Virulence factors of *Burkholderia cepacia*

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**Introduction**

Originally named following its identification as a cause of soft rot in onions, *Burkholderia cepacia* has also been known as *Pseudomonas multivorans* and *Pseudomonas kingae* [1,2], and until most recently, as *Pseudomonas cepacia* [3]. A proposal for the transfer of 7 species of the genus *Pseudomonas* RNA Homology group II to a new genus *Burkholderia* with the type-species *Burkholderia cepacia* has been validated [3]. For the purpose of this review the name *B. cepacia* will be used.

Once considered solely as a phytopathogen, *B. cepacia* is now recognized as an important pathogen in nosocomial infection and in patients with chronic granulomatous disease and particularly in those with cystic fibrosis (CF). CF is the most common autosomal recessive lethal disease in Caucasian populations with an incidence of approximately 1 in 2500 live births and a carrier frequency of 1 in 20. The basic cause of the pathophysiological symptoms of CF is a defect in epithelial ion transport which results in viscous dehydrated bronchopulmonary and gastrointestinal secretions. Build-up of viscid mucus is associated with impaired mucociliary clearance and susceptibility to bacterial colonization which in turn initiates a vicious cycle of chronic inflammatory reaction. The susceptibility of CF patients to pulmonary colonization has been recognised since the earliest descriptions of the disease when patients seldom survived infancy. Advances in management of CF have meant that today most patients survive to early adulthood. However, this increased longevity has in part created its own problems including the emergence of new opportunistic pathogens, including and most notably, *B. cepacia*.

The last decade has seen *B. cepacia* emerge as a particular problem amongst patients with CF, where colonization may be symptomless or associated with a slow decline in lung function. A more serious clinical outcome, not observed with other CF pathogens, in which some colonized CF patients experience *B. cepacia* bacteraemia and/
or succumb to an accelerated and fatal deterioration in pulmonary function [4–7], is central to the current concern over *B. cepacia* in the CF community.

Based on nucleic acid homology, *B. cepacia* is more closely related to *B. pseudomallei*, *B. mallei* and *B. gladioli* than to *P. aeruginosa* and other fluorescent pseudomonads, and was placed in the separate subgroup, *Pseudomonas* RNA homology group II. *B. cepacia* is nutritionally versatile, with minimal growth requirements and the ability to survive in unfavourable environments: it has been isolated from disinfectants and antiseptics and can even use penicillin G as a nutrient [8–11]. The organism is intrinsically resistant to most antibiotics, and even if individual strains show in vitro susceptibility to an antibiotic, there is little clinical response [10,12–15].

In contrast to the large amount of information on *P. aeruginosa* virulence factors, knowledge of the virulence factors and pathogenesis of *B. cepacia* is scanty. Animal models have indicated that *B. cepacia* is less virulent than *P. aeruginosa* [16]. The aim of this review is to discuss the main features and properties of *B. cepacia* and, in particular, to focus on those which may contribute to its ability to colonize patients with CF.

### Colonization and adherence

The ability of a potential pathogen to adhere to the host mucosal or epithelial cell surfaces is often pivotal in the subsequent establishment of infection. Few potential adhesins have been described for *B. cepacia*, and most attention to date has been focused on the adhesive properties of fimbriae.

Electron microscope studies have shown that approximately 60% of *B. cepacia* strains express peritrichous fimbriae (see Fig. 1) [17,58]. Other *B. cepacia* strains possess polar fimbriae, similar to those expressed by *B. aeruginosa* [18]. Kuehn et al. [17] showed that outer membrane protein preparations of *B. cepacia* were enriched with 3 proteins (16, 20 and 40 kDa) which were not present in a non-fimbriated strain. The fimbrial subunit was identified as the 16 kDa protein; the protein appeared similar to those seen in other bacteria and showed homology with PAK fimbriae of *P. aeruginosa* [17]. This data contrasts with that of Saiman et al. who found minimal cross-reactivity with anti-*P. aeruginosa* anti-pilin monoclonal antibodies and no homology between *P. aeruginosa* pilin gene probes and *B. cepacia* genomic DNA [18,19]. It is possible that sequence variation exists among the pilin genes of different *B. cepacia* strains and that any individual pilin gene probe from *P. aeruginosa* may not reveal a specific *B. cepacia* gene [17].

The presence of fimbriae increases the ability of *B. cepacia* to adhere to pneumocytes in vitro [17]. In vitro binding experiments by Kirvan et al. [20] demonstrated that both *B. cepacia* and *P. aeruginosa* adhere to the same Galβ1–4GalNAc sequence present in many asialoglycolipids. The experiments of Saiman et al. [19] did not demonstrate competition for epithelial receptors, indicating that different epithelial receptors may be used preferentially by each of the *Pseudomonas* species or that the bacteria may bind to each other. Binding of 2 *B. cepacia* strains to epithelial monolayers increased in the presence of *P. aeruginosa* indicating a possible synergistic relationship whereby *P. aeruginosa* exo-products modify epithelial cell surfaces, exposing receptors and facilitating increased *B. cepacia* attachment [19]. It must be stressed, however, that not all CF patients are colonized with *P. aeruginosa* prior to acquisition of *B. cepacia*: in the Edinburgh CF clinic 38% of patients with *B. cepacia* are not co-colonized with *P. aeruginosa* [6].

Sajjan et al. [21] were able to demonstrate specific binding of *B. cepacia* isolates from patients with CF to both CF and non-CF mucins as well as to buccal epithelial cells. Unfortunately no typing data was available to exclude the possibility of clonal relationships between the strains [21]. The degree of binding observed with *B. cepacia* is considerably less than that observed with *P. aeruginosa* [58]. Deglycosylation of mucin indicated that the mucin receptors for *B. cepacia* include N-acetylgalactosamine and N-acetylgalactosamine. Isolates exhibiting the highest mucin binding values tended to correlate with those patients with severe illness leading to speculation...
that variability in the binding of different *B. cepacia* isolates to respiratory mucin may contribute to morbidity and mortality, and may explain why some *B. cepacia* strains colonize patients transiently whereas other strains, once acquired, are never lost. A sparsely distributed 22 kDa pilin-associated protein was identified as a mucin binding adhesin specific to piliated strains of *B. cepacia* [22].

**Siderophores**

Production of siderophores enables bacteria to compete for iron with host iron-binding proteins including transferrin and lactoferrin and has been correlated with the ability of various bacteria to establish and maintain infection. *B. cepacia* strains express at least 3 siderophore-mediated iron transport systems, including pyochelin, cepabactin and azurechelin [23–26]. Pyochelin produced by *B. cepacia* is chemically unrelated to the pyochelin siderophore of *P. aeruginosa* [23,24]. A 14 kDa ferripyochelin binding protein, present in increased amounts in the outer membrane of iron-starved *B. cepacia* cells, has also been described [23]. Morbidity and mortality in infected CF patients has been correlated to the production of pyochelin. Although such evidence may point to a role in pathogenicity, half of the clinical isolates investigated by Sokol [23] were pyochelin-negative. Pyochelin may increase the ability of *B. cepacia* to disseminate throughout the lungs and perhaps induce a greater inflammatory

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*Fig. 1. Pseudomonas cepacia* J1359 in the process of dividing into 2 separate cells which exhibit peritrichous pili. Staining is 2% w/v phosphotungstic acid. Magnification × 25 000.*
response due to the increased area of infection in the lung [27]. Exogenously supplied pyochelin enhanced the virulence of non-pyochelin producing \textit{B. cepacia} strains in a chronic pulmonary model in rats [27]. Meyer et al. [26] showed that \textit{B. cepacia} ATCC25416 excreted both pyochelin and a lower molecular mass compound, cepabactin, which strongly chelated Fe III and facilitated iron translocation. Azurechelin, another distinct iron-binding compound, has been identified in 88% of \textit{B. cepacia} strains isolated from the respiratory tract [25].

**Extracellular virulence factors**

\textit{B. cepacia} produces a number of extracellular products including protease, gelatinase, haemolysin and lipase, although no pathogenic role for these factors has been demonstrated [28,29]. Unlike \textit{P. aeruginosa}, \textit{B. cepacia} does not appear to produce toxin A, exoenzyme S, or other detectable extracellular factors capable of producing a cytotoxic effect in vitro [28]. In a study of putative pathogenic factors of \textit{B. cepacia} [30] a number of characteristics were demonstrated more frequently in isolates from CF patients than control isolates. These factors included production of catalase, ornithine decarboxylase, valine aminopeptidase, C14 lipase, alginate, and trypsin; reduction of nitrate to nitrite; hydrolysis of urea and xanthine and complete haemolysis on bovine red blood cells. The role of any of these factors in respiratory colonization or infection in CF patients is not clear [30]. Indeed, an epidemic strain of \textit{B. cepacia} CF5610 associated with fatal clinical outcome in CF does not produce C14 lipase or haemolysis [6].

Molecular studies of the \textit{Pseudomonas} exotoxin A gene by Vasil et al. [31] concluded that the production of exotoxin A and the presence of the exotoxin A gene are probably limited to \textit{P. aeruginosa} and is not found in other \textit{Pseudomonas} spp. Southern hybridization experiments under low, medium and high stringency conditions with an exotoxin A gene probe failed to produce a positive signal with any of 8 \textit{B. cepacia} strains tested. Similar experiments conducted by ourselves also failed to demonstrate the presence of the exotoxin A gene in 3 environmental strains of \textit{B. cepacia} although a positive band was obtained with \textit{B. cepacia} CF5610 strain isolated from a patient with CF. However, growth of this strain in both iron replete and depleted medium and subsequent analysis of the cell free culture supernate by polyacrylamide gel electrophoresis and immunoblot analysis with anti-\textit{P. aeruginosa} exotoxin A antisera, failed to confirm production of a 66 kDa protein equivalent to \textit{P. aeruginosa} toxin A. (J.W. Nelson, unpublished results).

The extracellular proteinase of \textit{B. cepacia}, a 34 kDa protein, has antigenic similarities to \textit{P. aeruginosa} elastase and cleaves gelatin, hide powder, collagen but not human immunoglobulin IgG, IgM, secretory IgA, or IgA [32]. Intratracheal instillation of purified proteinase into rat lungs produces a bronchopneumonia characterized by polymorphonuclear cell infiltration and proteinaceous exudation into large airways. Active immunization of rats with \textit{B. cepacia} proteinase elicits an immunological response although this is not protective against subsequent lung infection with \textit{B. cepacia} [32].

There is also evidence that lipases, particularly phospholipases, may play an important role in bacterial virulence [33,34]. Phospholipase C is an enzyme that cleaves phosphatidylcholine, a major lung surfactant, to yield phosphorylcholine and diacylglycerol, and has been associated with cytopathology of lung tissue. \textit{B. cepacia} has frequently been described as being lipolytic [28,33–35]. McKevitt and Woods [28] reported that 32 of 48 strains of \textit{B. cepacia} isolated from CF patients demonstrated lipase activity on egg-yolk agar whilst Carson et al. [35] showed that 32 of 48 strains of \textit{B. cepacia} isolated from CF patients demonstrated lipase activity on egg-yolk agar. In another study [34] 6 out of 10 clinical strains of \textit{B. cepacia} from the sputum of CF patients produced lecithinase by the egg-yolk reaction, whilst lipase activity on 4 different Tweens was strain-dependent. Purified enzyme had a molecular weight of 25 000 and was not cytotoxic for Hela cells or for mice injected intravenously with purified lipase. It has been reported, however, that lipase adversely affected the phagocytic function of rat pulmonary
alveolar macrophages in a dose-dependent manner [36]. Phagocytosis of B. cepacia by rat pulmonary alveolar macrophages was significantly reduced when the cells were either preincubated with lipase or when phagocytosis occurred in the presence of the lipase [36]. Scanning electron microscopy showed that the macrophages exposed to B. cepacia lipase had fewer pseudopodia, microvilli and other projections compared to untreated macrophages. Thus B. cepacia lipase may be an important virulence factor which allows the bacteria to evade the mammalian host defence system.

B. cepacia produces a heat-labile haemolysin which has both phospholipase C and sphingomyelinase activities [37]. Haemolytic and phospholipase C (lecithinase) expression in B. cepacia appears to be a complex phenomena. The study of Nakazawa et al. [29] found that only 4% of clinical isolates were β-haemolytic, whilst 67% of isolates produced lecithinase. Others have found higher percentages of haemolytic isolates of B. cepacia if a variety of erythrocyte types were tested, including a study of clinical isolates of B. cepacia from CF patients which found that 40% were haemolytic when erythrocytes from various animals were tested [37]. Unlike the PLC activity of P. aeruginosa the PLC activity in B. cepacia does not correlate with haemolytic activity [29,37]. However, all haemolytic strains produce detectable lecithinase activity, and strains of B. cepacia, whether haemolytic or non-haemolytic, appear to produce detectable amounts of extracellular PLC activity. In contrast to the consistent patterns observed in the PLC gene of P. aeruginosa there is hypervariability in genetic organization of the PLC gene of B. cepacia [37]. The variable manner in which a B. cepacia PLC specific gene probe hybridizes with restricted B. cepacia DNA, the variability in expression of haemolytic and PLC activities of different strains, and the association of DNA arrangements with conversion of an Hly + to an Hly – variant may be related to the relatively large number of distinct insertion sequences (IS) reported for B. cepacia (> 25) [37,38]: in contrast these elements have yet to be discovered in P. aeruginosa. Some of these IS elements of B. cepacia, can be found in multiple copies and have been shown to both activate or inactivate gene expression.

**Cell surface antigens**

**Lipopolysaccharide**

B. cepacia strains isolated from patients with CF may express either the rough (R) or smooth (S) lipopolysaccharide (LPS) phenotype, whereas the majority of B. cepacia strains isolated from other clinical conditions or from the environment express S-LPS (S.L. Butler, unpublished results). This is in agreement with the study of McKevitt and Woods [28] where 22 strains examined possessed S-LPS and 26 strains possessed R-LPS. The epidemic strain of B. cepacia isolated from a number of CF patients in the UK invariably has a R-LPS phenotype and is associated with the appearance of dry colonies [6]. There is no evidence to date to confirm that B. cepacia strains undergo a phenotypic change from S to R LPS within the CF lung as is observed with P. aeruginosa.

Western blotting and absorption studies demonstrated that a significant proportion of serum antibodies from B. cepacia-infected CF patients which reacted with the core LPS of B. cepacia did not react with the core LPS of P. aeruginosa [39]. These observations indicate differences in the structure and composition of core LPS between B. cepacia and P. aeruginosa confirming previous findings, including the lack of phosphorus in the core of B. cepacia LPS [40] and the inability of a monoclonal antibody reactive with P. aeruginosa and P. fluorescens core LPS to react with B. cepacia [41]. Core heterogeneity between different isolates of B. cepacia may also exist because immunoblotting demonstrated that serum from patients colonized with B. cepacia produced a band reactive with some but not all core LPS preparations [39].

Initial chemical analysis of B. cepacia LPS indicated the absence of detectable 3-deoxy-d-manno-2-octulosonic acid (KDO) in LPS from B. cepacia [42,43]. However, Straus et al. [44] reported the isolation of KDO from the culture supernate of 2 out of 10 strains of B. cepacia and
in a further study KDO was demonstrated in 6 clinical isolates of *B. cepacia* and all 6 LPS preparations were equally toxic for mice when injected intraperitoneally [45]. Compared with LPS from *P. aeruginosa* that from strains of *B. cepacia* has less phosphorus and more heptose. Glucose and rhamnose were the major saccharide components of LPS from the organisms tested [42]. An extracellular material isolated from a clinical *B. cepacia* consisted of a surface carbohydrate antigen, LPS and protein, the toxicity of which appeared to be associated with the LPS portion of the complex [46]. It has been proposed that this extracellular toxic complex produced by *B. cepacia* is responsible for the lethality and extensive pulmonary tissue necrosis associated with pneumonia produced by this organism.

**Outer membrane proteins**

*B. cepacia* produces 5 major outer membrane proteins A (56 kDa), B (38 kDa), C (37 kDa), D (28 kDa) and E (21 kDa). The C and D proteins have been identified as porin proteins [47,48], and appear to be antigenic in most patients with CF who are chronically colonized with *B. cepacia* [48,49]. In the study of Anwar et al. [55], outer membrane protein profiles of magnesium-depleted cells were much simpler than that of iron-depleted cells and nutrient broth grown cells. Synthesis of a 66 kDa outer membrane protein was induced when *B. cepacia* was grown under iron depletion. *B. cepacia* isolates from individual CF patients may exhibit marked phenotypic variability, including manifestation of different patterns of outer membrane proteins separated on a polyacrylamide gel: up to 5 OMP patterns have been identified from *B. cepacia* isolates derived from a single strain [50].

Various studies indicate that the outer membrane of *B. cepacia* is a major contributing factor in the β-lactam resistance of this species, retarding the diffusion of β-lactams to their penicillin-binding protein targets [47,51,52]. Resistance to aminoglycosides and hydrophobic compounds in *B. cepacia* is largely due to the low outer membrane permeability [51]. Loss of the major porin protein D and decreased expression of protein C may also be associated with high level β-lactam resistance in some CF isolates of *B. cepacia* [52]. Production of β-lactamases, including carbapenemases capable of hydrolyzing the most potent and broad spectrum of the β-lactam antibiotics, imipenem and meropenem, also contribute significantly to the resistance of *B. cepacia* [53].

**Exopolysaccharide**

Production of alginate by mucoid strains of *P. aeruginosa* is the major virulence determinant associated with strains which colonize the lungs of patients with CF. In contrast, *B. cepacia* does not appear to produce alginate. PCR studies with primers of the *P. aeruginosa algD* gene, encoding the essential enzyme GDP mannose dehydrogenase, indicate that this gene was absent in 10 *B. cepacia* strains studied and therefore that *B. cepacia* is unlikely to produce an alginate-like polymer (J.W. Nelson, unpublished results). Additional studies in our laboratory and by Sage et al. [54] showed that some *B. cepacia* strains do produce an exopolysaccharide comprising galactose, glucose, mannose, glucuronic acid and rhamnose, with lesser amounts of uronic acid: no mannnuronic or guluronic acid was detected. Surveys of clinical isolates from patients with CF indicate that there is no correlation between the ability of *B. cepacia* to colonize the respiratory tract and capacity to form exopolysaccharide [6,54]. In contrast, Straus et al. [46] observed that 1 strain of *B. cepacia* produced an alginate-like compound containing 72% guluronic acid with 1.75% acetylation.

**Evasion of the immune system**

Immunological studies on *B. cepacia* colonization of patients with CF indicate that the organism persists despite a considerable antibody response and suggest the possibility of immune-mediated damage. Aronoff et al. [48,49] demonstrated the presence of IgG antibodies to outer membrane antigens of *B. cepacia* in serum from patients with CF colonized with *B. cepacia* and/
or *P. aeruginosa*. These authors concluded that some *B. cepacia* outer membrane components may be antigenically related to those of *P. aeruginosa* and that colonization with *B. cepacia* occurs in the presence of antibodies specific for the outer membrane of the organism. Serum IgG and sputum IgA antibodies directed towards the core LPS of *B. cepacia* have also been described [39].

Investigations into the bactericidal effect of human serum have shown a large variation in the responses of the *B. cepacia* strains investigated. All strains expressing R-LPS were serum-sensitive under a variety of test conditions whilst strains expressing S-LPS exhibit a range of responses (S.L. Butler, unpublished results). Anwar et al. [55] showed that a *B. cepacia* strain grown in different nutrient depletions in batch culture showed varying degrees of sensitivity to engulfment and killing by human polymorphonuclear leucocytes (PMN) and to killing by human serum. The wide range of sensitivity shown by the organism may reflect the phenotypic variation in cell envelope composition caused by specific nutrient depletions. Patients with chronic granulomatous disease (GCD) are at particular risk of infection with *B. cepacia*, which is able to resist neutrophil-mediated non-oxidative bactericidal killing (D.P. Speert, personal communication). The ability of *B. cepacia* to survive a pronounced humoral response and other immunological defences is intriguing and requires further investigation. Indeed there is a suggestion that *B. cepacia* may invade and survive within respiratory epithelial cells, enabling the organism to persist within the CF lung [56].

Concluding remarks

Acquisition of *B. cepacia* is a major concern among patients with CF although the exact pathophysiological role of the organism remains controversial and unsolved. Epidemiological data and the use of phenotypic and genotypic typing systems for *B. cepacia* suggest that certain strains are particularly transmissible, although there is no evidence at present that some strains are more virulent. The role of any of the described virulence factors of *B. cepacia* relating to its pathogenesis in patients with CF remains unclear. Enhanced adhesion to mucin of certain *B. cepacia* strains may aid initial colonization whilst multi-resistance to antibiotics and possible intracellular localization may contribute to persistence of the organism. Production of anti-*B. cepacia* antibodies by the host and subsequent immune complex mediated damage, is probably responsible for pulmonary decline. The development of a CF mouse model carrying precise and clinically relevant mutations [57] will allow in vivo investigation of *B. cepacia* colonisation and virulence factors. Current concern over *B. cepacia* colonisation amongst CF patients has highlighted the urgent need to identify *B. cepacia* colonising factors and the pathophysiological and/or immunological factors which account for the rapid clinical deterioration in some CF patients.

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References


