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Burkholderia cepacia Complex as Human Pathogens[1]

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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 "pseudoepidemics" 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 "miniepidemics," 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 microbicidal 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 (ΔF508). 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

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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 (genomovars) 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-

plete description of the taxonomy of the *B. cepacia* complex is provided elsewhere in these proceedings—Vandamme and Mahenthiralingam (2002) see also the review by Coenye et al. (2001).

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, 1998b).

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 endemicity 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; DeSoyza 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

Agodi, A., E. Mahenthiralingam, M. Barchitta, V. Giannino, A. Sciacca, and S. Stefani. 2001. *Burkholderia cepacia* complex infection in Italian patients with cystic fibrosis: Prevalence, epidemiology, and genomovar status. Journal of Clinical Microbiology 39:2891–2896.

Aris, R. M., J. Routh, J. J. LiPuma, D. Heath, and P. H. Gilligan. 2001. *Burkholderia cepacia* complex in cystic fibrosis patients after lung transplantation: Survival linked to genomovar type. American Journal of Respiratory and Critical Care Medicine 164:2102–2106.

Bauernfeind, A., I. Schneider, R. Jungwirth, and C. Roller. 1999. Discrimination of *Burkholderia multivorans* and *Burkholderia vietnamiensis* from *Burkholderia cepacia genomovars I, III,* and *IV* by PCR. Journal of Clinical Microbiology 37:1335–1339.

Berkelman, R. L., S. Lewin, J. R. Allen, R. L. Anderson, L. D. Budnick, S. Shapiro, S. M. Friedman, P. Nicholas, R. S. Holzman, and R. W. Haley. 1981. Pseudobacteremia attributed to contamination of povidone-iodine with *Pseudomonas cepacia.* Annals of Internal Medicine 95:32–36.

Blessing, J., J. Walker, and B. Maybury. 1979. *Pseudomonas cepacia* and maltophilia in the cystic fibrosis patient. American Review of Respiratory Diseases Supplement 119:262.

Brown, P., S. Butler, and J. Nelson. 1993. *Pseudomonas cepacia* in adult cystic fibrosis: Accelerated decline in lung function and increased mortality. Thorax 48:425.

Burkholder, W. H. 1950. Sour skin, a bacterial rot of onion bulbs. Phytopathology 40:115–118.

Burns, J. L., D. M. Lien, and L. A. Hedin. 1989. Isolation and characterization of dihydrofolate reductase from trimethoprim-susceptible and trimethoprim-resistant *Pseudomonas cepacia.* Antimicrobial Agents and Chemotherapy 33:1247–1251.

Burns, J. L., C. D. Wadsworth, J. J. Barry, and C. P. Goodall. 1996. Nucleotide sequence analysis of a gene from *Burkholderia (Pseudomonas) cepacia* encoding an outer membrane lipoprotein involved in multiple antibiotic resistance. Antimicrobial Agents and Chemotherapy 40:307–313.

Chen, J. S., K. A. Witzmann, T. Spilker, R. J. Fink, and J. J. LiPuma. 2001. Endemicity and inter-city spread of *Burkholderia cepacia* genomovar III in cystic fibrosis. Journal of Pediatrics 139:643–649.

Chiesa, C., P. H. Labrozzi, and S. C. Aronoff. 1986. Decreased baseline beta-lactamase production and inducibility associated with increased piperacillin susceptibility of *Pseudomonas cepacia* isolated from children with cystic fibrosis. Pediatric Research 20:1174–1177.

Chiu, C.-H., S. Wong, R. E. W. Hancock, and D. P. Speert. 2001. Adherence of *Burkholderia cepacia* to respiratory tract epithelial cells and inhibition with dextrans. Microbiology 147:2651–2658.

Coenye, T., P. Vandamme, J. R. W. Govan, J. J. LiPuma. 2001. Taxonomy and identification of the *Burkholderia cepacia* complex. Journal of Clinical Microbiology 39:3427–3436.

Craven, D. E., B. Moody, M. G. Connolly, N. R. Kollisch, K. D. Stottmeier, and W. R. McCabe. 1981. Pseudobacteremia caused by povidone-iodine solution contaminated with *Pseudomonas cepacia.* New England Journal of Medicine 305:621–623.

Dailey, R. H., and E. J. Benner, E. J. 1968. Necrotizing pneumonitis due to the pseudomonad "eugonic oxidizer-group 1." New England Journal of Medicine 279:361.

DeSoyza, A., A. McDowell, L. Archer, J. H. Dark, S. J. Elborn, E. Mahenthiralingam, K. Gould, and P. A. Corris. 2001. *Burkholderia ce-*

pacia complex genomovars and pulmonary transplantation outcomes in patients with cystic fibrosis. Lancet 358:1780–1781.

Dinwiddie, R. 2000. Pathogenesis of lung disease in cystic fibrosis. Respiration 67:3–8.

Ederer, G. M., and J. M. Matsen. 1972. Colonization and infection with *Pseudomonas cepacia.* Journal of Infectious Diseases 125:613–618.

Gilligan, P. H., P. A. Gage, L. M. Bradshaw, D. V. Schidlow, and B. T. DeCicco. 1985. Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis. Journal of Clinical Microbiology 22:5–8.

Goldmann, D. A., and J. D. Klinger. 1986. *Pseudomonas cepacia:* Biology, mechanisms of virulence, epidemiology. Journal of Pediatrics 108:806–812.

Goldstein, R., L. Sun, R. Z. Jiang, U. Sajjan, J. F. and Forstner, and C. Campanelli. 1995. Structurally variant classes of pilus appendage fibers coexpressed from *Burkholderia (Pseudomonas) cepacia.* Journal of Bacteriology 177:1039–1052.

Gotschlich, A., B. Huber, O. Geisenberger, A. Togl, A. Steidle, K. Riedel, P. Hill, B. Tummler, P. Vandamme, B. Middleton, M. Camara, P. Williams, A. Hardman, and L. Eberl. 2001. Synthesis of multiple N-acylhomoserine lactones is widespread among the members of the *Burkholderia cepacia* complex. Systematic and Applied Microbiology 24:1–14.

Govan, J. R., P. H. Brown, J. Maddison, C. J. Doherty, J. W. Nelson, M. Dodd, A. P. Greening, and A. K. Webb. 1993. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. Lancet 342:15–19.

Hagedorn, C., W. D. Gould, T. R. Bardinelli, and D. R. Gustavson, D. R. 1987. A selective medium for enumeration and recovery of *Pseudomonas cepacia* biotypes from soil. Applied and Environmental Microbiology 53:2265–2268.

Hancock, R. E. 1998. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. Clinical Infectious Diseases 27 (Suppl 1):S93–S99.

Henry, D. A., M. E. Campbell, J. J. LiPuma, and D. P. Speert. 1997. Identification of *Burkholderia cepacia* isolates from patients with cystic fibrosis and use of a simple new selective medium. Journal of Clinical Microbiology 35:614–619.

Henry, D., M. Campbell, C. McGimpsey, A. Clarke, L. Louden, J. L. Burns, M. H. Roe, P. Vandamme, and D. Speert. 1999. Comparison of isolation media for recovery of Burkholderia cepacia complex from respiratory secretions of patients with cystic fibrosis. Journal of Clinical Microbiology 37:1004–1007.

Hughes, J. E., J. Stewart, G. R. Barclay, and J. R. Govan. 1997. Priming of neutrophil respiratory burst activity by lipopolysaccharide from *Burkholderia cepacia.* Infection and Immunity 65:4281–4287.

Isles, A., I. Maclusky, M. Corey, R. Gold, C. Prober, P. Fleming, and H. Levison. 1984. *Pseudomonas cepacia* infection in cystic fibrosis: An emerging problem. Journal of Pediatrics 104:206–210.

Keig, P. M., E. Ingham, and K. G. Kerr. 2001. Invasion of human type II pneumocytes by *Burkholderia cepacia.* Microbial Pathogenesis 30:167–170.

Kiska, D. L., A. Kerr, M. C. Jones, J. A. Caracciolo, B. Eskridge, M. Jordan, S. Miller, D. Hughes, N. King, and P. H. Gilligan. 1996. Accuracy of four commercial systems for identification of *Burkholderia cepacia* and other gram-negative nonfermenting bacilli recovered from patients, with cystic fibrosis. Journal of Clinical Microbiology 34:886–891.

Laraya-Cuasay, L. R., M. Lipstein, and N. N. Huang. 1977. *Pseudomonas cepacia* in the respiratory flora of patients with cystic fibrosis. Pediatric Pulmonology 11:502 (Abstr.).

Larson, J. E., and J. C. Cohen. 2000. Cystic fibrosis revisited. Molecular Genetics and Metabolism 71:470–477.

Ledson, M. J., M. J. Gallagher, M. Jackson, C. A. Hart, and M. J. Walshaw. 2002. Outcome of *Burkholderia cepacia* colonization in an adult cystic fibrosis centre. Thorax 57:142–145.

Lewenza, S., B. Conway, E. P. Greenberg, and P. A. Sokol. 1999. Quorum sensing in *Burkholderia cepacia:* Identification of the LuxRI homologs CepRI.. Journal of Bacteriology 181:748–756.

Lewin, L. O., P. J. Byard, and P. B. Davis. 1990. Effect of *Pseudomonas cepacia* colonization on survival and pulmonary function of cystic fibrosis patients. Journal of Clinical Epidemiology 43:125–131.

LiPuma, J. J. 1998a. *Burkholderia cepacia.* Management issues and new insights. Clinics in Chest Medicine 19:473–486.

LiPuma, J. J. 1998b. *Burkholderia cepacia* epidemiology and pathogenesis: Implications for infection control. Current Opinion in Pulmonary Medicine 4:337–341.

LiPuma, J. J., S. E. Dasen, D. W. Nielson, R. C. Stern, and T. L. Stull. 1990. Person-to-person transmission of *Pseudomonas cepacia* between patients with cystic fibrosis. Lancet 336:1094–1096.

LiPuma, J. J., B. J. Dulaney, J. D. McMenamin, P. W. Whitby, T. L. Stull, T. Coenye, and P. Vandamme. 1999. Development of rRNA-based PCR assays for identification of *Burkholderia cepacia* complex isolates recovered from cystic fibrosis patients. Journal of Clinical Microbiology 37:3167–3170.

LiPuma, J. J., K. A. Marks-Austin, D. S. Holsclaw, Jr., G. B. Winnie, P. H. Gilligan, and T. L. Stull. 1994. Inapparent transmission of *Pseudomonas (Burkholderia) cepacia* among patients with cystic fibrosis. Pediatric Infectious Disease Journal 13:716–719.

LiPuma, J. J., J. E. Mortensen, S. E. Dasen, T. D. Edlind, D. V. Schidlow, J. L. Burns, and T. L. Stull. 1988. Ribotype analysis of *Pseudomonas cepacia* from cystic fibrosis treatment centers. Journal of Pediatrics 113:859–862.

LiPuma, J. J., T. Spilker, L. H. Gill, P. W. Campbell III, L. Liu, and E. Mahenthiralingam. 2001. Disproportionate distribution of *Burkholderia cepacia* complex species and transmissibility markers in cystic fibrosis. American Journal of Respiratory and Critical Care Medicine 164:92–96.

Mahenthiralingam, E., J. Bischof, S. K. Byrne, C. Radomski, J. E. Davies, Y. Av-Gay, and P. Vandamme. 2000. DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis, Burkholderia multivorans, Burkholderia stabilis,* and *Burkholderia cepacia* genomovars I and III. Journal of Clinical Microbiology 38:3165–3173.

Mahenthiralingam, E., D. A. Simpson, and D. P. Speert. 1997. Identification and characterization of a novel DNA marker associated with epidemic *Burkholderia cepacia* strains recovered from patients with cystic fibrosis. Journal of Clinical Microbiology 35:808–816.

Martin, D. W., and C. D. Mohr. 2000. Invasion and intracellular survival of *Burkholderia cepacia.* Infection and Immunity 68:24–29.

McMenamin, J. D., T. M. Zaccone, T. Coenye, P. Vandamme, and J. J. LiPuma. 2000. Misidentification of *Burkholderia cepacia* in U.S. cystic fibrosis treatment centers: An analysis of 1,051 recent sputum isolates. Chest 117:1611–1615.

Muhdi, K., F. P. Edenborough, L. Gumery, S. O'Hickey, E. G. Smith, D. L. Smith, and D. E. Stableforth. 1996. Outcome for patients colonized with *Burkholderia cepacia* in a Birmingham adult cystic fibrosis clinic and the end of an epidemic. Thorax 51:374–377.

Nelson, J. W., S. L. Butler, D. Krieg, and J. R. Govan. 1994. Virulence factors of *Burkholderia cepacia.* FEMS Immunology and Medical Microbiology 8:89–97.

Nolan, G., P. Moivor, H. Levison, P. C. Fleming, M. Corey, and R. Gold. 1982. Antibiotic prophylaxis in cystic fibrosis: Inhaled cephaloridine as an adjunct to oral cloxacillin. Journal of Pediatrics 101: 626–630.

Palleroni, N. J., R. Kunisawa, and R. Contopoulou. 1973. Nucleid acid homologies in the genus *Pseudomonas.* International Journal of Systematic Bacteriology 23:333.

Phillips, I., S. Eykyn, M. A. Curtis, and J. J. Snell. 1971. *Pseudomonas cepacia (multivorans)* septicemia in an intensive-care unit. Lancet 1: 375–377.

Poe, R. H., H. R. Marcus, and G. L. Emerson. 1977. Lung abscess due to *Pseudomonas cepacia.* American Review of Respiratory Diseases 115:861–865.

Rosenstein, B. J., and D. E. Hall. 1980. Pneumonia and septicemia due to *Pseudomonas cepacia* in a patient with cystic fibrosis. Johns Hopkins Medical Journal 147:188–189.

Saini, L. S., S. B. Galsworthy, M. A. John, and M. A. Valvano. 1999. Intracellular survival of *Burkholderia cepacia* complex isolates in the presence of macrophage cell activation. Microbiology 145:3465–3475.

Sajjan, U. S., L. Sun, R. Goldstein, and J. F. Forstner. 1995. Cable (cb1) type II pili of cystic fibrosis-associated *Burkholderia (Pseudomonas) cepacia:* Nucleotide sequence of the cb1A major subunit pilin gene and novel morphology of the assembled appendage fibers. Journal of Bacteriology 177:1030–1038.

Sajjan, U. S., F. A. Sylvester, and J. F. Forstner. 2000. Cable-piliated *Burkholderia cepacia* binds to cytokeratin 13 of epithelial cells. Infection and Immunity 68:1787–1795.

Segonds, C., T. Heulin, N. Marty, and G. Chabanon. 1999. Differentiation of *Burkholderia* species by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene and application to cystic fibrosis isolates. Journal of Clinical Microbiology 37:2201–2208.

Shaw, D., I. R. Poxton, and J. R. Govan. 1995. Biological activity of *Burkholderia (Pseudomonas) cepacia* lipopolysaccharide. FEMS Immunology and Medical Microbiology 11:99–106.

Shelly, D. B., T. Spilker, E. J. Gracely, T. Coenye, P. Vandamme, and J. J. LiPuma. 2000. Utility of commercial systems for identification of *Burkholderia cepacia* complex from cystic fibrosis sputum culture. Journal of Clinical Microbiology 38:3112–3115.

Simmonds, E. J., S. P. Conway, A. T. Ghoneim, H. Ross, and J. M. Littlewood. 1990. *Pseudomonas cepacia:* A new pathogen in patients with cystic fibrosis referred to a large centre in the United Kingdom. Archives of Disease in Childhood 65:874–877.

Sobel, J. D., N. Hashman, G. Reinherz, and D. Merzbach. 1982. Nosocomial *Pseudomonas cepacia* infection associated with chlorhexidine contamination. American Journal of Medicine 73:183–186.

Speert, D. P., M. Bond, R. C. Woodman, and J. T. Curnutte. 1994. Infection with *Pseudomonas cepacia* in chronic granulomatous disease: Role of nonoxidative killing by neutrophils in host defense. Journal of Infectious Disease 170:1524–1531.

Speert, D. P., D. Henry, P. Vandamme, M. Corey, and E. Mahenthiralingam. 2002. Epidemiology of *Burkholderia cepacia* complex in patients with cystic fibrosis, Canada. Emerging Infectious Diseases 8:181–187.

Steere, A. C., J. H. Tenney, D. C. Mackel, M. J. Snyder, S. Polakavetz, M. E. Dunne, and R. Dixon. 1977. Pseudomonas species bacteremia caused by contaminated normal human serum albumin. Journal of Infectious Diseases 135:729–735.

Tablan, O. C., T. L. Chorba, D. V. Schidlow, J. W. White, K. A. Hardy, P. H. Gilligan, W. M. Morgan, L. A. Carson, W. J. Martone, and J. M. Jason. 1985. *Pseudomonas cepacia* colonization in patients with cystic fibrosis: Risk factors and clinical outcome. Journal of Pediatrics 107:382–387.

Tablan, O. C., W. J. Martone, C. F. Doershuk, R. C. Stern, M. J. Thomassen, J. D. Klinger, J. W. White, L. A. Carson, and W. R. Jarvis. 1987. Colonization of the respiratory tract with *Pseudomonas cepacia* in cystic fibrosis. Risk factors and outcomes. Chest 91:527–532.

Taylor, R. F., H. Gaya, and M. E. Hodson. 1993. *Pseudomonas cepacia:* Pulmonary infection in patients with cystic fibrosis. Respiratory Medicine 87:187–192.

Thomassen, M. J., C. A. Demko, C. F. Doershuk, R. C. Stern, and J. D. Klinger. 1986. *Pseudomonas cepacia:* Decrease in colonization in patients with cystic fibrosis. American Review of Respiratory Diseases 134:669–671.

Thomassen, M. J., C. A. Demko, J. D. Klinger, and R. C. Stern. 1985. *Pseudomonas cepacia* colonization among patients with cystic fibrosis: A new opportunist. American Review of Respiratory Diseases 131:791–796.

Vandamme, P., B. Holmes, M. Vancanneyt, T. Coenye, B. Hoste, R. Coopman, H. Revets, S. Lauwers, M. Gillis, K. Kersters, J. R. W. Govan. 1997. Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. International Journal of Systematic Bacteriology 47:1188–1200.

Vandamme, P., and E. Mahenthiralingam. 2002. Strains from the *Burkholderia cepacia* complex: Relationship to opportunistic pathogens. Journal of Nematology.

Welch, D. F., M. J. Muszynski, C. H. Pai, M. J. Marcon, M. M. Hribar, P. H. Gilligan, J. M. Matsen, P. A. Ahlin, B. C. Hilman, and S. A. Chartrand. 1987. Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. Journal of Clinical Microbiology 25:1730–1734.

Whiteford, M. L., J. D. Wilkinson, J. H. McColl, F. M. Conlon, J. R. Michie, T. J. Evans, and J. Y. Paton. 1995. Outcome of *Burkholderia (Pseudomonas) cepacia* colonization in children with cystic fibrosis following a hospital outbreak. Thorax 50:1194–1198.

Winkelstein, J. A., M. C. Marino, R. B. Johnston, Jr., J. Boyle, J. Curnutte, J. L. Gallin, H. L. Malech, S. M. Holland, H. Ochs, P. Quie, R. H. Buckley, C. B. Foster, S. J. Chanock, and H. Dickler. 2000. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore) 79:155–169.

Yabuuchi, E., Y. Kosako, H. Oyaizu, I. Yano, H. Hotta, Y. Hashimoto, T. Ezaki, and M. Arakawa. 1992. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia.* Microbiology and Immunology 36:1251–1275.

Zeitlin, P. L. 1999. Novel pharmacologic therapies for cystic fibrosis. Journal of Clinical Investigations 103:447–452.