REVIEW ARTICLE

Burkholderia cepacia complex infection in patients with cystic fibrosis

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The word ‘complex’ has several meanings and synonyms such as composite, obsession, heterogeneous, mixed and network, can all be used in its place. Our obsession with bacteria from the Burkholderia cepacia complex started in the early 1990s. In less than 10 years, we have seen the status of this bacterium move from: (i) a lesser known pseudomonad opportunist pathogen, (ii) to devastating infections transmitted between patients with cystic fibrosis (CF), (iii) through divisions into several new species, and (iv) now on towards one of the largest gram-negative genome sequencing projects. For microbiologists, hospital infection control officers, caregivers, and most of all the CF community, the changes in our understanding of the taxonomy, epidemiology and pathogenesis of the bacterium ‘B. cepacia’ are complex.

The complex

In 1992, the species Pseudomonas cepacia was reclassified as Burkholderia cepacia and it was assigned as the type species for the new genus Burkholderia [1]. Several new species were added to the genus in the following 5 years but it was not until 1997 that isolates classified as ‘B. cepacia’ were re-examined by polyphasic taxonomic approaches. Bacteria biochemically identified as B. cepacia were found to consist of at least five genetically distinct species or genomovars [2]. In hindsight, it was ironic that the species B. cepacia was designated as a reference for the genus [1] when its own taxonomy was still very uncertain. Further work has identified at least nine genomovars which constitute the B. cepacia complex; each of these is listed in Fig. 1. The taxonomy and identification of the B. cepacia complex have recently been reviewed in detail elsewhere [3] and the authors provided a summary of both biochemical and genetic means of genomovar identification. Six of the published genomovars have been assigned the following species names [3]: B. cepacia (the original specific epithet is preserved for genomovar I), B. multivorans (formerly genomovar II), B. vietnamiensis (formerly genomovar V), B. stabilis (formerly genomovar IV) and B. ambifaria (formerly genomovar VII). B. cepacia genomovars III and VI await full species names if simple differential tests can be found [3]. The description of genomovars VIII and IX is currently underway (P. Vandamme and E. Mahenthiralingam, unpublished data) (Fig. 1). The name ‘B. anthina’ has been proposed for strains of genomovar VIII, as a number of different tests enabling the identification of this species have been found (P. Vandamme and E. Mahenthiralingam, unpublished data). The reference strain for genomovar IX, LMG 14191, had already been formerly named as the species B. pyrrocinia [2]. It is now clear from polyphasic taxonomic approaches that this strain and other closely related isolates constitute a distinct genomovar within the current B. cepacia complex (Fig. 1) (P. Vandamme and E. Mahenthiralingam, unpublished data). To assist researchers and microbiologists studying B. cepacia complex bacteria, a panel of strains representative of the first five genomovars was published 2 years ago [4]. With the identification of four further genomovars this useful strain panel is already in need of updating and expansion.

Composite genomovar tests and identification of genomovars based on the recA gene

The B. cepacia complex genomovars are very closely related, with few if any biochemical reactions able to separate them and multiple tests often required for accurate identification [3]. While seeking rapid molecular tests for identification of B. cepacia complex bacteria, we discovered that there was sufficient
variation in nucleotide sequence of the recA gene to enable discrimination of the first five genomovars described [5]. We have subsequently found that analysis of recA sequence variation, in general, correlates well with the genomovar taxonomy. Restriction fragment length polymorphism (RFLP) analysis of the recA gene can serve as a primary means of identifying taxonomic diversity among isolates [5]. 50 B. cepacia complex RFLP types have now been found when the gene is cut with the restriction enzyme HaeIII (E. Mahenthiralingam and P. Vandamme, unpublished data). Novel recA gene RFLP types that do not correlate with a known genomovar can then be subjected to nucleotide sequence analysis to enable phylogenetic predication of genomovar status [3, 5].

Phylogenetic analysis of recA gene sequences from strains representative of each genomovar is indicated in Fig. 1. All nine B. cepacia complex genomovars separate into distinct arms of the phylogenetic tree. Interestingly, sequence variation in the recA gene also appears to separate strains of genomovar III into two distinct clusters: III-A and III-B (Fig. 1); the prevalence of each lineage of genomovar III varies between different CF populations (see below). A similar subdivision of B. cepacia genomovar I strains (defined by polyphasic taxonomic analysis) into two distinct recA phylogenetic clusters has also been observed (P. Vandamme and E. Mahenthiralingam, unpublished data).

The PCR primers, BCR1 and BCR2, originally designed to amplify the full length recA gene remain highly specific for B. cepacia complex bacteria and do not cross-react with closely related Burkholderia spp. or other common CF pathogens such as P. aeruginosa [5]. This specificity has led to the successful application of recA PCR directly to CF sputum [6]. Such direct testing for suspected B. cepacia complex infection may prove very useful in hospital infection control and the clinical management of CF patients. Other genomovar-specific recA-based PCR tests [5] appear to be less specific in light of the identification of further taxonomic diversity. For example, the PCR

![Phylogenetic tree of recA gene sequences](image-url)

Fig. 1. A phylogenetic tree of recA gene sequences indicating the presence of nine B. cepacia complex genomovars. Nucleotide sequences were determined, aligned and used to construct the neighbour joining tree as described previously [5]. The strains representative of each genomovar are indicated and the GenBank accession no. for published and novel recA gene sequences is provided in brackets. Sequence data for the B. pseudomallei strain K6243 and B. cepacia genomovar III strain J2315 recA genes were generated by the Pathogen Sequencing Unit at the Sanger Institute, and can be obtained from http://www.sanger.ac.uk/Projects/Microbes. The genomovar or species name for each distinct cluster is indicated on the right. Genetic distance and bootstrap values >70% for each node are shown.
primers designed to be specific for B. cepacia geno-
movar I [5] cross-react with B. pyrrocinia and fail to
detect some genovar I isolates which fall into the
second recA genovar I cluster mentioned above (E. Mahenthiralingam, unpublished data). Hence, even
with molecular genovar identification, multiple tests
(RFLP, sequence analysis and genovar-specific PCR)
may be required, with no single diagnostic approach
possessing absolute specificity.

Genovar prevalence in CF infection

From the initial description of five genovars within
the B. cepacia complex, it was clear that strains from
each genovar may cause infection in patients with
CF [2]. This basic pathogenic trait has continued to be
true for all further genovars described and strains
from CF infection may be found in each evolutionary
arm of the diverse B. cepacia complex phylogenetic
tree (Fig. 1). (E. Mahenthiralingam and P. Vandamme,
unpublished data). Because of the development of rapid
and widely applicable genovar identification tests,
national systematic analysis of the prevalence of each
B. cepacia complex species has now been examined in
the USA [7] and Canada [8]. A smaller study
examining the prevalence and epidemiology of B. cepacia complex bacteria among CF patients attending
treatment centres in Italy has also been published
[9]. A summary of the findings of the latter studies is
presented in Table 1 [7–9]. Studies based on the
examination of collections of B. cepacia complex isolates had indicated that genovar III and B. multivorans
were the predominant CF pathogens [2, 5]. These findings have been validated by the
systematic studies of prevalence of CF (Table 1).

B. cepacia genovar III is the most prevalent
genovar in CF, causing >50% of CF infections in
all CF populations examined (Table 1). Interestingly, if
strains of genovar III are divided into their separate
recA lineages of III-A and III-B (see Fig. 1), distinct
differences between the USA, Canadian and Italian CF
cultures can be observed. Within the USA, strains of B. cepacia genovar III-B represent 75% of
genovar III infections [7], whereas in Canada and
the four Italian CF centres examined, III-A strains were
dominant, accounting for >70% of genovar III
infections in each population (Table 1) [8, 9]. The basis
for these distinct differences is unknown as yet.

B. multivorans is the second most predominant CF
pathogen after B. cepacia genovar III (Table 1).
Once again though, there were differences between the
three CF populations examined. In the USA, B. multivorans CF infection occurs almost as widely as
genovar III infection (38% of cases) [7]. In Canada
and Italy, B. multivorans caused <10% of B. cepacia
complex infections (Table 1) [8, 9]. The prevalence of
all the remaining B. cepacia genovars in CF was at
most 5% in the populations examined (Table 1). Hence,
taxonomic classification of predominant CF species
appears nearly complete, with B. cepacia genovar III and B. multivorans accounting for 95% of the
infections (Table 1). So, which species are the most
problematic from a clinical standpoint?

Molecular epidemiology

Spread of respiratory infections in patients with CF,
although a controversial area of research, had not been
a significant clinical problem until the early 1990s.
Reports in the UK and USA indicated that P. cepacia
as it was known then, was capable of nosocomial
transmission [10, 11]. In addition to risk of spread,
infection was also linked to a rapid decline in clinical
condition in certain CF patients, which became known
as ‘B. cepacia’ syndrome. These hazards resulted in
the cohorting of CF patients colonised with B. cepacia
in many treatment centres.

During the early 1990s concern about the increasing
incidence of B. cepacia-infected patients at a treatment
centre in Vancouver, Canada, led to the application of
random amplified polymorphic DNA (RAPD) finger-
printuing to the epidemiological analysis of ‘B. cepacia’
infection [12]. Several B. cepacia strain types were
each found to infect multiple patients [12]. Moreover,
epidemiological observations suggested that transmis-
sion had been due to direct patient-to-patient contact
both within and outside the hospital setting, as had
been observed in earlier studies [9]. One of the strains
encountered in the Vancouver CF patient population
was designated as RAPD strain type 2 [12]. This strain
type was a member of a clonal lineage of strains which
first infected CF patients in Toronto, subsequently
spread across Canada and was also introduced into the
UK CF population, probably as a result of patient
contact during CF summer camps [13]. This highly
infectious strain had at the time been recently identified

Table I. The prevalence of B. cepacia complex geno-
movars among three CF populations

<table>
<thead>
<tr>
<th>Genovar or species</th>
<th>USA</th>
<th>Canada</th>
<th>Italy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(606 patients)</td>
<td>(475 patients)</td>
<td>(59 patients)</td>
</tr>
<tr>
<td>Genovar I</td>
<td>2.6</td>
<td>0.2</td>
<td>4.8</td>
</tr>
<tr>
<td>B. multivorans</td>
<td>37.8</td>
<td>9.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Genovar III</td>
<td>50.0</td>
<td>80.0</td>
<td>72.6</td>
</tr>
<tr>
<td>B. stabilis</td>
<td>8.2</td>
<td>3.8</td>
<td>3.2</td>
</tr>
<tr>
<td>B. vietnamiensis</td>
<td>5.1</td>
<td>1.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Genovor VI</td>
<td>2.0</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>Genovor VII</td>
<td>0.7</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>1.6</td>
<td>1.8</td>
<td>14.5</td>
</tr>
</tbody>
</table>

7 Data from LiPuma et al. (2001) [7].
8 Data from Speert et al. (2002) [8].
9 Data adapted from Agodi et al. (2001) [9] and representative of 59 patients attending four CF treatment centres.
and was known as the ET12 (electrophoretic type 12) [14] or cable pilus-encoding strain [15].

Our success with the RAPD typing technique was not just limited to accurate molecular epidemiological analysis. We were also able to identify a very useful DNA marker, the _B. cepacia_ epidemic strain marker (BCESM) [15]. The BCESM DNA was associated with _B. cepacia_ strains that were known to have spread among CF patients in Vancouver and other parts of Canada [15]. Hence it could be used as a rapid diagnostic marker to alert CF patients and clinicians to the potential risks associated with infection by these strains. By examining the genomovar status of BCESM-positive isolates, we now understand that this marker is found exclusively in strains of _B. cepacia_ genomovar III [5], but is not carried by all strains. Among Canadian CF patients, BCESM-positive strains account for >80% of all genomovar III infections [8]. Several of these strain types, not just the dominant ET12 strain, were capable of epidemic spread among CF patients [5, 12]. Within the USA, the picture in terms of incidence of BCESM-encoding strains is very different, with positive strains accounting for only 23% of all _B. cepacia_ genomovar III strains encountered [7].

The BCESM region of the genome is unstable, particularly in strains of genomovar III-B, and can be lost after passage in vitro (E. Mahenthiralingam, unpublished data). In the USA, genomovar III-B strains most frequently lacked the marker (64% negative) and were the predominant genomovar III strain lineage encountered (Table 1) [7]. The BCESM DNA is much more stable in _B. cepacia_ genomovar III-A strains [5], suggesting that they may be the ‘natural’ hosts for this unusual genomic DNA element. The instability of the BCESM element, its size and the potential virulence genes encoded within this region are currently under investigation (E. Mahenthiralingam and A. Baldwin, unpublished data). Overall, while it is clear that BCESM DNA is not an absolute marker of the ability to cause infection or spread among CF patients [7], in CF populations where BCESM-positive strains dominate, they have proved to be highly virulent and very problematic [16; see below].

Although _B. cepacia_ genomovar III strains have been implicated in the majority of the published accounts of patient-to-patient spread in CF [4], the ability of all the other genomovars to cause outbreaks of infection cannot be ignored. A common phenotypic trait of all _B. cepacia_ complex bacteria is their intrinsic resistance to multiple antibiotics. _B. cepacia_ complex bacteria appear to share the same ability for nosocomial spread as other drug-resistant bacterial infections such as methicillin-resistant _Staphylococcus aureus_ and vancomycin-resistant enterococci. Outbreaks of _B. multivorans_ infection, affecting large numbers of patients, have been reported in the UK [17] and France [18]. Current UK infection control guidelines recommend individual segregation of CF patients infected with _B. cepacia_ complex bacteria; because of the mixed epidemiology which has been observed with _B. cepacia_ complex bacteria, these strict measures are the best current means of preventing strain transmission.

Clinical outcome in relation to genomovar

Few studies have systematically examined clinical outcome in relation to _B. cepacia_ complex genomovar. Strains isolated from CF patients attending the Vancouver treatment centres had been collected since 1981 and this enabled a retrospective examination of epidemiology and clinical outcome to be determined for this CF patient population [16]). Initial infection control procedures implemented at the CF clinics involved cohorting of all ‘_B. cepacia_’-positive patients, and their separation from _P. aeruginosa_-positive and non-colonised CF patients. The _B. cepacia_ genomovar status of the Vancouver CF population was heterogeneous. Strains of genomovar III-A (four different strain types, including ET12) accounted for the majority of cases of infection (46 of 52 patients). Distinct _B. multivorans_ strains were isolated from 19 of 62 patients, and the remaining three patients were infected with _B. stabilis_, _B. vietnamiensis_ and an indeterminate _B. cepacia_ complex isolate, respectively [16].

Epidemiology and clinical outcome associated with _B. cepacia_ genomovar III and _B. multivorans_ infection were strikingly different [16]). Patient-to-patient spread of four different genomovar III-A strains (each encoding the BCESM) had occurred until the introduction of _B. cepacia_ complex-infected patients in 1995. No spread of _B. multivorans_ strains (apart from transient strain-sharing between two CF siblings) was observed during the entire 17 years of study. Genomovar III infections were more likely to be chronic, whereas the majority of cases of _B. multivorans_ infection were transient. Patients with genomovar III infection suffered the greatest mortality (20 of 46 patients died), but only three of the 19 _B. multivorans_-infected patients died (notably two of these were co-colonised with genomovar III at the time of death). From an infection control stance, the most worrying feature of _B. cepacia_ genomovar III infection was the ability of these strains to replace infection with _B. multivorans_, which occurred in six cases of infection. However, with the introduction of segregation of _B. cepacia_ complex-infected patients as a result of the strain-typing observations, spread of infection and the incidence of _B. cepacia_ genomovar III infection were significantly reduced after 1995 [16].

Is the Vancouver experience representative of what may happen with _B. cepacia_ complex infection in patients with CF? Experience gained from lung transplantation
of *B. cepacia* complex-infected CF patients also shows that infection with genomovar III is associated with significant postoperative mortality, whereas infection by other genomovars is less problematic [19, 20]. These findings correlate with the greater mortality linked to *B. cepacia* genomovar III infection in Vancouver [16]. However, the absence of spread and virulence of *B. multivorans* is not shown by other studies [17, 18]. Significant transmission was observed during an outbreak of infection with a *B. multivorans* strain among Glasgow CF patients [17]. Fatal *B. multivorans* septicaemias and multiple patients infected with single strains have been observed in French CF patients [18]. Phylogenetic comparison of the Glasgow *B. multivorans* strain (represented by strain C1576) indicates that it is from a different strain lineage when compared with the soil-isolated reference strain ATCC 17616, which is representative of the same recA lineage of *B. multivorans* strains encountered in Vancouver (see Fig. 1). Further study may uncover whether there are discrete epidemiological differences among *B. multivorans* strains that are similar to those observed for BCESM-positive *B. cepacia* genomovar III strains. Overall, although infection with *B. cepacia* genomovar III bacteria represents a significant clinical risk to patients with CF [16], *B. multivorans* and all the remaining genomovars are capable of causing devastating infections within any given patient.

**References**


