MOLECULAR EPIDEMIOLOGY OF \textit{BURKHOLDERIA} SPECIES

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1. ABSTRACT

Although most species in the genus \textit{Burkholderia} are not pathogenic for healthy persons, a few are capable of causing severe, life threatening infection. \textit{B. mallei} and \textit{B. pseudomallei} are the causative agents of glanders and melioidosis, respectively. Interest in these species has increased recently owing to their potential for use as agents of bioterrorism. \textit{B. cepacia} emerged during the past two decades as an important opportunistic pathogen among persons with certain underlying diseases. Persons with chronic granulomatous disease, a primary immunodeficiency, or cystic fibrosis (CF), the most common lethal inherited disorder in Caucasians, are at particular risk. In CF, respiratory tract infection may be chronic or associated with a rapid deterioration in pulmonary function. Studies in the early 1990s utilized a variety of genotyping techniques to provide compelling evidence of person-to-person transmission of \textit{B. cepacia} among CF patients. This prompted the institution of rigorous infection control measures that have placed a heavy burden on persons with CF. More recent work has demonstrated that several distinct bacterial species actually exist among bacteria previously identified merely as \textit{B. cepacia}. How these species, collectively referred to as the \textit{B. cepacia} complex, differ with respect to their epidemiology, natural history, and pathology in CF is the subject of ongoing investigation.

2. INTRODUCTION

The genus \textit{Burkholderia} consists of several bacterial species, most of which are soil commensals and phytopathogens that rarely cause human infection (1). There are, however, notable exceptions. \textit{B. mallei} is the causative agent of glanders, an acute infection characterized by either pneumonia and necrosis of the tracheobronchial tree if the organism is inhaled, or pustular skin lesions, multiple abscesses, and sepsis if skin is the portal of entry (2). \textit{B. pseudomallei}, the causative agent of melioidosis, is endemic in northern Australia and eastern Asia; in Thailand it is a major cause of morbidity and mortality, responsible for approximately one-fifth of all community-acquired septicemias (3). Interest in \textit{B. mallei} and \textit{B. pseudomallei} has increased recently, as these are agents with potential for use in bioterrorism (http://www.bt.cdc.gov/Agent).

The \textit{B. cepacia} complex is a subset of several closely related, yet distinct, genomic species (or
Burkholderia sp.

genomovars) within the genus *Burkholderia* (1). Although generally not pathogenic for healthy persons, these are opportunistic human pathogens, capable of causing chronic and often severe infection in certain vulnerable populations. Persons with chronic granulomatous disease, a primary immune deficiency, or cystic fibrosis (CF), the most common inherited lethal disease in Caucasians, are particularly susceptible (4,5). The broad-spectrum antibacterial resistance demonstrated by most isolates severely limits therapeutic options. The observation that certain *B. cepacia* complex strains may be spread among CF patients has driven the institution of stringent infection control measures that now place a severe financial and psychosocial burden on CF care (6).

At the same time that *B. cepacia* complex species emerged as human pathogens, they have also gained attention for their potential commercial use as biocontrol agents useful in both agriculture and bioremediation programs (7,8). This has stimulated additional efforts to better understand the taxonomy of the genus *Burkholderia* and the epidemiology of human infection due to these organisms.

This review will provide an overview of the epidemiology of *Burkholderia* species as agents of human disease. The primary focus will be on the *B. cepacia* complex and infection in persons with CF. Brief mention will also be made of the other *Burkholderia* species that are capable of causing human infection. Additional information regarding *B. cepacia* complex infection in CF can be found in several recent reviews (4,5,6,9,10), as can updates on *B. cepacia* complex taxonomy and identification (1), cellular aspects of infection (11), antimicrobial resistance (12) and risk assessment of biological control strains (13). Up-to-date information regarding *B. cepacia* can also be found on the website of the International *Burkholderia cepacia* Working Group, http://go.to/cepacia.

3. TAXONOMY OF BURKHOLDERIA SPECIES

*Pseudomonas cepacia* was originally described by Burkholder in 1950 as the causative agent of bacterial rot of onion bulbs (14). Since then, however, the taxonomy of the genus *Pseudomonas* has changed dramatically (reviewed in reference 15). Due to its broad and vague phenotypic definition, many incompletely characterized, polarly flagellated, rod-shaped, aerobic, Gram-negative bacteria were initially placed in the genus *Pseudomonas*. However, rRNA-DNA hybridization analyses during the early 1970s demonstrated considerable genetic diversity among members of this genus, which was thus divided into five so-called rRNA homology groups (16). Subsequent genotypic analyses confirmed that these five groups were actually only distantly related to each other. Consequently, the genuine genus *Pseudomonas* was restricted solely to homology group I, containing the type species, *P. aeruginosa* (17). The seven species belonging to rRNA homology group II (*P. solanacearum, P. pectinii, P. cepacia, P. gladioli, P. mallei, P. pseudomallei* and *P. caryophyllii*) were transferred to the novel genus *Burkholderia* in 1992 (18). This genus resides in rRNA superfamily III *sensu* De Ley (19) or subgroup Beta-3 of the Beta-Proteobacteria *sensu* Woese (20). In recent years, the number of species included in this genus has increased dramatically (reviewed in reference 1); there are currently 24 validly described *Burkholderia* species.

During the past few years, several polyphasic taxonomic studies by Vandamme and colleagues (21-25) have indicated that strains identified as *B. cepacia* based primarily on phenotypic analysis actually represent a complex of several closely related genomic species or genomovars. This group, collectively referred to as the *B. cepacia* complex, currently consists of nine species including, *B. cepacia* genomovar I, *Burkholderia multivorans* (genomovar II), *B. cepacia* genomovar III, *Burkholderia stabilis* (genomovar IV), *Burkholderia vietnamiensis* (genomovar V), *B. cepacia* genomovar VI, *Burkholderia ambifaria* (genomovar VII), *Burkholderia anthina* (genomovar VIII) and *Burkholderia pyrrocinia* (genomovar IX).

4. METHODS FOR EPIDEMIOLOGIC STUDY OF BURKHOLDERIA

4.1. Molecular epidemiological tools

Numerous typing methods have been used during the past 30 years to establish relationships between *Burkholderia* strains. Initially, these included phenotypic methods, such as serotyping, antimicrobial susceptibility typing, phage typing, and bacteriocin typing (26-28). In general, these methods suffered from poor reproducibility and/or relatively low discriminatory power. In a comparative study of typing methods in 1989, Rabkin et al found that several phenotypic methods were inferior to chromosomal analysis in confirming epidemiological relatedness among 101 *B. cepacia* isolates (27). Thus, in recent years, phenotypic methods have been largely replaced by genotypic methods (28-30).

Among the genotyping methods that have been applied to *B. cepacia* are ribotyping, pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) typing, and various PCR-based techniques that target repetitive elements (so-called ERIC, REP or BOX sequences). In ribotyping, chromosomal restriction fragment length polymorphisms (RFLP) are detected by probing restriction digested genomic DNA with rRNA sequences. In ribotyping, chromosomal restriction fragment length polymorphisms (RFLP) are detected by probing restriction digested genomic DNA with rRNA genes. A modification of ribotyping, referred to as PCR-ribotyping, employs PCR to amplify the 16S-23S intergenic spacer region of the bacterial rRNA operon to detect sequence length polymorphisms therein (33). Both ribotyping and PCR-ribotyping were used in early investigations of *B. cepacia* epidemiology (34-37), but have since been replaced by methods with higher discriminatory power.

Macrorestriction digestion of chromosomal DNA followed by PFGE, now considered by many to be the gold standard in bacteriological typing (28,30,38), has been applied to numerous studies of the molecular epidemiology of *B. cepacia*. The data are generally reproducible and
Burkholderia sp.

portable between laboratories. The major drawbacks of this method, however, are that it is time-consuming and requires specialized equipment. PCR-based fingerprinting using short random primers (RAPD) or primers directed against repetitive elements (ie, ERIC, REP or BOX-sequences) in the bacterial genome (28-30,39,40) are increasingly being used as alternatives to PFGE for the typing of bacterial pathogens in general, and B. cepacia in particular.

4.2. Analysis of population structure

Most of the methods described above are well suited to compare relatively small sets of isolates obtained during outbreak investigations; their utility for answering questions regarding long-term epidemiology or population structure, however, is limited (41,42). To date, the tool most used in studies addressing these questions has been multilocus enzyme electrophoresis (MLEE). In this method allelic variation in sets of randomly selected housekeeping genes is indexed through the electrophoretic mobility of the corresponding enzymes (43). Several studies have documented the utility of MLEE to assess the molecular epidemiology of B. cepacia (44-47), but these were performed before the recognition that multiple species constitute the B. cepacia complex and most studies were limited to environmental isolates only. Recently, multilocus sequence typing (MLST) has emerged as a powerful and portable replacement to MLEE (41). With MLST allelic variation at chromosomal loci is examined by direct nucleotide sequencing. The development of MLST schemes for B. pseudomallei and B. cepacia has been initiated (http://www.mlst.net/mlst-info/pseudomallei/pseudomallei-info.htm). An alternative to MLST is multilocus restriction typing (MLRT) in which variation at several loci is indexed by restriction analysis of PCR-amplified genes. A MLRT scheme has been developed for B. cepacia genomovar III (48) that will allow further investigation of the global epidemiology and population structure of B. cepacia complex species.

4.3. Discussion

The use of the methods described above in studies of B. cepacia complex epidemiology has advanced our understanding of these species as agents of human disease. Nevertheless, a number of potential pitfalls exist that could limit the use of these methods. In addition to considering cost and ease of use, attention must be paid to how each method performs in terms of reproducibility, discriminatory power, and ease of interpretation of the data obtained.

Reproducibility of results is especially important if DNA fingerprinting patterns are being stored in a database for comparison with profiles generated in several different experiments. PFGE generally offers excellent reproducibility, whereas the PCR based methods are variable in this regard (49). Because of the low stringency conditions employed, reproducibility of RAPD typing may be particularly problematic. Although some investigators have found good reproducibility of RAPD patterns (50), others have observed significant day-to-day variation that has limited use of RAPD in large-scale studies (49, 51, 52).

The lack of pattern reproducibility between laboratories also has been an obstacle to implementing a uniform RAPD typing scheme with broad utility.

Discriminatory power must be carefully considered when choosing a typing method. It is important to appreciate that the degree to which a given method is able to detect differences between isolates is often a function of the species being investigated. In a recent comparative assessment of typing methods for B. cepacia genomovar III, we observed that PFGE and RAPD had higher discriminatory power than BOX-PCR (49). It is also important to understand that different degrees of discrimination may be appropriate for different epidemiologic questions. In other words, higher discriminatory power does not necessarily always result in a more accurate representation of epidemiologic relatedness. For example, when investigating a hospital-outbreak, a genotyping method with high discriminatory power may be needed to distinguish between outbreak-related and non-related strains. On the other hand, the same genotyping method may be too discriminatory for studies assessing long term or global epidemiology wherein single genetic events that may alter DNA banding profiles (eg, insertions, deletions and inversions) may obscure similarity among epidemiologically related isolates.

Finally, the interpretation of data derived from molecular typing can be far from straightforward, especially in the absence of epidemiological data. This is illustrated by the finding of unexpected similarity in PFGE and RAPD patterns obtained from a diverse collection of B. stabilis (B. cepacia genomovar IV) isolates (22). Based on the results of genotyping alone it could be concluded that there was extensive inter-patient spread of a single strain. However, most isolates in this study were epidemiologically unrelated suggesting that the similarities observed in banding patterns were due to a low level of genomic diversity within this species. Thus, interpreting similarity of DNA profiles for epidemiological investigation must be done cautiously, and, whenever possible, within the context of the available epidemiological data.

5. EPIDEMIOLOGY OF B. CEPACIA COMPLEX SPECIES

5.1. B. cepacia complex in CF
5.1.1. Introduction

Cystic fibrosis (CF) is the most common lethal genetic disorder among whites, affecting approximately 1 in 2750 live births. Approximately one person in 25 is an asymptomatic carrier. There are currently some 30,000 persons with CF in the U. S.; an equal number can be found in Europe. CF 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. Nearly 1000 cftr mutations have been identified, the most common being a deletion of phenylalanine at amino acid position 508 (ΔF508). The consequences of cftr mutations are complex (53,54), but the resultant altered respiratory
epithelial surface fluid in some way predisposes the lungs to bacterial infection (55). Progressive pulmonary deterioration secondary to recurrent or chronic infection is the leading cause of death in CF; the median survival age is approximately 32 years (Cystic Fibrosis Foundation, Patient Registry Annual Data Report, 1999).

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. A much smaller proportion of patients becomes infected with B. cepacia complex species. Nevertheless, B. cepacia complex infection has a significant impact on survival in CF. Clinical outcomes studies employing multivariate regression analyses have consistently identified B. cepacia infection as a significant independent risk factor for morbidity and mortality in CF (56-61). In addition, many CF treatment centers consider infection with B. cepacia an absolute contraindication to lung transplantation, which at present offers the only therapeutic option for successful intermediate-term survival in persons with end-stage pulmonary disease (62).

5.1.2. The emergence of B. cepacia in CF

The first reports of B. cepacia infection in CF patients appeared in the late 1970s and early 1980s (63-67). In 1984 Isles et al. documented the increasing prevalence of B. cepacia infection among patients receiving care in the Toronto CF center, and described the occurrence in some of a rapidly progressive deterioration in respiratory function (68). This so-called “cepacia syndrome” is characterized by necrotizing pneumonia, bacteremia and sepsis, and was observed in as many as 20% of infected patients. Similar increases in incidence of B. cepacia infection were noted subsequently in other CF treatment centers in North America (69-71). In these early studies, risk factors for acquisition of B. cepacia by persons with CF included increasing age, underlying severe lung disease, use of aminoglycoside antibiotics, previous hospitalisation and the presence of a B. cepacia colonized sibling.

5.1.3. Evidence for person-to-person transmission

These risk factors, along with the clustering of cases in some centers and the relative sparing of others, and the dramatic decrease in incidence of new infection after the institution of strict infection control (72) suggested that B. cepacia could be transmitted between CF patients. By using ribotyping, LiPuma et al. demonstrated the existence of strains common to several patients in each of three large CF treatment centers in North America (34). Shortly thereafter ribotyping was also used to document person-to-person transmission of B. cepacia by CF patients attending an educational program (35,36). Numerous other reports subsequently provided strong epidemiologic and genotyping evidence for inter-patient spread of B. cepacia between CF patients via simultaneous hospital admission or social contact outside of the hospital (73-78). In other CF centers no evidence for person-to-person transmission of B. cepacia was found (79-81).

The precise mechanisms by which B. cepacia may be transmitted between CF patients are not entirely clear. Infected patients can contaminate their hands during coughing and this could provide ample opportunity for direct transmission to another patient (82). Indirect transmission is also likely. B. cepacia can survive for long periods in respiratory droplets on environmental surfaces typically found in CF clinics (83); if drying is prevented, survival for several weeks is possible (4). B. cepacia has been isolated at low levels from air in rooms occupied by infected patients (84,85), and several studies have shown that infected patients can contaminate hospital rooms and inhalation equipment (86-88). Hospital sink drains may also become contaminated (89,90). B. cepacia was not isolated in throat cultures from non-CF health care workers, and so spread via transient colonization of non-CF individuals does not seem likely (91).

5.1.4. Evidence for environmental acquisition

The observation that strict infection control measures have reduced but not eliminated new infection has raised speculation that the environment serves as the reservoir for acquisition of novel B. cepacia complex strains. A limited number of studies during the early 1990s did not recover B. cepacia complex strains in significant numbers from select environmental sources (92-94). More recent studies, however, employing improved isolation and identification techniques, have demonstrated that B. cepacia complex organisms, including genomovar III, may be readily recovered from a variety of agricultural soils (95-98). Most recently, we identified from agricultural soil a B. cepacia genomovar III strain that infects a large number of CF patients in the same region of the U. S. (99). In contrast, genomovar III was found infrequently among B. cepacia complex recovered from urban soil samples (100). Although typically not animal pathogens, B. cepacia complex has been detected in unpasteurized milk (101), and an outbreak of subclinical mastitis in sheep due to B. cepacia complex species has been reported (102). The preferred environmental niche of each species of the B. cepacia complex, as well as the existence of other possible animal reservoirs remains to be elucidated. Only then will it be possible to determine if these sources constitute a risk for persons with CF.

5.1.5. Species and strain-specific differences in epidemiology

The recognition that several distinct species comprise the B. cepacia complex, prompted a reassessment of the epidemiology of B. cepacia infection in CF. Although all nine B. cepacia complex species have been identified in CF sputum culture, recent large-scale surveys indicate that the distribution of these species among CF patients is quite disproportionate. Surveys from the U. S., Canada and Italy all indicate that most infected CF patients harbor either B. cepacia genomovar III or B. multivorans (103-105). The implications of this finding for infection control, clinical outcome assessment and virulence studies are discussed in the following section.

In addition to the differences noted in species distribution in CF, genotyping analyses have provided
Burkholderia sp.

important insights as to the occurrence of specific strain types at a subspecies level. As noted above, ribotyping studies in the late 1980s and early 1990s identified several B. cepacia strains that were shared by multiple persons with CF (34,35). In 1994, Johnson et al used multilocus enzyme electrophoresis and ribotyping to identify a B. cepacia strain, designated electrophoretic type (ET) 12, that predominated among CF patients in Ontario, Canada (45). This so-called “epidemic” strain apparently spread via CF summer camp to patients in the United Kingdom (75), where it was later recognized as accounting for nearly one-half of B. cepacia-infected CF patients (106). ET12 is characterized by the expression of unusual “cable pili” that mediate adherence to respiratory epithelial cells (107). In another study, Kumar et al used cellular fatty acid methyl ester and PFGE analyses to demonstrate that 29 of 32 B. cepacia isolates from five CF centers in Michigan were the same strain type (108). Mahenthiralingam et al identified several other B. cepacia strains that also infected more than one CF patient (109). These, however, involved relatively small numbers of patients and although not described in detail, most were characterized by a genomic fragment referred to as the “B. cepacia epidemic strain marker” (BCESM). This 1.4 kb sequence contains an open reading frame with homology to negative transcriptional regulators of other species. The role of this element in virulence or strain transmissibility has not been determined.

As discussed in detail above, in 1997 the taxonomic studies of Vandamme et al. identified several distinct species among bacteria previously identified merely as “B. cepacia” based on phenotype (21). The ET12 strain was recognized as belonging to B. cepacia genomovar III, as were other strains that were apparently spread between CF patients during outbreaks in Manchester and Newcastle (110). Subsequent analysis of the Michigan-dominant strain described by Kumar et al and the “epidemic” strains described by Mahenthiralingam et al indicated that these too were genomovar III (unpublished observations).

We recently described another B. cepacia genomovar III strain, designated PHDC, that is recovered from nearly every B. cepacia-infected CF patient in the mid-Atlantic region of the US (111). By using a variety of genotyping methods, including RAPD typing, BOX-PCR and PFGE, we identified the 20-year persistence of PHDC in one large CF treatment center. We also demonstrated the spread of this strain to a second center, apparently via an infected patient who transferred care between centers. In contrast to ET12, PHDC lacks cable pili and does not contain BCESM.

The degree to which non-genomovar III strains may be shared among multiple CF patients is not clear. B. multivorans strains common to several patients have been reported (78,112), and the strain involved in the first report of inter-patient transmission (35) is now known to be B. cepacia genomovar VI.

5.1.6. Dynamics of B. cepacia complex infection

Much remains unknown regarding the natural history of B. cepacia complex infection in CF. A study by LiPuma et al in 1994 indicated that after acquisition of B. cepacia, some patients may remain sputum culture negative for prolonged periods of time. The frequency of such “inapparent” infection is not clear (36). However, B. cepacia DNA has been detected by PCR in sputum from several patients who were sputum culture negative (113).

Nevertheless, after initial acquisition of B. cepacia, it appears that most patients remain infected for prolonged periods of time. The conventional wisdom that dictates “once infected, always infected,” however, has been challenged by several recent observations indicating that B. cepacia complex infection may be more dynamic than previously thought. For example, Mahenthiralingam et al., identified several patients in whom genomovar III strains apparently supplanted initial infection with B. multivorans (114). A similar observation was made by Ledson et al., who demonstrated superinfection with B. cepacia ET12 in patients who were previously infected with another B. cepacia complex strain (115). These data suggest that in some patients a change in infecting strain may occur during ‘chronic’ B. cepacia complex infection.

In a more comprehensive study, we recently investigated serial isolates from 379 CF patients receiving care in 112 CF treatment centers in the USA (unpublished). Overall, a change in infecting species or strain was found in approximately 7% of patients infected with B. cepacia complex species. Several patients were also identified who were likely co-infected, at least transiently, with more than one B. cepacia complex strain.

5.1.7. Discussion: Facts, fiction, and implications for infection control, outcome and virulence

The observation that some B. cepacia complex species (and, at a subspecies level, some specific strains) are disproportionately represented among isolates recovered from infected CF patients could have important implications with respect to infection control, clinical outcome and studies of bacterial virulence and host susceptibility. However, conclusions and recommendations based on extrapolation of the existing data must be made cautiously. Terms such as “epidemic” and “highly transmissible” have been used rather loosely and interchangeably applied to strains common to multiple CF patients. But consensus definitions as to what constitutes such strains have not been proposed (is any strain found in more than one patient an “epidemic” strain?). Further, these terms imply a biologic property outside the context of infection control (is an “epidemic, highly transmissible” strain in center A, with relatively lax infection control measures, any more “transmissible” than any other strain in center B wherein stringent infection control measures are practiced?). Until there is a better definition of terms and / or the biologic underpinnings of inter-patient transmission are determined, it is best to avoid these descriptors.

These concerns notwithstanding, it is certainly clear that among the nine B. cepacia complex species, genomovar III and B. multivorans are most frequently recovered from infected CF patients. It is also clear that a
few genomovar III strains (eg, ET12 and PHDC) have been identified as infecting large numbers of patients. These findings strongly suggest an enhanced capacity for human infection by these species and these specific strains, the biologic basis of which remains to be elucidated. The identification of cable pili expression by ET12 initially raised speculation that this phenotype accounted for and could prospectively identify “highly transmissible” strains (107,116). Screening for cable pili could, in turn, enable CF centers to selectively apply infection control measures, isolating only those patients harboring an epidemic strain. In fact, recommendations for such stratification of infection control have been made more recently (117). Such recommendations are, however, premature. Although cable pili probably mediate events important in the pathogenesis of infection by ET12, it is not yet clear whether cable pili expression confers an enhanced capacity for inter-patient transmission, per se. Furthermore, other ‘epidemic’ B. cepacia complex strains do not contain cblA sequences (109, 118). Strain PHDC contains neither BCESM (described above) nor cblA (111). The B. multivorans strain responsible for a hospital-associated outbreak among CF patients in the U. K. (78) and the genomovar VI strain involved in the first report of inter-patient spread (35) similarly lack BCESM and cblA (118). Thus, neither of these markers (nor any others identified to date) is sufficiently sensitive or specific for the identification of strains with an apparent increased ability for spread in CF.

The distinction between frequency of isolation and virulence in CF has been blurred in recent literature. B. cepacia complex species and specific strains that are more frequently recovered from CF patients (ie, genomovar III and ET12, respectively) are often described as more virulent than other strains. However, there are currently only limited outcomes data to support this claim. Two recent studies among lung transplant recipients demonstrate greater six-month post-operative mortality in patients infected with genomovar III than with other B. cepacia complex species (119,120). Ledson et al recently showed higher mortality rates among CF patients infected with ET12 than among patients without B. cepacia complex infection (61). But, rigorous comparisons of clinical outcome between persons infected with different B. cepacia complex species or specific strains have not been undertaken. Thus, claims that genomovar III in general or ET12 in particular are more virulent, per se, than other strains in CF are, at this time, unsubstantiated.

5.2. B. cepacia complex in non-CF patients

B. cepacia can cause life-threatening infection in persons with chronic granulomatous disease (CGD) (121-124). In this inherited primary immunodeficiency disease white blood cells are unable to kill some bacterial and fungal species after phagocytosis (125-126). The underlying defect is an inability of phagocytic cells to generate superoxide and reactive oxidants that are necessary for intracellular microbial activity. As a result, CGD patients suffer from recurrent life-threatening infections, such as severe pneumonia and bacteremia caused by some 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 also mediate pathogenicity in this condition (127). Fortunately, CGD is a relatively rare disease having an average annual incidence of approximately 1/200,000 live births in the U. S.; this means there are approximately 20 persons with CGD born each year in the U. S.

Although not typically pathogenic for healthy humans, B. cepacia has been reported as causing a variety of infections in persons without CF. Most often, infection occurs in hospitalized patients with an underlying disabling illness (128-131). However, severe community-acquired infections, such as endocarditis (132), brain abscesses (133) and pneumonia (134), also have been reported. Nosocomial infections have been associated with contamination of sterile solutions such as anesthetics or mouthwash, or disposable equipment such as nebulizers and biopsy needles (135-142). In some cases the source of an outbreak has remained obscure despite extensive investigation (143). Nosocomial “pseudoepidemics” of B. cepacia infection have occurred as a result of contamination of disinfectant solutions, including benzalkonium chloride and quaternary ammonium products (144,145).

Ledson et al. described chronic respiratory colonization with a B. cepacia complex strain in a person without CF who most likely acquired the organism from one of her two infected CF children, a phenomenon not described previously (146). Holmes et al described a large nosocomial outbreak of B. cepacia infection among CF and non-CF patients (147). A strain common to both patient groups was described. Infection was transient in the non-CF patients, while persons with CF remained infected for prolonged periods.

6. EPIDEMIOLOGY OF OTHER BURKHOLDERIA SPECIES

6.1. B. gladioli

B. gladioli is a plant pathogen typically recovered from Gladiolus sp., Iris sp. and rice (148). B. gladioli are capable of infecting certain vulnerable human hosts, and again CF and CGD patients seem to be especially susceptible (149-154). Although little is known about the epidemiology of human disease caused by this species, it is clear that CF patients may remain infected with the same B. gladioli strain for prolonged periods (unpublished observation). It is also clear that compared to B. cepacia complex, the prevalence of B. gladioli infection in the CF population is rather low. This low prevalence and the difficulty in accurate identification by routine phenotypic analysis (110, 155) have precluded more comprehensive study of the epidemiology of B. gladioli. For example, isolates initially identified as B. gladioli based on phenotypic analyses and implicated in inter-patient spread (156) were subsequently found by genotypic testing to be B. cepacia genomovar III (157). The development of PCR-based species identification will be a useful asset to further study (158).
Burkholderia sp.

6.2. B. mallei

B. mallei is primarily a pathogen in horses, in which it causes equine glanders, a disease characterized by fever, inflammation of the nasal mucosa, necrosis and obstruction of the oropharynx. In humans, infection can be limited to subcutaneous tissues or can disseminate to cause sepsis. If inhaled B. mallei can cause pneumonia with necrosis of the tracheobronchial tree (2). B. mallei can be spread via contact with infected animals or through exposure in research laboratories (2,159). The highly contagious and potentially lethal nature of human infection with B. mallei makes this species well suited for use as an agent of bioterrorism. In fact, B. mallei was one of the first biologic weapons of the 20th century, being used by Germany during World War I (160). Glanders has been virtually eliminated in the Western world due to stringent infection control measures, including the immediate slaughter of affected animals. However, research interest in this species due to recent concerns about biological warfare may result in an increasing risk of occupational exposure.

6.3. B. pseudomallei

B. pseudomallei is the etiologic agent of melioidosis, an infection characterized by a broad spectrum of clinical manifestations, ranging from asymptomatic colonization to fulminant sepsis. The most common presentations of melioidosis include pneumonia, soft-tissue infection, abscesses of liver and spleen and septicemia (161-162). B. pseudomallei is a saprophytic organism, broadly distributed in soil and water in Southeast Asia and northern Australia. It is an important cause of morbidity and mortality in Thailand, where the frequency of disease is most likely underestimated due to lack of access to adequate health care (3). The organism can also cause infections in a wide variety of animals, including livestock wherein it is capable of causing economically significant losses (162). It is thought that most infected humans and animals acquired the organism through percutaneous inoculation on exposure to contaminated soil or water, although the possibility of inhalation or ingestion as modes of infection requires further investigation (163-165). Sporadic cases have resulted from person-to-person or animal-to-person spread (163). Exposure in endemic areas is quite frequent due to the organism’s ubiquity, and latent infections are common. Thus, it is difficult to accurately determine what sort of environmental exposure poses the greatest risk of melioidosis. Sporadic cases of human melioidosis occur in regions outside the endemic area, such China, Korea, the Philippines, Indonesia, India and West Africa. Most cases in Europe and North America are thought to be imported by immigrants or international travelers (167,168). Two recent reports describe the occurrence of B. pseudomallei in European CF patients; in both cases B. pseudomallei was most likely acquired during travel to Thailand (169-170). In a review of reported cases of confirmed melioidosis in North America, Dorman et al. report systemic lupus erythematosus and CGD as predisposing factors (167). In nearly all those cases B. pseudomallei was initially misidentified (due to a lack of familiarity with the disease and/or inappropriate identification schemes). This suggests that in cases of febrile respiratory illness in patients at risk who are returning from endemic areas, referral of the organism to a reference laboratory for confirmation seems advisable.

7. ROLE OF REFERENCE LABORATORIES

Epidemiologic studies of Burkholderia species rely, of course, on the accurate identification of these and related taxa. Unfortunately, this has often proven to be quite challenging and species misidentification is common (171). Because these species are generally not pathogenic for healthy persons, they are underrepresented in some commercial bacterial identification systems (155). The poorly defined taxonomy of these species has also confounded accurate identification based on phenotypic analyses alone. To circumvent these problems, a number of genomic-based assays have been developed in recent years for identification of these species. Polymerase chain reaction (PCR) based assays targeting ribosomal DNA and recA gene sequences have demonstrated excellent sensitivity and specificity for B. cepacia complex species (1, 172). PCR based assays have also been developed for identification of B. gladioli (158), and other species recovered from CF sputum culture and commonly confused with Burkholderia sp, including Stenotrophomonas maltophilia (173), Pandoraea sp (174), Achromobacter xylosidans (175) and Ralstonia sp (176). The availability of reference laboratories capable of augmenting routine phenotypic analyses with these genomic based assays has proven an important adjunct to both clinical care of infected patients and large scale epidemiologic studies.

8. CONCLUDING REMARKS

Studies employing bacterial genotyping have added a great deal to our understanding of the epidemiology of human infection due to Burkholderia species. This is particularly true regarding B. cepacia complex infection in CF, wherein a variety of genotypic methods have proved useful. Because these methods differ with respect to discriminatory power, reproducibility, and ease of interpretation, the choice of method in future studies will depend on the specific questions being addressed. The recognition that several distinct species comprise the B. cepacia complex provides a critical platform for further study. These efforts will better determine the preferred environmental niches, potential for inter-patient spread, and pathogenic potential of strains within each species. The ability to reliably genotype large numbers of isolates recovered from clinical specimens and the natural environment is fundamental to these efforts. Renewed interest in B. mallei and B. pseudomallei will most likely prompt additional study of the epidemiology of these species as agents of human infection.

9. ACKNOWLEDGEMENTS

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**Burkholderia sp.**

### 10. REFERENCES

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