Burkholderia cepacia Complex as Human Pathogens

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Abstract: Although sporadic human infection due to Burkholderia cepacia has been reported for many years, it has been only during the past few decades that species within the B. cepacia complex have emerged as significant opportunistic human pathogens. Individuals with cystic fibrosis, the most common inherited genetic disease in Caucasian populations, or chronic granulomatous disease, a primary immunodeficiency, are particularly at risk of life-threatening infection. Despite advances in our understanding of the taxonomy, microbiology, and epidemiology of B. cepacia complex, much remains unknown regarding specific human virulence factors. The broad-spectrum antimicrobial resistance demonstrated by most strains limits current therapy of infection. Recent research efforts are aimed at a better appreciation of the pathogenesis of human infection and the development of novel therapeutic and prophylactic strategies.

Key words: Burkholderia cepacia, cystic fibrosis, human infection.

Until relatively recently, Burkholderia cepacia had been considered a phytopathogenic or saprophytic bacterial species with little potential for human infection. However, reports of sporadic human infection have appeared in the biomedical literature, generally describing infection in persons with some underlying disease or debilitation (Dailey and Benner, 1968; Poe et al., 1977). Indeed, an early review of one medical center’s experience with B. cepacia infection during the years 1968–1969 indicated that essentially all infections occurred in patients with a chronic disease that predisposed them to opportunistic infection (Ederer and Matsen, 1972). Other reports described “pseudoeipidemics” among hospitalized patients, most often attributed to contamination of disinfectants used in the preparation of blood culture systems (Berkelman et al., 1981; Craven et al., 1981; Sobel et al., 1982). Contamination of antiseptic and anesthetic solutions also has resulted in true nosocomial infection and “mini-epidemics,” particularly in intensive care units (Phillips et al., 1971; Steere et al., 1977).

Chronic Granulomatous Disease: In addition to hospital-acquired infection, persons with certain chronic diseases are susceptible to infection by B. cepacia. Among these disorders is chronic granulomatous disease (CGD). In this inherited primary immunodeficiency disease, white blood cells are unable to kill some bacterial and fungal species after phagocytosis (Winkelstein et al., 2000). The underlying defect is an inability of phagocytic cells to generate superoxide and reactive oxidants that are necessary for intracellular microbiidal activity. As a result of this defect, CGD patients suffer from recurrent life-threatening infections, such as severe pneumonia and bacteremia caused by certain catalase-positive species. The observation that not all catalase-positive bacteria are capable of causing severe infection in CGD suggests that some species, including B. cepacia, possess other factors that remain to be elucidated that also mediate pathogenicity in this condition (Speert et al., 1994). Fortunately, CGD is a relatively rare disease, having an average annual incidence of approximately 1/200,000 live births in the United States; this means there are approximately 20 persons with CGD born each year in the United States.

Cystic fibrosis: Cystic fibrosis (CF) is another inherited disorder in which B. cepacia can cause severe infection (LiPuma 1998a). In contrast to CGD, CF is relatively common. It is, in fact, the most common lethal genetic disorder among Caucasians, affecting approximately 1/2,750 live births. One person in 25 is an asymptomatic carrier. There are currently some 30,000 persons with CF in the United States, and an equal number can be found in Europe. Cystic fibrosis is a multisystem disease that is believed to result primarily from a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-dependent chloride channel. The consequences of this defect are complex (Larson and Cohen, 2000; Zeitlin, 1999), but the resultant altered respiratory epithelial surface fluid in some way predisposes to chronic pulmonary infection. Nearly 1,000 mutations have been identified in the CF gene—the most common being a deletion of phenylalanine at amino acid position 508 (AF508). Despite the presence of this mutation among the majority of persons with CF, there is a wide spectrum of disease severity. Most persons have some degree of respiratory dysfunction and are prone to chronic respiratory tract infection (Dinwiddie, 2000). Common bacterial pathogens in young CF patients include Staphylococcus aureus and Haemophilus influenzae. During adolescence Pseudomonas aeruginosa infection becomes common, and by adulthood nearly 80% of CF patients are chronically infected with P. aeruginosa. Progressive lung deterioration secondary to recurrent or chronic infection is the leading cause of death in CF; the median survival age is approximately 32 years. Nevertheless, it is important to point out that many persons with CF are in relatively good health, infrequently hospitalized, and lead productive and active lives.

History of B. cepacia infection in CF: The first reports of
B. cepacia infection in persons with CF appeared in the late 1970s (Blessing et al., 1979; Laraya-Cuasay et al., 1977). Shortly thereafter, a report described severe pneumonia, sepsis, and death due to B. cepacia in a CF patient (Rosenstein and Hall, 1980), and a study of prophylactic antibiotic use in CF from Toronto in 1982 reported that 45% of enrolled patients were infected with B. cepacia (Nolan et al., 1982). The seminal report by Isles et al. (1984) subsequently described in greater detail the clinical significance of B. cepacia infection in the Toronto CF center. In addition to documenting a steadily increasing prevalence of B. cepacia infection during the previous decade, these investigators described a syndrome of severe progressive respiratory failure with bacteremia that occurred in several patients. Soon thereafter, this so-called “cepacia syndrome” was also described in reports from other North American CF treatment centers that had witnessed similar increases in incidence of B. cepacia infection among their patients (Tablan et al., 1985; Thomassen et al., 1985). A number of subsequent studies further defined the impact of B. cepacia infection in CF and identified several risk factors for infection, including hospitalization and having an infected sibling (Goldmann and Klinger, 1986; Tablan et al., 1987).

Virulence of B. cepacia: Several case-controlled studies have demonstrated an association between infection with B. cepacia and poor prognosis in CF (Brown et al., 1993; Ledson et al., 2002; Lewin et al., 1990; Taylor et al., 1993; Whiteford et al., 1995). In fact, although many individuals may remain infected with B. cepacia for prolonged periods, up to 20% succumb to a rapidly progressive necrotizing pneumonia soon after infection is recognized (Isles et al., 1984; Tablan et al., 1987; Simmonds et al., 1990). Despite this association, the precise role B. cepacia plays in the pathology of CF lung disease is not clear. This uncertainty has fueled speculation that B. cepacia is merely a marker of pulmonary deterioration in a subpopulation of individuals with more severe underlying disease. This hypothesis is challenged by the observations that fatalities have occurred in adults with mild pulmonary disease prior to infection (Govan et al., 1993) and that infection frequently occurs in persons who have had no apparent antecedent decline in lung function (Muhdi et al., 1996).

Unfortunately, the lack of clearly defined virulence factors and the limitations of current models of human infection have precluded a better understanding of the mechanisms by which B. cepacia acts as a human pathogen. Several extracellular products known to contribute to virulence in other bacterial species, including proteases, lipases, siderophores, and hemolysins, have been identified in B. cepacia (Nelson et al., 1994). At least five different classes of pili that may mediate bacterial adherence to respiratory mucins or epithelial cells also have been described (Goldstein et al., 1995). However, the role of these factors in virulence is yet to be firmly established. There is increasing evidence that the lung damage seen with B. cepacia infection results from a marked host inflammatory response (Hughes et al., 1997). For example, B. cepacia lipopolysaccharide is a potent stimulator of neutrophil respiratory burst responses and induces significantly more production of tumor necrosis factor alpha from monocytes in vitro than does lipopolysaccharide from P. aeruginosa (Shaw et al., 1995). The ability of B. cepacia to invade and survive within respiratory epithelial cells (Chiu et al., 2001; Keig et al., 2001; Martin and Mohr, 2000) and resist intracellular killing by phagocytic cells (Saini et al., 1999) may play a role in evasion of host immune response and persistence of infection. Finally, N-acylhomoserine lactone-dependent quorum-sensing systems that most likely regulate biofilm production by B. cepacia in vivo have been described (Gotschlich et al., 2001; Lewenza et al., 1999).

Antimicrobial resistance: The broad-spectrum antibiotic resistance demonstrated by most strains of B. cepacia severely limits effective therapy of human infection. In fact, identification of strains resistant to all currently available antibiotics, particularly in CF patients, frequently renders infection refractory to antimicrobial therapy. The sparse phosphorylation of B. cepacia lipopolysaccharide is believed to be responsible for intrinsic resistance to polycationic peptides including aminoglycoside antibiotics (Hancock, 1998). Inducible chromosomal β-lactamases are present in the majority of strains (Chiesa et al., 1986) as are antibiotic efflux pumps that mediate resistance to chloramphenicol, quinolone antibiotics, and trimethoprim (Burns et al., 1996). Altered dihydrofolate reductase is yet another mechanism by which some strains may exhibit trimethoprim resistance (Burns et al., 1989).

Taxonomy and clinical microbiology: Although B. cepacia was described 50 years ago (Burkholder, 1950), the complex taxonomy of this and closely related species was not fully appreciated until recently. Originally designated Pseudomonas cepacia, this species, along with several others (including the closely related P. gladioli), was placed in Pseudomonas RNA homology group II (Palleroni et al., 1973). Based on subsequent molecular analyses that demonstrated significant differences with other pseudomonads, this entire group became members of the new genus Burkholderia in 1992 (Yabuuchi et al., 1992).

More recently, Vandamme et al. (1997) employed a polyphasic approach including whole-cell protein and fatty acid analyses together with DNA-DNA and DNA:rRNA hybridization to demonstrate several distinct species among presumed B. cepacia isolates recovered from CF sputum culture. Initially, five genomic species (genovars) were identified and collectively referred to as the “B. cepacia complex.” During the past few years four additional species have been described that are also considered members of this group. (A more com-
Accurate identification of *B. cepacia* complex species may be problematic. Misidentification is relatively common and likely results from the taxonomic complexity described previously. In recent studies employing polymerase chain reaction (PCR)-based analyses, approximately 10% of putative *B. cepacia* isolates referred from clinical microbiology laboratories had been misidentified based on phenotypic assessment alone (McMenamin et al., 2000; Shelly et al., 2000). The use of selective media, including TB-T (Hagedorn et al., 1987), PC agar (Gilligan et al., 1985), and OFPBL (Welch et al., 1987), which take advantage of these species’ broad antibiotic resistance, is important in recovery of *B. cepacia* complex from clinical specimens. However, these media may allow the growth of other related bacteria such as *B. gladioli*, *Alcaligenes* spp., *Comamonas* spp., *Flavobacterium* spp., and *Stenotrophomonas* maltophilia. A more recently described medium, *B. cepacia* selective agar (BCSA), is better able to inhibit related species while supporting the growth of all *B. cepacia* strains examined (Henry et al., 1997, 1999). Commercial test systems specifically developed for the identification of gram-negative, non-fermenting bacilli offer another important adjunct in identification, but these do not always yield unequivocal results (Kiska et al., 1996; Shelly et al., 2000). A number of PCR-based assays targeting *B. cepacia* complex species-specific 16S rDNA or *recA* gene sequences have been developed (Bauernfeind et al., 1999; LiPuma et al., 1999; Mahenthiralingam et al., 2000) and provide the most accurate tools in current identification schemes (Coenye et al., 2001).

**Epidemiology of B. cepacia complex infection:** During the 1980s, the clustering of *B. cepacia* infection at some CF treatment centers with the sparing of others, and the dramatic reduction in incidence of infection after institution of strict infection-control measures (Thomassen et al., 1986), suggested nosocomial acquisition or person-to-person transmission of *B. cepacia*. Studies employing isolate ribotyping analysis demonstrated that, within several CF treatment centers, the majority of *B. cepacia*-colonized patients harbored the same strain (LiPuma et al., 1988). Inter-patient spread of *B. cepacia* was documented in 1990 (LiPuma et al., 1990), and a number of studies since have provided compelling evidence of person-to-person transmission of *B. cepacia* through nosocomial and social contact (LiPuma, 1999b).

More recent studies have applied a variety of genotyping methods including random amplified polymorphic DNA (RAPD) typing, pulsed field gel electrophoresis (PFGE), and repetitive extragenic palindromic PCR (rep-PCR) typing to further investigate the epidemiology of *B. cepacia* complex infection in CF. These efforts confirm that patients receiving care in the same CF treatment center are frequently infected with the same so-called “epidemic” *B. cepacia* complex strain. In fact, in one center the same genomovar III strain, termed PHDC, has been recovered from the majority of infected patients for the past 20 years (Chen et al., 2001). This endemicty was punctuated by the spread of this strain between CF treatment centers in two cities, presumably via re-location of an infected patient.

Bacterial features specific for *B. cepacia* complex strains with an apparent enhanced capacity for human infection or transmission have been sought. Mahenthiralingam et al. (1997) found that several strains recovered from multiple patients contained a conserved 1.4-kb genomic fragment not found in strains recovered from single patients. This fragment, termed the *B. cepacia* epidemic strain marker (BCESM), encodes an approximately 834-bp open reading frame, *esmR*, with homology to negative transcriptional regulators; however, the role of this putative gene in virulence remains unknown. ET12, a genomovar III strain that dominates among CF patients in Ontario, Canada, and is associated with inter-patient spread in the United Kingdom, has been the most completely studied epidemic lineage. In addition to *esmR*, this strain elaborates large peritrichous pili, termed cable pili. The gene encoding cable pili, *cblA*, has been characterized, as has the epithelial cell receptor for the cable pili associated adhesin (Sajjan et al., 1995, 2000). Although *cblA*-bearing ET12 are common among CF patients in Canada and the United Kingdom, a recent study of *B. cepacia* complex isolates recovered from more than 600 United States CF patients demonstrated that only one contained the complete *cblA* sequence (LiPuma et al., 2001). Strain PHDC (described above) contains neither *esmR* nor *cblA* sequences (Chen et al., 2001). Therefore, while having potential roles in the virulence of some epidemic strains, the presence of these markers clearly is not essential in all epidemic lineages.

**Distribution of B. cepacia complex species:** The appreciation that several distinct species comprise bacteria previously identified merely as *B. cepacia* has provided an opportunity to reassess the natural history and epidemiology of “*B. cepacia*” infection in CF. In the study noted above, *B. cepacia* complex isolates from 606 CF patients receiving care at 132 treatment centers in 105 cities in the United States were assessed to determine species distribution within the *B. cepacia* complex. Isolates were also examined for the presence of *esmR* and *cblA* (LiPuma et al., 2001). Fifty percent of patients were infected with *B. cepacia* complex genomovar III, 38% with *B. multivorans* (genomovar II), and 5% with *B. vietnamiensis* (genomovar V); fewer than 5% of patients were infected with either genomovar I, *B. stabilis* (genomovar IV), genomovar VI, *B. ambifaria* (genomovar VII), *B. anthina* (genomovar VIII), or *B. pyrrocinia* (genomovar IX). The *esmR* locus was found in 46% of
genomovar III isolates and not in any other species. Only one isolate, from a patient infected with the ET12 epidemic lineage, contained the complete cblA pilin subunit gene.

Recent studies from Canada (Speert et al., 2002) and Italy (Agodi et al., 2001) similarly demonstrate the dominance of genomovar III among B. cepacia complex-infected CF patients. In addition, most strains described to date as being involved in inter-patient spread are genomovar III. However, multiple patients infected with the same B. multivorans strain have been found (Segonds et al., 1999), and the “epidemic” strain involved in the first description of inter-patient spread of B. cepacia is now known, in fact, to be genomovar VI (LiPuma et al., 1990, 1994). Whether these differences in epidemiology translate into differences in virulence, per se, remains to be determined. Recent observations among CF patients undergoing lung transplantation have demonstrated substantially greater rates of postoperative mortality among persons infected with genomovar III compared with other B. cepacia complex species (Aris et al., 2001; DeSoysa et al., 2001). Nevertheless, bacteremia and death among CF patients infected with non-genomovar III species certainly occurs (unpubl. obs.).

In summary, these data indicate that although all nine species currently constituting the B. cepacia complex are capable of causing infection in CF, their distribution is quite disproportionate, suggesting a differential capacity for human infection among these phylogenetically closely related species. The low frequency of esmR and cblA indicates that they are not sufficient markers of B. cepacia complex virulence or transmissibility in human infection.

Conclusions

Although species of the B. cepacia complex are generally not pathogenic for healthy humans, sporadic human infection and outbreaks among debilitated hospitalized patients have been recognized for many years. More importantly, for reasons that remain to be elucidated, persons with certain underlying disorders, particularly CGD and CF, are susceptible to life-threatening infection. In both conditions infection can result in acute illness and death or remain chronic for many years. Unfortunately, effective therapy is severely limited by the inherent broad-spectrum antibiotic resistance exhibited by most strains.

Comprehensive taxonomic studies that have defined several closely related species within the B. cepacia complex provide a critical platform for further study of the pathogenesis, epidemiology, and natural history of human infection due to “B. cepacia.” Within this context, recent investigation indicates a high rate of misidentification of B. cepacia complex species based on phenotype alone. Recent work also indicates that although all

B. cepacia complex species are capable of causing infection, some (i.e., B. multivorans and genomovar III) are much more frequently involved than are others. Furthermore, some specific strains, especially within genomovar III, seem to possess a particular predilection for human infection and/or person-to-person transmission. Ongoing study is aimed at defining the specific human features and bacterial virulence factors involved. Such studies are prerequisites for the development of novel therapeutic and preventive strategies.

Literature Cited


