burkholderia* [16]. It is known from the literature that social contacts in CF summer camps led to intercontinental spread of epidemic strains of *B. cenocepacia* in CF patients [16, 17]. In the study conducted by Govan *et al.* in Edinburgh and Manchester between 1986 and 1992, it was shown that social contacts outside the hospital, e.g., attendance of fitness class, camps and other social events, were strongly implicated in transmission of epidemic strains [17]. In our study, most of the patients acquired Bcc in the years 2001-2006, two of the patients acquired Bcc in 1997 and 1998, respectively. Three out of eight patients were known to have attended CF summer camps that time. The genomic data of the 18 *B. cenocepacia* ST-250 isolates from the eight CF patients in Switzerland demonstrated a high relatedness and strongly supports the potential for transmission between patients. For at least three of the infected CF cohort, it is possible that *B. cenocepacia* was acquired through person-to-person transmission during the summer camps. In Switzerland, CF children tested positive with *B. cenocepacia* by culture methods were excluded from participation in summer camps, however, false-negative testing results may happen. After transmission events of *B. cenocepacia* in summer camps, Swiss pneumologists recommended to disband such camps in Switzerland after 2003. This was in line with the Canadian recommendations where CF summer camps were disbanded since 1998 [18].

Two patients were siblings, in this case it is possible that an intra-family transmission occurred. Besides social or family contacts, spread of epidemic strains of *B. cenocepacia* has also been described in a hospital setting. Blanchard *et al.* have shown nosocomial transmission of the epidemic *B. cenocepacia* strain ET12 in adults with cystic fibrosis [4]. Because of limited data, we were not able to investigate whether the patients of our study were hospitalized at the same time in the same unit, which would have explained a possible transmission.

Phylogenetic analyses showed closely related isolates and suggests a recent common ancestor of this lineage in 1985. Substantial within-host evolution is apparent over the sampling timescale of up to nine years per patient. The estimated mutation rate of 8.28 SNPs per year is high compared to previously estimated within-host rates of 2.08 of *B. cenocepacia* in a *B. multivorans* co-infected CF patient [19]. The high mutation rate may be a result of the antibiotic pressure over several years and adaptation within the CF lung. Given the patient histories, specific patient-to-patient transmission cannot be inferred, but shared sources are highly likely, particularly between the sibling pair in the study. It is not known, where the source of this outbreak originated. We can speculate that the common ancestor derived from an environmental reservoir, as it was described by Lood *et al.* of an endemic *B. multivorans* strain infecting multiple CF patients, however, without evidence for cross-infection [20]. Although an industrial *B. cenocepacia* ST-250 strain was described in the literature, we have no indications of transmission by contaminated industrial products as this was described for a hospital outbreak caused by *Burkholderia stabilis* contaminated gloves in Switzerland [21,22]. There is no genomic data of environmental or product-associated strains of *B. cenocepacia* ST-250 in Switzerland, which could be source for transmission.

Most of the *B. cenocepacia* ST-250 isolates in our study showed high resistance to the tested antibiotics. The acquisition of new resistances over time within one patient was visible for ceftazidime (patients 6 and 8) and minocycline (patient 8). Both patients were exposed to ceftazidime therapy, patient 8 received additionally minocycline. This is in accordance with the resistance profile of the *B. cenocepacia* ST-250 isolates described by Lupo *et al.* [14]. It is known that Bcc have an innate resistance to multiple antibiotics, e.g., aminoglycosides, which challenges the treatment [23]. We were not able to link the mutations in

the genomes of the *B. cenocepacia* ST-250 strains with corresponding phenotypic resistance profiles or patient clinical outcomes. Clinical breakpoints in Bcc are difficult to ascribe because of limited reproducibility of antibiotic susceptibility testing of Bcc strains [24].

The clinical outcome of the eight patients infected with *B. cenocepacia* ST-250 varied widely: two of the patients are still alive whereas six patients died within 5-14 years after *B. cenocepacia* infection. One of the surviving patients received modulator therapy which might have also a positive effect on the *B. cenocepacia* colonization despite the pathogenic potential of *B. cenocepacia* ST-250. Studies investigating the microbiome of the respiratory tract of CF patients under modulator therapy have the potential to answer this important question [25]. Three patients underwent lung transplantation. In one patient *B. cenocepacia* was still detected after transplantation. This shows the high persistence of *B. cenocepacia* ST-250 and difficulty of eradication despite aggressive therapy. There is evidence of strain relatedness based on genomic analyses and given the pathogenicity of the *B. cenocepacia* ST-250 strain, it can be considered an epidemic lineage for the people with CF in Switzerland.

Taken together, we can speculate about the most possible transmission route in our study. As three of the patients were known having participated in summer camps, which resulted in new-onset of *B. cenocepacia* infection in two patients, it is highly suggestive that there was person-to-person transmission at this location. A limitation is that we have only a limited number of isolates of each patient and it was not possible to access the primary *B. cenocepacia* isolate of each patient. Nevertheless, for most patients, we have an isolate from the first two years since the onset of the *B. cenocepacia* infection, and the phylogeny provides an inference of the primary isolate. In patients with multiple isolates, we observed an intrapatient *B. cenocepacia* evolution. Another limitation is that we could not reconstruct the wider outbreak, with data from the eight patients. Therefore the inference of direct transmissions must be treated with caution.

Our study shows the advantages of the application of WGS technology in elucidating isolate relatedness on a genomic level. The identification of a genomic cluster of the *B. cenocepacia* ST-250 isolates in this CF cohort and the epidemiological investigations of probable social contacts, e.g., attendance of summer camps suggest transmission by direct patient-to-patient contact. The majority of the patients in our study had a fatal outcome due to *B. cenocepacia* ST-250 infection, thus early recognition of colonization and prevention of transmission is critical for CF patients. Although modulator therapy has revolutionized the management of CF patients, our study shows the importance of infection control measures to prevent the transmission of *B. cenocepacia* in CF patients, in particular in patients not responding or eligible for modulator therapy.

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CRediT authorship contribution statement

Andrea Zbinden: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Helena M.B. Seth-Smith:** Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Investigation, Formal analysis. **Vanessa Beltrami:** Resources, Data curation. **Stefano Mancini:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Sara Droz:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Urs Bürgi:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **David Melillo:** Formal analysis, Data curation. **Mace M. Schuurmans:** Writing – original draft, Writing – review & editing, Data curation, Formal analysis.

Bernhard Schwizer: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Iris Schmid:** Writing – original draft, Data curation. **Carmen Casaulta:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Jürg Barben:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Nicolas J Mueller:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Frank Imkamp:** Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.diagmicrobio.2024.116429](https://doi.org/10.1016/j.diagmicrobio.2024.116429).

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