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MOLECULAR EPIDEMIOLOGY OF *BURKHOLDERIA* SPECIES

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1. ABSTRACT

Although most species in the genus *Burkholderia* are not pathogenic for healthy persons, a few are capable of causing severe, life threatening infection. *B. mallei* and *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. *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 *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 *B. cepacia*. How these species, collectively referred to as the *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 *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. *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). *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 *B. mallei* and *B. pseudomallei* has increased recently, as these are agents with potential for use in bioterrorism (http://www.bt.cdc.gov/Agent).

The *B. cepacia* complex is a subset of several closely related, yet distinct, genomic species (or

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. pickettii*, *P. cepacia*, *P. gladioli*, *P. mallei*, *P. pseudomallei* and *P. caryophylli*) 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 (31,32). RFLPs result from nucleotide sequence variation in regions flanking 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

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 (?F508). 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

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 microbicidal 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).

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 characterised 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 xylosoxidans* (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.

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10. REFERENCES

1. Coenye T., P. Vandamme, J. R. W. Govan & J .J. LiPuma: Taxonomy and identification of the *Burkholderia cepacia* complex. *J Clin Microbiol* 39, 3427-3436 (2001)
2. Srinivasan A., C. N. Kraus, D. DeShazer, P. M. Becker, J. D. Dick, L. Spacek, J. G. Bartlett, W. R. Byrne & D. L. Thomas: Glanders in a military research microbiologist. *N Engl J Med* 345, 256-258 (2001)
3. Chaowagul W., N. J. White, D. A. Dance, Y. Wattanagoon, P. Naigowit, T. M. Davis, S. Looareesuwan & N. Pitakwatchara: Melioidosis: a major cause of community-acquired septicemia in northeastern Thailand. *J Infect Dis* 159, 890-899 (1989)
4. Govan J.R.W., J. E. Hughes & P. Vandamme: *Burkholderia cepacia*: medical, taxonomic and ecological issues. *J Med Microbiol* 45, 395-407 (1996)
5. LiPuma J. J.: *Burkholderia cepacia*: management issues and new insights. *Clin Chest Med* 19, 473-486 (1998)
6. LiPuma J. J.: *Burkholderia cepacia* epidemiology and pathogenesis: implications for infection control. *Curr Op Pulm Med* 4, 337-341 (1998)
7. LiPuma J. J. & E. Mahenthiralingam: Commercial use of *Burkholderia cepacia*. *Emerg Infect Dis* 5, 305-306 (1999)
8. Holmes A., J. Govan, R. Goldstein: Agricultural use of *Burkholderia (Pseudomonas) cepacia*: a threat to human health? *Emerg Infect Dis* 4, 221-227 (1998)
9. Govan J.R.W. & V. Deretic: Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* 60, 539-574 (1996)
10. Jones A. M., M. E. Dodd & A. K. Webb: *Burkholderia cepacia*: current clinical issues, environmental controversies and ethical dilemmas. *Eur Respir J* 17, 295-301 (2001)
11. Mohr C. D., M. Tomich & C. A. Herfst: Cellular aspects of *Burkholderia cepacia* infection. *Microbes Infect* 3, 425-435 (2001)
12. Hancock R. E.: Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. *Clin Infect Dis* 27, S93-S99 (1998)
13. Parke J. L. & D. Gurian-Sherman: Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. *Annu Rev Phytopathol* 39, 225-258 (2001)
14. Burkholder W. H.: Sour skin, a bacterial rot of onion bulbs. *Phytopathol* 40, 115-117 (1950)
15. Kersters K., W. Ludwig, M. Vancanneyt, P. De Vos, M. Gillis & K. H. Schleifer: Recent changes in the classification of the pseudomonads: an overview. *Syst Appl Microbiol* 19, 465-477 (1996)
16. Palleroni N. J., R. Kunisawa, R. Contopolous & M. Doudoroff: Nucleic acid homologies in the genus *Pseudomonas*. *Int J Syst Bact* 23, 333-339 (1973)
17. De Vos P., M. Goor, M. Gillis & J. De Ley: Ribsomal ribonucleic acid cistron similarities of phytopathogenic *Pseudomonas* species. *Int J Syst Bact* 35, 169-184 (1985)
18. Yabuuchi E., Y. Kosako, H. Oyaizu, I. Yano, H. Hotta, Y. Hashimoto, T. Ezaki & M. Arakawa: Proposal of *Burkholderia* gen. nov.; and transfer of seven species of the *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. *Microbiol Immunol* 39, 897-904 (1992)
19. De Ley J.: The Proteobacteria: ribosomal RNA cistron similarities and bacterial taxonomy. In: The Prokaryotes, 2nd edition. Eds: Balows A, Truper H, Dworkin M, Harder W, Schleifer KH, Springer-Verlag, NY (1992)
20. Woese C. R.: Bacterial evolution. Microbiol Rev 51, 221-271 (1987)
21. Vandamme P., B. Holmes, M. Vancanneyt, T. Coenye, B. Hoste, R. Coopman, H. Revets, S. Lauwers, M. Gillis, K. Kersters & J. R. W. Govan: Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. *Int J Syst Bact* 47, 1188-1200 (1997)
22. Vandamme P., E. Mahenthiralingam, B. Holmes, T. Coenye, B. Hoste, P. De Vos, D. Henry & D. P. Speert: Identification and population structure of *Burkholderia stabilis* sp. nov. (formerly *Burkholderia cepacia* genomovar IV). *J Clin Microbiol* 38, 1042-1047 (2000)
23. Coenye T., J. J. LiPuma, D. Henry, B. Hoste, K. Vandemeulebroecke, M. Gillis, D. P. Speert & P. Vandamme: *Burkholderia cepacia* genomovar VI, a new member of the *Burkholderia cepacia* complex isolated from cystic fibrosis patients. *Int J Syst Evol Microbiol* 51, 271- 279 (2001)
24. Coenye T., E. Mahenthiralingam, D. Henry, J. J. LiPuma, S. Laevens, M. Gillis, D. P. Speert & P. Vandamme: *Burkholderia ambifaria* sp. nov., a novel member of the *Burkholderia cepacia* complex including biocontrol and cystic fibrosis-related isolates. *Int J Syst Evol Microbiol* 51, 1481-1490 (2001)
25. Vandamme P., D. Henry, T. Coenye, S. Nzula, M. Vancanneyt, J. J. LiPuma, D. P. Speert, J. R. W. Govan & E. Mahenthiralingam: *Burkholderia anthina* sp. nov. and *Burkholderia pyrrocinia*, two additional *Burkholderia cepacia* complex bacteria, may confound test results of new molecular diagnostic tools. *FEMS Immunol Med Microbiol* 33,143-149 (2002)
26. Rabkin C. S., W. R. Jarvis & W. J. Martone: Current status of *Pseudomonas cepacia* typing systems. *Eur J Epidemiol* 3, 343-346 (1987)
27. Rabkin C. S., W. R. Jarvis, R. L. Anderson, J. Govan, J. Klinger, J. J. LiPuma, W. J. Martone, H. Monteil, C. Richard, S. Shigeta, A. Sosa, T. Stull, J. Swenson & D. Woods: *Pseudomonas cepacia* typing systems: collaborative study to assess their potential in epidemiologic investigations. *Rev Infect Dis* 11, 600-607 (1989)
28. Arbeit R. D.: Laboratory procedures for the epidemiologic analysis of microorganisms. In: Manual of clinical microbiology. 6th edition. Eds: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, ASM Press, Washington DC, (1995)
29. LiPuma J. J.: Molecular tools for epidemiological study of infectious diseases. *Pediatr Infect Dis J* 17, 667-675 (1998)
30. Olive D. M & P. Bean: Principles and applications of methods for DNA-based typing of microbial organisms. *J Clin Microbiol* 37, 1661-1669 (1999)
31. Stull T. L., J. J.LiPuma & T. D. Edlind: A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. *J Infect Disease* 157, 280-286 (1988)

32. Bingen E. H., E. Denamur & J. Elion: Use of ribotyping in epidemiological surveillance of nosocomial outbreaks. *Clin Microbiol Rev* 7, 311-327 (1994)
33. Kostman J. R., M. B. Alden, M. Mair, T. D. Edlind, J. J. LiPuma & T. L. Stull: A universal approach to bacterial molecular epidemiology by polymerase chain reaction ribotyping. *J Infect Dis* 171, 204-208 (1995)
34. LiPuma J. J., J. E. Mortensen, S. E. Dasen, T. D. Edlind, D. V. Schidlow, J. L. Burns & T. L. Stull: Ribotype analysis of *Pseudomonas cepacia* from cystic fibrosis treatment centers. *J Pediatr* 113, 859-862 (1988)
35. LiPuma J. J., S. E. Dasen, D. W. Nielson, R. C. Stern & T. L. Stull: Person-to-person transmission of *Pseudomonas cepacia* between patients with cystic fibrosis. *Lancet* 336, 1094-1096 (1990)
36. LiPuma, J. J., K. A. Marks-Austin, D. S. Holsclaw, G. B. Winnie, P. H. Gilligan & T. L. Stull: Inappararent transmission of *Pseudomonas (Burkholderia) cepacia* among patients with cystic fibrosis. *Pediatr Infect Dis J* 13, 716-719 (1994)
37. Dasen S. E., J. J. LiPuma, J. R. Kostman & T. L. Stull: Characterization of PCR-ribotyping for *Burkholderia (Pseudomonas) cepacia*. *J Clin Microbiol* 32, 2422-2424 (1994)
38. Tenover F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing & B. Swaminathan: Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33, 2233-2239 (1995)
39. van Belkum A.: DNA fingerprinting of medically important microorganisms by use of PCR. *Clin Microbiol Rev* 7, 174-184 (1994)
40. Power E. G.: RAPD typing in microbiology – a technical review. *J Hosp Infect* 34, 247-265 (1996)
41. Maiden M. C. J., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman & B. G. Spratt: Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA* 95, 3140-3145 (1998)
42. Enright M. C. & B. G. Spratt: Multilocus sequence typing. *Trends Microbiol* 7, 482-487 (1999)
43. Musser J. M.: Molecular population genetic analysis of emerged bacterial pathogens: selected insights. *Emerg Infect Dis* 2, 1-17 (1996)
44. McArthur J. V., D. A. Kovacic & M. H. Smith: Genetic diversity in natural populations of a soil bacterium across a landscape gradient. *Proc Natl Acad Sci USA* 85, 9621-9624 (1988)
45. Johnson W. M., S. D. Tyler & K. R. Rozee: Linkage analysis of geographic and clinical clusters in Pseudomonas cepacia infections by multilocus enzyme electrophoresis and ribotyping. *J Clin Microbiol* 32, 924-930 (1994)
46. Wise M. G., L. J. Shimkets & J. V. McArthur: Genetic structure of a lotic population of *Burkholderia (Pseudomonas) cepacia*. *Appl Env Microbiol* 61, 1791-1798 (1995)
47. Wise M. G., J. V. McArthur, C. Wheat & L. J. Shimkets: Temporal variation in genetic diversity and structure of a lotic population of *Burkholderia (Pseudomonas) cepacia*. *Appl Env Microbiol* 62, 1558-1562 (1996)
48. Coenye T. & J. J. LiPuma: Multilocus restriction typing, a novel tool for studying global epidemiology of *Burkholderia cepacia* complex infection in cystic fibrosis. *J Infect Dis* 185:1454-1462 (2002)
49. Coenye T., T. Spilker, A. Martin, J. J. LiPuma: Comparative assessment of genotyping methods for epidemiologic study of *Burkholderia cepacia* genomovar III. *J Clin Microbiol* (in press)
50. Mahenthiralingham E., M. E. Campbell, D. A. Henry & D. P. Speert: Epidemiology of *Burkholderia cepacia* infection in patients with cystic fibrosis: analysis by randomly amplified polymorphic DNA fingerprinting. *J Clin Microbiol* 34, 2914-2920 (1996)
51. Bingen E. H., M. Weber, J. Derelle, N. Brahimi, N. Y. Lambert-Zechovsky, M. Vidailhet, J. Navarro & J. Elion: Arbitrarily primed polymerase chain reaction as a rapid method to differentiate crossed from independent *Pseudomonas cepacia* infections in cystic fibrosis patients. *J Clin Microbiol* 31, 2589-2593 (1993)
52. Liu P. Y., Z. Y. Shi, Y. J. Lau, B. S. Hu, J. M. Shyr, W. S. Tsai, Y. H. Lin & C. Y. Tseng: Comparison of different PCR approaches for characterization of *Burkholderia (Pseudomonas) cepacia* isolates. *J Clin Microbiol* 33, 3304-3307 (1995)
53. Zeitlin, P. L.: Novel pharmacologic therapies for cystic fibrosis. *J Clin Invest* 103, 447-452 (1999)
54. Larson J. E. & J. C. Cohen: Cystic fibrosis revisited. *Mol Genet Metabol* 71, 470-477 (2000)
55. Dinwiddie R.: Pathogenesis of lung diseases in cystic fibrosis. *Respiration* 67, 3-8 (2000)
56. Lewin L. O., P. J. Byard & P. B. Davis: Effect of *Pseudomonas cepacia* colonization on survival and pulmonary function of cystic fibrosis patients. *J Clin Epidemiol* 43, 125-131 (1990)
57. Corey M. & V. Farewell: Determinants of mortality from cystic fibrosis in Canada, 1970-1989. *Am J Epidemiol* 143, 1007-1017 (1996)
58. Muhdi K., F. P. Edenborough, L. Gumery, S. O'Hickey, E. G. Smith, D. L. Smith & D. E. Stableforth: Outcome for patients colonized with *Burkholderia cepacia* in a Birmingham adult cystic fibrosis clinic and the end of an epidemic. *Thorax* 51, 374-377 (1996)
59. Liou T. G., F. R. Adler, S. C. FitzSimmons, B. C. Cahill, J. R. Hibbs & B. C. Marshall: Predictive 5-year survivorship model of cystic fibrosis. *Am J Epidemol* 153, 345-352 (2001)
60. McCloskey M., J. McCaughan, A. O. B. Redmond & J. S. Elborn: Clinical outcome after acquisition of *Burkholderia cepacia* in patients with cystic fibrosis. *Ir J Med Sci* 170, 28-31 (2001)
61. Ledson M. J., M. J. Gallagher, M. Jackson, C. A. Hart & M. J. Walshaw: Outcome of *Burkholderia cepacia* colonisation in an adult cystic fibrosis centre. *Thorax* 57, 142-145 (2002)
62. LiPuma J. J.: *Burkholderia cepacia* complex: A contraindication to lung transplantation in CF? *Transpl Infec Dis* 3, 150-161 (2001)
63. Blessing J., J. Walker, B. Maybury: *Pseudomonas cepacia* and *maltophilia* in the cystic fibrosis patient. *Am Rev Respir Dis Suppl* 119, 262 (1979)

64. Laraya-Cuasay L. R., M. Lipstein, N. N. Huang: *Pseudomonas cepacia* in the respiratory flora of patients with cystic fibrosis. *Pediatr Pulmonol* 11, 502 (1977)
65. Rosenstein B. J. & D. E. Hall: Pneumonia and septicemia due to *Pseudomonas cepacia* in a patient with cystic fibrosis. *Johns Hopkins Med J* 147, 188-189 (1980)
66. Nolan G., P. Moivor, H. Levison, P. C. Fleming, M. Corey & R.Gold: Antibiotic prophylaxis in cystic fibrosis: inhaled cephaloridine as an adjunct to oral cloxacillin. *J Pediatr* 101, 626-630 (1982)
67. Gold R., E. Jin, H. Levison, A. Isles & P. C. Fleming: Ceftazidime alone and in combination in patients with cystic fibrosis: lack of efficacy in treatment of severe respiratory infections caused by *Pseudomonas cepacia. J Antimicrob Chemother* 12 *Suppl* A, 331-336 (1983)
68. Isles A., I. Maclusky, M. Corey, R. Gold, C. Prober, P. Fleming & H. Levison: *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J Pediatr* 104, 206-210 (1984)
69. Thomassen M. J., C. A. Demko, J. D. Klinger & R. C. Stern: *Pseudomonas cepacia* colonization among patients with cystic fibrosis. A new opportunist. *Am Rev Respir Dis* 131, 791-796 (1985)
70. Tablan O. C., T. L. Chorda, D. V. Schidlow, J. W. White, K. A. Hardy, P. H. Gilligan, W. M. Morgan, L. A. Carson, W. J. Martone & J. M. Jason: *Pseudomonas cepacia* colonization in patients with cystic fibrosis: risk factors and clinical outcome. *J Pediatr* 107, 382-387 (1985)
71. Tablan O. C., W. J. Martone, C. F. Doerschuk, R. C. Stern, M. J. Thomassen, J. D. Klinger, J. W. White, L. A. Carson & W. R. Jarvis: Colonization of the respiratory tract with *Pseudomonas cepacia* in cystic fibrosis. Risk factors and outcomes. *Chest* 91, 527-532 (1987)
72. Thomassen M. J., C. A. Demko, C. F. Doerschuk, R. C. Stern & J. D. Klinger: *Pseudomonas cepacia*: decrease in colonization in patients with cystic fibrosis. *Am Rev Respir Dis* 134, 669-671 (1986)
73. Millar-Jones L., A. Paull, Z. Saunders & M. C. Goodchild: Transmission of *Pseudomonas cepacia* among cystic fibrosis patients. *Lancet* 340, 491 (1992)
74. Smith D. L., L. B. Gummery, E. G. Smith, D. E. Stableforth, M. E. Kaufmann & T. L. Pitt: Epidemic of *Pseudomonas cepacia* in an adult cystic fibrosis unit: evidence of person-to-person transmission. *J Clin Microbiol* 31, 3017-3022 (1993)
75. Govan J. R. W., P. H. Brown, J. Maddison, C. J. Doherty, J. W. Nelson, M. Dodd, A. P. Greening & A. K. Webb: Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis patients. *Lancet* 342, 15-19 (1993)
76. John M., E. Ecclestone, E. Hunter, P. Couroux & Z. Hussain: Epidemiology of *Pseudomonas cepacia* colonization among patients with cystic fibrosis. *Pediatr Pulmon* 18, 108-113 (1994)
77. Pegues D. A., L. A. Carson, O. C. Tablan, S. C. FitzSimmons, S. B. Roman, J. M. Miller & W. R. Jarvis: Acquisition of *Pseudomonas cepacia* at summer camps for patients with cystic fibrosis. Summer Camp Study Group. *J Pediatr* 124, 694-702 (1994)
78. Whiteford M. L., J. D. Wilkinson, J. H. McColl, F. M. Conlon, J. R. Michie, T. J. Evans & J. Y. Paton: Outcome of *Burkholderia (Pseudomonas) cepacia* colonisation in children with cystic fibrosis following a hospital outbreak. *Thorax* 50, 1194-1198 (1995)
79. Hardy A. K., K. L. McGowan, M. C. Fisher & D. V. Schidlow: *Pseudomonas cepacia* in the hospital setting: lack of transmission between cystic fibrosis patients. *J Pediatr* 109, 51-54 (1986)
80. Taylor C. J., R. Howden, T. Smith & R. C. Spencer: *Pseudomonas cepacia. Arch Dis Child* 70, 358 (1994)
81. Ryley H. C., B. Ojeniyi, N. Hoiby & J. Weeks: Lack of evidence of nosocomial cross-infection by *Burkholderia cepacia* among Danish cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 15, 755-758 (1996)
82. Pegues D. A., D. V. Schidlow, O. C. Tablan, L. A. Carson, N. C. Clark & W. R. Jarvis: Possible nosocomial transmission of *Pseudomonas cepacia* in patients with cystic fibrosis. *Arch Ped Adolesc Med* 148, 805-812 (1994)
83. Drabick J. A., E. J. Gracely, G. J. Heidecker & J. J. LiPuma: Survival of *Burkholderia cepacia* on environmental surfaces. *J Hosp Infect* 32, 267-276 (1996)
84. Hymphreys H., D. Peckham, P. Patel & A. Knox: Airborne dissemination of *Burkholderia (Pseudomonas) cepacia* from adult patients with cystic fibrosis. Thorax 49, 1157-1159 (1994)
85. Ensor E., H. Humphreys, D. Peckham, C. Webster & A. J. Knox: Is *Burkholderia (Pseudomonas) cepacia* disseminated from cystic fibrosis patients during physiotherapy? *J Hosp Infect* 32, 9-15 (1996)
86. Pitchford K. C., M. Corey, A. K. Highsmith, R. Perlman, R. Bannatyme, R. Gold, H. Levinson, E. L. Ford-Jones: *Pseudomonas* species contamination of cystic fibrosis patients' home inhalation equipment. *J Pediatr* 111, 212-216 (1987)
87. Burgde D. R., E. M. Nakielna & M. A. Noble: Case-control and vector studies of nosocomial acquisition of *Pseudomonas cepacia* in adult patients with cystic fibrosis. *Infect Control Hosp Epidemiol* 14, 127-130 (1993)
88. Hutchinson G. R., S. Parker, J. A. Pryor, F. Duncan-Skingle, P. N. Hoffman, M. E. Hodson, M. E. Kaufmann & T. L. Pitt: Home-use nebulizers : a potential primary source of *Burkholderia cepacia* and other colistin-resistant, gram-negative bacteria in patients with cystic fibrosis. *J Clin Microbiol* 34, 584-587 (1996)
89. Doring G., S. Jansen, H. Noll, H. Grupp, F. Frank, K. Botzenhart, K. Magdorf & U. Wahn: Distribution and transmission of Pseudomonas aeruginosa and *Burkholderia cepacia* in a hospital ward. *Pediatr Pulmon* 21, 90-100 (1996)
90. Moore J. E., I. Thompson, M. Crowe, J. Xu, A. Shaw, B. C. Millar, A. O. B. Redmond, A. J. M. Reid, C. Clarke & J. S. Elborn: *Burkholderia cepacia* from a sink drain. *J Hosp Infect* 50, 235-237 (2002)
91. Dy M. E., J. A. Nord, V. LaBombardi, J. Germana & P. Walker: Lack of throat colonization with *Burkholderia cepacia* among cystic fibrosis healthcare workers. *Infect Control Hosp Epidemiol* 20, 90 (1999)
92. Fisher M. C., J. J. LiPuma, S. E. Dasen, G. C. Caputo, J. E. Mortensen, K. L. McGowan & T. L. Stull: Source of *Pseudomonas cepacia*: ribotyping of isolates from patients and from the environment. *J Pediatr* 123, 745-747 (1993)
93. Mortensen J. E., M. C. Fisher & J. J. LiPuma: Recovery of *Pseudomonas cepacia* and other

Pseudomonas species from the environment. *Infect Control Hosp Epidemiol* 16, 30-32 (1995)
94. Butler S. L., C. J. Doherty, J. E. Hughes, J. W. Nelson & J. R. W. Govan: *Burkholderia cepacia* and cystic fibrosis: do natural environments present a potential hazard? *J Clin Microbiol* 33, 1001-1004 (1995)
95. Gonzalez C. F., G. L. Mark, E. Mahenthiralingam & J. J. LiPuma: Isolation of soilborne genomovar III *Burkholderia cepacia* and lytic phages with inter-genomovar host range. *Pediatr Pulmonol* S20, 288-289 (2000)
96. Balandreau J., V. Viallard, B. Cournoyer, T. Coenye, S. Laevens & P. Vandamme: *Burkholderia cepacia* genomovar III is a common plant-associated bacterium. *Appl Env Microbiol* 67, 982-985 (2001)
97. Fiore A., S. Laevens, A. Bevivino, C. Dalmastri, S. Tabbacchioni, P. Vandamme & L. Chiarini: *Burkholderia cepacia* complex: distribution of genomovars among isolates from the maize rhizosphere in Italy. *Environ Microbiol* 3, 137-143 (2001)
98. Bevivino A., C. Dalmastri, S. Tabacchioni, L. Chiarini, M. L. Belli, S. Piana, A. Materazzo, P. Vandamme & G. Manno: *Burkholderia cepacia* complex bacteria from clinical and environmental sources in Italy: genomovar status and distribution of traits related to virulence and transmissibility. *J Clin Microbiol* 40, 846-851 (2002)
99. LiPuma J. J., T. Spilker, T. Coenye & C. F. Gonzalez: An epidemic *Burkholderia cepacia* complex strain identified in soil. *Lancet* 359, 2002-2003 (2002)
100. Miller S. M., J. L. Parke, S. Bies & J. J. LiPuma: Detection, recovery and identification of *Burkholderia cepacia* from the natural environment. *Pediatr Pulmonol* S20, 288 (2000)
101. Moore J. E., B. McIlhatton, A. Shaw, P. G. Murphy & J. S. Elborn: Occurrence of *Burkholderia cepacia* in foods and waters: clinical implications for patients with cystic fibrosis. *J Food Prot* 7, 1076-1078 (2001)
102. Berriatua E., I. Ziluaga, C. Miguel-Virto, P. Uribarren, R. Juste, S. Laevens, P. Vandamme & J. R. W. Govan: Outbreak of subclinical mastitis in a flock of dairy sheep associated with *Burkholderia cepacia* complex infection. *J Clin Microbiol* 39, 990-994 (2001)
103. LiPuma J. J., T. Spilker, L. H. Gill, P. W. Campbell III, L. Liu & E. Mahenthiralingam: Disproportionate distribution of *Burkholderia cepacia* complex species and transmissibility markers in cystic fibrosis. *Am J Respir Crit Care Med* 164, 92-96 (2001)
104. Agodi A., E. Mahenthiralingam, M. Bachitta, V. Giannino, A. Sciacca & S. Stefani: *Burkholderia cepacia* complex infection in Italian patients with cystic fibrosis: prevalence, epidemiology, and genomovar status. *J Clin Microbiol* 39, 2891-2896 (2001)
105. Speert D. P., D. Henry, P. Vandamme, M. Corey & E. Mahenthiralingam: Epidemiology of *Burkholderia cepacia* complex in patients with cystic fibrosis, Canada. *Emerg Infect Dis* 8, 181-187 (2002)
106. Pitt T. L., M. E. Kaufmann, P. S. Patel, L. C. Benge, S. Gaskin & D. M. Livermore: Type characterisation and antibiotic susceptibility of *Burkholderia (Pseudomonas) cepacia* isolates from patients with cystic fibrosis in the United Kingdom and the Republic of Ireland. *J Med Microbiol* 44, 203-210 (1996)
107. Sun L., R. Z. Jiang, S. Steinbach, A. Holmes, C. Campanelli, J. Forstner, U. Sajjan, Y. Tan, M. Riley & R. Goldstein: The emergence of a highly transmissible lineage of *cbl+ Pseudomonas (Burkholderia) cepacia* causing CF centre epidemics in north America and Britain. *Nat Med* 1, 661-666 (1995)
108. Kumar A., S. Dietrich, W. Schneider, R. Jacobson, F. Pouch Downes, B. E. Robinson-Dunn, R. Honicky, J. Smith & R. Martin: Genetic relatedness of *Burkholderia (Pseudomonas) cepacia* isolates from five cystic fibrosis centers in Michigan. *Resp Med* 91, 485-492 (1997)
109. Mahenthiralingam E., D. A. Simpson & D. P. Speert: Identification and characterization of a novel DNA marker associated with epidemic *Burkholderia cepacia* strains recovered from patients with cystic fibrosis. *J Clin Microbiol* 35, 808-816 (1997)
110. Simpson I. N., J. Finlay, D. J. Winstanley, N. Dewhurst, J. W. Nelson, S. L. Butler & J. R. W. Govan: Multi-resistance isolates possessing characteristics of both *Burkholderia (Pseudomonas) cepacia* and *Burkholderia gladioli* from patients with cystic fibrosis. *J Antmicrob Chemother* 34, 353-361 (1994)
111. Chen J., K. A. Witzmann, T. Spilker, R. J. Fink & J. J. LiPuma: Endemicity and inter-city spread of *Burkholderia cepacia* genomovar III in cystic fibrosis. *J Pediatr* 139, 643-649 (2001)
112. Segonds C., T. Heulin, N. Marty & G. Chabanon: Differentiation of *Burkholderia* species by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene and application to cystic fibrosis isolates. *J Clin Microbiol* 37, 2201-2208 (1999)
113. Whitby P. W., H. L. N. Dick, P. W. Campbell III, D. E. Tullis, A. Matlow & T. L. Stull: Comparison of culture and PCR for detection of *Burkholderia cepacia* in sputum samples of patients with cystic fibrosis. *J Clin Microbiol* 36, 1642-1645 (1998)
114. Mahenthiralingam E., P. Vandamme, M. E. Campbell, D. A. Henry, A. M. Gravelle, L. T. K. Wong, A. G. F. Davidson, P. G. Wilcox, B. Nakielna & D. P. Speert: Infection with *Burkholderia cepacia* complex genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace *Burkholderia multivorans*. *Clin Infect Dis* 33, 1469-1475 (2001)
115. Ledson M. J., M. J. Gallagher, J. E. Corkill, C. A. Hart & M. J. Walshaw: Cross infection between cystic fibrosis patients colonised with Burkholderia cepacia. *Thorax* 53, 432-436 (1998)
116. Hearst J. E. & K. E. Elliott: Identifying the killer in cystic fibrosis. *Nat Med* 1, 626-627 (1995)
117. Clode F. E., M. E. Kaufmann, H. Malnick & T. L. Pitt: Distribution of genes encoding putative transmissibility factors among epidemic and nonepidemic strains of *Burkholderia cepacia* from cystic fibrosis patients in the United Kingdom. *J Clin Microbiol* 38, 1763-1766 (2000)
118. Mahenthiralingam E., T. Coenye, J. W. Chung, D. P. Speert, J. R. Govan, P. Taylor & P. Vandamme: Diagnostically and experimentally useful panel of strains from the *Burkholderia cepacia* complex. *J Clin Microbiol* 38, 910-913 (2000)
119. Aris R. M., J. C. Routh, J. J. LiPuma, D. G. Heath & P. H. Gilligan: Lung transplantation for cystic fibrosis patients with *Burkholderia cepacia* complex: survival

linked to genomovar type. *Am J Respir Crit Care Med* 164, 2102-2106 (2001)
120. De Soyza A., A. McDowell, L. Archer, J. H. Dark, S. J. Elborn, E. Mahenthiralingam, K. Gould & P. A. Corris: *Burkholderia cepacia* complex genomovars and pulmonary transplantation outcomes in patients with cystic fibrosis. *Lancet* 358, 1780-1781 (2001)
121. Bottone E. J., S. D. Douglas, A. R. Rausen & G. T. Keusch: Association of *Pseudomonas cepacia* with chronic granulomatous disease. *J Clin Microbiol* 1, 425-428 (1975)
122. Siber O. F. & V. A. Fulginiti: *Pseudomonas cepacia* pneumonia in a child with chronic granulomatous disease and selective IgA deficiency. *Acta Paediatr Scand* 65, 519-520 (1976)
123. Lacy D. E., D. A. Spencer, A. Goldstein, P. H. Weller & P. Darbyshire: Chronic granulomatous disease presenting in childhood with *Pseudomonas cepacia* septicaemia. *J Infect* 27, 301-304 (1993)
124. Ahlin A., M. De Boer, D. Roos, J. Leusen, C. I. Smith, U. Sundin, H. Rabbani, J. Palmblad & G. Elinder: Prevalence, genetics and clinical presentation of chronic granulomatous disease in Sweden. *Acta Paediatr* 84, 1386-1394 (1995)
125. Johnston R. B.: Clinical aspects of chronic granulomatous disease. *Curr Opin Hematol* 8, 17-22 (2001)
126. Winkelstein J. A., M. C. Marino, R. B. Johnston, J. Boyle, J. Curnutte, J. I. Gallin, H. L. Malech, S. M. Holland, H. Ochs, P. Quie, R. H. Buckley, C. B. Foster, S. J. Chanock & H. Dickler: Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine* 79, 155-169 (2000)
127. Speert D. P., M. Bond, R. C. Woodman & J. T. Curnutte: Infection with *Pseudomonas cepacia* in chronic granulomatous disease: role of nonoxidative killing by neutrophils in host defense. *J Infect Dis* 170, 1524-1531 (1994)
128. Pegues D. A., L. A. Carson, R. L. Anderson, M. J. Norgard, T. A. Argent, W. R. Jarvis & C. H. Woernle: Outbreak of *Pseudomonas cepacia* bacteremia in oncology patients. *Clin Infect Dis* 16, 407-411 (1993)
129. Yamagishi Y., J. Fujita, K. Takigawa, K. Negayama, T. Nakazawa & J. Takahara: Clinical features of *Pseudomonas cepacia* pneumonia in an epidemic among immunocompromised patients. *Chest* 103, 1706-1709 (1993)
130. van Laer F., D. Raes, P. Vandamme, C. Lammens, J. P. Sion, C. Vrints, J. Snoeck & H. Goossens: An outbreak of *Burkholderia cepacia* with septicaemia on a cardiology ward. *Infect Control Hosp Epidemiol* 19, 112-113 (1998)
131. Kaitwatcharachai C., K. Silpapojakul, S. Jitsurong & S. Kalnauwakul: An outbreak of *Burkholderia cepacia* bacteremia in hemodialysis patients: an epidemiological and molecular study. *Am J Kidney Dis* 36, 199-204 (2000)
132. Mandell I. N., H. D. Feiner, N. M. Price & M. Simberkoff: *Pseudomonas cepacia* endocarditis and ecthyma gangrenosum. *Arch Dermatol* 113, 199-202 (1977)
133. Hobson R., I. Gould & J. Govan: *Burkholderia (Pseudomonas) cepacia* as a cause of brain abscesses secondary to chronic suppurative otitis media. *Eur J Clin Microbiol Infect Dis* 14, 908-911 (1995)
134. Waterer G. W., C. B. Jones & R. G. Wunderink: Bacteremic community-acquired pneumonia in an immunocompetent adult due to *Burkholderia cepacia. Chest* 116, 1842-1843 (1999)
135. Steere A. C., J. H. Tenney, D. C. Mackel, M. J. Snyder, S. Polakavetz, M. E. Dunne & R. Dixon: *Pseudomonas* species bacteremia caused by contaminated normal human serum albumin. *J Infect Dis* 135, 729-735 (1977)
136. Sobel J. D., N. Hashman, G. Reinherz & D. Merzbach: Nosocomial *Pseudomonas cepacia* infection associated with chlorhexidine contamination. *Am J Med* 73, 183-186 (1982)
137. Keizur J. J., B. Lavin & R. B. Leidich: Iatrogenic urinary tract infection with *Pseudomonas cepacia* after transrectal ultrasound guided needle biopsy of the prostate. *J Urol* 149, 523-526 (1993)
138. Weems J. J.: Nosocomial outbreak of *Pseudomonas cepacia* associated with contamination of reusable electronic ventilator temperature probes. *Infect Control Hosp Epidemiol* 14, 583-586 (1993)
139. Gravel-Tropper D., M. L. Sample, C. Oxley, B. Toye, D. E. Woods & G. E. Garber: Three-year outbreak of pseudobacteremia with *Burkholderia cepacia* traced to a contaminated blood gas analyzer. *Infect Control Hosp Epidemiol* 17, 737-740 (1996)
140. Reboli A. C., R. Koshinski, K. Arias, K. Marks-Austin, D. Stieritz & T. L. Stull: An outbreak of Burkholderia cepacia lower respiratory tract infection associated with contaminated albuterol nebulization solution. *Infect Control Hosp Epidemiol* 17, 741-743 (1996)
141. Matrician L., G. Ange, S. Burns, W. L. Fanning, C. Kioski, G. D. Cage & K. K. Komatsu: Outbreak of nosocomial *Burkholderia cepacia* infection and colonization associated with intrinsically contaminated mouthwash. *Infect Control Hosp Epidemiol* 21, 739-741 (2000)
142. Gravel D., M. L. Sample, K. Ramotar, B. Toye, C. Oxley & G. Garber: Outbreak of *Burkholderia cepacia* in the adult intensive care unit traced to contaminated indigo-carmine dye. *Infect Control Hosp Epidemiol* 23, 103-106 (2002)
143. Siddiqui A. H., M. E. Mulligan, E. Mahenthiralingam, J. Hebden, J. Brewink, S. Qaiyumi, J. A. Johnson & J. J. LiPuma: An episodic outbreak of genetically related *Burkholderia cepacia* among non-cystic fibrosis patients at a university hospital. *Infect Control Hosp Epidemiol* 22, 419-422 (2001)
144. Berkelman R. L., S. Lewin, J. R. Allen, R. L. Anderson, L. D. Budnick, S. Shapiro, S. M. Friedman, P. Nicholas, R. S. Holzman & R. W. Haley: Pseudobacteremia attributed to contamination of povidone-iodine with *Pseudomonas cepacia. Ann Intern Med* 95, 32-36 (1981)
145. Craven D. E., B. Moody, M. G. Connolly, N. R. Kollisch, K. D. Stottmeier & W. R. McCabe: Pseudobacteremia caused by povidone-iodine solution contaminated with *Pseudomonas cepacia. N Engl J Med* 305, 621-623 (1981)
146. Ledson M. J., M. J. Gallagher & M. J. Walshaw: Chronic *Burkholderia cepacia* bronchiectasis in a non-cystic fibrosis individual. *Thorax* 53, 430-432 (1998)
147. Holmes A., R. Nolan, R. Taylor, R. Finley, M. Riley, R. Jiang, S. Steinbach, & R. Goldstein. An epidemic of *Burkholderia cepacia* transmitted between patients with and without cystic fibrosis. *J Infect Dis* 179, 1197-1205 (1997)
148. Palleroni N. J.: Genus I Pseudomonas Migula 1894, 237AL. In: Bergey's Manual of Systematic Bacteriology

Volume 1. Eds: Krieg NR, Holt JG, Williams and Wilkins, Baltimore, MD (1984)
149. Christenson J. C., D. F. Welch, G. Mukwaya, M. J. Muszynsky, R. E. Weaver & D. J. Brenner: Recovery of *Pseudomonas gladioli* from respiratory tract specimens of patients with cystic fibrosis. *J Clin Microbiol* 27, 270-273 (1989)
150. Ross J. P., S. M. Holland, V. J. Gill, E. S. DeCarlo & J. I. Gallin: Severe *Burkholderia (Pseudomonas) gladioli* infection in chronic granulomatous disease: report of two successfully treated cases. *Clin Infect Dis* 21, 1291-1293 (1995)
151. Hoare S. & A. J. Cant: Chronic granulomatous disease presenting as severe sepsis due to *Burkholderia gladioli*. *Clin Infect Dis* 23, 441 (1996)
152. Khan S. U., S. M. Gordon, P. C. Stilwell, T. J. Kirby & A. C. Arroliga: Empyema and bloodstream infection caused by *Burkholderia gladioli* in a patient with cystic fibrosis after lung transplantation. *Pediatr Infect Dis J* 15, 637-639 (1996)
153. Graves M., T. Robin, A. M. Chipman, J. Wong, S. Khashe & J. M. Janda: Four additional cases of *Burkholderia gladioli* infection with microbiological correlates and review. *Clin Infect Dis* 25, 838-842 (1997)
154. Jones A. M., T. N. Stanbridge, B. J. Isalska, M. E. Dodd & A. K. Webb: *Burkholderia gladioli*: recurrent abscesses in patient with cystic fibrosis. *J Infect* 42, 69-71 (2001)
155. Shelly D. B., T. Spilker, E. Gracely, T. Coenye, P. Vandamme & J. J. LiPuma: Utility of commercial test systems for identification of *Burkholderia cepacia* complex from cystic fibrosis sputum culture. *J Clin Microbiol* 38, 3112-3115 (2000)
156. Wilsher M. L., J. Kolbe, A. J. Morris & D. F. Welch. Nosocomial acquisition of *Burkholderia gladioli* in patients with cystic fibrosis. *Am J Respir Crit Care Med* 155, 1436-1440 (1997)
157. Clode F. E., L. A. Metherell & T. L. Pitt. Nosocomial acquisition of *Burkholderia gladioli* in patients with cystic fibrosis. *Am J Respir Crit Care Med* 160, 374-375 (1999)
158. Whitby P. W., L. C. Pope, K. B. Carter, J. J. LiPuma & T. L. Stull: Species-specific PCR as a tool for the identification of *Burkholderia gladioli*. *J Clin Microbiol* 38, 282-285 (2000)
159. Centers for Disease Control and Prevention: Laboratory-acquired human glanders – Maryland, May 2000. *MMWR* 49, 532-535 (2000)
160. Wheelis M.: First shots fired in biological warfare. *Nature* 395, 213 (1998)
161. Gilligan P.: *Pseudomonas* and *Burkholderia*. In: Manual of Clinical Microbiology. Eds.: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, ASM Press, Washington DC (1995)
162. Dance D. A.: Melioidosis: the tip of the iceberg? *Clin Microbiol Rev* 4, 52-60 (1991)
163. Dance D. A.: Ecology of *Burkholderia pseudomallei* and the interactions between environmental *Burkholderia* spp. and human-animal hosts. *Acta Trop* 74, 159-168 (2000)
164. Haase A., H. Smith-Vaughan, A. Melder, Y. Wood, A. Janmaat, J. Gilfedder, D. Kemp & B. Currie: Subdivision of *Burkholderia pseudomallei* ribotypes into multiple types by random amplified polymorphic DNA analysis provides new insights into epidemiology. *J Clin Microbiol* 33, 1687-1690 (1995)
165. Currie B. J., M. Mayo, N. M. Anstey, P. Donohoe, A. Haase & D. J. Kemp: A cluster of melioidosis cases from an endemic region is clonal and is linked to the water supply using molecular typing of *Burkholderia pseudomallei* isolates. *Am J Trop Med Hyg* 65, 177-179 (2001)
166. Currie B. J., D. A. Fisher, D. M. Howard, J. N. Burrow, S. Selvanayagam, P. L. Snelling, N. M. Anstey & M. J. Mayo: The epidemiology of melioidosis in Australia and Papua New Guinea. *Acta Trop* 74, 121-127 (2000)
167. Dorman S. E., V. J. Gill, J. I. Gallin & S. M. Holland: *Burkholderia pseudomallei* infection in a Puerto Rican patient with chronic granulomatous disease: case report and review of occurrences in the Americas. *Clin Infect Dis* 26, 889-894 (1998)
168. Dance D. A., M. D. Smith, H. M. Aucken & T. L. Pitt: Imported melioidosis in England and Wales. *Lancet* 353, 208 (1999)
169. Visca P., G. Cazzola, A. Petrucca & V. Vraggion: Travel-associated *Burkholderia pseudomallei* infection (melioidosis) in a patient with cystic fibrosis: a case report. *Clin Infect Dis* 32, e15-e16 (2001)
170. Schulin T. & I. Steinmetz: Chronic melioidosis in a patient with cystic fibrosis. *J Clin Microbiol* 39, 1676-1677 (2001)
171. McMenamin J. D., T. M. Zaccone, T. Coenye, P. Vandamme & J. J. LiPuma. Misidentification of *Burkholderia cepacia* in U.S. cystic fibrosis treatment centers: An analysis of 1051 recent sputum isolates. *Chest* 117,1661-1665 (2000).
172. Mahenthiralingam E., J. Bischof, S. K. Byrne, C. Radomski, J. E. Davies, Y Av-Gay & P. Vandamme. DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J Clin Microbiol* 38,3165-3173 (2000)
173. Whitby P. W., K. B. Carter, J. L. Burns, J. A. Royall, J. J. LiPuma & T. L. Stull. Identification and detection of *Stenotrophomonas maltophilia* by rRNA-directed PCR. *J Clin Microbiol* 38,4305-4309 (2000)
174. Coenye T, L. Liu, P. Vandamme & J. J. LiPuma. Identification of *Pandoraea* species by 16S ribosomal DNA-based PCR assays. *J Clin Microbiol* 39,4452-4455 (2001)
175. Liu L., T. Coenye, J. L. Burns, P. Whitby, T. L. Stull & J. J. LiPuma. Ribosomal DNA-directed PCR for identification of *Achromobacter* (*Alcaligenes*) *xylosoxidans* recovered from sputum samples from cystic fibrosis patients. *J Clin Microbiol* 40,1210-1213 (2002)
176. Coenye T, P. Vandamme & LiPuma JJ. Infection by *Ralstonia* species in cystic fibrosis patients: Identification of *R. pickettii* and *R. mannitolilytica* by polymerase chain reaction. *Emerg Infect Dis* 8,692-696 (2002)

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