Chapter 4

Burkholderia cepacia Complex Infections Among Cystic Fibrosis Patients: Perspectives and Challenges

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Abstract

The *Burkholderia cepacia* complex (Bcc) is a group of closely related bacterial species that emerged in the 1980s as the etiological agents of severe and often lethal respiratory infections among cystic fibrosis (CF) patients. After several outbreaks in CF centers in Europe and North America, segregation measures were introduced to avoid patient-to-patient transmission. Presently, the prevalence of Bcc infections among CF patients worldwide is below 5% in the majority of CF centers, although exceptions are registered in some European countries. Infections by these pathogens remain problematic due to the high resistance to antimicrobials, the easy patient-to-patient transmission, and the unpredictable outcome of infections that range from asymptomatic carriage to the cepacia syndrome, a fulminating pneumonia often associated with septicemia that can lead to the decease of patients within a period of time as short as 1 week. In this chapter, we review the evolving epidemiology of Bcc infections in CF patients, the virulence traits and mechanisms used by these bacteria, and the recent developments in vaccine and vaccine components research to prevent Bcc infections.

Keywords: *Burkholderia cepacia* complex, emerging species, evolving epidemiology, virulence determinants, immunoreactive proteins, vaccine development

1. Introduction

The *Burkholderia cepacia* complex (hereafter referred to as Bcc) is a group of closely related bacteria that emerged in the 1980s as problematic pathogens to cystic fibrosis (CF) patients [1]. Infections by Bcc are particularly feared due to (1) the easy patient-to-patient transmission of

specific strains; (2) the ability to resist to multiple antibiotics; and (3) the unpredictable outcome of infections, which ranges from asymptomatic carriage to the so-called cepacia syndrome, an often lethal necrotizing pneumonia accompanied with septicemia [1, 2]. Initially described in the 1950s by Burkholder [3] as the cause of soft rot in onions, the species then named *Pseudomonas cepacia* was moved into the new genus *Burkholderia* after the work of Yabuuchi and colleagues in 1992 [4]. However, the most impressive developments on the taxonomy of this group of bacteria have been achieved after the seminal work of Vandamme and colleagues who proposed the division of the species into distinct genomovars [5]. Presently, the Bcc comprises 20 species (**Table 1**), and the genome sequence of several strains is publicly available in databases such as the Burkholderia Genome DB and the Integrated Microbial Genomes & Microbiomes [6, 7].

Bcc species	Genome sequence availability	Reference
B. ambifaria	4 complete genomes (strains AMMD, MC40-6, MEX-5, IOP-120)	[8]
B. anthina	In progress	[9]
B. arboris	In progress	[10]
B. cenocepacia	18 complete genomes (strains J2315, H111, AU1054, B1, MCO-3, PC184, HI2424, DDS 22E-1, DWS 37E-2, ST32, 842, 895, MSMB384 WGS, 6, 7, CEIB, 869T2, TAtl-371)	[11]
B. cepacia	8 complete genomes (strains 383, AMMD, ATCC 25416; Bu72, DDS 7H-2, GG4, JBK9, LO6)	[4]
B. contaminans	1 complete genome (strain MS14)	[12]
B. diffusa	In progress	[10]
B. dolosa	1 complete genome (strain AU0158)	[13]
B. lata	1 complete genome (strain 383)	[12]
B. latens	In progress	[10]
B. metallica	No information	[10]
B. multivorans	3 complete genomes (ATCC17616, ATCC BAA-247, DDS 15A-1)	[5]
B. pseudomultivorans	In progress	[14]
B. pyrrocinia	1 complete genome (strain DSM 10685)	[9]
B. seminalis	In progress	[10]
B. stabilis	No information	[15]
B. stagnalis	In progress	[16]
B. territorii	In progress	[16]
B. ubonensis	1 complete genome (strain MSMB22)	[17]
B. vietnamiensis	3 complete genomes (strains G4, LMG10929, WPB)	[18]

Databases were assessed by the end of July 2016.

Table 1. *Burkholderia cepacia* complex species names and genome sequence availability in the databases Burkholderia Genome DB and Integrated Microbial Genomes & Microbiomes [6, 7].

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2. Evolving epidemiology of Bcc infections

All Bcc species are virtually potential pathogens to CF patients. However, epidemiology studies have shown an uneven geographical and regional distribution of clinical isolates among the Bcc species, with the predominance of *Burkholderia cenocepacia*, followed by *Burkholderia multivorans*. Early studies performed during the 1980s and 1990s have shown that in addition to cases of chronic infection due to specific strains, many outbreaks reported in Europe and North America were due to the spread of particularly virulent strains that easily disseminated within a given CF center [1]. Although the environment is thought to be the natural reservoir of these strains, a definitive proof is still lacking.

A few particularly epidemic strains became notorious for the worst reasons. Perhaps, the best-known strain is the Edinburgh-Toronto lineage also known as the ET12 clone, an intercontinental clone responsible for several infections and fatalities in CF centers in the UK and Canada [19]. The best-known representative strain of this highly transmissible clone is the *B. cenocepacia* J2315 strain, the first Bcc strain with its genome sequence publicly available (**Table 1**) and one of the best studied Bcc strains [20]. Another example of a strain that disseminated within centers and even among centers is the PHDC strain. The strain, responsible for almost 20% prevalence in one CF center in the USA, was later found in another CF center, where an increase in Bcc prevalence was experienced. The dissemination of the strain was associated with the transfer of an infected patient from the initial center to the second one [21]. A later study by Coenye et al. [22] showed that the PHDC strain was also present in European patients (namely in France, Italy, and the UK), concluding that the PHDC strain was the second-identified Bcc transatlantic clone. Interestingly, both intercontinental clones belong to the *B. cenocepacia* species, although the ET12 belongs to subgroup IIIA and the PHDC belongs to subgroup IIIB. The *B. cenocepacia* species includes other clones that spread among CF centers, namely the Midwest American clone and the CZI Czech epidemic clone [23, 24]. Evidence of transmission of particularly epidemic strains of *B. cenocepacia* led to the introduction of segregation measures in CF centers in Europe and America, with a significant reduction of prevalence of infections [1, 25–27]. However, these segregation policies had a devastating impact on patients infected with Bcc due to social isolation and stigma and negative psychological impacts [28]. Although effective in interrupting strain transmission, segregation measures do not prevent new acquisitions. Nevertheless, these measures led to a reduction of prevalence of Bcc infections from more than 20% in several centers to less than 5% both in the USA and the majority of European countries [29, 30]. However, prevalence of chronic Bcc infections is still ranging 5–10% in Denmark, Portugal, Slovak Republic, Russian Federation, and Latvia, reaching values of 15 and 23% in Serbia and Lithuania, respectively [30].

Although the Bcc strains responsible for the vast majority of infections both in Europe and North America belong to the *B. cenocepacia* species, recent evidence indicates a changing epidemiology. *B. multivorans* emerged as the dominant species in France by 2004 and as the second most important species in the USA [31, 32]. Recent reports also indicate *Burkholderia contaminans* as an emerging Bcc species associated with CF infections. Early reports of a high incidence of the species among CF patients came from Portugal and Argentina [33–35].

Interestingly, in the case of the Portuguese CF population, two *B. contaminans* clones infecting CF patients were found as indistinguishable from two *B. contaminans* strains isolated from nonsterile nasal saline solutions of commercial origin during routine surveillance by the Portuguese Medicines and Health Products Authority [36]. A recent work by Medina-Pascual and colleagues on the surveillance of Bcc infections in Spanish CF patients also reported a *B. contaminans* overall incidence of 36.5% in the period 2008–2012, surpassing the previously dominant species *B. cenocepacia* and *B. multivorans* [37]. The emergence of *B. contaminans* among Spanish CF patients was hypothesized to be due to unspecified ecological advantages that enable the species to increase its presence in hospitals or in the environment [37]. In the case of Swiss CF-patients, *B. cenocepacia* was the most frequently isolated species in the period 1998–2013, but *B. multivorans* and *B. contaminans* emerged during the last years of the study period [38]. A 30-year study of Bcc infections among CF patients from British Columbia (Canada) evidenced a major impact of segregation measures in Bcc epidemiology; while *B. cenocepacia* was dominant before the introduction of these measures, *B. multivorans* strains became dominant after implementation of novel infection control measures in 1995 [39]. This study and others highlight the impact of infection control measures on Bcc species recovered from CF patients. It is now apparent that while epidemic *B. cenocepacia* strains dominated in early years, nonclonal *B. multivorans* and *B. contaminans* strains are emerging.

3. Bcc virulence factors and traits

Over the last 20 years, substantial progress has been achieved on the knowledge of Bcc virulence factors and determinants, although the exact contribution of some of them to the success of infection remains to be fully understood. It is currently accepted that Bcc virulence does not rely on a single virulence factor, being multifactorial. Bacterial structures such as flagella, the cable pili, and the 22-kDa adhesin are considered virulence factors since they play important roles in the initial steps of interaction with the host cell, promoting the adherence to the lung surface and the invasion of lung epithelial cells [39–41]. In addition, the majority of *B. cenocepacia* strains are able to survive and replicate intracellularly in airway epithelial cells and macrophages, evading the primary cellular defense mechanisms of the lung and avoiding clearance. The factors involved in this ability, exopolysaccharide (EPS) biosynthesis, biofilm formation, resistance to antibiotics, and oxidative stress resistance, as well as the iron acquisition ability are also among virulence determinants described for Bcc [20, 42, 43]. Some of these virulence factors are further detailed below.

3.1. Alternative sigma factors

RpoE and RpoN are two alternative sigma factors involved in the regulation of the ability of intracellular *B. cenocepacia* to delay phagolysosomal fusion in murine macrophages [44, 45]. RpoE is the extra-cytoplasmic stress response regulator required by *B. cenocepacia* to grow under conditions of high osmolarity and high temperature [44]. RpoN, or sigma factor σ^{54}, is best known for its involvement in nitrogen-related gene regulation. In *B. cenocepacia*, σ^{54} is involved

in motility and biofilm formation [45]. Results from the mapping of σ^{54} regulon and the characterization of a *B. cenocepacia* H111-derived σ^{54} mutant suggest that this alternative sigma factor plays an important role in the control of nitrogen metabolism, in the metabolic adaptation of *B. cenocepacia* H111 to stressful and nutrient-limited environments and in virulence toward the nematode *Caenorhabditis elegans* [46]. In addition, it was also reported that RpoN regulates genes involved in exopolysaccharide production, biofilm formation, motility, and virulence [46]. A *B. cenocepacia* mutant defective in a gene encoding a putative σ^{54}-related transcription regulator (BCAL1536) was found as attenuated in the rat agar bead infection model [47].

3.2. Lipopolysaccharides and extracellular polysaccharides

One of the central components of the outer membrane in Gram-negative bacteria is the lipopolysaccharide (LPS), a complex molecule composed by the lipid A, the core oligosaccharide, and the O-antigen moieties (reviewed in Ref. [48]). The genes involved in LPS production by *B. cenocepacia* are located in chromosome I, organized in three main clusters, one for each LPS component (lipid A: *BCAL1929* to *BCAL1935*; core: *BCAL2402* to *BCAL2408*; O antigen: *BCAL3110* to *BCAL3125*) together with additional genes encoding sugar modification enzymes [49, 50]. Bcc bacteria LPS differs from other Gram-negative bacteria LPS due to the complete lack of negatively charged residues and the presence of the heterodimeric disaccharide D-glycero-D-talo-oct-2-ulosonic acid-(2–4)-3-deoxy-D-manno-oct-2-ulosonic acid (Ko-(2–4)-Kdo) in the core region; the presence of a 4-amino-4-deoxyarabinose (Ara4N) residue, either in the core or in lipid A; and the structure of O-antigen [50, 51]. This particular composition changes the bacterial surface charge, inhibiting the binding and successful action of antibiotics, contributing to the persistence of bacterial infection [51]. Recently, it was demonstrated that although L-Ara4N modifications do not affect recognition, they are critical for the establishment of infection [52]. Several studies have demonstrated that when neutrophils interact with Bcc LPS, the expression of CD11b on their surface increases, stimulating neutrophil respiratory burst response [53]. In addition, macrophages and human blood cells are also stimulated by Bcc LPS, producing pro-inflammatory cytokines such as TNF-α, IL-6, and IL-8 [54, 55].

B. cenocepacia J2315 is unable to produce the O-antigen. In this particular strain, this is due to an interruption in the *wbcE* gene-encoding BCAL 3125 [56]. The expression of O-antigen by Bcc strains was demonstrated to reduce phagocytosis by macrophages without interfering with the intracellular survival of bacteria [56].

The production of exopolysaccharides (EPSs) was described for several *Burkholderia* species. EPS production by Bcc is regarded as playing an important role in the chronicity of Bcc infections [57–62]. Cepacian is the most common EPS produced by Bcc and non-Bcc species, both from clinical and environmental sources [59, 63]. Cepacian interferes with phagocytosis by human neutrophils, facilitating the bacterial persistence in a mouse model of infection [64, 65]. The EPS was shown to inhibit the production of ROS by neutrophils and to scavenge reactive oxygen species (ROS), playing a role in the survival of cepacian-producing strains in different environments [64–67]. As a result of a frameshift mutation in the *bceB* gene (*BCAM0856*) encoding a putative

glycosyltransferase, Cepacian is not produced by the *B. cenocepacia* ET12 representative strain J2315 [49, 62].

3.3. Biofilms

Bcc bacteria were found to persist in biofilms *in vitro*. Biofilm formation and maturation depend on many factors, including EPS production, motility, iron availability, and multiple gene regulatory systems, such as quorum sensing, alternative sigma factors, or global regulators such as the ShvR and AtsR [45, 58, 68–73]. In addition, Bcc can form small colony variants *in vitro*, a colony morphology that is associated with enhanced biofilm formation, antibiotic resistance, and persistence [74].

Several studies have been performed to understand the importance and relevance of biofilm formation in Bcc biology. Bcc bacteria growing in biofilms are usually more tolerant to multiple antibiotics, although similar susceptibilities were reported for plancktonic and biofilm cells to the antibiotics kanamycin, amikacin, and ciprofloxacin [75, 76]. Recently, Bcc biofilms were shown to contain persister cells that are able to survive in the presence of high concentrations of antibiotics by avoiding production of reactive oxygen species [77]. In addition, using neutrophil-like dHL60 cells, it was shown that the presence of these immune system cells enhanced biofilm formation that protected Bcc bacteria against neutrophils by inducing their necrosis, acting as a barrier to the migration of neutrophils, and masking the bacteria from being recognized by neutrophils [78]. Although some evidence suggests that biofilm formation plays a role in bacterial persistence in the CF airways, this topic needs to be further studied.

3.4. Quorum sensing

Quorum sensing is a mode of regulation of gene expression that is dependent on the density of the bacterial population. Bcc bacteria have at least four quorum sensing systems. The CepIR quorum sensing system is homologous to the LuxIR system of *Vibrio fischeri* (reviewed in Ref. [79]). The CepIR system positively regulates the virulence of *B. cenocepacia* toward model organisms like *C. elegans*, *Galleria mellonella*, rodents, zebrafish, alfalfa, and onions [80–83]. In addition to the CepIR, *B. cenocepacia* encodes the CciIR, the CepR2, and the BDSF quorum sensing systems [84, 85]. While the CepIR and CciR quorum sensing systems rely on acyl homoserine lactones as signaling molecules, the BDSF system uses cis-2-dodecenoic acid as the signaling molecule, and the CepR2 is an orphan quorum sensing system [85]. An arsenal of genes regulated by quorum sensing in Bcc bacteria was described, including the negatively regulated siderophore synthesis and the positively regulated expression of the genes encoding zinc metalloproteases (Zmps), swarming motility and biofilm formation, all thought to have an impact when the bacterium is infecting the CF patient [71, 80, 86, 87].

3.5. Protein secretion systems

Both Gram-negative and positive bacteria use protein secretion systems to secrete toxins or other proteins, either directly into the environment or into host cells. These systems are

particularly well studied in the CF pathogens Bcc and *Pseudomonas aeruginosa*. For instance, Bcc strains of the ET12 lineage and *Burkholderia vietnamiensis* harbor type I and II secretion systems (T1SS, T2SS) implicated, for instance, in the secretion of hemolytic proteins [88, 89]. The T2SS is also involved in *B. cenocepacia* secretion of two zinc metalloproteases, ZmpA and ZmpB, which play a role in virulence [80, 90]. Two T4SSs are encoded by *B. cenocepacia*; the T4SS-1 encoded in a plasmid, and the T4SS-2 encoded in chromosome 2 [91]. Until now, only the T4SS-1 was identified in *B. cenocepacia* strains as necessary for virulence in onions and intracellular survival in phagocytes [92].

In a mouse agar bead infection model, the T3SS has been shown to be important for bacterial pathogenesis [93]. Although the precise mechanism is still not clear, T3SS seems to play no role in intracellular survival of *B. cenocepacia* [94].

Four type V secretion systems are encoded within the genome of *B. cenocepacia* J2315 [49]. Proteins transported by this type of transporters contain pertactin and hemagglutinin domains and are thought to play a role in bacterial adhesion [49].

B. cenocepacia also encodes a T6SS, which was shown to affect the actin cytoskeleton of macrophages and the assembly of the reduced nicotinamide adenine dinucelotide phosphate (NADPH) oxidase complex in *B. cepacia*-containing vacuoles (BcCV's) by inactivation of Rac1 and Cdc42 [73, 95, 96]. *B. cenocepacia* was found to efficiently activate the inflammasome by a yet uncharacterized T6SS effector [97]. Consequently, monocytes and THP-1 cells release IL-1β in a pyrin-, Asc-, and T6SS-dependent manner [97]. The T6SS also enhances caspase-1 activation, negatively regulated by the sensor kinase-response regulator AtsR [73]. In addition, a recent paper suggests that the T6SS might be important for the secretion of T2SS effectors into the host cytoplasm, such as ZmpA and ZmpB, revealing an unanticipated role for type II secretion systems in intracellular survival and replication of *B. cenocepacia* [96]. Although membrane vesicles cannot be considered a canonical secretion system, they can effectively allow the secretion of several hydrolytic enzymes and toxins [98]. **Table 2** summarizes and compares the most relevant information available about secretion systems of Bcc bacteria and their counterparts in the major CF pathogen *P. aeruginosa*.

3.6. Iron uptake

In order to carry out iron chelation and uptake, members of the Bcc can produce up to four distinct siderophores: ornibactin, pyochelin, cepabactin, and cepaciachelin [122]. Ornibactin appears to be the most important and abundant siderophore produced by *B. cenocepacia* strains [123, 124]. The pathways and regulatory mechanisms of ornibactin synthesis and uptake are relatively well known [87, 125–127]. The requirement of this siderophore for *B. cenocepacia* virulence was demonstrated in different infection models, including the rat agar bead, *G. mellonella,* and *C. elegans* [82, 125, 127].

The competition for available iron by Bcc bacteria and other CF lung colonizing organisms such as *P. aeruginosa* was reported to occur in the CF lung, although it is not completely clear how Bcc organisms acquire iron from host proteins [128, 129].

Secretion system	*Burkholderia cepacia* complex	*P. aeruginosa*
T1SS	Hemolytic proteins [88, 89]	HasAp (heme-binding) [99]; AprA and AprX (alkaline proteases) [100, 101]
T2SS	ZmpA and ZmpB [80, 90]	LasB (Major extracellular protease) [102], Staphylolysin LasA [102], Aminopeptidase PaAP [103], Protease IV [104], Lipases LipA, LipC, phospholipase C, PlcH, and PlcN [105, 106], CbpD Chitin-binding protein CbpD [107]; Exotoxin A [108]
T3SS	No effector described yet, plays a role in evasion of the host immune system [93, 94]	GTPase-activator ExoS and ADP-ribosyltransferase ExoT [109], adenylate cyclase ExoY [110], phospholipase A2 ExoU and ExoS [111]
T4SS	T4SS-1: Plant cytotoxic proteins, T4SS-2: Plasmid mobilization [91]	Integrative and conjugative elements (ICEs): ICE*clc* [112], Pathogenicity islands: pKLC102 (includes the type IV sex pili-encoding pil cluster and the *chvB* gene encoding a virulence factor) [113], and PAP-I (includes several virulence factors, such as CupD type fimbriae, and the PvrSR/RcsCB regulatory system) [114]
T5SS	Four T5SS: two containing pertactin domains involved in adhesion, other two contain haemagglutinin repeats [49]	Autotransporter: EstA (esterase activity) [115]; Two-partner secretion systems LepA/LepB [116] and CupB [117], and the PdtA/PdtB system [118]
T6SS	Hcp and VgrGs [73, 95, 96]	Hcp and VgrGs [119, 120]
Membrane vesicles (MV)	MV-associated (metallo)proteases, (phospho)lipases, peptidoglycan-degrading enzymes [98]	Multiple virulence factors: Alkaline phosphatase, hemolytic phospholipase C; the Cif toxin that inhibits CFTR-mediated chloride secretion in the airways [121]

Table 2. Summary of secretion systems from Bcc and the respective counterparts from the CF major pathogen *P. aeruginosa*.

3.7. Resistance to antimicrobials

Difficulties in eradicating Bcc infections mainly result from their intrinsic resistance to multiple antibiotics, including polymyxins, aminoglycosides, and most β-lactams. In addition, these bacteria have the ability to develop *in vivo* resistance to virtually all classes of antibiotics [20, 130, 131]. Antibiotics administration to CF patients was also reported to affect resistance profiles of Bcc bacteria [132]. Various mechanisms involved in the resistance of Bcc to multiple antibiotics have been described and include enzymatic inactivation (β-lactamases, aminoglycoside-inactivating enzymes, dihydrofolate reductase), alteration of drug targets, integrons, cell wall impermeability, and active efflux pumps [88, 133–140]. However, major contributions to intrinsic and acquired multidrug resistance by Bcc seem to be due to efflux pumps of the resistance nodulation cell division (RND) family. In fact, the *B. cenocepacia* J2315 genome encodes at least 16 efflux systems of the RND family [141]. At least six of these RND efflux pumps were implicated in drug resistance—RND-1, RND-3, RND-4, RND-8, RND-9, and RND-10 [138–140, 142,

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143]. RND-3 and RND-4 efflux pumps were described as being involved in the resistance to various antimicrobial drugs including tobramycin and ciprofloxacin; the RND-3, RND-8, and RND-9 efflux systems protect biofilm-grown cells against tobramycin; the RND-8 and RND-9 efflux pumps are not involved in ciprofloxacin resistance; and RND-10 efflux pump seems to confer resistance to chloramphenicol, fluoroquinolones, and trimethoprim [140, 143]. It was suggested that mutations in the RND-3 regulator-encoding gene may be responsible for the prevalent overexpression of this efflux pump in clinical Bcc isolates, contributing to their high levels of antibiotics resistance [144].

3.8. Motility

Genes involved in the synthesis and assembly of *B. cenocepacia* flagella are located in chromosome I, distributed within five clusters, with two additional genes found on chromosomes 2 and 3 [49]. These genes were found as being upregulated when the organism was incubated in CF sputum, contributing to its virulence in a murine agar bead infection model [145, 146]. More recently, flagellin expression and flagellar morphology of *B. cenocepacia* grown in a medium mimicking the CF sputum was analyzed [147]. Those nutritional conditions led to increased motility and flagellin expression, by inducing the synthesis of multiple flagella on the cell surface of *B. cenocepacia* K56-2 [147]. A link between the loss of bacterial motility and the development of the cepacia syndrome was recently established based on a transcriptomics analysis comparing the *B. cenocepacia* ST32 CF isolates recovered from bloodstream, at the time of cepacia syndrome, with their sputum counterparts, recovered prior to the development of this syndrome, revealing that flagellar genes were downregulated in isolates recovered from the bloodstream [148].

3.9. Intracellular survival

Infection assays using free-living amoeba demonstrated that *B. cenocepacia* can survive in an acidified intracellular compartment [94, 149]. These bacteria were also demonstrated to have the ability to delay the maturation of phagolysosomes in murine macrophages [94–96, 150]. Although the *B. cenocepacia* containing vacuoles (BcCVs) progress normally to the early phagosomal stage, the fusion of the BcCV's with late endosomes and subsequent maturation is significantly delayed comparing with vacuoles containing heat-killed bacteria [94]. In contrast to heat-killed bacteria that ended up in phagolysosomes with a pH of 4.5, BcCVs did not acidify normally maintaining a luminal pH around 6.4 [94]. This ability of *B. cenocepacia* to alter the acidification of the vacuole seems to be correlated with the delay in recruitment or assembly on the BcCV membrane of both the 16-kDa subunit of the phagosomal vacuolar ATPase (vATPase) and the NADPH phagocyte oxidase [96, 151]. In contrast, Al-Khodor and colleagues demonstrated that *B. cenocepacia* J2315 only transiently interacts with the endocytic pathway, event after which the bacterium is able to rapidly escape to the cytosol [152]. Escaped bacteria are afterward targeted by the host autophagy pathway, through the recruitment to the bacterial vicinity of the ubiquitin conjugation system, the autophagy adaptors p62 and NDP52, and the autophagosome membrane-associated protein LC3B. However, apparently, this host cell control through autophagy ultimately fails in a high proportion of infected cells,

being *B. cenocepacia* able to block the autophagosome completion and replicate in the cytosol of the host cell [152].

To better understand the intracellular behavior of *B. cenocepacia* in CF infected patients, studies have also been performed in Cystic fibrosis transmembrane conductance regulator (CFTR)-defective macrophages. Remarkably, the delayed maturation arresting of BcCV's is more exaggerated in CFTR-defective macrophages than in normal macrophages and is specific to live *B. cenocepacia* [153]. Although it is not clear how the CFTR defect enhances the *B. cenocepacia* intracellular survival, there is evidence of a link between the defective CFTR with autophagy deficiency and decreased clearance of protein aggregates and inflammation [154]. The elucidation of these survival details, especially the ability of *B. cenocepacia* to synergize with the CFTR defect and its consequences on the mechanism of autophagy will provide new avenues to explore novel therapeutic approaches for CF patients [155].

4. Toward a vaccine to prevent Bcc infections

No objective guidelines for eradication strategies are available for Bcc infections, as these pathogens are intrinsically resistant to the majority of the clinical available antimicrobials [156]. Currently, no immunotherapeutic strategy to protect CF patients from Bcc infections is available. Several studies on the immune response elicited by Bcc species in CF patients have been performed; however, they are challenging due to the ability of this bacteria to modulate and overcome the host immune responses and the ability to survive intracellularly in phagocytes and epithelial cells [157, 158].

An important aspect to consider during vaccine design is the optimal balance of Th1 and Th2 responses required for effective pathogen clearance. For example, a Th1 bias elicits a cell-mediated response, while Th2 induces a humoral immune response [159]. In the case of CF, their immune phenotype appears to be skewed toward Th2 responses [160]. In the case of Bcc, the type of host response necessary to clear the pathogen is still not fully understood, making it difficult to develop a protective vaccine (**Table 3**). Recently, BALB/c mice immunized intraperitoneally with the proteins Linocin and OmpW showed a significant reduction of *B. cenocepacia* and *B. multivorans* cells in the lung and lower dissemination of bacteria to the spleen [161]. While Linocin led to a robust Th1 response, the OmpW led to a mixed Th1/Th2 response [161]. The protection achieved with these proteins was greater against *B. cenocepacia* infection, and OmpW immunization was more efficient in reducing the lung bacterial load [161].

Nonpurified outer membrane proteins (OMP) from *B. multivorans*, supplemented with the mucosal adjuvant adamantylamide dipeptide (AdDP) that promotes a robust Th2 response, were tested for immunization of BALB/c mice [162]. A statistically significant increase in IgG and in mucosal IgA OMP-specific antibodies was observed, together with a reduction of *B. multivorans* burden and lung pathology, but only a moderate cross protection to *B. cenocepacia* was reported. The specificity of the immune response was found to be against

90, 72, 66, and 60 kDa proteins. Elicitation of specific IgA antibodies by mucosal immunization was also reported to be important to prevent the colonization of the respiratory tract by Bcc bacteria. In another study, the intranasal immunization of CD-1 mice with outer membrane proteins (OMP) from *B. cenocepacia* was described to originate a Th2-biased response with the maintenance of the bacterial burden, while mice immunized with OMP and the noninflammatory mucosal adjuvant nanoemulsion (NE) elicited a Th1/Th2-balanced response that led to a significant reduction of the *B. cenocepacia* cell burden [163]. The serum derived from mice vaccinated with OMP-NE could also inhibit *B. multivorans* growth by 80.1%, showing that induction of cross-reactive antibodies occurred after mice immunization. Additionally, a highly conserved 17-kDa OmpA-like protein was recently identified as a new immunedominant epitope in mucosal immunization [163].

Metalloproteases are also considered as potential effective candidates for vaccine development [90]. It was demonstrated that immunizations of rats using a conserved zinc metalloprotease peptide 15 (PSCP) decreased the severity of *B. cenocepacia* infection and the lung damage was reduced by 50% upon challenge with a *B. cenocepacia* strain after immunization [90].

In 2012, it was shown that the bacterial surface polysaccharide poly-β-(1-6)-N-acetyl-glucosamine (PNAG) confers protective immunity against Bcc infection in a lethal peritonitis mice model [164]. In this study by Skurnik and colleagues using opsophagocytic assays, it was observed that goat-raised antibodies against PNAG could kill Bcc strains (>80%) of the *B. ceno-*

Antigen	Immune response	Bcc animal model	*In vitro* models	References
OmpW	Mixed Th1/Th2	BALB/c mice immunosuppressed with cyclophosphamide	Spleen cells from mice	[161]
Linocin	Th1	BALB/c mice immunosuppressed with cyclophosphamide	Spleen cells from mice	[162]
OMP plus NE	Mixed Th1/Th2	CD-1 mice	Murine splenocytes	[163]
OMP plus AdDP	Higher IgG and IgA titers	BALB/c mice immunosuppressed with cyclophosphamide	ND	[162]
PNAG	ND	FVB/N mice	Opsonophagocytic assay	[164]
Zinc metalloprotease peptide 15 (PSCP)	Higher IgG and IgA titers	Sprague-Dawley rat agar bead model	ND	[90]
FliC	ND	ND	T cell hybridoma assays	[165]
BCAL2958	High IgG titers in human CF serum samples	ND	Human neutrophils	[166]

ND—Not determined.

Table 3. Summary of vaccine development against Bcc infections.

cepacia, *Burkholderia dolosa* and *B. multivorans* species. Furthermore, bacterial killing was found to depend of the presence of the complement [164].

Other proteins of putative immunogenic activity have been reported as potential vaccine candidates. However, studies in a Bcc infection animal model are still lacking (**Table 3**). One of these promising antigens is the OmpA-like BCAL2958 protein that was shown to be highly conserved in Bcc, to elicit IgG antibodies in CF patients and to elicit an increase of TNFα, elastase, NO, and MPO in neutrophils [166].

Musson and colleagues have shown that T-cell hybridomas against the *Burkholderia pseudomallei* flagellar protein FliC epitope cross-reacted with orthologous FliC sequences from *B. multivorans* and *B. cenocepacia* [165]. FliC epitopes were accessible for processing and presentation from live or heat-killed *B. cenocepacia* bacteria, demonstrating that flagellin enters the HLA class II Ag presentation pathway during infection of macrophages with *B. cenocepacia*.

Studies referred above revealed that subunit vaccines that only produce an antibody response cannot fully prevent an infection caused by Bcc bacteria [157, 161, 164]. Therefore, Bcc vaccines containing multiple antigens that elicit a balanced Th1 and Th2 response are expected to be effective in preventing Bcc infections. With this aim, immunoproteomics approaches have been performed. For instance, Mariappan and colleagues identified 18 immunogenic proteins from culture supernatants of *B. cepacia* that reacted with mice antibodies raised against inactivated *B. cepacia* whole cells [167]. More recently, the analysis of the imunoproteome of two clinical relevant strains of *B. cenocepacia* and *B. multivorans* revealed 15 common immunoreactive proteins that reacted with CF human serum samples [168].

5. Concluding remarks

An overview of Bcc infections in CF from early 1980s until the more recent available data was presented. The prevalence of Bcc species in CF patients worldwide is still evolving, most probably as a result of infection control measures and segregation policies. Many virulence factors have been identified, and the resulting wealth of information prompted the establishment of new research lines envisaging the development of novel protective strategies and products, namely vaccines and vaccine components.

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References

[1] Govan JR, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Microbiol Rev. 1996; 60:539-574.

[2] Isles A, Maclusky I, Corey M, Gold R, Prober C, Fleming P, Levison H. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. J Pediatr. 1984; 104:206-210. DOI:10.1016/S0022-3476(84)80993-2.

[3] Burkholder WH. Sour skin, a bacterial rot of onion bulbs. Phytopathology. 1950; 40: 115-117.

[4] Yabuuchi E, Kosako Y, Oyaizu H, Yano I, Hotta H, Hashimoto Y, Ezaki T, Arakawa M. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. Microbiol Immunol. 1992; 36:1251-1275. DOI:10.1111/j.1348-0421.1992.tb02129.x.

[5] Vandamme P, Holmes B, Vancanneyt M, Coenye T, Hoste B, Coopman R, Revets H, Lauwers S, Gillis M, Kersters K, Govan JR. Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. Int J Syst Bacteriol. 1997; 47:1188-1200. DOI:10.1099/00207713-47-4-1188.

[6] Burkholderia Genome DB. Available from: http://www.burkholderia.com/ [Accessed 2016-07-31].

[7] Integrated Microbial Genomes & Microbiomes. Available from: https://img.jgi.doe.gov/. [Accessed 2016-07-31].

[8] Coenye T, Mahenthiralingam E, Henry D, LiPuma JJ, Laevens S, Gillis M, Speert DP,Vandamme P. *Burkholderia ambifaria* sp. nov., a novel member of the *Burkholderia cepacia* complex including biocontrol and cystic fibrosis-related isolates. Int J Syst Evol Microbiol. 2001; 51:1481-1490. DOI:10.1099/00207713-51-4-1481.

[9] Vandamme P, Henry D, Coenye T, Nzula S, Vancanneyt M, LiPuma JJ, Speert DP, Govan JR, Mahenthiralingam E. *Burkholderia anthina* sp. nov. and *Burkholderia pyrrocinia*, two additional *Burkholderia cepacia* complex bacteria, may confound results of new molecular

diagnostic tools. FEMS Immunol Med Microbiol. 2002; 33:143-149. DOI:10.1111/j.1574-695X.2002.tb00584.x.

[10] Vanlaere E, Lipuma JJ, Baldwin A, Henry D, De Brandt E, Mahenthiralingam E, Speert D, Dowson C, Vandamme P. *Burkholderia latens* sp. nov., *Burkholderia diffusa* sp. nov., *Burkholderia arboris* sp. nov., *Burkholderia seminalis* sp. nov. and *Burkholderia metallica* sp. nov., novel species within the *Burkholderia cepacia* complex. Int J Syst Evol Microbiol. 2008; 58:1580-1590. DOI:10.1099/ijs.0.65634-0.

[11] Vandamme P, Holmes B, Coenye T, Goris J, Mahenthiralingam E, LiPuma JJ, Govan JR. *Burkholderia cenocepacia* sp. nov.--a new twist to an old story. Res Microbiol. 2003; 154:91-96. DOI:10.1016/S0923-2508(03)00026-3.

[12] Vanlaere E, Baldwin A, Gevers D, Henry D, De Brandt E, LiPuma JJ, Mahenthiralingam E, Speert DP, Dowson C, Vandamme P. Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. Int J Syst Evol Microbiol. 2009; 59:102-111. DOI:10.1099/ijs.0.001123-0.

[13] Vermis K, Coenye T, LiPuma JJ, Mahenthiralingam E, Nelis HJ, Vandamme P. Proposal to accommodate *Burkholderia cepacia* genomovar VI as *Burkholderia dolosa* sp. nov. Int J Syst Evol Microbiol. 2004; 54:689-691. DOI:10.1099/ijs.0.02888-0.

[14] Peeters C, Zlosnik JE, Spilker T, Hird TJ, LiPuma JJ, Vandamme P. *Burkholderia pseudomultivorans* sp. nov., a novel *Burkholderia cepacia* complex species from human respiratory samples and the rhizosphere. Syst Appl Microbiol. 2013; 36:483-489. DOI:10.1016/j.syapm.2013.06.003.

[15] Vandamme P, Mahenthiralingam E, Holmes B, Coenye T, Hoste B, De Vos P, Henry D, Speert DP. Identification and population structure of *Burkholderia stabilis* sp. nov. (formerly *Burkholderia cepacia* genomovar IV). J Clin Microbiol. 2000; 38:1042-1047.

[16] De Smet B, Mayo M, Peeters C, Zlosnik JE, Spilker T, Hird TJ, LiPuma JJ, Kidd TJ, Kaestli M, Ginther JL, Wagner DM, Keim P, Bell SC, Jacobs JA, Currie BJ, Vandamme P. *Burkholderia stagnalis* sp. nov. and *Burkholderia territorii* sp. nov., two novel *Burkholderia cepacia* complex species from environmental and human sources. Int J Syst Evol Microbiol. 2015; 65:2265-2271. DOI:10.1099/ijs.0.000251.

[17] Yabuuchi E, Kawamura Y, Ezaki T, Ikedo M, Dejsirilert S, Fujiwara N, Naka T. and Kobayashi K. *Burkholderia ubonensis* sp. nov., L-arabinose-assimilating but different from *Burkholderia thailandensis* and *Burkholderia vietnamiensis*. Microbiol Immunol. 2000; 44:307-317. DOI:10.1111/j.1348-0421.2000.tb02500.x.

[18] Gillis M, Van TV, Bardin R, Goor M, Hebbar P, Willems A, Segers P, Kersters K, Heulin T, Fernandez MP. Polyphasic taxonomy in the genus *Burkholderia* leading to an amended description of the genus and proposition of *Burkholderia vietnamiensis* sp. nov. for N_2-fixing isolates from rice in Vietnam. Int J Syst Bacteriol. 1995; 45:274-289. DOI:10.1099/00207713-45-2-274.

[19] Johnson WM, Tyler SD, Rozee KR. Linkage analysis of geographic and clinical clusters in *Pseudomonas cepacia* infections by multilocus enzyme electrophoresis and ribotyping. J Clin Microbiol. 1994; 32:924-930.

[20] Mahenthiralingam E, Urban TA, Goldberg JB. The multifarious, multireplicon *Burkholderia cepacia* complex. Nat Rev Microbiol. 2005; 3:144-156. DOI:10.1038/nrmicro1085.

[21] Chen JS, Witzmann KA, Spilker T, Fink RJ, LiPuma JJ. Endemicity and inter-city spread of *Burkholderia cepacia* genomovar III in cystic fibrosis. J Pediatr. 2001; 139 :643-649. DOI:10.1067/mpd.2001.118430.

[22] Coenye T, Spilker T, Van Schoor A, LiPuma JJ, Vandamme P. Recovery of *Burkholderia cenocepacia* strain PHDC from cystic fibrosis patients in Europe. Thorax. 2004; 59:952-954. DOI:10.1136/thx.2003.019810.

[23] Coenye T, LiPuma JJ. Multilocus restriction typing: a novel tool for studying global epidemiology of *Burkholderia cepacia* complex infection in cystic fibrosis. J Infect Dis. 2002; 185:1454-1462. DOI:10.1086/340279.

[24] Drevinek P, Mahenthiralingam E. *Burkholderia cenocepacia* in cystic fibrosis: epidemiology and molecular mechanisms of virulence. Clin Microbiol Infect. 2010; 16:821-830. DOI:10.1111/j.1469-0691.2010.03237.x.

[25] Thomassen MJ, Demko CA, Klinger JD, Stern RC. *Pseudomonas cepacia* colonization among patients with cystic fibrosis. A new opportunist. Am Rev Respir Dis. 1985; 131:791-796. DOI:10.1164/arrd.1985.131.5.791.

[26] Whiteford ML, Wilkinson JD, McColl JH, et al. Outcome of *Burkholderia* (*Pseudomonas*) *cepacia* colonisation in children with cystic fibrosis following a hospital outbreak. Thorax. 1995:50; 1194-1198. DOI:10.1136/thx.50.11.1194.

[27] Muhdi K, Edenborough FP, Gumery L, et al. Outcome for patients colonised with *Burkholderia cepacia* in a Birmingham adult cystic fibrosis clinic and the end of an epidemic. Thorax. 1996; 51:374-377. DOI:10.1136/thx.51.4.374.

[28] Lynch JP 3rd. *Burkholderia cepacia* complex: impact on the cystic fibrosis lung lesion. Semin Respir Crit Care Med. 2009; 30:596-610. DOI:10.1055/s-0029-1238918.

[29] Cystic Fibrosis Foundation 2014 report. Available from: (https://www.cff.org/2014-Annual-Data-Report.pdf) [Accessed 2016-09-8].

[30] European Cystic Fibrosis Society Patient Registry Annual Data report 2013. Available from: (https://www.ecfs.eu/sites/default/files/images/ECFSPR_Report2013_02.2016.pdf). [Accessed 2016-09-8].

[31] Brisse S, Cordevant C, Vandamme P, Bidet P, Loukil C, Chabanon G, Lange M, Bingen E. Species distribution and ribotype diversity of *Burkholderia cepacia* complex isolates from French patients with cystic fibrosis. J Clin Microbiol. 2004; 42:4824-4827. DOI:10.1128/JCM.42.10.4824-4827.2004.

[32] Reik R, Spilker T, Lipuma JJ. Distribution of *Burkholderia cepacia* complex species among isolates recovered from persons with or without cystic fibrosis. J Clin Microbiol. 2005; 43:2926-2928. DOI:10.1128/JCM.43.6.2926-2928.2005.

[33] Cunha MV, Leitão JH, Mahenthiralingam E, Vandamme P, Lito L, Barreto C, et al. Molecular analysis of *Burkholderia cepacia* complex isolates from a Portuguese cystic fibrosis center: a 7-year study. J Clin Microbiol. 2003; 41(9):4113-4120. DOI:10.1128/JCM.41.9.4113-4120.2003.

[34] Jordá-Vargas L, Degrossi J, Castañeda NC, D'Aquino M, Valvano MA, Procopio A, Galanternik L, Centrón D. Prevalence of indeterminate genetic species of B*urkholderia cepacia* complex in a cystic fibrosis center in Argentina. J Clin Microbiol. 2008; 46:1151-1152. DOI:10.1128/JCM.01595-07.

[35] Martina P, Bettiol M, Vescina C, Montanaro P, Mannino MC, Prieto CI, Vay C, Naumann D, Schmitt J, Yantorno O, Lagares O, Bosch A. Genetic diversity of *Burkholderia contaminans* isolates from cystic fibrosis patients in Argentina. J Clin Microbiol. 2013; 51:339-344. DOI:10.1128/JCM.02500-12.

[36] Cunha MV, Pinto-de-Oliveira A, Meirinhos-Soares L, Salgado MJ, Melo-Cristino J, Correia S, Barreto C, Sá-Correia I. Exceptionally high representation of *Burkholderia cepacia* among *B. cepacia* complex isolates recovered from the major Portuguese cystic fibrosis center. J Clin Microbiol. 2007; 45:1628-1633. DOI:10.1128/JCM.00234-07.

[37] Medina-Pascual MJ, Valdezate S, Carrasco G, Villalón P, Garrido N, Saéz-Nieto JA. Increase in isolation of *Burkholderia contaminans* from Spanish patients with cystic fibrosis. Clin Microbiol Infect. 2015; 21:150-156. DOI:10.1016/j.cmi.2014.07.014.

[38] Lupo A, Isis E, Tinguely R, Endimiani A. Clonality and antimicrobial susceptibility of *Burkholderia cepacia* complex isolates collected from cystic fibrosis patients during 1998-2013 in Bern, Switzerland. New Microbiol. 2015; 38:281-288. DOI:10.7892/boris.81553.

[39] Zlosnik JEA, Zhou G, Brant R, Henry DA, Hird TJ, Mahenthiralingam E, Chilvers MA, Wilcox P, Speert DP. *Burkholderia* species infections in patients with cystic fibrosis in British Columbia, Canada. 30 Years' Experience. Ann Am Thorac Soc. 2015; 12:70-78. DOI:10.1513/AnnalsATS.201408-395OC.

[40] Sajjan US, Forstner JF. Role of a 22-kilodalton pilin protein in binding of *Pseudomonas cepacia* to buccal epithelial cells. Infect Immun. 1993; 61:3157-3163.

[41] Tomich M, Herfst CA, Golden JW, Mohr CD. Role of flagella in host cell invasion by *Burkholderia cepacia*. Infect Immun. 2002; 70(4):1799-1806. DOI:10.1128/IAI.70.4.1799-1806.2002.

[42] Lefebre M, Valvano M. In vitro resistance of *Burkholderia cepacia* complex isolates to reactive oxygen species in relation to catalase and superoxide dismutase production. Microbiology. 2001; 147:97-109. DOI:10.1099/00221287-147-1-97.

[43] Miethke M, Marahiel MA. Siderophore-based ion acquisition and pathogen control. Microbiol Mol Biol Rev. 2007; 71:413-451. DOI:10.1128/MMBR.00012-07.

[44] Flannagan RS, Valvano MA. *Burkholderia cenocepacia* requires RpoE for growth under stress conditions and delay of phagolysosomal fusion in macrophages. Microbiology. 2008; 154:643-653. DOI:10.1099/mic.0.2007/013714-0.

[45] Saldías MS, Lamothe J, Wu R, Valvano MA. *Burkholderia cenocepacia* requires the RpoN sigma factor for biofilm formation and intracellular trafficking within macrophages. Infect Immun. 2008; 76:1059-1067. DOI:10.1128/IAI.01167-07.

[46] Lardi M, Aguilar C, Pedrioli A, et al. σ(54)-dependent response to nitrogen limitation and virulence in *Burkholderia cenocepacia* strain H111. Appl Environ Microbiol. 2015; 81:4077-4089. DOI:10.1128/AEM.00694-15.

[47] Hunt TA, Kooi C, Sokol PA, Valvano MA. Identification of *Burkholderia cenocepacia* genes required for bacterial survival in vivo. Infect Immun. 2004; 72(7):4010-4022. DOI:10.1128/IAI.72.7.4010-4022.2004.

[48] Vinion-Dubiel AD, Goldberg JB. Lipopolysaccharide of *Burkholderia cepacia* complex. J Endotoxin Res. 2003; 9:201-213. DOI:10.1177/09680519030090040101.

[49] Holden MTG, Seth-Smith HMB, Crossman LC, et al. The genome of *Burkholderia cenocepacia* J2315, an epidemic pathogen of cystic fibrosis patients. J Bacteriol. 2009; 191(1):261-277. DOI:10.1128/JB.01230-08.

[50] Loutet SA, Flannagan RS, Kooi C, Sokol PA, Valvano MA. A complete lipopolysaccharide inner core oligosaccharide is required for resistance of *Burkholderia cenocepacia* to antimicrobial peptides and bacterial survival in vivo. J Bacteriol. 2006; 188:2073-2080. DOI:10.1128/JB.188.6.2073-2080.2006.

[51] De Soyza A, Silipo A, Lanzetta R, Govan JR, Molinaro A. Review: chemical and biological features of *Burkholderia cepacia* complex lipopolysaccharides. Innate Immun. 2008; 14(3):127-144. DOI:10.1177/1753425908093984.

[52] Khodai-Kalaki M, Andrade A, Fathy Mohamed Y, Valvano MA. *Burkholderia cenocepacia* lipopolysaccharide modification and flagellin glycosylation affect virulence but not innate immune recognition in plants. MBio. 2015; 6(3):e00679-e00615. DOI:10.1128/mBio.00679-15.

[53] Hughes JE, Stewart J, Barclay GR, Govan JR. Priming of neutrophil respiratory burst activity by lipopolysaccharide from *Burkholderia cepacia*. Infect Immun. 1997; 65:4281-4287.

[54] Hutchison ML, Bonell EC, Poxton IR, Govan JRW. Endotoxic activity of lipopolysaccharides isolated from emergent potential cystic fibrosis pathogens. FEMS Immunol Med Microbiol. 2000; 27(1):73-77. DOI:10.1111/j.1574-695X.2000.tb01414.x.

[55] Shimomura H, Matsuura M, Saito S, Hirai Y, Isshiki Y, Kawahara K. Lipopolysaccharide of *Burkholderia cepacia* and its unique character to stimulate murine macrophages with relative lack of interleukin-1β-inducing ability. Infect Immun. 2001; 69:3663-3669. DOI:10.1128/IAI.69.6.3663-3669.2001.

[56] Saldías MS, Ortega X, Valvano MA. *Burkholderia cenocepacia* O antigen lipopolysaccharide prevents phagocytosis by macrophages and adhesion to epithelial cells. J Med Microbiol. 2009; 58:1542-1548. DOI:10.1099/jmm.0.013235-0.

[57] Richau JA, Leitão JH, Correia M, Lito L, Salgado MJ, Barreto C, Cescutti P, Sá-Correia I. Molecular typing and exopolysaccharide biosynthesis of *Burkholderia cepacia* isolates from a Portuguese cystic fibrosis center. J Clin Microbiol. 2000; 38:1651-1655.

[58] Cunha M V, Sousa SA, Leitão JH, Moreira LM, Videira PA, Sá-Correia I. Studies on the involvement of the exopolysaccharide produced by cystic fibrosis-associated isolates of the *Burkholderia cepacia* complex in biofilm formation and in persistence of respiratory infections. J Clin Microbiol. 2004; 42(7):3052-3058. DOI:10.1128/JCM.42.7.3052-3058.2004.

[59] Ferreira AS, Leitão JH, Silva IN, et al. Distribution of cepacian biosynthesis genes among environmental and clinical *Burkholderia* strains and role of Cepacian exopolysaccharide in resistance to stress conditions. Appl Environ Microbiol. 2010; 76(2):441-450. DOI:10.1128/AEM.01828-09.

[60] Goldberg JB. Polysaccharides of *Burkholderia* spp. In: Coenye T, Peter V, eds. *Burkholderia* Molecular Microbiology and Genomics. Wymondham: Horizon Bioscience; 2007:93-110.

[61] Herasimenka Y, Cescutti P, Impallomeni G, et al. Exopolysaccharides produced by clinical strains belonging to the *Burkholderia cepacia* complex. J Cyst Fibros. 2007; 6(2):145-152. DOI:10.1016/j.jcf.2006.06.004.

[62] Moreira LM, Videira PA, Sousa SA, Leitão JH, Cunha M V, Sá-Correia I. Identification and physical organization of the gene cluster involved in the biosynthesis of *Burkholderia cepacia* complex exopolysaccharide. Biochem Biophys Res Commun. 2003; 312(2):323-333. DOI:10.1016/j.bbrc.2003.10.118.

[63] Chiarini L, Cescutti P, Drigo L, et al. Exopolysaccharides produced by *Burkholderia cenocepacia* recA lineages IIIA and IIIB. J Cyst Fibros. 2004; 3(3):165-172. DOI:10.1016/j.jcf.2004.04.004.

[64] Bylund J, Burgess LA, Cescutti P, Ernst RK, Speert DP. Exopolysaccharides from *Burkholderia cenocepacia* inhibit neutrophil chemotaxis and scavenge reactive oxygen species. J Biol Chem. 2006; 281:2526-2532. DOI:10.1074/jbc.M510692200.

[65] Conway BAD, Chu KK, Bylund J, Altman E, Speert DP. Production of exopolysaccharide by *Burkholderia cenocepacia* results in altered cell-surface interactions and altered bacterial clearance in mice. J Infect Dis. 2004; 190:957-966. DOI:10.1086/423141.

[66] Cuzzi B, Cescutti P, Furlanis L, et al. Investigation of bacterial resistance to the immune system response: Cepacian depolymerisation by reactive oxygen species. Innate Immun. 2012; 18(4):661-671. DOI:10.1177/1753425911435954.

[67] Zlosnik JEA, Hird TJ, Fraenkel MC, Moreira LM, Henry DA, Speert DP. Differential mucoid exopolysaccharide production by members of the *Burkholderia cepacia* complex. J Clin Microbiol. 2008; 46:1470-1473. DOI:10.1128/JCM.02273-07.

[68] Messiaen A-S, Nelis H, Coenye T. Investigating the role of matrix components in protection of *Burkholderia cepacia* complex biofilms against tobramycin. J Cyst Fibros. 2014; 13:56-62. DOI:10.1016/j.jcf.2013.07.004.

[69] Huber B, Riedel K, Köthe M, Givskov M, Molin S, Eberl L. Genetic analysis of functions involved in the late stages of biofilm development in *Burkholderia cepacia* H111. Mol Microbiol. 2002; 46(2):411-426. DOI:10.1046/j.1365-2958.2002.03182.x.

[70] Berlutti F, Morea C, Battistoni A, et al. Iron availability influences aggregation, biofilm, adhesion and invasion of *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*. Int J Immunopathol Pharmacol. 2005; 18:661-670. DOI:10.1177/039463200501800407.

[71] Tomlin KL, Malott RJ, Ramage G, Storey DG, Sokol PA, Ceri H. Quorum-sensing mutations affect attachment and stability of *Burkholderia cenocepacia* biofilms. Appl Environ Microbiol. 2005; 71:5208-5218. DOI:10.1128/AEM.71.9.5208-5218.2005.

[72] Bernier SP, Nguyen DT, Sokol PA. A LysR-type transcriptional regulator in *Burkholderia cenocepacia* influences colony morphology and virulence. Infect Immun. 2008; 76:38-47. DOI:10.1128/IAI.00874-07.

[73] Aubert DF, Flannagan RS, Valvano MA. A novel sensor kinase-response regulator hybrid controls biofilm formation and type VI secretion system activity in *Burkholderia cenocepacia*. Infect Immun. 2008; 76(5):1979-1991. DOI:10.1128/IAI.01338-07.

[74] Häussler S, Lehmann C, Breselge C, et al. Fatal outcome of lung transplantation in cystic fibrosis patients due to small-colony variants of the *Burkholderia cepacia* complex. Eur J Clin Microbiol Infect Dis. 2003; 22(4):249-253. DOI:10.1007/s10096-003-0901-y.

[75] Peeters E, Nelis HJ, Coenye T. In vitro activity of ceftazidime, ciprofloxacin, meropenem, minocycline, tobramycin and trimethoprim/sulfamethoxazole against planktonic and sessile *Burkholderia cepac*ia complex bacteria. J Antimicrob Chemother. 2009; 64:801-809. DOI:10.1093/jac/dkp253.

[76] Caraher E, Reynolds G, Murphy P, McClean S, Callaghan M. Comparison of antibiotic susceptibility of *Burkholderia cepacia* complex organisms when grown planktonically or as biofilm in vitro. Eur J Clin Microbiol Infect Dis. 2007; 26:213-216. DOI:10.1007/s10096-007-0256-x.

[77] Van Acker H, Sass A, Bazzini S, et al. Biofilm-grown *Burkholderia cepacia* complex cells survive antibiotic treatment by avoiding production of reactive oxygen species. PLoS One. 2013; 8:e58943. DOI:10.1371/journal.pone.0058943.

[78] Murphy MP, Caraher E. Residence in biofilms allows *Burkholderia cepacia* complex (Bcc) bacteria to evade the antimicrobial activities of neutrophil-like dHL60 cells. Pathog Dis. 2015; 73(8):ftv069. DOI: 10.1093/femspd/ftv069.

[79] Venturi V, Friscina A, Bertani I, Devescovi G, Aguilar C. Quorum sensing in the *Burkholderia cepacia* complex. Res Microbiol. 2004; 155(4):238-244. DOI:10.1016/j.resmic.2004.01.006.

[80] Kooi C, Subsin B, Chen R, Pohorelic B, Sokol PA. *Burkholderia cenocepacia* ZmpB is a broad-specificity zinc metalloprotease involved in virulence. Infect Immun. 2006; 74(7):4083-4093. DOI:10.1128/IAI.00297-06.

[81] Sokol PA, Sajjan U, Visser MB, Gingues S, Forstner J, Kooi C. The CepIR quorum-sensing system contributes to the virulence of *Burkholderia cenocepacia* respiratory infections. Microbiology. 2003; 149(Pt 12):3649-3658. DOI:10.1099/mic.0.26540-0.

[82] Uehlinger S, Schwager S, Bernier SP, et al. Identification of specific and universal virulence factors in *Burkholderia cenocepacia* strains by using multiple infection hosts. Infect Immun. 2009; 77:4102-4110. DOI:10.1128/IAI.00398-09.

[83] Vergunst AC, Meijer AH, Renshaw SA, O'Callaghan D. *Burkholderia cenocepacia* creates an intramacrophage replication niche in Zebrafish embryos, followed by bacterial dssemination and establishment of systemic infection. Infect Immun. 2010; 78:1495-1508. DOI:10.1128/IAI.00743-09.

[84] Baldwin A, Sokol PA, Parkhill J, Mahenthiralingam E. The *Burkholderia cepacia* epidemic strain marker is part of a novel genomic island encoding both virulence and metabolism-associated genes in *Burkholderia cenocepacia*. Infect Immun. 2004; 72(3):1537-1547. DOI:10.1128/IAI.72.3.1537-1547.2004.

[85] Subramoni S, Sokol PA. Quorum sensing systems influence *Burkholderia cenocepacia* virulence. Future Microbiol. 2012; 7(12):1373-1387. DOI:10.2217/fmb.12.118.

[86] Huber B, Riedel K, Hentzer M, et al. The cep quorum-sensing system of *Burkholderia cepacia* H111 controls biofilm formation and swarming motility. Microbiology. 2001; 147(Pt 9):2517-2528. DOI:10.1099/00221287-147-9-2517.

[87] Lewenza S, Conway B, Greenberg EP, Sokol PA. Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. J Bacteriol. 1999; 181:748-756.

[88] Fehlner-Gardiner CC, Hopkins TMH, Valvano MA. Identification of a general secretory pathway in a human isolate of *Burkholderia vietnamiensis* (formerly *B. cepacia* complex genomovar V) that is required for the secretion of hemolysin and phospholipase C activities. Microb Pathog. 2002; 32(5):249-254. DOI:10.1006/mpat.2002.0503.

[89] Whitby PW, Vanwagoner TM, Springer JM, Morton DJ, Seale TW, Stull TL. *Burkholderia cenocepacia* utilizes ferritin as an iron source. J Med Microbiol. 2006; 55:661-668. DOI:10.1099/jmm.0.46199-0.

[90] Corbett CR, Burtnick MN, Kooi C, Woods DE, Sokol PA. An extracellular zinc metalloprotease gene of *Burkholderia cepacia*. Microbiology. 2003; 149:2263-2271. DOI:10.1099/mic.0.26243-0.

[91] Zhang R, LiPuma JJ, Gonzalez CF. Two type IV secretion systems with different functions in *Burkholderia cenocepacia* K56-2. Microbiology. 2009; 155(Pt 12):4005-4013. DOI:10.1099/mic.0.033043-0.

[92] Sajjan SU, Carmody LA, Gonzalez CF, LiPuma JJ. A type IV secretion system contributes to intracellular survival and replication of *Burkholderia cenocepacia*. Infect Immun. 2008; 76:5447-5455. DOI:10.1128/IAI.00451-08.

[93] Tomich M, Griffith A, Herfst CA, Burns JL, Mohr CD. Attenuated virulence of a *Burkholderia cepacia* type III secretion mutant in a murine model of infection. Infect Immun. 2003; 71:1405-1415. DOI:10.1128/IAI.71.3.1405-1415.2003.

[94] Lamothe J, Huynh KK, Grinstein S, Valvano MA. Intracellular survival of *Burkholderia cenocepacia* in macrophages is associated with a delay in the maturation of bacteria-containing vacuoles. Cell Microbiol. 2007; 9(1):40-53. DOI:10.1111/j.1462-5822.2006.00766.x.

[95] Flannagan RS, Jaumouillé V, Huynh KK, et al. *Burkholderia cenocepacia* disrupts host cell actin cytoskeleton by inactivating Rac and Cdc42. Cell Microbiol. 2012; 14:239-254. DOI:10.1111/j.1462-5822.2011.01715.x.

[96] Rosales-Reyes R, Skeldon AM, Aubert DF, Valvano MA. The Type VI secretion system of *Burkholderia cenocepacia* affects multiple Rho family GTPases disrupting the actin cytoskeleton and the assembly of NADPH oxidase complex in macrophages. Cell Microbiol. 2012; 14:255-273. DOI:10.1111/j.1462-5822.2011.01716.x.

[97] Gavrilin MA, Abdelaziz DHA, Mostafa M, et al. Activation of the pyrin inflammasome by intracellular *Burkholderia cenocepacia*. J Immunol. 2012; 188:3469-3477. DOI:10.4049/jimmunol.1102272.

[98] Allan ND, Kooi C, Sokol PA, Beveridge TJ. Putative virulence factors are released in association with membrane vesicles from *Burkholderia cepacia*. Can J Microbiol. 2003; 49(10):613-624. DOI:10.1139/w03-078.

[99] Letoffe S, Redeker V, Wandersman C. Isolation and characterization of an extracellular haem-binding protein from *Pseudomonas aeruginosa* that shares function and sequence similarities with the *Serratia marcescens* HasA haemophore. Mol Microbiol. 1998; 28(6):1223-1234. DOI:10.1046/j.1365-2958.1998.00885.x.

[100] Guzzo J, Pages JM, Duong F, Lazdunski A, Murgier M. *Pseudomonas aeruginosa* alkaline protease: evidence for secretion genes and study of secretion mechanism. J Bacteriol. 1991; 173(17):5290-5297. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC208238/.

[101] Duong F, Bonnet E, Géli V, Lazdunski A, Murgier M, Filloux A. The AprX protein of *Pseudomonas aeruginosa*: a new substrate for the Apr type I secretion system. Gene. 2001; 262(1-2):147-153. DOI:10.1016/S0378-1119(00)00541-2.

[102] Olson JC, Ohman DE. Efficient production and processing of elastase and LasA by *Pseudomonas aeruginosa* require zinc and calcium ions. J Bacteriol. 1992; 174(12):4140-4147. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC206126/.

[103] Cahan R, Axelrad I, Safrin M, Ohman DE, Kessler E. A secreted aminopeptidase of *Pseudomonas aeruginosa*: identification, primary structure, and relationship to other aminopeptidases. J Biol Chem. 2001; 276(47):43645-43652. DOI:10.1074/jbc.M106950200.

[104] Engel LS, Hill JM, Caballero AR, Green LC, O'Callaghan RJ. Protease IV, a unique extracellular protease and virulence factor from *Pseudomonas aeruginosa*. J Biol Chem. 1998; 273(27):16792-16797. DOI:10.1074/jbc.273.27.16792.

[105] Diaz-Laviada I, Larrodera P, Diaz-Meco MT, et al. Evidence for a role of phosphatidylcholine-hydrolysing phospholipase C in the regulation of protein kinase C by *ras* and *src* oncogenes. EMBO J. 1990; 9(12):3907-3912. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC552161/.

[106] Ostroff RM, Vasil AI, Vasil ML. Molecular comparison of a nonhemolytic and a hemolytic phospholipase C from *Pseudomonas aeruginosa*. J Bacteriol. 1990; 172(10):5915-5923. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC526912/.

[107] Folders J, Tommassen J, van Loon LC, Bitter W. Identification of a chitin-binding protein secreted by *Pseudomonas aeruginosa*. J Bacteriol. 2000; 182(5):1257-1263. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC94410/.

[108] Allured VS, Collier RJ, Carroll SF, McKay DB. Structure of exotoxin A of *Pseudomonas aeruginosa* at 3.0-Angstrom resolution. Proc Natl Acad Sci U S A. 1986; 83(5):1320-1324. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC323067/.

[109] Barbieri JT, Sun J. *Pseudomonas aeruginosa* ExoS and ExoT. In: Reviews of Physiology, Biochemistry and Pharmacology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2005:79-92. DOI:10.1007/s10254-004-0031-7.

[110] Yahr TL, Vallis AJ, Hancock MK, Barbieri JT, Frank DW. ExoY, an adenylate cyclase secreted by the *Pseudomonas aeruginosa* type III system. Proc Natl Acad Sci U S A. 1998; 95(23):13899-13904. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC24955/.

[111] Sato H, Frank DW. ExoU is a potent intracellular phospholipase. Mol Microbiol. 2004; 53(5):1279-1290. DOI:10.1111/j.1365-2958.2004.04194.x.

[112] Juhas M, Crook DW, Dimopoulou ID, et al. Novel type IV secretion system involved in propagation of genomic islands. J Bacteriol. 2007; 189(3):761-771. DOI:10.1128/JB.01327-06.

[113] Klockgether J, Würdemann D, Reva O, Wiehlmann L, Tümmler B. Diversity of the abundant pKLC102/PAGI-2 family of genomicislands in *Pseudomonas aeruginosa*. J Bacteriol. 2007; 189(6):2443-2459. DOI:10.1128/JB.01688-06.

[114] Mikkelsen H, Hui K, Barraud N, Filloux A. The pathogenicity island encoded PvrSR/RcsCB regulatory network controls biofilm formation and dispersal in *Pseudomonas aeruginosa*. Mol Microbiol. 2013; 89(3):450-463. DOI:10.1111/mmi.12287.

[115] Wilhelm S, Gdynia A, Tielen P, Rosenau F, Jaeger K-E. The autotransporter esterase EstA of *Pseudomonas aeruginosa* is required for rhamnolipid production, cell motility, and biofilm formation. J Bacteriol. 2007; 189(18):6695-6703. DOI:10.1128/JB.00023-07.

[116] Kida Y, Higashimoto Y, Inoue H, Shimizu T, Kuwano K. A novel secreted protease from *Pseudomonas aeruginosa* activates NF-kappaB through protease-activated receptors. Cell Microbiol. 2008; 10(7):1491-1504. DOI:10.1111/j.1462-5822.2008.01142.x.

[117] Ruer S, Ball G, Filloux A, de Bentzmann S. The "P-usher", a novel protein transporter involved in fimbrial assembly and TpsA secretion. EMBO J. 2008; 27(20):2669-2680. DOI:10.1038/emboj.2008.197.

[118] Faure LM, Garvis S, de Bentzmann S, Bigot S. Characterization of a novel two-partner secretion system implicated in the virulence of *Pseudomonas aeruginosa*. Microbiology. 2014; 160:1940-1952. DOI:10.1099/mic.0.079616-0.

[119] Ballister ER, Lai AH, Zuckermann RN, Cheng Y, Mougous JD. In vitro self-assembly of tailorable nanotubes from a simple protein building block. Proc Natl Acad Sci U S A. 2008; 105(10):3733-3738. DOI:10.1073/pnas.0712247105.

[120] Leiman PG, Basler M, Ramagopal UA, et al. Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin. Proc Natl Acad Sci U S A. 2009; 106(11):4154-4159. DOI:10.1073/pnas.0813360106.

[121] Bomberger JM, MacEachran DP, Coutermarsh BA, Ye S, O'Toole GA, Stanton BA. Long-distance delivery of bacterial virulence factors by *Pseudomonas aeruginosa* outer membrane vsicles. Ausubel FM, ed. PLoS Pathog. 2009; 5(4):e1000382. DOI:10.1371/journal.ppat.1000382.

[122] Thomas MS. Iron acquisition mechanisms of the *Burkholderia cepacia* complex. BioMetals. 2007; 20:431-452. DOI:10.1007/s10534-006-9065-4.

[123] Darling P, Chan M, Cox AD, Sokol PA. Siderophore production by cystic fibrosis isolates of *Burkholderia cepacia*. Infect Immun. 1998; 66:874-877.

[124] Agnoli K, Lowe CA, Farmer KL, Husnain SI, Thomas MS. The ornibactin biosynthesis and transport genes *of Burkholderia cenocepacia* are regulated by an extracytoplasmic function σ factor which is a part of the Fur regulon. J Bacteriol. 2006; 188(10):3631-3644. DOI:10.1128/JB.188.10.3631-3644.2006.

[125] Sokol PA, Darling P, Woods DE, Mahenthiralingam E, Kooi C. Role of Ornibactin biosynthesis in the virulence of *Burkholderia cepacia*: characterization of *pvdA*, the gene encoding l-ornithine N(5)-oxygenase. Infect Immun. 1999; 67(9):4443-4455.

[126] Sokol PA, Darling P, Lewenza S, Corbett CR, Kooi CD. Identification of a siderophore receptor required for ferric ornibactin uptake in *Burkholderia cepacia*. Infect Immun. 2000; 68(12):6554-6560.

[127] Visser MB, Majumdar S, Hani E, Sokol PA. Importance of the ornibactin and pyochelin siderophore transport systems in *Burkholderia cenocepacia* lung infections. Infect Immun. 2004; 72:2850-2857. DOI:10.1128/IAI.72.5.2850-2857.2004.

[128] Høiby N, Ciofu O, Bjarnsholt T. *Pseudomonas aeruginosa* biofilms in cystic fibrosis. Future Microbiol. 2010; 5(11):1663-1674. DOI:10.2217/fmb.10.125.

[129] Imperi F, Tiburzi F, Visca P. Molecular basis of pyoverdine siderophore recycling in *Pseudomonas aeruginosa*. Proc Natl Acad Sci. 2009; 106(48):20440-20445. DOI:10.1073/pnas.0908760106.

[130] Nikaido H, Pagès J-M. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. FEMS Microbiol Rev. 2012; 36(2):340-363. DOI:10.1111/j.1574-6976.2011.00290.x.

[131] Rushton L, Sass A, Baldwin A, Dowson CG, Donoghue D, Mahenthiralingam E. Key role for efflux in the preservative susceptibility and adaptive resistance of *Burkholderia cepacia* complex bacteria. Antimicrob Agents Chemother. 2013; 57:2972-2980. DOI:10.1128/AAC.00140-13.

[132] Leitão JH, Sousa SA, Cunha MV, Salgado MJ, Melo-Cristino J, Barreto MC, Sá-Correia I. Variation of the antimicrobial susceptibility profiles of *Burkholderia cepacia* complex clonal isolates obtained from chronically infected cystic fibrosis patients: a five-year survey in the major Portuguese treatment center. Eur J Clin Microbiol Infect Dis. 2008; 27:1101-1111. DOI:10.1007/s10096-008-0552-0.

[133] Pope CF, Gillespie SH, Pratten JR, McHugh TD. Fluoroquinolone-resistant mutants of *Burkholderia cepacia*. Antimicrob Agents Chemother. 2008; 52(3):1201-1203. DOI:10.1128/AAC.00799-07.

[134] Crowley D, Daly M, Lucey B, et al. Molecular epidemiology of cystic fibrosis-linked *Burkholderia cepacia* complex isolates from three national referral centres in Ireland. J Appl Microbiol. 2002; 92(5):992-1004. DOI:10.1046/j.1365-2672.2002.01612.x.

[135] Ramírez MS, Vargas LJ, Cagnoni V, Tokumoto M, Centrón D. Class 2 Integron with a novel cassette array in a *Burkholderia cenocepacia* isolate. Antimicrob Agents Chemother. 2005; 49(10):4418-4420. DOI:10.1128/AAC.49.10.4418-4420.2005.

[136] Aronoff SC. Outer membrane permeability in *Pseudomonas cepacia*: diminished porin content in a beta-lactam-resistant mutant and in resistant cystic fibrosis isolates. Antimicrob Agents Chemother. 1988; 32:1636-1639. DOI:10.1128/AAC.32.11.1636.

[137] Moore RA, Hancock RE. Involvement of outer membrane of *Pseudomonas cepacia* in aminoglycoside and polymyxin resistance. Antimicrob Agents Chemother. 1986; 30(6):923-926. DOI:10.1128/AAC.30.6.923.

[138] Bazzini S, Udine C, Sass A, et al. Deciphering the role of RND efflux transporters in *Burkholderia cenocepacia*. PLoS One. 2011; 6:e18902. DOI:10.1371/journal.pone.0018902.

[139] Buroni S, Pasca MR, Flannagan RS, et al. Assessment of three resistance-nodulation-cell division drug efflux transporters of *Burkholderia cenocepacia* in intrinsic antibiotic resistance. BMC Microbiol. 2009; 9:1-11. DOI:10.1186/1471-2180-9-200.

[140] Nair BM, Cheung KJ, Griffith A, Burns JL. Salicylate induces an antibiotic efflux pump in *Burkholderia cepacia* complex genomovar III (*B. cenocepacia*). J Clin Invest. 2004; 113:464-473. DOI:10.1172/JCI19710.

[141] Podnecky NL, Rhodes KA, Schweizer HP. Efflux pump-mediated drug resistance in *Burkholderia*. Front Microbiol. 2015; 6:305. DOI:10.3389/fmicb.2015.00305.

[142] Coenye T, Van Acker H, Peeters E, et al. Molecular mechanisms of chlorhexidine tolerance in *Burkholderia cenocepacia* biofilms. Antimicrob Agents Chemother. 2011; 55:1912-1919. DOI:10.1128/AAC.01571-10.

[143] Buroni S, Matthijs N, Spadaro F, et al. Differential roles of RND efflux pumps in antimicrobial drug resistance of sessile and planktonic *Burkholderia cenocepacia* cells. Antimicrob Agents Chemother. 2014; 58:7424-7429. DOI:10.1128/AAC.03800-14.

[144] Tseng SP, Tsai WC, Liang CY, et al. The contribution of antibiotic resistance mechanisms in clinical *Burkholderia cepacia* complex isolates: an emphasis on efflux pump activity. PLoS One. 2014; 9(8):e104986. DOI:10.1371/journal.pone.0104986.

[145] Drevinek P, Holden MTG, Ge Z, et al. Gene expression changes linked to antimicrobial resistance, oxidative stress, iron depletion and retained motility are observed when *Burkholderia cenocepacia* grows in cystic fibrosis sputum. BMC Infect Dis. 2008; 8:121. DOI:10.1186/1471-2334-8-121.

[146] Urban TA, Griffith A, Torok AM, Smolkin ME, Burns JL, Goldberg JB. Contribution of *Burkholderia cenocepacia* flagella to infectivity and inflammation. Infect Immun. 2004; 72:5126-5134. DOI:10.1128/IAI.72.9.5126-5134.2004.

[147] Kumar B, Cardona ST. Synthetic cystic fibrosis sputum medium regulates flagellar biosynthesis through the *flhF* gene in *Burkholderia cenocepacia*. Front Cell Infect Microbiol. 2016; 6:65. DOI:10.3389/fcimb.2016.00065.

[148] Kalferstova L, Kolar M, Fila L, Vavrova J, Drevinek P. Gene expression profiling of *Burkholderia cenocepacia* at the time of cepacia syndrome: loss of motility as a marker of poor prognosis? J Clin Microbiol. 2015; 53:1515-1522. DOI:10.1128/JCM.03605-14.

[149] Lamothe J, Thyssen S, Valvano MA. *Burkholderia cepacia* complex isolates survive intracellularly without replication within acidic vacuoles of *Acanthamoeba polyphaga*. Cell Microbiol. 2004; 6(12):1127-1138. DOI:10.1111/j.1462-5822.2004.00424.x.

[150] Keith KE, Hynes DW, Sholdice JE, Valvano MA. Delayed association of the NADPH oxidase complex with macrophage vacuoles containing the opportunistic pathogen *Burkholderia cenocepacia*. Microbiology. 2009; 155(Pt 4):1004-1015. DOI:10.1099/mic.0.026781-0.

[151] Rosales-Reyes R, Aubert DF, Tolman JS, Amer AO, Valvano MA. *Burkholderia cenocepacia* type VI secretion system mediates escape of type II secreted proteins into the cytoplasm of infected macrophages. PLoS One. 2012; 7(7):e41726. DOI:10.1371/journal.pone.0041726.

[152] Al-Khodor S, Marshall-Batty K, Nair V, Ding L, Greenberg DE, Fraser IDC. *Burkholderia cenocepacia* J2315 escapes to the cytosol and actively subverts autophagy in human macrophages. Cell Microbiol. 2014; 16:378-395. DOI:10.1111/cmi.12223.

[153] Lamothe J, Valvano MA. *Burkholderia cenocepacia*-induced delay of acidification and phagolysosomal fusion in cystic fibrosis transmembrane conductance regulator (CFTR)-defective macrophages. Microbiology. 2008; 154(Pt 12):3825-3834. DOI:10.1099/mic.0.2008/023200-0.

[154] Luciani A, Villella VR, Esposito S, et al. Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. Nat Cell Biol. 2010; 12:863-875. DOI:10.1038/ncb2090.

[155] Valvano MA. Intracellular survival of *Burkholderia cepacia* complex in phagocytic cells. Can J Microbiol. 2015; 61:607-615. DOI:10.1139/cjm-2015-0316.

[156] Regan KH, Bhatt J. Eradication therapy for *Burkholderia cepacia* complex in people with cystic fibrosis. Cochrane Database Syst Rev. 2014; 10:1-21. CD009876. DOI:10.1002/14651858.CD009876.pub2.

[157] Pradenas, GA, Ross BN, Torres AG. *Burkholderia cepacia* Complex Vaccines: Where Do We Go from here? Vaccines. 2016; 4, 10:1-14. doi:10.3390/vaccines4020010.

[158] Schwab U, Abdullah LH, Perlmutt OS, et al. Localization of *Burkholderia cepacia* complex bacteria in cystic fibrosis lungs and interactions with *Pseudomonas aeruginosa* in hypoxic mucus. Infect Immun. 2014; 82:4729-4745. DOI:10.1128/IAI.01876-14.

[159] Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology. 2008; 123:326-338. DOI:10.1111/j.1365-2567.2007.02719.x.

[160] Moser C, Kjaergaard S, Pressler T, Kharazmi A, Koch C, Høiby N. The immune response to chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients is predominantly of the Th2 type. APMIS. 2000; 108:329-335. DOI:10.1034/j.1600-0463.2000.d01-64.x.

[161] McClean S, Healy ME, Collins C, Carberry S, O'Shaughnessy L, Dennehy R, Adams Á, Kennelly H, Corbett JM, Carty F, et al. Linocin and OmpW are involved in attachment of the cystic fibrosis associated pathogen *Burkholderia cepacia* complex to lung epithelial cells and protect mice against infection. Infect Immun. 2016; 84:1424-1437. DOI:10.1128/IAI.01248-15.

[162] Bertot GM, Restelli MA, Galanternik L, Aranibar Urey RC, Valvano MA, Grinstein S. Nasal immunization with *Burkholderia multivorans* outer membrane proteins and the mucosal adjuvant adamantylamide dipeptide confers efficient protection against experimental lung infections with *B. multivorans* and *B. cenocepacia*. Infect Immun. 2007; 75:2740-2752. DOI:10.1128/IAI.01668-06.

[163] Makidon PE, Knowlton J, Groom JV, Blanco LP, LiPuma JJ, Bielinska AU, Baker JR, Jr. Induction of immune response to the 17 kDa OMPA *Burkholderia cenocepacia* polypeptide and protection against pulmonary infection in mice after nasal vaccination with an OMP nanoemulsion-based vaccine. Med Microbiol Immunol. 2010; 199:81-92. DOI:10.1007/s00430-009-0137-2.

[164] Skurnik D, Davis MR Jr, Benedetti D, Moravec KL, Cywes-Bentley C, Roux D, Traficante DC, Walsh RL, Maira-Litràn T, Cassidy SK, Hermos CR, Martin TR, Thakkallapalli EL, Vargas SO, McAdam AJ, Lieberman TD, Kishony R, Lipuma JJ, Pier GB, Goldberg JB, Priebe GP. Targeting pan-resistant bacteria with antibodies to a broadly conserved surface polysaccharide expressed during infection. J Infect Dis. 2012; 205:1709-1718. DOI:10.1093/infdis/jis254.

[165] Musson JA, Reynolds CJ, Rinchai D, et al. CD4+ T cell epitopes of FliC conserved between strains of *Burkholderia* - implications for vaccines against melioidosis and cepacia complex in Cystic Fibrosis. J Immunol (Baltimore, Md : 1950). 2014; 193(12):6041-6049. DOI:10.4049/jimmunol.1402273.

[166] Sousa SA, Morad M, Feliciano JR, Pita T, Nady S, El-Hennamy RE, Abdel-Rahman M, Cavaco J, Pereira L, Barreto C, Leitão JH. The *Burkholderia cenocepacia* OmpA-like protein BCAL2958: identification, characterization, and detection of anti-BCAL2958 antibodies in serum from *B. cepacia* complex-infected Cystic Fibrosis patients. AMB Express. 2016; 6(1):41. DOI:10.1186/s13568-016-0212-1.

[167] Mariappan V, Vellasamy KM, Thimma JS, Hashim OH, and Vadivelu J. Identification of immunogenic proteins from *Burkholderia cepacia* secretome using proteomic analysis. Vaccine. 2010; 28:1318-1324. DOI:10.1016/j.vaccine.2009.11.027.

[168] Shinoy M, Dennehy R, Coleman L, Carberry S, Schaffer K, Callaghan M, Doyle S, McClean S. Immunoproteomic analysis of proteins expressed by two related pathogens, *Burkholderia multivorans* and *Burkholderia cenocepacia*, during human infection. PLoS One. 2013; 8(11):e80796. DOI:10.1371/journal.pone.0080796.

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