REVIEW ARTICLE

Burkholderia cepacia: medical, taxonomic and ecological issues

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The increasing challenge posed by multiresistant saprophytes in medical microbiology is strikingly demonstrated by the emergence of Burkholderia (formerly Pseudomonas) cepacia as an opportunist pathogen in immunocompromised patients, particularly individuals with chronic granulomatous disease and cystic fibrosis (CF). Best known previously as a phytopathogen and the cause of soft rot of onions, B. cepacia presents three major problems for the CF community: innate multiresistance to antimicrobial agents; person-to-person transmission of epidemic strains through nosocomial or social contacts; and ‘cepacia syndrome’, a fulminating fatal pneumonia, sometimes associated with septicemia, that occurs in approximately 20% of colonised patients, including those with previously mild disease. Accumulated evidence to dispel earlier suggestions that the organism is avirulent and merely a marker of existing lung disease includes: case-controlled studies in CF patients; reports of serious infections in non-CF patients; in-vitro and in-vivo evidence that B. cepacia induces production of pro-inflammatory markers, including the major cytokine TNFa; and histopathological evidence that exposure of transgenic CF mice to B. cepacia results in pneumonia. By the early 1990s, the use of selective culture media and DNA-based bacterial fingerprinting confirmed suspicions of epidemic person-to-person spread of B. cepacia. This evidence provided scientific justification for draconian and controversial measures for infection control, in particular, segregation of B. cepacia-colonised patients during treatment at CF centres and their exclusion from social gatherings and national conferences. Recently, molecular analyses of type strains and clinical isolates have revealed that isolates identified previously as B. cepacia belong to at least three distinct species and have increased concern regarding the reliability of current laboratory detection and identification systems. Clarification of the taxonomy of B. cepacia-like organisms and the pathogenic potential of environmental isolates remains a high priority, particularly when the organism’s antifungal and degradative properties have created interest in its potential use as a biological control agent to improve crop yields and its use for the bioremediation of contaminated soils.

Introduction

‘The development of multiresistance in major microbial pathogens is well-recognised; in contrast, little attention has been paid to the pathogenic potential of naturally resistant environmental saprophytes’.

Known originally as a phytopathogen, Burkholderia cepacia (previously Pseudomonas cepacia, P. multivorans, P. kingii, ‘Eugonic oxidiser 1’) exhibits impressive nutritional versatility. Some microbes have an inherent or acquired ability to degrade antibiotics, but few have the ability to use penicillin as a sole carbon source [1] or to reduce onions to a macerated pulp! The earlier name, P. multivorans, reflected the organism’s omnivorous appetite, but it was not until 1950 that its pathogenic potential was recognised when Burkholder identified the organism as the cause of soft rot of onions—particularly ‘compromised’ onions damaged during harvesting—and provided an appropriate species epithet (Latin: cepia = onion) [2]. In the early 1990s, following taxonomic re-appraisal, the RNA group II pseudomonads were recognised as...
the new genus *Burkholderia*, with *B. cepacia* as the type species [3]. At present, the genus *Burkholderia* comprises *B. cepacia*, *B. gladioli*, *B. mallei*, *B. pseudomallei*, *B. caryophylli*, and recently added to the group, *B. plantarii*, *B. glumae*, *B. vandii* [4], *B. cocovenenans* [5] and *B. vietnamiensis* [6].

The general characteristics of *B. cepacia* include the following: gram-negative, non-sporulating, aerobic bacillus; motile with a respiratory metabolism and typically catalase- and oxidase-positive; various non-fluorescent pigments may be produced and poly-β-hydroxyalkanoates can be accumulated as reserve materials; the optimal temperature for growth is 30–35°C [7]. Recently, elegant molecular analyses have provided scientific evidence that may account for the organism's impressive versatility, including multilocus linkage disequilibrium analysis of environmental populations [8]—which suggested an extraordinarily high rate of recombination in *B. cepacia* relative to binary fission—and demonstration of multiple replicons and insertion sequences in type strains [9, 10].

The natural habitats of *B. cepacia* have been described as soil, water and vegetation [11]. However, it is a common but erroneous belief that *B. cepacia* is a ubiquitous saprophyte sharing similar environmental habitats with *Pseudomonas aeruginosa* and other pseudomonads. Extensive surveillance studies have shown that culture of *B. cepacia* from natural sources, including soil, water and plants, or from hospitals, foodstores, restaurant salad bars and patients' homes is surprisingly difficult, with detection rates of only 1–16% [12–16].

In agricultural microbiology, ecological awareness and an increasing incidence of pesticide-resistant pathogens have led to interest in *B. cepacia* as a potential agent for biological control and soil decontamination. *B. cepacia* produces several antimicrobial agents, including pyrrolnitrins, alotericins, cepalyccins and bacteriocin-like agents [17–20], that inhibit bacterial and fungal phytopathogens and suppress tobacco wilt and other plant diseases [21]. *B. cepacia* is also capable of degrading industrial waste and herbicides, including 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), the principal ingredient of the highly potent 'agent orange' [22]. Indeed, *B. cepacia* has been shown to degrade 2,4,5-T in heavily contaminated soils at a rate up to 20 000-fold greater than other known degradative bacteria [23].

In contrast to its potential agricultural benefits, *B. cepacia* has also emerged as a multiresistant opportunistic human pathogen, leading to concern about the relationship between environmental and clinical isolates [14, 24–26] and the potential hazards of releasing *B. cepacia* as a biological control agent [14, 24]. This review will provide an update on microbes currently described as *B. cepacia*, with particular focus on clinical, taxonomic and ecological issues (Table 1) associated with pulmonary infection in patients with cystic fibrosis (CF).

### The emergence of *B. cepacia* as a human pathogen

Before the early 1980s, reports of human infections caused by *B. cepacia* were sporadic and generally restricted to hospitalised patients exposed to contaminated disinfectant and anaesthetic solutions in which this nutritionally adaptable saprophyte survives for long periods. Infections included those of soft tissues and the respiratory and urinary tracts, but bacteraemia also occurred, sometimes associated with endocarditis and septic shock [27–31]. A rising incidence of *B. cepacia* infection was noted during the early 1980s and, although in some cases culture of *B. cepacia* was thought to reflect mere colonisation or contamination rather than infection [11, 32], extensive analyses of USA databases of nosocomial infections confirmed a significant increase in clinically significant *B. cepacia* infections [33, 34]. The apparent propensity of *B. cepacia* to cause fatal pulmonary infections, as suggested by these analyses, is emphasised in patients with chronic granulomatous disease (CGD)—in whom *B. cepacia* pneumonia and septicaemia are life-threatening [35, 36]—and in its emergence as a major pathogen in patients with CF [37–39]. By the 1990s, disturbing reports also emerged of fatal cases of *B. cepacia* pneumonia and septicaemia in previously

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<thead>
<tr>
<th>Table 1. Major issues associated with <em>B. cepacia</em> and cystic fibrosis (CF)</th>
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<tr>
<td><strong>Is there convincing evidence to confirm that <em>B. cepacia</em> has pathogenic potential and is not merely a marker of pulmonary disease?</strong></td>
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<td><strong>Based on the success, but unpopularity, of segregation and advances in clarifying the taxonomy of the genus <em>Burkholderia</em>, should all <em>B. cepacia</em> be treated as equal? Can phenotypic or genomic markers be found which would identify highly transmissible or virulent clones?</strong></td>
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<td><strong>To what degree do natural environments represent a reservoir for <em>B. cepacia</em> and a hazard for CF patients? What hazards are associated with the development and use of <em>B. cepacia</em> as a biological control agent?</strong></td>
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<td><strong>Could an improved understanding of the host immune response, including enhanced cytokine induction by bacterial surface components, clarify the immunopathology of <em>B. cepacia</em> and lead to innovative forms of immunotherapy?</strong></td>
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<td><strong>At present, is it not possible to forecast the clinical outcome of <em>B. cepacia</em> colonisation. Can host and bacterial factors responsible for initial colonisation and poor clinical outcome be identified?</strong></td>
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<td><strong>Recently, it has been demonstrated that CF airway epithelia contain bactericidal activity that is inhibited reversibly by high NaCl concentrations. Does this killing potential include <em>B. cepacia</em> and is it host or strain specific?</strong></td>
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<td><strong>Ultimately, the identification of bacterial and host factors associated with transmission and virulence would assist greatly in the rational design of an effective <em>B. cepacia</em> vaccine.</strong></td>
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B. cepacia and cystic fibrosis

During the last decade, the major clinical interest in B. cepacia has focused on its addition to the relatively narrow spectrum of microbial pathogens responsible for debilitating and ultimately fatal pulmonary infections in patients with CF [26, 39, 48, 49]. In the late 1980s, surveillance studies in the UK indicated a maximum prevalence of 7% [39, 50–52]; however, in some CF centres this later increased to approach the prevalence of 40% described in contemporary North American studies [53]. The three major issues concerning B. cepacia can be summarised as follows: 1, the clinical risk of rapid and fatal pulmonary decline, even in patients with previously mild disease; 2, patient-to-patient spread of epidemic strains within and between regional CF centres and between centres in the UK and North America; and 3, the innate multiresistance of most B. cepacia isolates to available antibiotics—which deprives patients of effective antimicrobial therapy [46, 54]—combined with the failure to reduce the bacterial population in sputum and a relatively poor clinical response even when the colonising strain exhibits in-vitro susceptibility.

The clinical significance of B. cepacia in CF patients was first described in 1984 in a seminal report by Isles et al. [37]. In addition to noting the increased prevalence of B. cepacia colonisation in patients attending Toronto clinics, Isles et al. described a rapid and unexpected clinical decline, including necrotising pneumonia and bacteraemia, that occurred in 20% of colonised patients. This acute clinical decline is sometimes referred to as ‘cepacia syndrome’ [37]. It is important to note that acute clinical deterioration and bacterial spread to sites other than the lung is not observed with the other major CF pathogens, Staphylococcus aureus, Haemophilus influenzae and P. aeruginosa.

The second major issue relating to B. cepacia arose in the mid 1980s as an increasing—but scientifically unproven—conviction held by some CF carers that the clustering of cases in some large North American clinics had arisen from cross-infection. At that time, an alternative explanation for clustering was the difficulty in culturing this relatively new pathogen from CF sputa [48]. As evidence, in a controlled study involving 115 North American CF centres, only 36 (31%) cultured the organism successfully from a seeded sputum specimen [55]. However, by the early 1990s, the availability of selective culture media [48] and awareness of the organism’s cultural idiosyncrasies [56] indicated that regional variation in the prevalence of B. cepacia colonisation could not be explained simply by laboratory methodology. Furthermore, the development and use of bacterial fingerprinting techniques—including multilocus enzyme electrophoresis (MLEE), pyrolysis mass spectroscopy, PCR-ribotyping and pulsed-field gel electrophoresis (PFGE)—provided compelling evidence for person-to-person spread of B. cepacia through nosocomial and social contacts (Table 2) [25, 37, 46, 57–75] and, occasionally, in the absence of proven sputum colonisation [67]. Epidemiological data also provided scientific justification for the introduction of guidelines by national CF organisations to improve personal and hospital hygiene and, more controversially, for the implementation of segregation policies to limit contact between colonised and non-colonised individuals [76]. Surveillance studies show that segregation undoubtedly reduces the incidence of B. cepacia cross-infection [38, 62, 71, 77], but the strategy has not eliminated acquisition. Furthermore, the logistic and social consequences of draconian infection control measures reminiscent of mediaeval approaches to leprosy have not been accepted universally. In particular, the need for such measures has been questioned fiercely by patients and care-givers in CF centres where intensive surveillance has not revealed a high incidence or prevalence of B. cepacia colonisation.

A pathogen or a marker of lung disease?

In the 1970s, some microbiologists and clinicians considered S. aureus to be the only true microbial pathogen in CF patients and viewed P. aeruginosa as merely a marker of disease. A similar doubt has accompanied the emergence of B. cepacia and has exacerbated the controversy surrounding segregation of colonised individuals. In discussions of any potential opportunistic pathogen, it is easy to find evidence of asymptomatic carriage; even Salmonella typhi and Vibrio cholerae do not invariably exhibit pathogenicity!

Clarification of the clinical relevance of B. cepacia is also thwarted by the fact that the available scientific evidence requires particularly careful analysis. There is an inclination to link bacterial transmissibility and virulence, and to categorise individual B. cepacia...
strains as either transmissible and virulent, or non-transmissible and avirulent. There is no scientific justification for this view. In epidemic outbreaks in which patients are colonised by the same strain, some patients may remain asymptomatic whilst other individuals succumb to rapid and unexpected fatal deterioration [37, 62]. In the case of transmission, epidemiological evidence has clearly identified lineages with enhanced transmissibility [25, 46, 62, 69]; however, based on present knowledge, it cannot be stated with confidence that a strain inherently lacks the ability for epidemic spread. Furthermore, apparently 'non-transmissible' strains that have not spread even to a patient's CF sibling have been responsible for fatal infection [72]. Finally, it could be argued that transmission is not strain-dependent, but is associated with nosocomial or social opportunities for transmission. This hypothesis is certainly not supported by the behaviour of the particular *B. cepacia* lineage with a notorious ability to spread in CF centres in the UK [46, 62] and North America [25, 69], referred to as the Edinburgh/Toronto lineage [69] or ET12 intercontinental clone (multilocus enzyme electrophoresis type 12) [25]. For convenience, this particular *B. cepacia* lineage will be referred to as the ET12 lineage in the remainder of this review.

Some CF carers who have experienced transmission of *B. cepacia* amongst small numbers of their patients have argued against segregation on the grounds that no significant clinical deterioration was observed and that implementation of such draconian measures stigmatises patients and prevents valuable social contacts with other CF patients [70]. However, the hypothesis that *B. cepacia* is transmissible but merely a marker of pulmonary deterioration can be challenged. A recent retrospective study of the clinical status of *B. cepacia*-colonised adults in the 24-month period before colonisation found no difference in their lung function, number of days in hospital or outpatient visits [77]. Furthermore, in numerous case-controlled studies involving large numbers of patients, *B. cepacia* colonisation has been associated in some but not all patients with an accelerated decline in pulmonary function and a poor prognosis [71, 77–81]. Most studies have reported that the risk of clinical deterioration on acquisition of *B. cepacia* is increased in adult patients with severe disease [78–80]. This contrasts with an epidemic outbreak of *B. cepacia* among children, in whom the dominant impact on respiratory function was greater in patients with better levels of respiratory function [71]. Explanations for the range of clinical responses associated with *B. cepacia* colonisation and inability to predict the clinical outcome in individual patients could include: 1, differences in strain virulence; 2, the relatively low 20% 'strike rate' of cepacia syndrome; 3, the influence of co-colonisation by other pathogens; 4, the age at which colonisation occurs; 5, individual host immune responses; and 6, the severity of underlying CF disease.

The hypothesis that *B. cepacia* colonisation is merely a marker of severe lung disease is also undermined by the fact that fatalities have occurred in CF adults with mild CF disease, including individuals not already harbouring *P. aeruginosa* [62]. Finally, one of the most striking results from the first microbiological studies in transgenic CF mice showed that 70% of CF mice exposed to *B. cepacia* succumbed to more severe broncho-pulmonary infection than control animals [82].

### Table 2. Evidence for and against person-to-person transmission of *B. cepacia*

<table>
<thead>
<tr>
<th>Reference</th>
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<tr>
<td>A. Evidence in favour of person-to-person transmission</td>
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<tr>
<td>Isles et al. [37]</td>
<td>Seminal paper: noted rising incidence of <em>B. cepacia</em> and cepacia syndrome in Canadian clinics</td>
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<td>Thomassen et al. [57]</td>
<td>Fall in incidence after segregation</td>
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<tr>
<td>LiPuma et al. [58]</td>
<td>Prevalence of one ribotype in individual clinics</td>
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<td>LiPuma et al. [59]</td>
<td>Ribotyping demonstrates person-to-person spread between two patients at a CF camp</td>
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<td>Anderson et al. [60]</td>
<td>Nosocomial outbreak</td>
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<tr>
<td>Millar-Jones et al. [61]</td>
<td>UK nosocomial outbreak</td>
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<tr>
<td>Govan et al. [62]</td>
<td>Genotypic fingerprinting and extensive epidemiological data provides compelling evidence of person-to-person spread through social contact in and between two UK CF centres</td>
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<tr>
<td>Smith et al. [63]</td>
<td>Further UK outbreak with transmission in clinical and social settings</td>
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<tr>
<td>Bingen et al. [64]</td>
<td>International consensus confirming <em>B. cepacia</em> transmissibility</td>
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<td>Corkill et al. [65]</td>
<td>Highlights transmission particularly at UK CF events</td>
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<tr>
<td>Pegues et al. [66]</td>
<td>Demonstration of transmission at USA CF camps</td>
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<tr>
<td>Johnson et al. [25]</td>
<td>Intercontinental spread of Edinburgh/Toronto strain ET12</td>
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<tr>
<td>LiPuma et al. [67]</td>
<td>Inapparent transmission from culture-negative patient (?)</td>
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<td>Ryley et al. [68]</td>
<td>Further UK outbreak</td>
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<td>Sun et al. [69]</td>
<td>Cable pili demonstrated on intercontinental strain (ET12)</td>
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<tr>
<td>Reverts et al. [70]</td>
<td>Prevalent strain in Belgian clinic</td>
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<td>Whiteford et al. [71]</td>
<td>Outbreak in UK paediatric clinic</td>
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<tr>
<td>Pitt et al. [46]</td>
<td>Strain ET12 prevalent in UK clinics: accounting for 38% of cases</td>
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<tr>
<td>B. Cases with no evidence of person-to-person transmission</td>
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<tr>
<td>Glass and Govan [72]</td>
<td>No transmission of pathogenic strain between siblings</td>
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<tr>
<td>Hardy et al. [73]</td>
<td>No transmission to uncolonised patients during hospitalisation</td>
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<tr>
<td>Taylor et al. [74]</td>
<td>No transmission in UK unit before segregation</td>
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<tr>
<td>Steinbach et al. [75]</td>
<td>No transmission in large CF unit despite no segregation of hospitalised patients</td>
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The Edinburgh/Toronto/ET12 epidemic lineage

In reviewing the emergence of *B. cepacia* in CF populations in Europe and North America, it is necessary to emphasise the influence of epidemic lineages on the incidence and prevalence of *B. cepacia* within CF centres. Evidence shows that the incidence in a centre can be influenced greatly by the epidemic spread of a single lineage, and that if such spread is discounted then the prevalence of *B. cepacia* in most CF centres remains relatively low at 5–10%. Transient colonisation by *B. cepacia* also influences prevalence and occurs in c. 5% of CF patients; however, transient colonisation is observed very rarely with the ET12 lineage (authors' unpublished observations), perhaps reflecting the high colonisation potential of this clone. From a clinical, epidemiological and evolutionary viewpoint, the influence of this single clone on the CF community is considerable. In the UK alone, it has been isolated in eight (50%) of 16 CF centres and from 68 (38%) of 178 *B. cepacia*-colonised patients [46]. Attempts to identify its origins have been frustrated by a lack of stored isolates; however, investigation of available isolates allows several conclusions to be reached. Based on evidence from MLEE and ribotyping [25] and PFGE [46, 62], the first known isolates of this epidemic lineage were cultured from Ontario paediatric patients in the latter half of the 1980s [25]. In the UK, the first recorded isolate of the same lineage was in August 1989 [62] from a patient who had never been out of the UK nor shown any evidence of *B. cepacia* colonisation during previous bacteriological investigations. The patient had previous contacts with other UK patients colonised by *B. cepacia*, but the isolates from these patients were not available.

From the available evidence, it appears that the Edinburgh/Toronto/ET12 lineage was established in Canada before its appearance in the UK, and that at some stage in the late 1980s, intercontinental spread occurred between UK and Canadian patients whilst attending summer camps in Ontario, followed by interregional spread in the UK during social contacts at meetings [25, 62]. It is tempting to conclude that this highly transmissible strain is clonally related to the isolates cultured during the first documented outbreak of *B. cepacia* in CF patients in Ontario, reported in 1984 [37].

Potential pathogenic mechanisms of *B. cepacia*

Although *B. cepacia* produces several putative virulence determinants—including haemolysins, proteases, lipases, siderophores and catalase—a major clinical role for these factors has not been demonstrated convincingly in CF [83, 84]. However, catalase is associated with the organism's ability to resist killing by professional phagocytes and to produce serious infection in patients with CGD [85].

Intracellular survival

Several puzzling clinical and scientific observations have led to speculation that *B. cepacia* can survive and grow within pulmonary phagocytes or respiratory epithelial cells. First, clinical resistance to antimicrobial therapy despite demonstration of an isolate's susceptibility *in vitro*; second, isolation of serum-sensitive isolates in bacteraemic infection [86]; third, chronic pulmonary colonisation despite a pronounced antibody response [87]; and fourth, the close taxonomic relationship between *B. cepacia* and the intracellular pathogen, *B. pseudomallei*. However, to date, the scientific evidence for intracellular survival or growth of *B. cepacia* is not convincing. Studies of intracellularity in bacterial pathogens can be difficult and, in the case of *B. cepacia*, are complicated further by the organism's innate resistance to antibiotics, including aminoglycosides, which are used commonly in intracellular assays to kill extracellular organisms. As it is known that *B. pseudomallei* survives and multiplies within professional phagocytes [88], studies within our group have focused on monocytes, with *Listeria monocytogenes* and *P. aeruginosa* as positive and negative controls, respectively. However, it was not possible to demonstrate either enhanced uptake or survival of *B. cepacia* in monocytes. Previously, Burns [89] reported the observation of *B. cepacia* within CF post-mortem respiratory epithelial cells by electron microscopy, but no further data have been published to validate this important finding. Low-level invasion *in vitro* of a respiratory epithelial cell line by the epidemic ET12 lineage has been demonstrated [90], but the significance of limited epithelial invasion by bacteria remains unclear [91]. A recent and potentially seminal publication has even suggested that enhanced uptake of CF pathogens by epithelial cells expressing surface cystic fibrosis transmembrane conductance regulator (CFTR), followed by epithelial desquamation, may be an important host defence mechanism rather than a bacterial virulence determinant [92].

Overall, the role of intracellularity in the pathogenesis of *B. cepacia* infection in CF patients is still in doubt. As a caveat, the demonstration of its intracellular survival and growth within amoebae raises the possibility that these free-living protozoa may act as an environmental reservoir from which CF patients could acquire the organism [93].

*B. cepacia* and host immune responses

Colonisation with *B. cepacia* is associated with a pronounced and specific humoral response, including raised serum IgG and IgA and sputum IgA titres against *B. cepacia* lipopolysaccharide (LPS) and outer-membrane protein (OMP) components [87, 94]. Anti-*B. cepacia* antibodies have also been detected in non-colonised CF patients, and particularly in patients colonised with *P. aeruginosa* [87, 95]. Studies with pre-absorbed sera have failed to demonstrate an appreciable
degree of cross-reactivity between the two species, either for OMP or LPS components [87, 96], suggesting that the response to *P. aeruginosa* is not the source of pre-colonisation anti-*B. cepacia* antibody. Generally, levels of anti-*B. cepacia* immunoglobulin in non-colonised patients are low, but the demonstration of substantially raised titres in a subset of patients may reflect previous exposure to *B. cepacia* where an appropriate antibody response has prevented the occurrence of colonisation. On the other hand, the demonstration of antibody in stored pre-colonisation sera from patients who subsequently became colonised, indicates that antibody does not always play a preventative role. Similarly, the role of antibody in patients once they are colonised is unclear; for example, clinical outcome is independent of the magnitude of anti-*B. cepacia* responses [87]. A recent study [97] with immunoblotting techniques has suggested that IgG antibodies against a 30-kDa OMP, identified presumptively as the major immunodominant porin, OMP D [95, 98], are associated with a better prognosis in colonised patients. If these results are confirmed, it raises the possibility of using this OMP as a target for immunotherapy.

The association of *B. cepacia* with CGD, an inherited defect in neutrophil oxidative killing pathways, and the role of neutrophils as the predominant immune effector cell in the CF lung [99], have led to speculation that the interaction between *B. cepacia* and neutrophils may be important in the evasion of host defences by this organism. Speert et al. [85] demonstrated that, unlike *P. aeruginosa*, *B. cepacia* is resistant to non-oxidative neutrophil killing mechanisms; hence the role of *B. cepacia* in CGD. Evasion of the normal neutrophil oxidative burst would aid the survival of *B. cepacia* in the presence of a pronounced immune response. Within the CF lung, normal opsonisation processes are compromised severely through the disruption of immune effector molecules by bacterial and host proteases [100, 101]. In particular, cleavage of complement receptors and immunoglobulin molecules within the respiratory tract may neutralise the humoral immune response to *B. cepacia* and enable the organism to persist in the lungs of colonised patients. However, this observation does not explain the ability of rough, LPS-deficient, serum-sensitive *B. cepacia* to cause invasive pneumonitis and septicemia in patients with elevated anti-*B. cepacia* immunoglobulin titres [86].

**Inflammatory damage**

Increasing evidence has emerged to suggest that host immune responses are important in the pathogenesis of *B. cepacia* infection. A UK multicentre study has shown that levels of the inflammatory markers, C-reactive protein and neutrophil elastase α1-antiproteinase complex, are significantly higher during *B. cepacia*-associated exacerbations than in exacerbations caused by *P. aeruginosa* alone. Aggressive antibiotic treatment with the most active agents available did not eliminate *B. cepacia*, but in most cases was associated with a decline in inflammatory markers to pre-exacerbation levels [102]. In addition, anecdotal evidence indicates that patients who exhibit rapid pulmonary decline and pronounced inflammatory symptoms, but who do not respond to antibiotic therapy, nevertheless respond to treatment with commercial preparations of immunoglobulin. The relative absence of *B. cepacia* antibodies in healthy human donors [87], from whom these immunoglobulins are obtained, suggests that such preparations contain potentially useful anti-inflammatory activity.

An unexpected but informative result from our own studies has demonstrated that LPS from clinical and environmental isolates of *B. cepacia* induces pro-inflammatory cytokines, including the major cytokine tumour necrosis factor α (TNFα), to a level 10-fold that induced by *P. aeruginosa* LPS and matching the inflammatory power of *Escherichia coli* endotoxin [103, 104]. The mechanism involved in *B. cepacia* cytokine stimulation is unclear, but is independent of CD14 receptors. Of interest, induction of TNFα by *B. cepacia* LPS is reduced in the presence of *P. aeruginosa* LPS, suggesting that the diversity of clinical outcomes in patients colonised with *B. cepacia* may be influenced in part by the presence or absence of *P. aeruginosa* and other CF pathogens [105].

**What is a true *B. cepacia*?**

Further research to establish a gold standard for laboratory identification of *B. cepacia* has assumed high priority. Reliable identification is important not only in attempts to clarify the organism's pathogenic potential, but also because of the clinical, social, psychological and potentially litigious consequences for patients, carers and diagnostic laboratories associated with the organism's acquisition and transmission. Selective media and laboratory protocols for culture and presumptive identification of *B. cepacia* from clinical or environmental sources have been described and their value in microbiological surveillance established [14, 48, 56, 106]. However, existing selective media also support the growth of other gram-negative non-fermenting bacilli [46, 48, 56] and unequivocal identification of *B. cepacia* by multiltest commercial systems can present difficulties [44, 56, 106, 107].

There is increasing evidence that organisms presently identified as *B. cepacia* by standard laboratory procedures exhibit such diverse genotypic and phenotypic properties that attempts to generalise on virulence, transmission and antibiotic susceptibility are ill-founded. Simpson et al. [44] speculated that epidemic strains may represent a *B. cepacia* sub-
population, arising as bacterial hybrids or through horizontal transfer of virulence genes from the closely related pseudomonads *B. gladioli* and the highly dangerous intracellular pathogen *B. pseudomallei*. Recently, isolates identified as *B. cepacia* were characterised further by analysis of cellular proteins and fatty acid components and clustered by means of computer-assisted numerical comparison of the profiles. Representative isolates from individual clusters were selected to determine genotypic relatedness within and between clusters by means of DNA–DNA and DNA–rRNA hybridisation assays. These molecular phylogenetic studies revealed that organisms identified by conventional tests as *B. cepacia* comprised several new *Burkholderia* spp. [108].

According to taxonomic conventions, new species names should not be given to bacteria that cannot be identified reliably by phenotypic characteristics; instead, such groups can be described by the terms genomovar I, II, etc. [109]. Following this convention, isolates identified as *B. cepacia* by conventional multistest systems such as the API 20NE system (API-bioMerieux, Marcy l’Etoile, France) constitute at least four different genomovars of *B. cepacia*; other presumed *B. cepacia* strains are identified as the nitrogen-fixing organism *B. vietnamiensis*. Preliminary studies on a small number of isolates have indicated that the majority of CF isolates from Belgium and the UK tend to cluster in genomovar III [70, 108]. Subsequent ongoing analyses of a larger collection of environmental, phytopathogenic and clinical isolates in our laboratories have confirmed the potential importance of genomovar identification. For example, the isolate responsible for the first UK report of cepacia syndrome [72], and three individual epidemic clones including the highly transmissible ET12 lineage [25, 44, 62, 69] each belong to genomovar III. It should be stressed that *B. vietnamiensis* and the remaining *B. cepacia* genomovars were also identified amongst isolates from CF patients, and that genomovar III status is not synonymous with high transmissibility [72]. Of the 150 ‘*B. cepacia*’ isolates studied to date, most environmental isolates (including the phytopathogenic type strain ATCC 25416) belong to genomovar I; in contrast, isolates associated with acute clinical decline in CF patients are restricted to genomovar III. These results confirm the complex taxonomic heterogeneity within the genus *Burkholderia* and have important diagnostic implications for infection control in the CF community.

**Unique bacterial clones and *B. cepacia* transmission factors**

Epidemiological data and genomic fingerprinting suggest that the variable incidence of *B. cepacia*—in particular, the lack of cross-infection in some centres [75, 81], and the contrasting epidemic spread in others—reflects the behaviour of a relatively small number of highly transmissible clones [46, 69, 110–112].

It seems reasonable to speculate that *B. cepacia* strains responsible for epidemic spread may harbour a common colonising factor whose identification could be exploited for diagnostic and therapeutic purposes. At present, the most significant of these factors is adhesion to respiratory mucin [53, 113–115], associated with giant intertwined fibres referred to as cable pili [53, 114]. The gene responsible for cable pili, *cbl*, has been detected in the highly transmissible ET12 lineage, represented by the Edinburgh isolate CF5610 (J2315) [16, 25, 62, 69, 115], and responsible for *B. cepacia* colonisation in 38% of UK patients [46]. In a slightly different form, *cbl* has also been associated with epidemic transfer of *B. cepacia* from CF to non-CF patients in a Mississippi outbreak [16, 69, 115]. However, studies with a *cbl* DNA probe indicated that *cbl* is not present in all epidemic clones, suggesting that other bacterial and host factors need to be identified [69]. Interestingly, a recent study [116] has described enhanced binding of the ET12 lineage to lipid receptors, particularly the galactolipid globotri-sylceramide (GB3), and led to speculation that upregulation of GB3, mediated through the infection process and TNF stimulation within the lung, may provide an alternative receptor for isolates in which cable pili are poorly expressed and a second receptor system for the epithelial attachment of bacteria that have migrated through the mucosal blanket.

Experimental proof of direct or indirect transmission of epidemic *B. cepacia* is not feasible and can be judged only by circumstantial evidence. However, epidemiological data has strikingly demonstrated such potential. Colonisation with more than one strain of *B. cepacia* is unusual and has been reported in <10% of patients [46]. During the Edinburgh outbreak, PFGE fingerprinting showed that one patient harboured two *B. cepacia* strains in his respiratory secretions, including the ET12 clone; however, only the epidemic strain was transmitted subsequently to his girlfriend [62].

**Modes of transmission and the risks of acquisition**

The potential risks of *B. cepacia* transmission, either directly by person-to-person spread or indirectly from contaminated fomites, continue to be a major concern to the CF community. Table 2 summarises the extensive documented evidence for direct transmission of *B. cepacia* between CF patients during close contacts within hospitals [61, 63, 65], at educational or summer camps [59, 66] and through other social contacts [62, 63]; in contrast, several reliable studies have found no evidence of cross-infection [72–75]. In
their initial report, LiPuma et al. [59] cited previous failures to culture B. cepacia from respiratory equipment and environmental surfaces as circumstantial evidence that direct person-to-person spread might be the primary means of transmission. However, a subsequent prospective study [117] with selective culture and DNA-based typing of isolates showed that colonised patients can contaminate their environment; thus indirect transmission might occur via contaminated surfaces. The intrinsic resistance of B. cepacia to many antibiotics also raised justifiable concern that the use of contaminated home-use nebulisers might present a special hazard for B. cepacia acquisition. Currently, evidence for nebuliser-associated transmission is scanty and equivocal. A case-controlled retrospective study of five CF patients undergoing treatment in a CF centre [118] showed a significant association between outpatient nebuliser use and B. cepacia colonisation. B. cepacia was also cultured from nebulisers used by colonised patients. Unfortunately, no bacterial typing was performed to confirm the clonal relationships of the human and nebuliser isolates. Recently, in a prospective study [119], B. cepacia was cultured from three of 35 home-use nebulisers. DNA macrorestriction analysis by PFGE revealed that one of two strains of B. cepacia recovered from the nebuliser of one patient was also present in the patient’s sputum. However, sputum cultures from the two other patients whose nebulisers harboured B. cepacia did not yield the organism, suggesting an environmental origin for the B. cepacia strain isolated from the nebuliser. Other studies of nosocomial acquisition of B. cepacia in non-CF patients have suggested that respiratory infection probably occurred by indirect transmission following use of contaminated nebuliser devices [31, 120]. Airborne dissemination may also present a small risk of B. cepacia acquisition. In a prospective study, B. cepacia was recovered from the room air during occupation by five of six patients, but to only a limited extent, with the number of bacteria ranging from 1 to 158 cfu/m³ [121]. Maximum yields were associated with episodes of coughing and, after a patient left the room, the organism persisted in room air for up to 45 min.

To conclude, ethical considerations prevent experi-

ments that could provide scientific data to assess the risks of B. cepacia acquisition, including clarification of the frequency of contact and the infectious dose required. Based on accumulated evidence (Tables 2 and 3), skin contact, respiratory aerosols, sharing food, contaminated equipment, co-habitation or undergoing physiotherapy in the same room as a B. cepacia-positive individual present reasonable risks of acquisition. However, epidemiological evidence [38, 62], including the high numbers (typically >10⁶ cfu/ml) of B. cepacia present in the saliva of colonised patients, suggests that the close and frequent social contact that occurs between siblings, the direct exchange of respiratory secretions associated with kissing, and the involvement of a highly transmissible B. cepacia lineage arguably present the greatest risks of acquisition.

### Environmental release of B. cepacia as a biological control agent

Whilst the CF community debates the clinical issues of B. cepacia colonisation and transmission, agricultural microbiologists continue to develop the organism as a biological control agent to exploit its antifungal activity (Fig. 1) for the enhancement of crop yields [122, 123] and its nutritional adaptability in the bioremediation of landfill sites, contaminated soils and ground water aquifers [124–126]. Deliberate environmental distribution of B. cepacia as field inoculants raises the issue of the phylogenetic relationship between B. cepacia of environmental and clinical origin and the potential hazard for human infection. The debate on this relationship has revealed the gulf that exists between different areas of interest and microbiological expertise and, as stated recently in an editorial comment on another contentious issue, bovine spongiform encephalopathy, ‘underscores the weakness of separating agricultural and medical science’ [127].

We have stated previously that the scientific evidence that environmental strains of B. cepacia present little hazard to man is weak [14] and is based on examination of only a few bacterial isolates and

### Table 3. Factors that may influence acquisition of B. cepacia

- In colonised individuals, B. cepacia saliva counts can exceed 10⁶ cfu/ml, suggesting that the highest risk of patient-to-patient spread is transmission of respiratory secretions during kissing or through sharing of eating or drinking utensils.
- Spirometer mouthpieces become heavily contaminated during lung function tests. Risk avoided by use of disposable mouthpieces.
- Recovery from the surface of lung function equipment is low.
- Recovery from antibiotic reservoirs of nebulisers has been reported, but incidence is low and the degree of risk appears secondary to the preceding factors.
- Aerosol recovery is low, suggesting low risk of aerosol transmission.
- Hands become contaminated after coughing and the organism can be transmitted by handshake. Survival on hands reduced to 10% after 30 min; this varies in different individuals and may account for variable recovery in surveillance studies.
- Gastrointestinal carriage has not been demonstrated, even in colonised individuals, suggesting that the risk of faecal-oral spread is minimal.
- After surface contamination with B. cepacia-containing sputum, viable bacteria can be recovered for several weeks.
- Surface contamination by B. cepacia sputum is eliminated by treatment with UV irradiation and with common hospital disinfectants, including Milton, Dettol, alcohol 70%, phenols, iodine and cetrimide. Careful drying is important after washing or disinfection.
- Recovery of B. cepacia from soil, plants, drains, lakes and surface waters is low, suggesting that natural environments present a possible but low risk for acquisition.
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Fig. 1. Inhibition of the phytopathogenic fungus Rhizoctonia solani by five isolates of B. cepacia. The fungus was inoculated in the centre of the plate and bacteria around the perimeter. Cultures were photographed after incubation for 14 days at room temperature.

inappropriate bacterial properties [24]. Although some studies have indicated that environmental and clinical isolates are distinct, no reliable phenotypic markers have been identified [25, 45, 108]. The suggestion that clinical isolates can be distinguished from soil isolates based on the former's lack of plant pathogenicity [45] is discounted by the fact that CF isolates of B. cepacia will readily macerate onion tissue (Fig. 2) [14]. In addition, the invasive B. cepacia foot lesions known as swamp foot [128], acquired by military personnel during jungle training, confirm the pathogenic potential of environmental strains of B. cepacia for man.

The potential hazard that some or all environmental B. cepacia strains present to the CF community is unclear and requires investigation. The fact that new cases of B. cepacia colonisation continue to occur with strains that show no genotypic relationship to other isolates within the same CF centre, points to the environment as a potential source. However, the extent of this risk is difficult to assess. Extensive microbiological safaris into supermarkets and domestic homes [15], and a range of botanical soils and cultivars [14], indicate that B. cepacia can be cultured from up to 20% of warm moist environmental sites, particularly soils, but that it is not as ubiquitous as other pseudomonads. Interestingly, in our studies to date, none of the environmental isolates of B. cepacia have been identified as belonging to genomovar III.

Conclusions and future prospects

B. cepacia is a striking example of a multiresistant soil saprophyte and phytopathogen that has emerged as an important threat to susceptible human hosts. In the CF community, the degree to which infection control measures should be implemented continues to arouse strong scientific and social debate. The validity of strict control is supported by circumstantial, but nevertheless compelling, evidence for direct person-to-person transmission of epidemic strains through nosocomial and social contact. In contrast, although the risk of indirect iatrogenic spread from contaminated fomites remains

Fig. 2. Soft rot of a segmented 'compromised' onion inoculated with a clinical B. cepacia isolate of the epidemic ET12 lineage (left) and an uninoculated control (right), both incubated at 30°C for 72 h. Reproduced with permission from Butler et al. [14].
unclear, available evidence suggests that this route is less important than direct transfer. An important caveat in attempts to generalise on *B. cepacia* transmission is evidence that the major epidemics of *B. cepacia* involve a subpopulation of highly epidemic lineages which might be re-allocated ultimately to new species; 'Burkholderia cefi' might be an appropriate but probably controversial choice! Ongoing microbiological surveillance in CF centres indicates that sporadic acquisition of epidemic lineages continues to occur when there is a failure to comply with infection control measures. For example, a striking demonstration of the continued potential for transmission of the ET12 lineage was its recent acquisition by an Edinburgh CF adult; extensive inquiries suggested that this patient had social contact for only 10 min whilst visiting another CF male who was hospitalised during an episode of *B. cepacia* septicaemia. Even when infection control appears effective in preventing spread of epidemic lineages, new cases of *B. cepacia* colonisation continue to occur with isolates that exhibit unique PCR ribotyping or PFGE profiles. Such sporadic acquisitions raise a fundamental question concerning the source and colonising potential of individual *B. cepacia* strains. For example, does the environment contain a subpopulation of *B. cepacia* clones that are innately primed for human colonisation, or does colonisation and virulence in man require in-vivo adaptation? Future improvements in laboratory identification of *B. cepacia* subpopulations associated with CF disease and identification of transmission factors, in addition to cable pili, may provide scientific justification for relaxation of segregation in the absence of known epidemic and potentially virulent lineages. Turning our attention to CF patients, we need to clarify why colonisation by the same strain of *B. cepacia* leads to variable clinical responses, ranging from asymptomatic colonisation to rapid fatal pulmonary deterioration. It could be argued that this particular problem is not unique to *B. cepacia*, and that applying Koch's postulates in an attempt to distinguish between sycophancy and pathogenic potential is difficult when dealing with any opportunist pathogen. Certainly, host factors cannot be ignored in attempts to understand the pathogenic processes involved in CF lung infections.

During the final preparation of this review, a deceptively simple and elegant study has illustrated how CFTR-associated defective Cl⁻ transport across airway epithelia might lead to bacterial colonisation in CF patients. Smith *et al.* [129] showed that the normal human apical epithelial surface is bactericidal for *P. aeruginosa* and *S. aureus*; in contrast, the bactericidal activity was inhibited reversibly in CF epithelia because of a high NaCl concentration. If this phenomenon varies in individual CF patients—or if individual *B. cepacia* strains differ in susceptibility to the defensin-like bactericidal agent—it might explain some of the host- and pathogen-specific anomalies associated with *B. cepacia* pulmonary infection and suggest novel strategies for infection control and therapy of this unusual and challenging opportunistic pathogen.

It is difficult to avoid a final comment on the irony that whilst *B. cepacia* continues to hold the CF community to ransom, agricultural microbiologists seek to develop the commercial and beneficial potential of this microbial Jekyll and Hyde in their search for biological control agents. This situation demonstrates the diversity of microbiology, but should also encourage attempts to reduce the present gulf between agricultural and medical science.

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Following submission of this review, the results of an epidemiological study of B. cepacia in a large series of CF patients attending the CF centre in Verona were published. Cazzola et al. concluded that their results are difficult to interpret. Nevertheless, data are essential if progress is to be made in unravelling B. cepacia epidemiology, and the results of this study are particularly relevant to the major issues discussed in our review.

Between Nov. 1991 and Dec. 1994, B. cepacia was cultured from 85 (11.0%) of 769 CF patients attending the Verona centre. Based on genomic fingerprinting, 32 (53.3%) patients were colonised by individual B. cepacia strains; the remaining 28 (46.7%) patients were divided into 10 subgroups, each colonised by a distinct strain. As previously encountered with the ET12 lineage, the outcome of B. cepacia colonisation in the Verona study varied from rapidly fatal septicaemia to maintenance of reasonably stable respiratory function, even in patients colonised by the same strain. Cazzola et al. provide further evidence for hypotheses discussed in our review that some B. cepacia strains exhibit and low transmissibility that the environment is a likely source of sporadic new cases: e.g., transmission was observed in only three of eight pairs of CF siblings; in unrelated patients, direct person-to-person transmission was evident in only 10 cases (16.7%); despite a strict segregation policy, whether as in- or out-patients, 15 new colonised patients were identified during 1993. Considering social implications and the paucity of previous data, it was particularly interesting to note that transmission was demonstrated between two unrelated CF schoolmates.