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Burkholderia cenocepacia in cystic fibrosis: epidemiology and molecular mechanisms of virulence

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Abstract

Burkholderia cepacia complex (Bcc) bacteria have gained notoriety as pathogens in cystic fibrosis (CF) because they are difficult to identify and treat, and also have the ability to spread between CF individuals. Of the 17 formally named species within the complex, *Burkholderia multivorans* and *Burkholderia cenocepacia* dominate in CF. Multilocus sequence typing has proven to be a very useful tool for tracing the global epidemiology of Bcc bacteria and has shown that *B. cenocepacia* strains with high transmissibility, such as the ET-12 strain (ST-28) and the Czech strain (ST-32), have spread epidemically within CF populations in Canada and Europe. The majority of research on the molecular pathogenesis of Bcc bacteria has focused on the *B. cenocepacia* ET-12 epidemic lineage, with gene mutation, genome sequence analysis and, most recently, global gene expression studies shedding considerable light on the virulence and antimicrobial resistance of this pathogen. These studies demonstrate that the ability of *B. cenocepacia* to acquire foreign DNA (genomic islands, insertion sequences and other mobile elements), regulate gene expression via quorum sensing, compete for iron during infection, and mediate antimicrobial resistance and inflammation via its membrane and surface polysaccharides are key features that underpin the virulence of different strains. With the wealth of molecular knowledge acquired in the last decade on *B. cenocepacia* strains, we are now in a much better position to develop strategies for the treatment of pathogenic colonization with Bcc and to answer key questions on pathogenesis concerning, for example, the factors that trigger the rapid clinical decline in CF patients.

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Introduction

Recurrent or chronic respiratory infection is one of the major factors that negatively affect the length of life in individuals with cystic fibrosis (CF). Infections result in a decline of pulmonary function and therapeutic options that can minimize their impact on patient health are still very limited. An unfavourable course of pulmonary disease and progression of a lung tissue damage is an inherent consequence of CF pathophysiology [1], yet infectious agents such as bacteria also play a major role in the process. They possess a multitude of virulence factors, which they can activate or suppress at different stages of infection, leading to both inflammation and damage of the lung.

Patients with CF are vulnerable throughout their lives to various bacterial infections that are caused in most instances by opportunistic pathogens. One of the most threatening pathogens in CF is *Burkholderia cenocepacia*, a member of a bacterial group collectively referred to as the *Burkholderia cepacia* complex (Bcc). The complex currently consists of 17 genetically closely related bacterial species [2–4] that may be isolated from sites of human infection as well as the natural environment [5]. Several Bcc species have been shown to be transmissible from one CF patient to another and are able to cause epidemic outbreaks [6]; however, it is *B. cenocepacia* and *Burkholderia multivorans* that predominate in CF, and these are found in a significantly higher percentage of respiratory specimens. These two species together account for approximately 85–97% of all Bcc infections in CF (the species distribution in selected CF populations is summarized in Table 1).

Although CF infection with any Bcc species may be associated with poor prognosis, *B. cenocepacia* is considered one of the most serious pathogens because it is frequently associated with reduced survival and highest risk

of developing fatal cepacia syndrome [2,7]. *B. cenocepacia* also contains the majority of epidemic strains described so far [8–12]. In light of this, research on the pathogenicity of Bcc bacteria has largely focused on *B. cenocepacia*, with the first sequenced Bcc genome being *B. cenocepacia* strain J2315 [13,14]. Herein, we provide an overview of the current knowledge on the epidemiology and virulence mechanisms of Bcc, with a primary focus on *B. cenocepacia*.

Epidemiology of *B. cenocepacia*: then and now

Epidemiological studies from the late 1990s demonstrated that *B. cenocepacia* (formerly Bcc genomovar III) was the most prevalent Bcc pathogen in most CF populations studied worldwide [15,16]. By using a *recA* gene sequence analysis [17], the species was subdivided into four phylogenetic clusters, IIIA to IIID, but almost all clinically relevant isolates resided in the IIIA and IIIB groups. One of the most notorious epidemic *B. cenocepacia* lineages is known as ET-12, comprising a group of strains that have been causing devastating infections in Canadian, UK and European CF populations [2] subsequent to the first descriptions of the virulence of '*Pseudomonas cepacia*' in CF [18]. The ET-12 and several other epidemic strains dominant in Canada [known as random amplified polymorphic DNA (RAPD) types 01, 04 and 06] [12] and Europe (e.g. the CZ1 Czech epidemic clone) [9,19] all belong to the *B. cenocepacia* IIIA subgroup [17,20]. By contrast, the dominant *B. cenocepacia* epidemic clones in the USA are part of the subgroup IIIB and include the Midwest clone [8] and PHDC strain [10], which has also been found in European CF patients [21].

In the last 5 years, the high prevalence of *B. cenocepacia* in CF has remained in certain populations, such as in Czech and Italian populations [9,22]. However, other countries have reported an increase in the proportion of *B. multivorans* causing Bcc infections in CF [23,24], with *B. cenocepacia* ranking second in dominance. This phenomenon may have resulted from a combination of several factors such as: (i) application of stringent infection control measures that prevented a spread of epidemic strains (belonging mostly to *B. cenocepacia*) [25] but were ineffective against acquisition of other Bcc species living in the natural environment and (ii) the high mortality rate within the group of patients who were infected with *B. cenocepacia* that has reduced their numbers. For example, at the Manchester adult CF centre [7], it was observed that only 66.6% of their patients survived 5 years after acquisition of *B. cenocepacia*. This 5-year survival rate was significantly lower compared to the rate calculated for patients infected with *Pseudomonas aeruginosa* (5-year survival = 85.3%).

However, it is worth noting that not all CF populations struggling with *B. cenocepacia* infection demonstrate such poor survival. Data from the Prague adult CF centre indicate that 91.4% of *B. cenocepacia*-positive patients reached a 5-year survival (L. Fila, Prague CF Centre, personal communication). Why the mortality rate differs among Bcc-infected populations is not known; however it is tempting to speculate that *B. cenocepacia* strain differences are responsible for the divergent clinical courses seen at these two CF centres. Strains of the ET-12 lineage accounted for the *B. cenocepacia* outbreak in the Manchester unit [7], whereas the Prague patients were infected with strain CZ1 [9]. Although both strains are very closely related as members of the *B. cenocepacia* IIIA subgroup, the differences in survival rates suggest that the strains have distinct virulence traits during infection.

TABLE 1. Distribution of individual *Burkholderia cepacia* complex species in selected populations of cystic fibrosis patients

Bcc species	USA [64] (1218 patients; 1997 –2004)[a]	Canada [12] (445 patients; 1994–2000)[a]	France [65] (153 patients; 1995–2000)[a]	Czech Rep. [66] (61 patients; 2001)[a]	Italy [67] (53 patients; 1985–1999)[a]	Portugal [68] (22 patients; 1995–2002)[a]
Burkholderia cepacia	3.1	0.2	0.0	0.0	2.9	36.4
Burkholderia multivorans	38.7	9.3	51.6	4.9	0.0	9.0
Burkholderia cenocepacia	45.6	80.0	45.1	91.8	86.8	52.2
Burkholderia stabilis	0.3	3.8	1.3	1.6	7.4	18.2
Burkholderia vietnamiensis	5.9	1.6	0.6	0.0	0.0	0.0
Burkholderia dolosa	3.8	0.0	0.0	0.0	0.0	0.0
Burkholderia ambifaria	0.8	0.0	0.0	0.0	0.0	0.0
Burkholderia anthina	0.2	–[b]	0.0	–[b]	0.0	0.0
Burkholderia pyrrocinia	0.3	–[b]	1.3	–[b]	2.9	0.0
Indeterminate	1.3	1.8	0.0	1.6	0.0	0.0

[a]The number of patients examined and the period of study.
[b]The species was not analyzed in the present study.

Contribution of multilocus sequence typing (MLST) to epidemiology

A new insight into the global epidemiology of *B. cenocepacia* has been offered by MLST, a recently developed typing technique [26] based on the comparison of nucleotide sequences of seven housekeeping genes. Individual strains are characterized by a sequence type (ST), an arbitrary number that represents a unique combination of seven sequenced alleles.

MLST analysis of the Bcc has led to the revision of the extent of genetic relatedness among and within individual epidemic lineages. Although some clones defined previously by typing methods, such as the RAPD assay, remained single STs (e.g. all RAPD group 06 isolates originally described in Canada [27] are ST-210), other *B. cenocepacia* epidemic clones have turned out to encompass several different STs. Another remarkable finding derived from MLST analysis is that isolates of the ET-12 lineage appear to belong to at least five different STs, with only ST-28 representing the ET-12 clone responsible for intercontinental spread between Canada and the UK (A. Baldwin, personal communication; Fig. 1).

New globally-distributed epidemic strain revealed

ET-12 (ST-28) was recognized as a globally spread *B. cenocepacia* epidemic strain prior to the application of MLST; however, the existence of other globally-distributed strains was confirmed using this powerful typing tool. Recently, it was shown that a Canadian epidemic strain type, RAPD 01 [27], and the Czech epidemic strain, CZ1 [9], were both sequence type ST-32, and that this ST is found in other countries around the world [19] (Fig. 1). How the ST-32 strain spread to multiple countries has not yet been elucidated. Intercontinental spread of the ET-12 strain has been linked to social contact at summer camps organized for CF patients [28]; however, no obvious epidemiological link between the location and source of ST-32 isolates is apparent.

We can speculate that ST-32 is either an inhabitant of a common environmental niche to which CF patients are exposed, or that it may have been spread as a result of contamination of healthcare or domestic products commonly used by individuals with CF. There is little epidemiological evidence to support a specific natural source of ST-32 because the only environmental isolate of this ST was cultivated from a radish in Mexico [5]. Indeed, a fascinating aspect of the ecology of *B. cenocepacia* IIIA strains is that they have been rarely found outside of human infections in comparison to other *B. cenocepacia recA* lineages and the Bcc as a whole [5]. Bcc isolates linked to industrial contamination have been shown to consist of various Bcc species and strains, but as yet not of ST-32 isolates (see MLST database at: http://pubmlst.org/bcc; [5]). Evidence to support the ability of ST-32 isolates to contaminate healthcare settings and to spread nosocomially has been recently provided by Graindorge *et al.* [29]. Their study showed that ST-32 isolates had caused an outbreak of *B. cenocepacia* infection among seven mechanically ventilated patients in a French intensive care unit, demonstrating the high transmissibility of this strain in outbreak scenarios outside of CF.

Although ST-28 and ST-32 are close relatives within the *B. cenocepacia* IIIA lineage, they differ from each other by polymorphisms in three MLST alleles. From a clinical point of

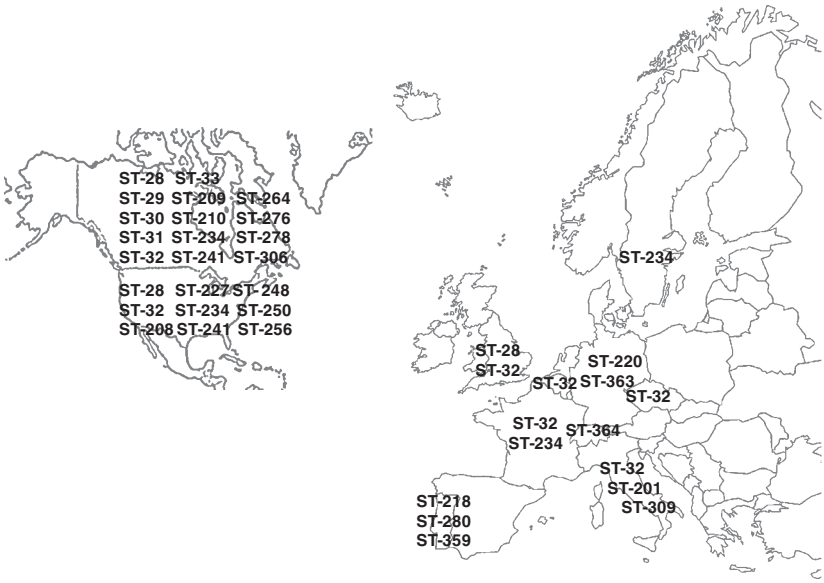

FIG. 1. Global distribution of *Burkholderia cenocepacia* IIIA strains that were analyzed by multilocus sequence typing (MLST) and deposited in the public MLST database.

view, ST-28 and ST-32 also differ in terms of the progression of the infections they cause, as well as with respect to 5-year survival, as mentioned above. The differences apparent in the virulence of each strain suggest they may have different responses to the conditions of chronic lung infection. We conducted a study that, for the first time, compared the behaviour of both epidemic strains under conditions of oxidative stress, an attribute of chronically inflamed CF lungs [19]. The exposure to reactive oxygen species had a considerable effect on genomic rearrangements in ST-32, as observed by pulsed-field gel electrophoresis, and it also enhanced the transposition activity of the mobile element IS*Bcen20*. By contrast, no changes were detected under the same stress conditions for ST-28. The results suggested that movement of mobile elements was strain-specific and the degree of genomic stability may differ substantially even between closely-related *B. cenocepacia* strains. The phenotypic impact of such rapid IS-mediated changes in the genome of *B. cenocepacia* remains to be studied, but may lead, for example, to the reduced virulence of ST-32 compared to ST-28.

Although many *B. cenocepacia* IIIA strains have been implicated in outbreaks of infection among CF patients [12,27], ST-28 and ST-32 strains are the only strains characterized by MLST that are present in multiple globally-distinct locations, and which, taken together, appear to account for a very large proportion of CF infections attributed to *B. cenocepacia* IIIA.

Virulence Factors from a Genomic and Transcriptomic Perspective

The most basic question as to why *B. cenocepacia* is able to colonize specifically and almost exclusively the respiratory tract of patients with CF remains unanswered. Although the pathogen is known also to be associated with immunodeficiency in chronic granulomatous disease (CGD) [30], no immune defect similar to CGD is present in CF.

A specific link between CF and infection has been postulated only for *P. aeruginosa* which takes advantage of an impaired epithelial cell protein that no longer functions as a receptor for internalization and clearance of the bacteria from the respiratory system [31]. Another equally important question to be answered relates to understanding why *B. cenocepacia* is an aggressive pathogen that, in many cases, ultimately leads to a patient's death.

Both issues are being addressed by intensive research concerning the pathogenesis of the *B. cenocepacia* infection, which includes: (i) the identification of bacterial virulence factors and (ii) the study of pathogen–host cell interaction. A recent review by McClean and Callaghan [32] summarized the mechanisms of the Bcc interaction with respiratory epithelium cells where virulence factors critical for bacterial adherence, cell invasion, intracellular survival and epithelial layer penetration are well described. Here, we provide an overview of *B. cenocepacia* virulence factors with a specific focus on the bacterial genome and transcriptome. The genome of *B. cenocepacia* IIIA strain J2315 belonging to ST-28 has been recently annotated [13] and its complete sequence information has allowed the design of a whole-genome DNA chip [33]. By using a microarray technology or cDNA sequencing, several global gene expression studies of *B. cenocepacia* that model environmental and infection-related interactions have been made available [34–37].

Using a mutation-based approach, many *B. cenocepacia* genes encoding putative virulence factors have also been successfully inactivated to study their virulence function (Table 2).

Genomic islands and mobile elements

The complete genome sequence analysis of *B. cenocepacia* J2315 revealed that it contained a staggering number of genomic islands occupying 9.3% of its 8.06 Mb chromosome [13]. Interestingly, of the fourteen islands described in strain J2315, none were found as conserved entities in the two genomes available for *B. cenocepacia* IIIB strains of the PHDC lineage (AU1054 and HI2424). Holden *et al.* [13] hypothesized that genomic islands may play a key role in the adaption of ST-28 to survival in the CF lung and contribute significantly to the overall pool of *B. cenocepacia* IIIA virulence factors.

Genomic island 11 is the most studied region of horizontally acquired DNA in *B. cenocepacia* and was originally designated as the *B. cenocepacia* island (cci) [38]. The cci encodes the *B. cepacia* epidemic strain marker (BCESM), a marker of epidemic strains characterized before the revision of species taxonomy within the Bcc [39]. Using comparative genomics, Holden *et al.* [13] revised the annotation of the cci, demonstrating it comprised a larger (44.1 kb) pathogenicity island located on chromosome 2 that contained a total of 43 coding sequences (BCAM 0233–BCAM 0281). Genes encoding an amidase (*amiI*), a porin (*opcI*) and a quorum-sensing system (*cciI* and *cciR*) that are part of genomic island 11 have been knocked out in previous studies, with the resulting mutants demonstrating attenuated virulence and inflammatory potential [38,40].

The island also contains a copy of IS*Bcen14*, one of 22 different IS elements that reside within the J2315 genome. We recently demonstrated that IS*Bcen20* lacked transposition

TABLE 2. Selected *Burkholderia cenocepacia* virulence genes that were studied by gene mutation

Gene name	ID in annotated J2315 genome	Product encoded	Mutated strain[a]	References
Quorum sensing				
cepR2	BCAM 0188	Response regulator	K56-2, H111	42
cciI	BCAM 0239a	*N*-acylhomoserine lactone synthase	K56-2	38,40
cciR	BCAM 0240	Response regulator	K56-2	40
cepR	BCAM 1868	Response regulator	K56-2	40,41
			H111	69
cepI	BCAM 1870	*N*-acylhomoserine lactone synthase	K56-2	40,41
			H111	69
Iron uptake				
orbS	BCAL 1688	RNA polymerase sigma factor	KLF1	70
orbI	BCAL 1696	Ornibactin biosynthesis peptide synthase	KLF1	70
orbJ	BCAL 1697	Ornibactin biosynthesis synthase	KLF1	70
pvdA	BCAL 1699	L-ornithine 5-oxygenase	K56-2	71
orbA	BCAL 1700	Ornibactin receptor precursor	K56-2	72
	BCAM 1187	TonB-dependent siderophore receptor	K56-2	73
fptA	BCAM 2224	Ferric-pyochelin outer membrane receptor	Pc715j	44
phuV (*hmuV*)	BCAM 2630	Haemin ATP-binding protein	K56-2	73
Lipopolysaccharide				
Core				
hldD	BCAL 2944	ADP-L-glycero-D-manno-heptose-6-epimerase	K56-2	46
hldA	BCAL 2945	D-β-D-heptose 7-phosphate kinase	K56-2	46
O antigen				
wbiI	BCAL 3119	Nucleotide sugar epimerase/dehydratase	K56-2	47
wbiG	BCAL 3121	Nucleotide sugar epimerase/dehydratase	K56-2	47
wbiF	BCAL 3122	Glycosyltransferase	K56-2	47
wbcE (*wbxE*)	BCAL 3125	Glycosyltransferase	K56-2	46,49
rmlD	BCAL 3132	dTDP-4-keto-L-rhamnose reductase	K56-2	47
Exopolysaccharide				
cpxA	BCAL 3240	Capsule polysaccharide export ATP-binding protein	K56-2	47
bceA	BCAM 0854	Mannose-1-phosphate guanylyltransferase	IST408	74
bceD	BCAM 0857	Protein-tyrosine-phosphatase	IST408	75
bceF	BCAM 0859	Tyrosine-protein kinase	IST408	75
Antimicrobial resistance				
amrA, *amrB*	BCAL 1674–BCAL 1676	RND family efflux transporter	J2315	57
oprM, *bpeA*	BCAL 2820–BCAL 2822	RND family efflux transporter	J2315	57
	BCAS 0591–BCAS 0593	RND family efflux transporter	J2315	57
Flagella and cable pilus				
fliC	BCAL 0114	Flagellin (type II)	K56-2	76
fliI	BCAL 0522	Flagellum-specific ATP synthase	J2315	58
fliG	BCAL 0524	Flagellar motor switch protein	J2315	58
adhA	BCAM 2143	Cable pilus-associated adhesin	BC7	77
cblS	BCAM 2758	Sensor histidine kinase	BC7	77
cblA	BCAM 2761	Giant cable pilus	BC7	77,78
Other virulence factors				
rpoE	BCAL 0998, BCAL 2872	RNA polymerase sigma-E factor	K56-2	61
mucD	BCAL 1001, BCAL 2869	Serine protease	K56-2	61
sodC	BCAL 2643	Superoxide dismutase	K56-2	79
	BCAL 2828	Putative exported protein	K56-2	63
htrA	BCAL 2829	Serine protease	K56-2	63
	BCAL 2831	Response regulator	K56-2	63
katB	BCAL 3299	Peroxidase/catalase	C5424	80
amiI	BCAM 0265	Putative amidase	K56-2	38
opcI	BCAM 0267	Putative porin	K56-2	38
atsR	BCAM 0379	Sensor histidine kinase/response regulator	K56-2	81
	BCAM 0392	Putative acetyltransferase	K56-2	73
scpB	BCAM 0957	Pepstatin-insensitive carboxyl proteinase	K56-2	73
mgtC	BCAM 1867	Putative Mg^{2+} transport ATPase	K56-2	62
				47
katA	BCAM 2107	Peroxidase/catalase	C5424	80
	BCAM 2160	Response regulator	K56-2	63
	BCAM 2631	Putative membrane protein	K56-2	73
aidA	BCAS 0293	Nematocidal protein	H111	82
			K56-2	73,83
zmpA	BCAS 0409	Zinc metalloprotease	K56-2	60

[a]Species and strains used for mutagenesis in these studies were as follows: *B. cenocepacia* BC7 (LMG 18826); *B. cenocepacia* K56-2 (LMG 18863); *B. cenocepacia* J2315 (LMG 16656); *B. cenocepacia* C5424 (LMG 18827); *B. cenocepacia* Pc715j; *B. cenocepacia* H111, *B. cenocepacia* KLF1 and *Burkholderia cepacia* IST408.

activity in strain J2315, whereas a homologous IS element in the Czech ST-32 *B. cenocepacia* strain responded to oxidative stress by causing genomic rearrangements. Whether any of the other 21 IS elements in the J2315 genome are capable of movement when the *B. cenocepacia* is stressed remains to be determined. The copy of IS*Bcen14* in genomic island 11 interrupts a cluster of fatty acid biosynthesis genes and it would be interesting to examine whether the virulence of strain J2315 is altered by this mutation in the cci pathogenicity island.

Quorum sensing

Quorum sensing is a form of bacterial cell-to-cell communication that regulates the expression of multiple genes, including virulence genes, and operates through two main components, an *N*-acyl-homoserine lactone (AHL) synthase and a regulator which is responsive to AHL. *B. cenocepacia* always possesses the CepI synthase with a CepR regulator [41]. Strains with genomic island 11 encode additional quorum-sensing components (i.e. the CciI synthase and the CciR regulator) [38,40]. Interestingly, a third regulator has been identified recently (designated CepR2) [42], although this has not been found to be associated with any adjacently encoded AHL synthase. An arsenal of genes have been identified that are regulated by quorum sensing and include those for siderophore synthesis, protease production, a type III secretion system, motility and biofilm formation [43]. Recently, a transcriptomic approach was applied to *B. cenocepacia* strain K56-2 aiming to examine the global gene responses mediated by both quorum-sensing systems [36]. The study demonstrated that expression of the majority of genes controlled by quorum sensing were regulated by CepR, the putatively ancestral regulator found in all Bcc bacteria. In addition, CepR functioned primarily as a positive regulator activating genes, whereas CciR was an inhibitor of gene expression. Nevertheless, interaction between CepIR and CciIR was also detected with approximately 200 genes co-regulated in a reciprocal way by both quorum-sensing systems.

Iron uptake

Under conditions of iron depletion, *B. cenocepacia* produces two main siderophores, ornibactin and pyochelin, acting to scavenge free iron from surrounding environment. Ornibactin has been reported as the biologically more important siderophore, and is able to compensate for the function of pyochelin [44]. It has also been postulated that pyochelin production may be defective in certain isolates such as *B. cenocepacia* J2315, which contains a frameshift mutation in *pchF*, one of the genes required for pyochelin biosynthesis [13]. Interestingly, when strain J2315 was incubated in a CF sputum-containing growth medium, several pyochelin synthesis genes (*pchR* and *pchD*) were found to be upregulated, but not ornibactin [35]. These expression data suggest that the pyochelin pathway may still be functional in strain J2315. Because CF sputum is an iron-deficient environment, it was not surprising that this study [35] also revealed significant upregulation of other genes involved in iron uptake and metabolism. These included a novel membrane ferric reductase gene (BCAL 0270) and *cyaY* (BCAL 0273), which may encode possible iron donors for the assembly of Fe-S intracellular storage molecules during infection.

Surface polysaccharides

One of the major components of the outer surface of *Burkholderia* bacteria is lipopolysaccharide (LPS). In certain *B. cenocepacia* strains, the expression of a capsular exopolysaccharide (EPS) that gives bacterial colonies mucoid appearance may also be seen; EPS production plays an important role in the chronicity of Bcc infections [45]. All the genes for LPS production are located on chromosome 1 with three main clusters for lipid A (BCAL 1929–BCAL 1935), core (BCAL 2402–BCAL 2408) and O antigen (BCAL 3110–BCAL 3125) [13], and additional sugar modification genes, *hldD*/*hldA* (BCAL 2944/BCAL 2945) [46] and *rmlD* (BCAL 3132) [47], which are also located on this replicon (Table 2). The O antigen is not expressed in some *B. cenocepacia* strains, such as J2315, where there is an interruption of the *wbcE* gene (BCAL 3125) by IS*Bcen20* [48]. *B. cenocepacia* strain K56-2 possesses a complete O antigen biosynthesis gene cluster and expresses the O antigen; inactivation of this pathway by mutation leads to a phenotype that is attenuated during infection [47,48]. These O antigen mutants were also found to be internalized into macrophages at significantly higher rates than the parental strain; however, their intracellular survival was not affected [49].

B. cenocepacia strain J2315 also appears unable to produce any EPS even though several gene clusters implicated in EPS biosynthesis were identified within its genome. Cepacian, the most abundant EPS among Bcc species [50], is not expressed in J2315 as a result of a frameshift mutation in *bceB* (BCAM 0856) encoding a putative glycosyltransferase [13,51]. Although Bcc strains producing cepacian are able to develop persistent infections in animal models [52], the role of EPS in virulence is similar to that of O antigen. Although expression of EPS is strain-specific and, when expressed, it appears to enhance the virulence of the producer isolate, the absence of EPS does not rule out that a Bcc strain may still cause a severe infection.

Antimicrobial resistance

B. cenocepacia is intrinsically resistant to polymyxins, aminoglycosides and most β-lactams, and can develop *in vivo* resistance to essentially all classes of antimicrobial drugs. It utilizes mechanisms of enzymatic inactivation (β-lactamases, aminoglycoside-inactivating enzymes, dihydrofolate reductase), alteration of drug targets, cell wall impermeability and active efflux pumps [53]. The genome of *B. cenocepacia* J2315 contains coding sequences for all five major families of efflux systems [13], with the resistance-nodulation-division family

(RND) arguably representing the best characterized efflux system.

Thirteen operons of RND efflux transporters were identified in J2315 [54] and one of the operons originally designated as the *ceo* cluster (BCAM 2549–2552, BCAM 2554) was demonstrated to confer resistance to chloramphenicol, trimethoprim and ciprofloxacin [55]. During growth in CF sputum [35], *B. cenocepacia* J2315 upregulated the expression of two RND efflux systems (BCAL 1675 and BCAM 1947) and downregulated expression of another RND system (BCAL 1813). However, this transcriptomic study did not demonstrate any alteration in the *ceo* RND system, suggesting that it is either not expressed in sputum or that insufficient levels of its substrate antimicrobials were present in the sputum samples examined to upregulate its expression. The activation of the other two efflux pumps in sputum may be linked to the presence of trace quantities of antibiotics because all the CF patients studied were on long-term antibiotic treatment. Alternatively, expression may have also been associated with the need of bacterial cells to acquire iron during infection because RND efflux pumps have been implicated in the transport of siderophores in *P. aeruginosa* [56]. The link between iron uptake and innate antimicrobial resistance is intriguing because it suggests that, by increasing siderophore secretion via RND pumps and adapting to the iron-starved conditions of the CF lung, *B. cenocepacia* simultaneously increases its level of antimicrobial resistance. Interestingly, two *B. cenocepacia* RND pumps (BCAL 1674–BCAL 1676 and BCAL 2822–BCAL 2820) have also been recently linked to secretion of the quorum-sensing signal AHL [57]. This observation indicates that the RND efflux systems in *B. cenocepacia* may transport multiple molecules and play a pivotal role in the virulence and antimicrobial resistance of this pathogen.

Motility and adherence

The flagellum is an important virulence factor that not only makes bacteria motile, but also serves as an adhesin and enables pathogens to invade host cells [58]. Genes for synthesis and assembly of flagella in *B. cenocepacia* J2315 are distributed within five clusters on chromosome 1, with two additional genes found on chromosomes 2 and 3 [13]. Increased transcriptional activity of flagellar genes was detected in *B. cenocepacia* when the organism was incubated in CF sputum [35]. This observation contrasts with the analysis of the transcription profile of *P. aeruginosa*, which is known to become non-motile during chronic CF lung infection. Retained motility in *B. cenocepacia* may account for the pathogen's ability to invade host cells and to cause life-threatening septicaemia. Another recent transcriptomic study demonstrated that regulation of flagellar gene transcription was also quorum sensing-dependent [36].

B. cenocepacia strains of ET-12 lineage also express specific fimbriae, the cable pili, which, in association with the 22-kDa adhesin AdhA (BCAM 2143), have been shown to play an essential role in the invasion of respiratory epithelium [59]. Recent transcriptomic studies demonstrated the decreased transcription of the cable pilus gene cluster (BCAM 2756–BCAM 2762) during rapid growth in CF sputum [35], suggesting that the whole fimbrial structure may not be an adhesive component as important as initially thought. However, the global gene expression analysis did shed light on a potentially new role for the cable pilus-associated adhesin AdhA during infection because its expression was significantly increased during the growth of *B. cenocepacia* in sputum [35].

Other virulence factors

Other classic pathogenicity factors described in *B. cenocepacia* include exoproducts such as zinc metalloproteases (ZmpA and ZmpB; BCAS 0409 and BCAM 2307, respectively) [60] and phospholipases C (BCAL 0443, BCAL 1046, BCAM 0408, BCAM 1969, BCAM 2429 and BCAM 2720) [13]. More recently, several studies have started to focus on the mechanisms that *B. cenocepacia* uses to adapt to the stressful conditions present in the CF lung environment and to investigate genes that are under the control of response regulators involved in the coordination of gene expression. Mutations in the genes encoding homologues of the alternative sigma factor RpoE (BCAL 0998 = *rpoE2* and BCAL 2872 = *rpoE1*; the gene is duplicated in J2315 genome) and in *mgtC* (BCAM 1867) are associated with the inability of *B. cenocepacia* to survive in macrophages [61,62]. In addition, RpoE was also found to be required for growth under conditions of osmotic stress and heat shock. Similarly, the HtrA protease (encoded by BCAL 2829) has been shown to be essential for survival at elevated temperatures and in the presence of high salt [63]. To resist the harmful effects of oxidative stress, *B. cenocepacia* may utilize several antioxidant enzymes, including superoxide dismutases (cytoplasmatic SodB and periplasmatic SodC; encoded by BCAL 2757 and BCAL 2643, respectively), catalases, catalase-peroxidases (*katA*, BCAM 2107 and *katB*, BCAL 3299) and alkyl-hydroperoxidase (BCAM 1216–BCAM 1217).

Concluding Remarks

Despite recent changes in the prevalence of the Bcc species, it is clear that *B. cenocepacia* remains one of the most dominant

and problematic CF pathogens from a worldwide perspective. Tracking by MLST of the global distribution of Bcc strains has revealed that, in addition to the well-characterized *B. cenocepacia* strains of the ET-12 lineage, other epidemic strains such as ST-32, most recently linked to the Czech CF population, are widely distributed in several other countries. The majority of studies on the molecular pathogenesis of *B. cenocepacia* have focused on strains that belong to the IIIA lineage (Table 2), and we know little about virulence determinants in IIIB isolates that dominate *B. cenocepacia* infection in the USA [15].

Factors involved in the pathogenesis of *B. cenocepacia* IIIA infection include surface polysaccharides, adhesins, flagella, siderophores, efflux pumps, quorum-sensing systems and metalloproteases, among others. The physical position and transcriptomic units of these virulence determinants have been mapped on the genome of *B. cenocepacia* J2315. Overall, it is clear that the number of virulence genes implicated in infection (Table 2) is far from complete and is likely to expand considerably with genomic analysis methods becoming available for several *B. cenocepacia* CF strains [14]. A thorough description of *B. cenocepacia* virulence is an essential prerequisite for the development of the next generation of antimicrobial drugs needed to treat life-threatening infections with multidrug-resistant strains. The progression of Bcc infection is both host- and strain-dependent, with different strains of *B. cenocepacia* potentially exploiting a variable armoury of virulence factors. Although our knowledge of the *B. cenocepacia* IIIA strain ST-28 has expanded substantially, the future characterization of other IIIA and of IIIB strains is vital to gain a balanced understanding of the virulence of *B. cenocepacia* in CF.

Transparency Declaration

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