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MINI-REVIEW

Pathogenicity, virulence factors, and strategies to fight against *Burkholderia cepacia* complex pathogens and related species

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Abstract The *Burkholderia cepacia* complex (Bcc) is a group of 17 closely related species of the β-proteobacteria subdivision that emerged in the 1980s as important human pathogens, especially to patients suffering from cystic fibrosis. Since then, a remarkable progress has been achieved on the taxonomy and molecular identification of these bacteria. Although some progress have been achieved on the knowledge of the pathogenesis traits and virulence factors used by these bacteria, further work envisaging the identification of potential targets for the scientifically based design of new therapeutic strategies is urgently needed, due to the very difficult eradication of these bacteria with available therapies. An overview of these aspects of Bcc pathogenesis and opportunities for the design of future therapies is presented and discussed in this work.

Keywords *Burkholderia cepacia* complex · Pathogenicity · Virulence factors · Antimicrobials

The *Burkholderia cepacia* complex and the cystic fibrosis host

Burkholderia cepacia was described in 1950 as *Pseudomonas cepacia*, the phytopathogen responsible for the sour skin disease in onions (Burkholder 1950). Molecular taxonomic analysis of *P. cepacia* and closely related species, recovered from diverse environments, including pathogens of plants, animals, and humans, led to their inclusion into the new genus *Burkholderia* (Yabuuchi et al. 1992), now comprising over 50 species. Since then, impressive advances have been achieved in the taxonomy of *B. cepacia* and related species, now collectively known as the *B. cepacia* complex (Bcc), comprising of at least 17 different but closely related species (Coenye et al. 2001; Vanlaere et al. 2008, 2009). There are presently nine Bcc strains from four species with their complete genome sequences publicly available and nine others in progress (data from January 2010). The genome of these strains consists of three chromosomes and many of them contain also plasmids. Their genome size varies between 6.2 Mbp in *Burkholderia dolosa* AUO158 and 8.7 Mbp in *Burkholderia lata* 383 (Table 1). The genome of *Burkholderia cenocepacia* strain J2315, a multidrug-resistant CF isolate belonging to the ET12 lineage was recently published (Holden et al. 2009). It is composed of three circular chromosomes and a plasmid, encoding for many metabolic and transport functions, as well as virulence factors and drug resistance determinants. A comparison of the genome of J2315 with other Bcc and non-Bcc genomes, revealed a high number of mobile genetic elements and genomic islands, highlighting the genomic plasticity of these highly versatile microorganisms (Holden et al. 2009).

The progress in the taxonomy of these bacteria has been mainly due to their emergence as important opportunistic pathogens, capable of causing life-threatening infections in immunocompromised patients, in patients with chronic granulomatous disease, and especially in patients suffering from cystic fibrosis (CF), the most common lethal inherited genetic disease among Caucasians. Although strains from all the Bcc species are capable of causing infections to CF

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Table 1 Genome features of Bcc strains with their genome projects completed (*Burkholderia ambifaria* MC40, *B. cenocepacia* J2315, *B. lata* 383, *B. multivorans* ATCC17616, and *B. vitnamiensis* G4) or unfinished (*B. dolosa* AUO158 and *Burkholderia ubonensis* Bu)

Chr chromosome, *p* plasmid, *nt* nucleotides, *NA* information not yet available

Species/strain	Genome size (nt)	Replicons	Protein coding genes	G+C (%)
B. ambifaria MC40-6	7642536	3 Chr+1 p	6878	66.4
B. cenocepacia J2315	8055782	3 Chr+1 p	7229	66.9
B. dolosa AUO158	6247594	NA	5441	66.8
B. lata 383	8676277	3 Chr+1 p	7725	66.8
B. multivorans ATCC 17616	7008622	3 Chr+1 p	6290	66.7
B. ubonensis Bu	6932532	NA	7192	67.3
B. vietnamiensis G4	8391070	3 Chr+5 p	7775	65.7

patients, their prevalence varies geographically and regionally. For example, while *B. cenocepacia* predominates in North America, *Burkholderia multivorans* is predominant in Europe (Govan et al. 2007). Illustrating this regional variation in Bcc species predominance, 85% of the Bcc isolates from patients who attended the major CF center in Lisbon (Portugal) belonged to the *B. cepacia* species (Cunha et al. 2007).

The genetic defect underlying the CF disease results from mutations in the gene encoding a membrane chloride channel (the CF transmembrane conductance regulator; Riordan et al. 1989), primarily found in apical membranes of epithelial cells. Although several organs are affected by the genetic defect, pulmonary manifestations of the disease result in defective mucocilliar clearance of bacterial pathogens, predisposing to respiratory infections which remain the main cause of morbidity and mortality among CF patients (Govan and Deretic 1996).

Although Bcc causes infections in only about 3.5% of CF patients worldwide (McClean and Callaghan 2009), these infections are of major concern to CF patients and their caregivers since the clinical outcome is highly variable and so far unpredictable. After colonization with a Bcc strain, few patients experience an asymptomatic carriage, while the majority experiences an increased decline of pulmonary function, associated with chronic infection and exacerbation episodes. Dramatically, a significant percentage of the Bcc-infected patients will develop a rapid and fatal necrotizing pneumonia known as the cepacia syndrome (Isles et al. 1984). In addition, several strains can be easily transmitted from patient-to-patient, leading to devastating infections. A particularly highly transmissible strain of the *B. cenocepacia* ET12 lineage was associated with numerous fatalities in CF patients in the United Kingdom and North America (Govan et al. 1993). Another major complication is the intrinsic resistance of Bcc bacteria to the clinically available antimicrobials, rendering chronic infections untreatable.

The correct identification of a given clinical isolate is therefore of critical importance, as a positive identification has a tremendous social and psychological consequence to the CF patient (Govan and Deretic 1996). Misidentification of Bcc by many diagnostic microbiology laboratories is still a problem, even when using the standard methodologies which includes the use of adequate selective growth media followed by phenotypic identification with commercial biochemical analysis kits (Coenye et al. 2001). In fact, after presumptive phenotypic identification, genetic methods based on specific genes such as the 16S rRNA and the recA-encoding genes are the most reliable for a positive diagnostic, as reviewed and discussed by Mahenthiralingam et al. (2008), although these methodologies are only accessible to reference laboratories. More recently, a multilocus sequence typing (MLST) scheme and a public database of MLST sequences for Bcc (http://pubmlst.org/bcc/) has been successfully implemented. The MLST scheme for Bcc bacteria is based on the nucleotide polymorphisms of the seven genes *atpD*, *gltB*, *gyrB*, *recA*, *lepA*, *phaC*, and *trpB*, encoding, respectively, the housekeeping enzymes ATP synthase β-chain, glutamate synthase large subunit, DNA gyrase B, recombinase A, GTP-binding protein, acetoacetyl-CoA reductase, and tryptophan synthase (Mahenthiralingam et al. 2008). To date, no failure of identification of a Bcc isolate has been registered using this methodology and therefore it has become the golden standard in Bcc identification, as it can be used by any laboratory worldwide (Mahenthiralingam et al. 2008).

Pathogenicity, virulence factors, and determinants of Bcc bacteria

Compared to the advances achieved in the taxonomy, knowledge on the molecular mechanisms of Bcc pathogenicity and progress on the development of new therapeutic agents are still limited. It is foreseen that the unveiling of the molecular mechanisms underlying the pathogenicity traits and of virulence factors and determinants of Bcc will allow the rational design of strategies to combat the infections caused by these bacteria.

In order to successfully establish an infection, after entering into the respiratory tract of the CF patient, bacteria have to adhere to host mucosal or epithelial surfaces. In the case of the CF lung, the thickened mucus layer provides an

ideal environment for microbial colonization, due to defective mucus clearance, reduced efficacy of antimicrobial peptides, and enhanced inflammatory response (Boucher 2007). The ability to cross the epithelial barrier and gain access to the blood stream seems to be restricted to Bcc strains, as other CF pathogens usually do not cause bacteremia. The airway epithelium plays a central role in the progression of CF lung disease via the production of numerous cytokines, chemokines, inflammatory enzymes, and adhesion molecules (Jacquot et al. 2008). Several studies have demonstrated invasion and survival within epithelial cells, although different mechanisms can be used by different Bcc bacteria, including invasion as a biofilm, rearrangement of the cytoskeleton, and penetration by paracytosis (Cieri et al. 2002; Schwab et al. 2002; Sajjan et al. 2006). Known mechanisms specifically used by Bcc bacteria to interact with and invade epithelial cells, and translocate to the basolateral side of the epithelium, have been recently reviewed by McClean and Callaghan (2009) and by Saldías and Valvano (2009).

During the interaction with the CF host, several virulence factors are thought to play critical roles for the success of the pathogen, although their precise contribution to the overall Bcc pathogenicity remains to be fully elucidated. Extracellular lipase, metalloproteases and serine proteases are thought to play roles directly related to the interaction with epithelial cells (McClean and Callaghan 2009). While metalloproteases and serine proteases seem to play a role on the proteolysis of the extracellular matrix and are produced by many but not all the Bcc species (Kooi et al. 2006), lipase production is thought to play a role in invasion and their production is broadly distributed among members of the Bcc (Mullen et al. 2007). Bacterial surface structures like the lipopolysaccharide (LPS), flagella and pili are also important in the interaction with the CF host. Flagella, pili, and a 22-kDa adhesin play important roles in motility and adherence to the host cell. The LPS of Bcc induces a strong immune response that can contribute to host cell damage (Hutchison et al. 2000). The structure of Bcc LPS differs from the LPS of other gram-negative bacteria. It contains less phosphate, the unusual sugar D-glycero-α-D-talo-oct-2-ulopyranosylonic acid (KO) is present in the inner core oligosaccharide, and 4-amino-4-deoxyarabinose residues are bound to phosphates of the lipid A. These modifications lower the anionic charge of the Bcc cell surface, inhibiting the binding and subsequent effects of cationic antibiotics (Vinion-Dubiel and Goldberg 2003). Bcc strains also present several distinct O-antigen structures (Vinion-Dubiel and Goldberg 2003).

All species of the Bcc possess, at least, one classical LuxIR quorum-sensing (QS) system, named CepIR, which provides a mechanism for rapid adaptation to environmental changes. In Bcc, QS regulates the expression of various virulence factors, like toxin, proteases, lipase, and siderophores. Swarming motility and biofilm formation are also regulated by QS in Bcc (reviewed in Venturi et al. 2004). Besides the conserved CepIR system, some Bcc strains have additional QS systems. For instance, the BviIR system was identified in *Burkholderia vietnamiensis*, while the CciIR system is encoded within a pathogenicity island of some *B. cenocepacia* strains (Malott and Sokol 2007; Malott et al. 2005). Two additional types of chemical signals used for cell-to-cell communication were found in *B. cenocepacia* strains, the 2-heptyl-4-quinolone (HHQ) and the *cis*-2-dodecenoic acid (BDSF) (Diggle et al. 2006; Deng et al. 2009). Loss of HHQ production in *Burkholderia pseudomallei* was shown to affect colony morphology and to increase elastase production (Diggle et al. 2006). BDSF was recently shown to be necessary for the virulence and normal physiology of *B. cenocepacia* (Deng et al. 2009). Interestingly, Bcc bacteria can recognize and respond to *Pseudomonas aeruginosa* quorum-sensing molecules (Riedel et al. 2001), highlighting a possible role for inter-species communication in the course of the disease of CF patients co-infected with *P. aeruginosa* and *B. cepacia*.

The production of siderophores such as pyochelin, salicylic acid, cepabactin, and ornibactin, also contribute to Bcc pathogenesis (Agnoli et al. 2006). In addition to its role in iron acquisition, pyochelin appears to play a role in tissue injury (Lamont et al. 2002). Iron bound to pyochelin has also been shown to be an efficient catalyst for hydroxyl radical ($OH^{\cdot}$) formation and to increased injury to pulmonary artery, endothelial cells and pulmonary epithelial cells, resulting from exposure to superoxide and hydrogen peroxide (Lewenza and Sokol 2001).

Protein secretion is also an important mechanism by which bacteria are able to deliver proteins to the environment and to host cells, being able to influence the host response and being crucial for virulence and survival. Several transport systems have been implicated in the secretion of many virulence factors by Bcc strains such as proteases, hemolysins, and adhesins, among others. Type I and type II secretion systems were shown to be responsible for the secretion of proteins with hemolytic activity in isolates of the *B. cenocepacia* ET12 lineage and *B. vietnamiensis* (Fehlner-Gardiner et al. 2002; Whitby et al. 2006). Mutations in a gene encoding an autotransporter adhesin belonging to type V secretion system, led to a hyper-colonization plant phenotype in *B. vietnamiensis* G4 (O'Sullivan et al. 2007). The importance of type IV and type VI secretion system have also been recently shown on Bcc strains. For instance, Aubert and co-authors (2008) described the presence of a functional type VI secretion system in *B. cenocepacia* K56-2 as playing a role in virulence. Two type IV secretion systems have been identified within *B. cenocepacia* strains: the Ptw system,

involved in the secretion of plant cytotoxic protein(s) that causes plant tissue water soaking and necessary for intracellular survival in phagocytes (Engledow et al. 2004; Sajjan et al. 2008), and the bc-VirB/D4, shown to play a role in DNA mobilization (Schulein and Dehio 2002; Zhang et al. 2009).

Another important feature of Bcc is their ability to form biofilms, communities within which bacteria live in a sessile lifestyle, protected from environmental insults and aggression from the immune system defenses of the host. In addition, Bcc bacteria in biofilms have been demonstrated to be more resistant to antibiotics than planktonic cells, contributing to their persistence in the CF lung (Caraher et al. 2007).

Although exopolysaccharide (EPS) production by Bcc was initially considered a rare phenomenon (Govan and Deretic 1996), about 80% of the Bcc clinical isolates from Portuguese CF patients were found to produce variable amounts of an EPS, named cepacian, in a study by Richau et al. (2000) and by subsequent studies (Cunha et al. 2004). Although not required for the initiation of biofilm formation, cepacian was also demonstrated to play a role in the establishment of thick biofilms (Cunha et al. 2004). The characterization of the chemical structure and composition of the EPS showed that it is composed of glucose, mannose, rhamnose, galactose, and glucuronic acid (Cescutti et al. 2000). Cepacian has been recently demonstrated to be a virulence factor produced by Bcc bacteria using $gp91^{phox-/-}$ mice as an infection model (Sousa et al. 2007a). In fact, no mortality was registered when mice were infected with an EPS-defective mutant carrying a plasposon insertion in the *bceF* gene encoding a tyrosine kinase (Sousa et al. 2007a). Reinforcing these observations, Conway et al. (2004) have also shown that the EPS produced by a *B. cenocepacia* clinical isolate interfered with phagocytosis of bacteria by human neutrophils and facilitated bacterial persistence in a mice model of infection. In addition, cepacian was also found to inhibit neutrophil chemotaxis and the production of oxygen reactive species (Bylund et al. 2006). In a survey of 560 Bcc clinical isolates from 100 CF patients Zlosnik et al. (2008) showed that all Bcc species represented were able to express the mucoid phenotype due to exopolysaccharide production. Nevertheless, the strains from *B. cenocepacia*, the most virulent species of the complex, were mostly nonmucoid. In addition, the frequency of phenotypic switching in sequential isolates was evaluated and 13 mucoid-to-nonmucoid and two nonmucoid-to-mucoid conversions were reported. The high frequency of nonmucoid isolates among strains of *B. cenocepacia* and the phenotypic switching typically from mucoid-to-nonmucoid raised the possibility of nonmucoid isolates being associated with augmented disease severity while the mucoid phenotype would be associated with persistence in the lungs (Zlosnik et al. 2008).

The genes required for cepacian biosynthesis by Bcc strains are located in clusters *bce*-I and *bce*-II of chromosome II of Bcc. Cluster *bce*-I was identified by random plasposon mutagenesis and selection of EPS-defective mutants (Moreira et al. 2003), while cluster *bce*-II was identified combining the use of comparative genomics and genetics (Ferreira et al. 2010). The functional characterization of some genes encoding enzymes involved in the synthesis of sugar nucleotides required for polymerization, as well as of genes involved in biosynthetic steps beyond activated sugar nucleotide formation have been performed (Sousa et al. 2007b; Sousa et al. 2008a; Ferreira et al. 2007; Videira et al. 2005; Loutet et al. 2009). Using comparative genomics in combination with genetics, cepacian biosynthetic genes were found to be widespread distributed among clinical and environmental strains of the Bcc, as well as in non-Bcc strains (Ferreira et al. 2010). In addition, cepacian was found to be required for survival to desiccation conditions and resistance to toxic ion metals, highlighting its role in bacterial survival in adverse environments (Ferreira et al. 2010).

Infection models to study virulence of Bcc bacteria

A wide range of hosts have been used as infection models of Bcc, including mammals, fishes, nematodes, insects, protozoa, and plants. Except for vertebrates which possess an adaptive immune system, the majority of the organisms rely exclusively on their innate immune systems to resist to Bcc pathogens. These models of infection, summarized in Table 2, have been used to assess the virulence of Bcc strains, to identify genes involved in the pathogenicity of Bcc strains, to study the host response, the effect of antimicrobial delivery, as well as in immunization studies. Despite their utility, all Bcc infection models present advantages and disadvantages, since a true CF model of infection is not available. In addition, one has to be careful on the choice of a given infection model because some Bcc virulence factors are host-specific, as shown by Uehlinger et al. (2009) when using a multihost pathogenesis system employing alfalfa, *Caenorhabditis elegans*, *Galleria mellonella*, and mice, as *B. cenocepacia* infection models.

Strategies to combat infections caused by Bcc bacteria

Currently, the eradication of infections caused by Bcc bacteria is very difficult and often impossible, due to their intrinsic resistance to the vast majority of clinically available antimicrobials. One of the few studies on the

Table 2 Models for *Burkholderia cepacia* complex infection studies

Infection Model	Relevant characteristics	Studies performed	References
Vertebrates			
Agar bead (rat or mice)	-Intratracheal inoculation	-Assessment of Bcc species virulence	Starke et al. 1987; Bernier et al. 2003; Cieri et al. 2002; Pirone et al. 2008; Tomich et al. 2003; Urban et al. 2004; Hunt et al. 2004; Marier et al. 2002
	-Chronic pulmonary infection	-Study of putative virulence factors: type III secretion system, flagella, and LPS -Evaluation of potential vaccine candidates, antibiotic formulations, and delivery methods	
CF mice	-Cftr^{tm1Unc} or Cftr^{tm1Hgu} mice	-Assessment of *B. cenocepacia* and *B. cepacia* strains virulence	Davidson et al. 1995; Sajjan et al. 2001; Sokol et al. 2003;
	-Repeated intranasal instillation	-Contribution of quorum sensing to *B. cenocepacia* virulence	
CGD mice	-gp91$^{phox-/-}$/intratracheal inoculation	-Assessment of Bcc species virulence -Contribution of quorum sensing to *B. cenocepacia* virulence -Contribution of EPS to *B. cepacia* virulence	Sousa et al. 2007a
Leukopenic mice	-Intranasal instillation	-Assessment of Bcc species-specific virulence	Chu et al. 2002; Chung et al. 2003
Zebrafish (*Danio rerio*)	-Intraperitoneal injection	-Contribution of the quorum-sensing signal BDSF and its synthase to Bcc virulence	Deng et al. 2009
	-Innate and adaptive immune system		
Invertebrates			
Caenorhabditis elegans	-Oral administration	-Assessment of Bcc species virulence	Kothe et al. 2003; Cardona et al. 2005; Sousa et al. 2008b
	-Slow and fast-killing assays	-Evaluation of excretable toxins production	
	-Innate immune system	-Evaluation of colonization ability	
	-Not viable at 37 °C		
Panagrellus redivivus	-Oral administration	-Assessment of *B. multivorans* strains virulence	Laws et al. 2005
	-Innate immune system		
	-Viable at 37 °C		
Galleria mellonella	-Injection of bacteria	-Assessment of Bcc species virulence	Seed and Dennis 2008; Seed and Dennis 2009; Mil-Homens et al. 2010
	-Innate immune system	-Evaluation of therapies against Bcc	
	-Viable at 37 °C		
Protozoa	-*Acanthamoeba* species	-Mechanisms of intracellular survival of Bcc in phagocytic cells	Marolda et al. 1999; Lamothe et al. 2004
	-Limited use as host model: most clinical Bcc strains are noninfective		
Plants			
Onion (*Allium cepa*)	-Inoculation of cultures on onion slices	-Taxonomic classification of Bcc strains	Gonzalez and Vidaver 1979; Gonzalez et al. 1997; Wigley and Burton 1999; Yohalem and Lorbeer 1994
	-Lack of an innate immune system	-Assessment of virulence of clinical and environmental strains	

Table 2 (continued)

Infection Model	Relevant characteristics	Studies performed	References
Alfalfa (*Medicago sativa*)	-Seedlings inoculation	-Assessment of Bcc species-specific and general virulence factors	Bernier et al. 2003; Bernier and Sokol 2005; Bernier et al. 2008; Uehlinger et al. 2009
	-Lack of an innate immune system	-Identification of virulence associated genes	
In vitro MODELS	-Macrophage cell lines: ANA-1, RAW264.7, PU5-1.8, J774A.1, and J774.2	-Identification of virulence factors	Aubert et al. 2008; Saldias et al. 2008; Saini et al. 1999; Hutchison et al. 1998; Cheung et al. 2007; Burns et al. 1996; Keig et al. 2001; Sajjan et al. 2004; Sousa et al. 2007a
	-Epithelial cell line A549	-Evaluation of specific pathogenicity mechanisms	
	-Human tissues: type II pneumocytes, lung explants and neutrophils	-Characterization of the host response	

characterization of antimicrobial resistance profiles of Bcc isolates characterized concerning their taxonomic status by molecular methodologies, revealed that 55% of the isolates were considered multidrug resistant (MDR; Leitão et al. 2008). The isolates belonged to four distinct Bcc species and an uneven distribution was observed, being 90% of the *B. cenocepacia* subgroup A considered MDR, while 65%, 45%, 35%, and 25% of the *B. cepacia*, *B. multivorans*, *Burkholderia stabilis*, and *B. cenocepacia* subgroup B, respectively, were considered as MDR (Leitão et al. 2008).

The characterization of the susceptibility profiles of Bcc isolates is determinant for the choice of the antimicrobials to be used. However, even after demonstration of in vitro susceptibility to a given antibiotic, in vivo efficacy is rarely observed, complicating the selection of appropriate antimicrobial therapies. Current therapies use combinations of two or three antibiotics. Gibson et al. (2003) have reviewed the combinations of antimicrobials in use for the management of outpatients and for the treatment of hospitalized patients with pulmonary exacerbations. The use of aerosolized antibiotics has emerged as an important strategy for the management of chronic lung infections in CF patients. However, it is worth to mention that Ball et al. (2010) have recently suggested that the use of nebulized amiloride and tobramycin may be effective for early eradication of Bcc within 2 months of initial infection, but do not appear effective to eradicate chronic Bcc infections.

Protective vaccines against Bcc are not currently available. Virulence factors such as metalloproteases are being studied for vaccine development against Bcc infections (Corbett et al. 2003). However, the metalloproteases ZmpB and ZmpA were not identified in *B. multivorans* and *B. dolosa* (Gingues et al. 2005). A trisaccharide repeating unit of the O-antigen fraction from the LPS of a clinical isolate of *B. cepacia* has been also studied as a potential vaccine candidate (Fauré et al. 2007). However, at least 16 LPS types were described until now (Vinion-Dubiel and Goldberg 2003), making the design of a LPS-based vaccine difficult. Recently, a protective immune response against *B. cenocepacia* pulmonary colonization in mice was reported after nasal vaccination with an outer membrane protein nanoemulsion-based vaccine (Makidon et al. 2010). It is quite possible that the identification of new antigenic proteins and structures of Bcc will lead to the development of immunization strategies to protect patients against Bcc infections.

Nowadays, the most successful measure to prevent infections of CF patients by Bcc is patient segregation (O´ Malley 2009). Although segregation of patients has devastating social consequences, clinics that have not implemented segregation policies experience ongoing transmission of Bcc.

Perspectives

Despite the tremendous progresses on the taxonomy of Bcc, the knowledge of the pathogenicity molecular mechanisms and virulence determinants used by these bacteria when infecting CF patients remains scarce. The deeper knowledge of those aspects is of critical importance to the future development of new strategies and/or the rational design of molecules to combat Bcc infections. Particular care has to be taken since the molecular mechanisms and virulence determinants should not be restricted to a single strain or species, but should be common to all Bcc species and, ideally, to all pathogenic species of the *Burkholderia* genus, as is the case of *B. pseudomallei* and *Burkholderia mallei*, the causative agents of melioidosis and glanders, respectively. This is the case of the essential gene *acp*, encoding a protein that is 100% conserved among sequenced strains of the *Burkholderia* genus and therefore can be considered a

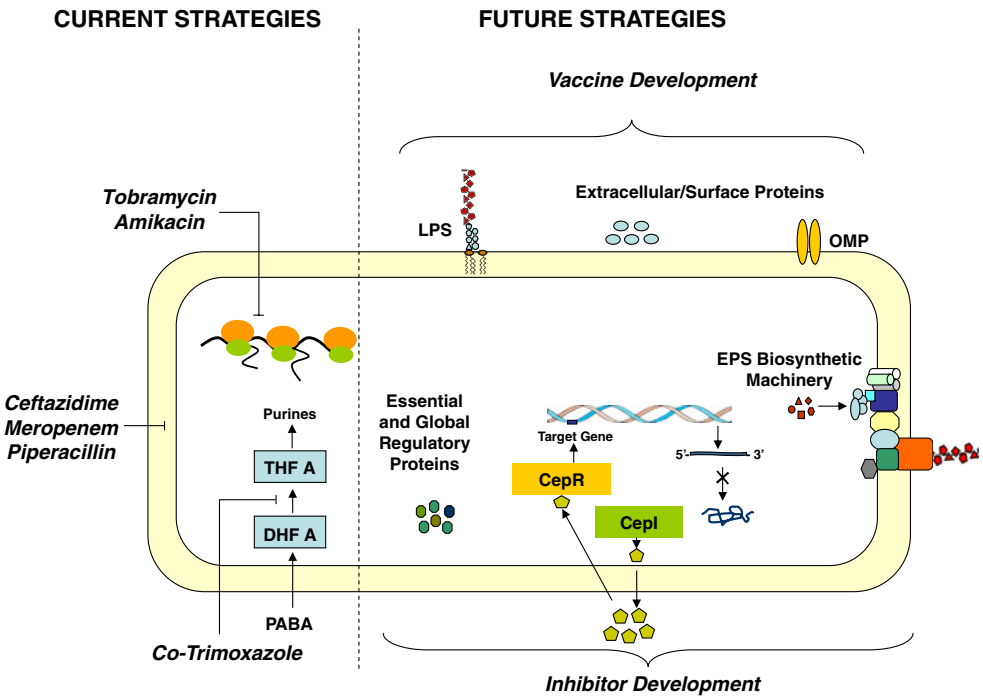

Fig. 1 Schematic representation of the main currently used antibiotic-based strategies to combat Bcc infections, and cellular targets, such as essential and global regulatory proteins, quorum-sensing systems, and specific mRNAs, that might be exploited to design new immunoprotective vaccines or new Bcc-specific inhibitory molecules. The EPS biosynthetic machinery is also exemplified as a potential target for specific inhibitors design. *PABA* p-aminobenzoic acid, *DHF A* dihydrofolic acid, *THF A* tetrahydrofolic acid, *LPS* lipopolysaccharide, *OMP* outer membrane protein

potential target to develop antibacterial agents to combat infections including those caused by *B. pseudomallei* and *B. mallei* (Sousa et al. 2008b). Interestingly, antisense phosphorodiamidate morpholino oligomers have been tested for the ability to inhibit the expression of the *Escherichia coli* and *Salmonella typhimurium acp* genes (Tilley et al. 2007). This is a promising methodology that is worth to explore in the silencing of essential genes or genes involved in virulence of Bcc bacteria.

Targeting of genes encoding components of the quorum-sensing systems regulating the expression of virulence factors (Sokol et al. 2007), or the development of inhibitors of proteins involved in the synthesis of key virulence factors such as the EPS cepacian, as is the case of type II phosphomannose isomerases (Sousa et al. 2008a), also represent attractive targets for the development of novel therapeutics. Major regulatory proteins, as is the case of the Hfq small noncoding RNA chaperone (Sousa et al. 2010), also represent attractive targets.

A highly valuable resource for the identification of those common pathogenicity traits and virulence determinants is the availability of several annotated genome sequences of strains from distinct Bcc species and also from *B. pseudomallei* and *B. mallei*. The combined use of post-genomics approaches like comparative genomics and proteomics, together with more classical tools such as genetics and models of infection, will certainly reveal in the near future novel and interesting targets for the development of new strategies to fight Bcc and closely related bacteria. An illustration of currently used antibiotic-based strategies to fight Bcc infections and cellular targets of potential interest for the development of vaccines or specific inhibitors is summarized in Fig. 1.

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