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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 epiphet 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.

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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

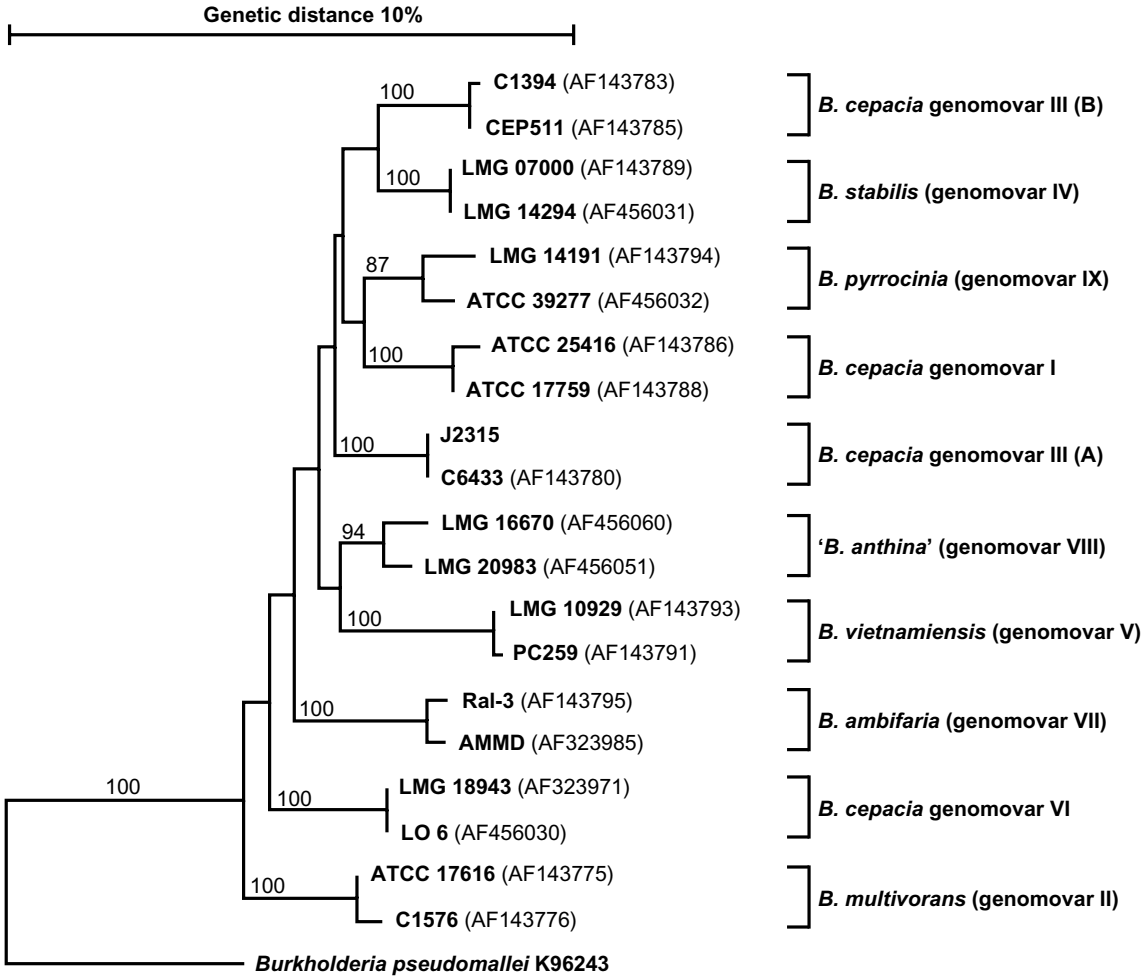

Fig. 1. A phylogenetic tree of *recA* gene sequences indicating the presence of nine *B. cepacia* complex genomovars. Nucleotide sequences were determined, aligned and used to construct the neighbour joining tree as described previously [5]. The strains representative of each genomovar are indicated and the GenBank accession no. for published and novel *recA* gene sequences is provided in brackets. Sequence data for the *B. pseudomallei* strain K96243 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.sangr.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.

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] and >50 *B. cepacia* complex RFLP types have now been found when the gene is cut with the restriction enzyme *Hae*III (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 all nine current *B. cepacia* complex genomovars is shown 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

primers designed to be specific for *B. cepacia* genomovar I [5] cross-react with *B. pyrrocinia* and fail to detect some genomovar I isolates which fall into the second *recA* genomovar I cluster mentioned above (E. Mahenthiralingam, unpublished data). Hence, even with molecular genomovar identification, multiple tests (RFLP, sequence analysis and genomovar-specific PCR) may be required, with no single diagnostic approach possessing absolute specificity.

Genomovar prevalence in CF infection

From the initial description of five genomovars within the *B. cepacia* complex, it was clear that strains from each genomovar may cause infection in patients with CF [2]. This basic pathogenic trait has continued to be true for all further genomovars 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 genomovar 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 four 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 genomovar III and *B. multivorans* were the predominant CF pathogens [2, 5]. These findings have been validated by the systematic studies of prevalence in CF (Table 1).

B. cepacia genomovar III is the most prevalent genomovar in CF, causing >50% of CF infections in all CF populations examined (Table 1). Interestingly, if strains of genomovar 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 populations can be observed. Within the USA, strains of *B. cepacia* genomovar III-B represent 75% of genomovar III infections [7], whereas in Canada and the four Italian CF centres examined, III-A strains were dominant, accounting for >70% of genomovar 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* genomovar 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 genomovar 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* genomovars 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* genomovar III and *B. multivorans* accounting for 95% of the infections (Table 1). So, which species are the most problematic from a clinical standpoint?

Table 1. The prevalence of *B. cepacia* complex genomovars among three CF populations

Genomovar or species	Percentage prevalence			Mean
	USA* (606 patients)	Canada† (475 patients)	Italy‡ (59 patients)	
Genomovar I	2.6	0.2	4.8	2.5
B. multivorans	37.8	9.3	4.8	17.3
Genomovar III	50.0	80.0	72.6	67.5
B. stabilis	0.2	3.8	3.2	2.4
B. vietnamiensis	5.1	1.6	0	2.2
Genomovar VI	2.0	0	0	0.7
Genomovar VII	0.7	0	0	0.2
Indeterminate	1.6	1.8	14.5	5.9

*Data from LiPuma *et al*. (2001) [7].
†Data from Speert *et al*. (2002) [8].
‡Data adapted from Agodi *et al*. (2001) [9] and representative of 59 patients attending four CF treatment centres.

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) fingerprinting 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 transmission 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

and was known as the ET12 (electrophoretic type 12) [14] or cable pilus-encoding strain [13].

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 new infection control procedures (individual segregation 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. mutivorans* 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.

B. cepacia complex virulence

Virulence in CF, perhaps more than any other area of *B. cepacia* biology, is still open to question and further research. What are the specific bacterial factors that lead to pathogenesis? Why do some patients succumb rapidly to infection while others remain healthy, despite being infected with the same strain of *B. cepacia*? Why does the outcome and prevalence of infection vary with *B. cepacia* genomovar and CF patient population? *B. cepacia* complex bacteria possess many of the bacterial factors known to play a role in *P. aeruginosa* CF infection [21]. Many of the answers to virulence lie within the fact that all *B. cepacia* complex bacteria have very large and unusual genomes [5, 22]. Average genome size is *c*. 8 Mb and the DNA is carried on at least two, or frequently three or four large replicons [5, 22]. Hence the potential for strain-to-strain variation in genomic content is enormous and perhaps contributes to the heterogeneity in virulence and pathogenesis. The *B. cepacia* genomovar III strain, J2315 (LMG16656), was recently chosen as the subject of genome sequence analysis (http://www.sanger.ac.uk/Projects/B_cepacia/). Strain J2315 was the index strain associated with spread of the ET12 cable pilus-positive strain among CF patients in Edinburgh [10]. The genomovar III-A CF strain lineage it represents (Fig. 1) is unique in being the only strain to date that possesses both the cable pilus gene and BCESM [5]. Strain J2315 and other clonal isolates (e.g., K56-2 or BC7) are amenable to various types of genetic manipulation [4] and, in combination with the genome sequence, these tools should provide an excellent means to uncover virulence factors within the *B. cepacia* complex.

An obsessed network

One group, the International *Burkholderia cepacia* Working Group (IBCWG; http://allserv.rug.ac.be/~ppvandam/cepacia/) has contributed considerably to our understanding of this group of micro-organisms. The IBCWG was created informally in 1995 after a small meeting in Washington, DC, where the focus was specifically on *B. cepacia* infection in patients with CF. The group now has annual meetings with around 50 participants and research within the network covers all aspects of *B. cepacia* biology, from clinical through ecological and industrial. With this broad base of knowledge and expertise, further explanations of the virulence and pathogenesis of the *B. cepacia* complex in patients with CF will be forthcoming.

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