Sequential burkholderia cenocepacia isolates from siblings with cystic fibrosis show increased lung cell attachment

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Sequential *Burkholderia cenocepacia* Isolates from Siblings with Cystic Fibrosis Show Increased Lung Cell Attachment

*To the Editor:*

*Burkholderia cepacia* complex (Bcc) is a group of 20 genetically distinct bacterial species (1) that has a severe impact on the quality of life of people with cystic fibrosis (CF) and is associated with a more rapid decline of lung function than *Pseudomonas aeruginosa* (2). *B. cenocepacia* is the most virulent species within the Bcc and is most frequently associated with septicemia, although other Bcc species have also been linked to bloodstream infections (BSI) (2, 3).

Many pathogens alter their phenotype during chronic infection in response to changing selection pressures, coinfecting species, and antimicrobial therapies (4, 5). Studies on bacterial adaptation in the CF context have predominantly focused on *P. aeruginosa*; however, the adaptive strategies of *B. cenocepacia* isolates have also been examined (4, 6, 7). Antimicrobial resistance, loss of motility, tolerance of iron limitation, and increased virulence to host cells over time of chronic infection were reported. In contrast, *P. aeruginosa* and another member of the Bcc, *B. multivorans*, showed reduced virulence over time of infection (5, 8). We have examined two series of sequential isolates from two adult male siblings with CF (referred to as P1 and P2). Both patients became infected with Bcc during their teens and were chronically infected when transitioning to adult care.

**Patient 1**

P1 has chronic *B. cenocepacia* infection confined to the lung and experienced reduced FEV1 values over 5 years, indicative of disease progression (Figures 1A and 1B). P1 was diagnosed with CF lung disease, pancreatic insufficiency, osteopenia, distal intestinal obstruction syndrome, and CF-related liver disease in childhood. Bcc was always grown in combination with *Candida* species from his sputum specimens. After transferring from the pediatric center, initial sputum specimens of P1 were positive with Bcc, antibiotic treatment was altered and the source of infection, port-a-cath (second BSI) or central venous catheter (final BSI), was removed. All sputum specimens taken over the 3-year period in P2 yielded a heavy Bcc growth in combination with *Candida* species. *S. aureus* and *P. aeruginosa* were only grown intermittently from sputum. Low FEV1 values (16–27%) were observed (Figure 1B), indicative of late-stage obstructive disease. He died of respiratory failure 3 years after his first *B. cenocepacia* blood culture isolation.

These sequential isolates provide a rare opportunity to examine the alterations that *B. cenocepacia* undergoes during the course of chronic lung infection and recurrent bacteremia. Five sputum isolates spanning 90 months were randomly selected from all P1 isolates designated P1S1 to P1S5. The first and third blood peripheral isolates (P2B1 and P2B3) together with three sputum isolates (P2S1 to P2S3) were selected from P2 isolates for investigation. The isolates were shown to be clonal by pulsed-field gel electrophoresis. Multilocus sequence typing analysis of the *B. cenocepacia* isolates was compared with the *Burkholderia cepacia* complex Multi Locus Sequence Typing website (http://pubmlst.org/bcc/), developed by Keith Jolley and sited at the University of Oxford (9). The isolates from both individuals shared the same unique sequence type on the basis of a new *trpB* allele 405 and were designated ST867. This novel sequence type clusters with *B. cenocepacia*, recA group IIIA.

We investigated whether the sequential isolates from both siblings differed in their ability to colonize lung epithelia by examining attachment to CF epithelial cells, CFBE410− (multiplicity of infection, 50:1), as previously described (10). The sputum isolates from P1 had an increased ability to adhere to CFBE410− cells over time of colonization (*P* = 0.0097), showing increased potential for host epithelial attachment (Figure 2A). P1S5 was the most adherent of all isolates examined. Although isolates from P2 were more than twofold less adherent than isolates from P1 (*P* = 0.003), the P2 sequential isolates also showed more attachment to CFBE410− cells with increasing time of colonization (*P* = 0.0012), regardless of origin of the isolate. The P2 isolates examined showed comparable epithelial cell attachment to positive controls, *B. cenocepacia* BC7 and K56-2, which are both *recA* group IIIA strains.

The increased host cell attachment of a selection of both siblings’ isolates over time of colonization was confirmed by confocal microscopy (Figure 2B). When bacteria were counted (10 fields per sample), attachment increased from 2.75 ± 0.07 (mean ± SD) to 27.8 ± 23.8 bacteria/100 host cells in the case of P1S1 and P1S4 isolates, respectively, and from 2.29 ± 0.08 to 8.79 ± 1.4 bacteria/100 host cells for P2B1 and P2B3 isolates, respectively (*P* < 0.01).

This is the first demonstration of an increased ability of sequential *B. cenocepacia* isolates to adhere to CF epithelial cells over time of infection. The increased attachment of blood isolates to epithelial cells over time is likely to be a consequence of bloodstream isolates being previously adapted lung isolates, and the phenotype being maintained during bloodstream infection. The increased epithelial attachment of *B. cenocepacia* ST867 intravenous antibiotic treatment. Once blood cultures became positive with Bcc, antibiotic treatment was altered and the source of infection, port-a-cath (second BSI) or central venous catheter (final BSI), was removed. All sputum specimens taken over the 3-year period in P2 yielded a heavy Bcc growth in combination with *Candida* species. *S. aureus* and *P. aeruginosa* were only grown intermittently from sputum. Low FEV1 values (16–27%) were observed (Figure 1B), indicative of late-stage obstructive disease. He died of respiratory failure 3 years after his first *B. cenocepacia* blood culture isolation.

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A detailed examination of the time-dependent alterations across both patients’ isolates leading to this increased attachment may provide a mechanism by which B. cenocepacia chronically persists, and more studies are required to determine whether enhanced attachment is common among larger cohorts of Bcc patient isolates. The mechanisms behind the phenotypic differences in bacterial isolates at a given time point can be complex, but understanding the key adaptations that differentiate Bcc blood and sputum isolate types should contribute to the prevention of potentially life-threatening BSI in these patients. Overall, the increased ability to attach to host cells is likely to

in both sets of isolates suggest that this may be a general strategy by which these isolates avoid clearance by the host and may contribute to the challenges of eradication of B. cenocepacia once chronic infection is established. Bcc can switch from a mucoid to nonmucoid phenotype, which correlates with a more rapid decline in FEV₁ (11); however, these isolates were predominantly nonmucoid (unpublished). Increased virulence and alterations in proteomic profile have previously been shown in sequential B. cenocepacia isolates (7). It is likely that these isolates alter their surface protein expression to improve host cell attachment.

A detailed examination of the time-dependent alterations across both patients’ isolates leading to this increased attachment may provide a mechanism by which B. cenocepacia chronically persists, and more studies are required to determine whether enhanced attachment is common among larger cohorts of Bcc patient isolates. The mechanisms behind the phenotypic differences in bacterial isolates at a given time point can be complex, but understanding the key adaptations that differentiate Bcc blood and sputum isolate types should contribute to the prevention of potentially life-threatening BSI in these patients. Overall, the increased ability to attach to host cells is likely to
conferring a bacterial survival advantage during infection, contributing to bacterial survival as a whole during chronic infection in CF.
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References


