Gram-negative bacteria in cystic fibrosis

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Cystic fibrosis (CF) is a recessive genetic disease caused by a mutation in the cystic fibrosis transmembrane regulator (*CFTR*) gene. One of the consequences of a dysfunctional CFTR protein is impaired mucocillary clearance, which allows the colonisation of microbial pathogens in the lung. The CF lung is colonised predominately by bacteria; with *Burkholderia cepacia* complex, *Pseudomonas aeruginosa* and *Staphylococcus aureus* being the most important pathogens. *Pseudomonas aeruginosa*, a Gram-negative bacterium, has the highest prevalence of these bacteria, occurring in up to 80% of adults infected with CF. During CF lung infection this bacterium will undergo phenotypic and genotypic adaptations allowing its persistence. The *Burkholderia cepacia* complex, the second most significant Gram-negative bacteria in the CF lung, is a lot less widespread than *P. aeruginosa*. However, this does not diminish the importance of the *B. cepacia* complex; as it is often associated with poor prognosis and can be transmitted from patient to patient. While the *B. cepacia* complex and *P. aeruginosa* are arguably the most important Gram-negative pathogens in CF, various other Gram-negative bacteria have been isolated from the CF lung but their role in infection has not been clarified.

Keywords: Cystic fibrosis; *Pseudomonas aeruginosa*; *Burkholderia cepacia* complex; Gram-negative

1. Introduction

Cystic fibrosis (CF) is a recessive genetic disease caused by a mutation in the cystic fibrosis transmembrane regulator (*CFTR*) gene [1]. The *CFTR* gene encodes for an epithelial ion channel protein (i.e. CFTR protein) which is responsible for transporting chloride and bicarbonate ions in and out of the cell [2]. A dysfunctional CFTR protein is unable to modulate ion transport causing an ionic imbalance [2]. The ionic imbalance results in a dehydrated mucus, impairing mucocillary clearance and allowing the colonisation of microbial pathogens in the lung [2, 3]. The CF lung is colonised predominantly by bacteria but can also be colonised by fungi, yeasts and viruses [4, 5]. The most important bacteria, associated with high morbidity and mortality, isolated from the CF lung are *Burkholderia cepacia* complex, *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

2. *Pseudomonas aeruginosa* in cystic fibrosis

Pseudomonas aeruginosa, a Gram-negative bacterium, has the highest prevalence of the CF-infecting bacteria, occurring in up to 80% of adults infected with CF [4]. The bacterium has a genome of 6.3 Mb with 5 570 open-reading frames (ORFs), resulting in a large number of genes encoding for outer membrane proteins (important for adhesion, motility and antibiotic resistance), transport systems and enzymes responsible for nutrient uptake and metabolism [6]. These genes are found as either part of the core genome (part of the genome that is highly conserved) or part of the accessory genome (a region of more plasticity) of *P. aeruginosa* [6].

Pseudomonas aeruginosa is usually acquired from an environmental source such as water or on occasion from a clinical setting such as from another patient [3,7]. The initial strain is non-mucoid and planktonic and uses its flagella activity to penetrate the mucus layer [1]. Once the flagella has entered the mucus layer, it recognises GalNAcβ1-4Gal, a receptor for pili and flagella [8]. This disaccharide (GalNAcβ1-4Gal) is found on asialylated glycolipids such as asialoGM1, which is highly abundant in CF cells (when compared to epithelial cells in a healthy lung) [8]. During the early stages of *P. aeruginosa* infection in the CF lung, its exhibits characteristics of an acute infection [9].

2.1 Acute infection

Once *P. aeruginosa* has adhered itself to the surface of the epithelial cells in the lung, the type III secretion system is activated [10]. The type III secretion system has the ability to deliver proteins directly into the cytosol of the epithelial cells and releases the type III effectors ExoS (exoenzyme S), ExoT (exoenzyme T), ExoU (exoenzyme U) and ExoY (exoenzyme Y), which play a role in the dissemination and invasion of *P. aeruginosa* in the CF lung [10,11]. The type II secretion system is another significant contributor to virulence of *P. aeruginosa* in CF; the type II secretion system secretes virulence factors such as, alkaline phosphatise, exotoxin A, elastase and phospholipase C [10]. These enzymes play a role in inflammation of the lung and aid *P. aeruginosa* invasion by destroying the respiratory epithelium cells [10]. However, the type II effectors are not the only enzymes to play a role in invasion and dissemination, pyocyanin (a blue pigment metabolite) is known to cause tissue damage and to further impair CFTR channels [10]. Pyoverdine, a siderophore is an important part of *P. aeruginosa* virulence; it regulates the secretion of both exotoxin and itself and is

required for biofilm formation [10]. The pyoverdine receptor acts as an entry target for pyocins (in addition to pyoverdine); a bactericidal phage-like molecule which is able to inhibit members of the same species as well as some closely related species such as *B. cepacia* complex [6,12,13].

2.2 Chronic infection

In order to persist in the CF lung, *P. aeruginosa* has to be able to evade the host's immune system (and survive any antibiotic treatment given) [14]. The modification of the LPS is one of the ways in which *P. aeruginosa* is able to persist in the CF lung. *Pseudomonas aeruginosa* has a typical Gram-negative LPS layer consisting of: i) lipid A moiety (the toxic part of the LPS), ii) central core of oligosaccharides attached to the lipid A and iii) O-antigen (a variable and non-essential polysaccharide) [15]. The lipid A moiety of chronic CF infection shows very different acylation patterns when compared to non-CF and environmental strains; the lipid A of CF strains is typically hexa- or hepta-acylated whereas the lipid A of the non-CF strains are typically penta-acylated [15]. The lipid A can be further modified with the addition of aminoarabinose [15]. However, the lipid A moiety is not the only component of the LPS to undergo modification; during the establishment of chronic infection the O-antigen (a highly immunogenic component) is lost as well, allowing *P. aeruginosa* to evade the host's immune response [15].

To further evade the host's immune system and to reduce energy consumption (thereby gaining a fitness advantage), *P. aeruginosa* modifies the expression of its genes [15]. This gene regulation results in the loss of several virulence factors which are important for acute infection such as: i) motility, ii) the type II secretion system (and its products), iii) the type III secretion system (and its products), iv) pyoverdine secretion and v) pyocin production [14,15]. Additionally, this gene regulation results in the differential expression of pyocyanin [14,15]. In addition to gene regulation, mutations in the DNA repair systems cause *P. aeruginosa* to be hypermutable during chronic infection [16]. These mutations result in defective DNA repair and proof-reading allowing the accumulation of spontaneous mutations [16]. Several of these mutations such as, the mutation in the *muc*A gene may result in a phenotypic change [8,15]. The *muc*A gene encodes for an anti-sigma factor controlling the expression of alginate (MucA) [15]. Normally the MucA inhibits the synthesis of alginate by binding to the sigma factor AlgT (also known as AlgU), the sigma factor for the *alg*D operon (which contains the genes required for alginate production) [15]. However, when the *muc*A gene acquires a mutation MucA is unable to bind AlgT and control the expression of alginate [15]. This results in the overproduction of alginate and the mucoid phenotype [15]. Alginate is an exopolysaccharide polymer of D-mannuronic and L-glucoronic acid, and is responsible for the mucoid phenotype (which provided protection against antibiotics and the immune system) [8,17,18]. Furthermore it is an important component of biofilm formation [8,17,18]. Another phenotypic characteristic of chronic infection with *P. aeruginosa* is the emergence of the small-colony variant (SCV) [18]. The SCV colonies are slow-growing, requiring 48 hours to grow, small in size (1 mm to 3 mm) and are selected by prolonged antibiotic usage [18]. The ability to auto-aggregate (which aids in the colonisation of abiotic surfaces) as well as biofilm formation are characteristics of SCVs [19].

Table 1 Summary of acute virulence factors and chronic virulence factors of *P. aeruginosa* [20–23]

Acute virulence factors	Chronic virulence factors
Type II secretion systems	Upregulation of type VI secretion system
Type III secretion systems	Loss of type III secretion systems
Flagella	Biofilm formation
Type IV pili	Loss of motility
QS-related virulence factors e.g. elastase, proteases and pyocyanin	Loss of QS
Pyoverdine	Extracellular polysaccharides
Pyocins	Small colony variants
	Antibiotic resistance
	LPS modification

QS- Quorum sensing; LPS-Lipolysaccharide layer

2.3 Biofilm formation

Biofilm formation is advantageous to CF pathogens for several reasons including: i) it aids in sequestering nutrients from the environment, ii) it offers protection from host defence e.g. phagocytosis and iii) it provides resistance to antibiotics (cells in a biofilm are up to 1 000 times more resistant to antibiotics) [24–26]. A biofilm can be defined as a sessile microbial community combined with a matrix of extracellular polysaccharides [24]. The extracellular matrix is composed of exopolysaccharides, proteins and extracellular DNA [27,28]. The biofilm can consist of a single species of bacteria (monomicrobial) or several species of bacteria together (polymicrobial) [8]. *Pseudomonas aeruginosa* has at least three different exopolysaccharides that contribute to biofilm formation: i) Psl (encoded by polysaccharide synthesis locus) which is required for adherence, ii) Pel (encoded by *pel* cluster), which is required for maintaining mature biofilm structures and iii) alginate (encoded by *alg*U), which is important for biofilm development [21,27,29,30]. Other components play a role in biofilm formation as well such as rhamnolipids (biosurfactants); rhamnolipids are important for the formation of microcolonies, play a role in biofilm dispersal, shield the bacteria (in

the biofilm) and destroy any polymorphonuclear neutrophilic leukocytes (PMNs) that come in contact with the biofilm [7,27].

The formation of biofilms in CF is dependent on quorum sensing (QS); typically the bacteria exist in a planktonic state until the critical mass (of the bacteria) is achieved whereupon the genes for biofilm formation are induced [8]. In order to determine when critical mass has been achieved, *P. aeruginosa* produces molecules such as acyl homoserine lactones (AHLs) [8]. These molecules diffuse in and out of the cell easily and thus the concentration outside the cell reflects the concentration inside the cell; this concentration is proportional to the cell density [3,8]. Once critical mass (quorum) has been achieved, these molecules (also known as autoinducers) bind to their respective regulator proteins and initiate transcription (of biofilm formation genes) [8,19]

2.4 Quorum sensing

Pseudomonas aeruginosa has two types of AHL-based quorum sensing (QS) systems, encoded by the *las* and *rhl* genes [7,31]. Additionally *P. aeruginosa* has a third QS system, *Pseudomonas* Quinolone Signal (PQS); PQS is a 2-alkyl-4-quinolone (AQ) based system [31]. These QS systems control a variety of virulence traits including: i) elastase, ii) exotoxin A, iii) pyocyanin, iv) pyoverdine, v) pyocins, vi) proteases, viii) rhamnolipids, ix) motility, x) biofilm formation and xi) type VI secretion system [18,21,27,31–35]. The AHL systems each have a autoinducer and regulator pair controlling gene expression [31]. The *las* system's autoinducer is N-(3-oxododecanoyl)-homoserine lactone (encoded by *las*I, a synthase) and it binds to the LasR regulator (encoded by *las*R) [31,36]. The *rhl* system's autoinducer is N-butryl-homoserine lactone (encoded by *rhl*I, a synthase) and it binds to the RhlR regulator (encoded by *rhl*R) [31,36]. These systems are hierarchical, with *las* system controlling both *rhl* and PQS systems and the *rhl* system controlling PQS [31]. Conversely the PQS system activates *rhl* expression [31]. During the establishment of chronic CF infection, *P. aeruginosa* acquires mutations in the *las*R and *rhl*R genes [7]. This results in first the *las* system and then the *rhl* system being lost [7]. As a result the virulence traits under the control of these system (such as elastase) are lost as well [37,38]. Nonetheless, the loss of the QS systems does offer some selective advantages such as increased β-lactamase (*in vitro*) activity [16].

2.5 Antibiotic resistance

Pseudomonas aeruginosa has the ability to be resistant to several classes of antibiotics including aminoglycosides, fluoroquinolone and β-lactams [39]. There are several different mechanisms thorough which *P. aeruginosa* acquires resistance such as, the low permeability of the outer membrane, the modification of antibiotic targets, the overproductions of efflux pumps and the enzymatic modification of the antibiotic [37,39–41]. Additionally modifications of the LPS (addition of aminoarabinose to lipid A moiety) and biofilm formation affect antibiotic resistance [37,39–41].

Table 2 Resistance mechanisms of *P. aeruginosa* [25,37,39–43]

Resistance mechanism	**Class of Antibiotic**			
	β-lactams	**Aminoglycosides**	**Fluoroquinolones**	**Polymyxin**
Efflux pump	MexAB-OprM, MexXY-OprM, MexCD- OprJ	MexXY-OprM as well as outer membrane proteins (OpmJ, OpmB and OpmG)	MexAB-OprM, MexXY-OprM, MexCD-OprJ. MexEF-OprN	N/A
Enzymatic	AmpC β-lactamase, Class A extended- spectrum β-lactamases (ESBLs), Class D β-lactamases (oxacillinases and carbapenemases), class B metallo- β-lactamases (MBLs)	Aminoglycoside acetyltransferases (AACs), aminoglycoside phosphoryltransferases (APHs), aminoglycoside nucleotidyltransferases (ANTs/AADs)	N/A	N/A
Target site modification	Modification of penicillin-binding proteins (PBPs)	Methylation of 16S rRNA	Point mutations in DNA gyrase (*gyr*A) and topoisomerase IV (*par*C) genes	N/A
Low permeability of outer membrane	Deficient OprD	Diminished permeability	N/A	N/A
LPS modification		Addition of aminoarabinose to lipid A moeity	N/A	Addition of aminoarabinose to lipid A moiety
Biofilm	Antibiotics such as imipenem induce β-lactamase production, lack of oxygen (and nutrients) drives the bacteria to a stationary phase	Alginate slows the movement of aminoglycoside through matrix	N/A	N/A

N/A- not applicable

In the CF lung, antibiotic resistance can occur through: i) biofilm formation, ii) mutations in target sites, iii) production of β-lactamases, iv) mutations in *opr*D, *gyr*A and *par*C genes, v) overproduction of AmpC β-lactamase, vi) MexCD-OprJ efflux pump, vii) MexEF-OprN efflux pump and viii) upregulation of MexXY-OprM efflux pump [42]. The over-expression of the MexXY-OprM pump occurs as a result of a mutation in the *mex*Z gene, which encodes for the repressor protein for the MexXY system i.e. MexZ [39,41,44].

3. *Burkholderia cepacia* complex in cystic fibrosis

Burkholderia cepacia complex has a relatively low prevalence when compared to *P. aeruginosa* (occurring in 12% of CF patients), however this does not diminish its importance in CF [45]. These bacteria are highly resistant to antibiotics, have the ability for person-to-person transfer and are associated with high morbidity and mortality [20,45,46]. Members of *B. cepacia* complex are very similar phenotypically but do differ genotypically [47]. The complex is comprised of 18 separate species, of which 17 species have been isolated from CF patients (only *Burkholderia ubonensis* has not been isolated from CF) [4,47]. The most commonly isolated members of the *B. cepacia* complex (in CF) are *Burkholderia cenocepacia*, *Burkholderia dolsa* and *Burkholderia multivorans* [4,47].

Burkholderia cepacia complex members have fairly large genomes, ranging from 6.2 Mb to 9 Mb in size, indicating the relatively genomic plasticity of the bacteria [48–50]. The genome is situated across three chromosomes, with chromosome one carrying the essential genes and chromosomes two and three carrying the virulence and accessory genes [49]. However, there is some debate to whether the third chromosome is in fact a chromosome; recent studies suggest that it may be a plasmid instead [49].

The initial (infecting) strain of *B. cepacia* complex is usually acquired from the environment but can also be acquired from other CF patients [4]. However, due to improved infection control, the spread of *B. cepacia* complex from patient to patient is becoming increasingly rarer [4]. Once in the CF lung *B. cepacia* complex adheres to the epithelial cells by recognising protein and glycolipid receptors [51]. Additionally *B. cepacia* complex is able to bind to secretory mucins; isolates that are able to bind to the secretory mucins also express the cable pilus phenotype [51]. These strains recognise cytokeratin 13 to which the cable pili-associated adhesion (a 22 kDa protein) binds [51]. The cable pilus is not required for binding to cytokeratin 13 but does enhance the binding of *B. cepacia* complex to it [51]. *Burkholderia cepacia* complex recognises the same glycolipid receptor as *P. aeruginosa*, asioloGM1 and additionally binds to asioloGM2 and globotriosylceramides (Gb2 and Gb3) [51]. This binding to the glycolipds may be mediated by *B. cepacia* complex's flagella (as with *P. aeruginosa*) [52]. In several instances after *B. cepacia* complex has bound to the epithelial cells, it is internalised [51]. This intracellular invasion can occur through several mechanisms (depending on the species) such as i) paracytosis, ii) rearrangement of the cytoskeleton and destruction of epithelial cells and iii) biofilm formation and is mediated by a membrane bound vacuole [48,51,53].

Burkholderia cepacia complex can survive within these epithelial cells and within macrophage and appears to be dependent on an unknown effector of the type IV secretion system [48,51,53]. In addition to intracellular survival, *B. cepacia* complex has the ability to transverse the epithelial cells in the lung and enter the bloodstream (causing bacteraemia); allowing not only the persistence of *B. cepacia* complex but also its dissemination [48,51,53].

The pathogenesis of *B. cepacia* complex in CF has not been as intensively studied as with *P. aeruginosa* [54]. The majority of the research conducted on the virulence factors of *B. cepacia* complex have focused on *B. cenocepacia*; in part due to its higher prevalence and increased transmissibility (when compared to other *B. cepacia* complex members) [55,56]. Several putative virulence factors for *B. cenocepacia* have been described and include: i) type II (secretion of haemolytic proteins) secretion systems, ii) type III secretion systems iii) type IV secretion systems iv) extracellular lipases (plays a role in invasion), v) zinc metalloproteases (lyse the extracellular matrix) vi) siderophores e.g. pyochelin and ornibactin (cause tissue injury) and vii) multiple QS systems [48,50]. Additionally structural components of *B. cenocepacia* play a role in virulence as such as the LPS, the cable pilus (when present) and the flagella [50,55]. The LPS of *B. cenocepacia* has a distinct core and may contain aminoarabinose in the lipid A moiety [57,58]. The O-antigen of some *B. cenocepacia* strains is not expressed and these strains are more likely to be internalised (into macrophages) [55]. *Burkholderia cepacia* complex produces several exopolysaccharides (EPSs) such as cepacian which play a role in biofilm formation and mucoidy [55,59]. Mucoidy appears early in CF infection and these strains are usually less virulent than the non-mucoid strains found in late infection [54,60]. There is very little known about biofilm formation of *B. cenocepacia* in CF, however it is well accepted that QS plays a role [55,59]. *Burkholderia cenocepacia* has two different AHL-based QS systems: i) Cep (CepI and CepR) and ii) Cci (CciI and CciR) [55,59]. Additionally *B. cepacia* complex has two other QS systems: i) 4-hydroxyl-2-alkylquinolines (HAQs) and ii) cis-2-dodecenoic acis ['*Burkholderia* diffusible signal factor' (BDSF)] [59]. These QS systems control the siderophore synthesis (ornibactin), the type III secretion system, motility, zinc metalloproteases production, lipase production, toxin production and efflux pumps [48,55,59].

Table 3 Comparison of chronic infection in *P. aeruginosa* and *B. cepacia* complex in CF [54]

Pseudomonas aeruginosa	***Burkholderia cepacia* complex**
Switch from non-mucoid to mucoid infection	Switch from mucoid to non-mucoid infection
Lose the QS systems	Maintain QS systems
Decreased virulence in chronic infection	Increased virulence in chronic infection
Lose motility	Retain motility

Burkholderia cepacia complex has the ability to become resistant to several classes of antibiotics including polymyxins (e.g. colistin), aminoglycosides, fluoroquinolones and β-lactams [55]. There are several different mechanism of resistance including low permeability of the outer membrane, target modification, efflux pump mediated, enzymatic modification of the antibiotics and modifications of the LPS [55].

Table 4 Resistance mechanisms of *B. cepacia* complex [58,61–63]

Resistance mechanism	**Class of Antibiotic**				
	β-lactams	**Aminoglycosides**	**Fluoroquinolones**	**Polymyxin**	**Trimethoprim**
Efflux pump	RND family efflux transporter	RND family efflux transporter	RND family efflux transporter	N/A	RND family efflux transporter
Enzymatic	AmpC β-lactamase, Class A β-lactamases, Class D β-lactamases, class C β-lactamases	Aminoglycoside acetyltransferases (AACs), aminoglycoside phosphoryltransferases (APHs), aminoglycoside nucleotidyltransferases (ANTs/AADs)	N/A	N/A	Dihydrofolate reductase
Target site modification	Modification of penicillin-binding proteins (PBPs)	N/A	Point mutations in DNA gyrase (*gyr*A) and topoisomerase IV (*par*C) genes	N/A	N/A
Low permeability of outer membrane	Diminished permeability	N/A	N/A	N/A	N/A
LPS modification	N/A	Unable to bind to LPS	N/A	Addition of aminoarabinose to lipid A moiety	N/A

N/A- Not applicable

This wide range of antibiotic resistance makes infection with *B. cepacia* complex difficult to treat and marks it as a formidable pathogen [55]. There are several other Gram-negative bacteria that have been isolated from the CF lung including *Haemophilus influenzae*, a known CF pathogen as well as bacteria that have been gaining increased attention such as *Stenetrophomonas maltophilia* [64].

4. *Haemophilus influenzae*

One of the most commonly isolated Gram-negative bacteria from the CF lung is *Haemophilus influenzae* [15]. *Haemophilus influenzae* is one of the first pathogens to colonise the CF lung and occurs up to 32% of CF patients [4,65] However, *H. influenzae* colonises the upper airways of healthy children as well and as a result in its role in disease progression of CF has been brough into question [15,66]. There are two types of *H. influenzae* strains, capsulated and non-encapsulated [66]. The majority of the *H. influenzae* strains isolated from CF patients are non-encapsulated [otherwise known as non-typeable *H. influenzae* (NTHi)] [67]. These NTHi strains bind to epithelial cells *via* adhesins, pili or *via* Hia and *Haemophilus* adhesion penetration (Hap) proteins [66,68]. Thereafter *H. influenzae* employs the use of proteases, microcolony formation, antigenic drift and intracellular survival to persist in the lung [66]. Biofilm formation and the hypermutable phenotype play a role in *H. influenzae* persistence in the CF lung as well [65,67].

5. *Stenotrophomonas maltophilia*

In addition to *H. influenzae*, a wide group of Gram-negative bacteria have been gaining increase attention [65]. Foremost of these bacteria is *Stenotrophomonas maltophilia*, a pathogen which has become increasingly more prevalent in CF patients, with a prevalence of 4% reported in 1996 to 12% in 2005 in USA [4]. *Stenotrophomonas maltophilia* is a member of the *Xanthomonadaceae* family and has been isolated from the environment (which may act as reservoir) [63]. Like *P. aeruginosa* and *B. cepacia* complex, *S. maltophilia* adheres to the epithelial cells to gain entry into host cells, mainly through flagella activity [69,70]. Pathogenesis of *S. maltophilia* in the CF lung is similar to *P. aeruginosa* and *B. cepacia* complex; i) motility(type I fimbriae), ii) alkaline proteases (cause tissue damage), iii) esterases and iv)

biofilm formation play a role in virulence [69]. Additionally hypermutable *S. maltophilia* isolates have been isolated from the CF lung [71].

While *Stenotrophomonas maltophilia* can cause chronic infection in the CF lung, this infection is less severe than infection with *P. aeruginosa* or *B. cepacia* complex [72,73]. In fact chronic infection with *P. aeruginosa* or *B. cepacia* complex decreases the risk of *S. maltophilia* infection [74].

7. *Achromobacter xylosoxidans*

Achromobacter xylosoxidans, is another CF pathogen which has been gaining increased attention [75]. Cystic fibrosis patients are infected with either an environmental strain or through person-to-person transmission [63,75]. In contrast to *P. aeruginosa*, *B. cepacia* complex and *S. maltophilia* infection, *A. xylosoxidans* infection is mostly transient, with less than three percent of patients being chronically infected [76]. Chronic infection of *A. xylosoxidans* in CF is associated with older age, an increased disease burden and chronic infection with *P. aeruginosa* [77]. The virulence factors of this bacteria is not well understood, however, it appears that biofilm formation and motility play a role in CF infection [76,77].

8. Other Gram-negative bacteria

Over 60 different genera from 29 different families have been isolated from the CF lung [4,5,15,35,47,63–65,77–119]. Several of these species have been isolated from other respiratory diseases, such as chronic obstructive pulmonary diseases (COPD) [120,121]. The role of these bacteria in causing disease in the CF lung is still unclear, however, several of the species may play a role in polymicrobial interactions in the lung [47].

8.1 *Enterobacteriaceae*

The family *Enterobacteriaceae* harbours the largest group of bacteria isolated from the CF lung including: i) *Klebsiella* spp. (*Klebsiella* pneumoniae, *Klebsiella terrigena*, *Klebsiella planticola* and *Klebsiella oxytoca*), ii) *Serratia marcescens* (and *Serratia proteamaculans*), iii) *Enterobacter clocae*, iv) *Proteus* spp., (*Proteus mirabilis*) v) *Citrobacter* spp., (*Citrobacter murlinae*) vi) *Hafnia* spp., vii) *Edwardsiella* spp., viii) *Dickeya* spp., ix) *Morganella* spp., x) *Pantoea* spp., (*Pantoea agglomerans*) xi) *Providencia* spp. and xii) *Escherichia coli* [5,15,65,77–85]. These bacteria have isolated from the CF lung, individually or with either *P. aeruginosa* or *S. aureus*, making it harder to determine their impact on disease progression in the CF lung [85,86]. However, *E. coli* isolated from the CF lung has shown the characteristic mucoid and SCV phenotypes as well as a higher prevalence of tissue damaging enzymes such as heamolysin and cytotoxic necrotising factor [85]

8.2 *Pasteurellaceae*

Haemophilus influenzae as well as the closely related *H. parainfluenzae* which have been isolated from CF patients, belong to this family [78,92]. Additionally members of the *Pasterella*, *Actinobacillus* and *Aggregatibacter* genera have been isolated from the CF lung [5,89].

8.3 *Alcaligenaceae*

Achromobacter xylosoxidans, an emerging CF pathogen is a member of this family along with *Achromobacter ruhlandii, Achromobacter insolitus* and *Alcaligenes faecalis* (all of which have been isolated from the CF lung) [81,90,91]. Other members of this family that have been isolated from the CF lung include *Bordetella* spp. (*Bordetella broncispecta, Bordetella avium, Bordetella hinzii, Bordetella holmesii* and *Bordetella petrii*) and *Advenella* spp. (*Advenellas incenata*) [4,64,65,81,87,88].

8.4 *Xanthomonadaceae*

In addition to *S. maltophilia*, other family members of the *Xanthomonas* and *Lysobacter* genera have been isolated from the CF lung [15,63,65,81]. This includes *Xanthomonas hyacinthi* and *Lysobacter enzymogenes* [15,65,81].

8.5 *Rhodospirillaceae*

Inquilinis limosus is the most well-known CF pathogen from this family [5,15,64,65,77,81,87,88,93–97]. This bacteria is multi-drug resistant, has the ability to convert to a mucoid phenotype and can form dual species biofilm with *P. aeruginosa*; which may account for its persistence in the CF lung [15,47,65,98,99]. The only other genus (from this family) to have members isolated from the CF lung is *Craurococcus* (*Craurococcus roseus*) [15,65].

8.6 *Burkholderiaceae*

Burkholderia cepacia complex as well as the other members of this genus that cause infection in CF patients such as *Burkholderia gladioli*, *Burkholderia pseudomallei* and *Burkholderia fungorum* belong to this family [63,100]. Additionally *Ralstonia* spp. (*Ralstonia pickettii, Ralstonia mannitolytica, Ralstonisa insidiosa, Ralstonisa gilardi, Ralstonia taiwanensis, Ralstonia respiraculi, Ralstonia paucula, Ralstonia metallidurans* and *Ralstonia basilensis*) , *Cuprivadus* spp. (*Cuprivadus gilardi, Cuprivadus taiwanensis, Cuprivadus respiraculi, Cuprivadus paucula* and *Cuprivadus metallidurans*), *Lautropia* spp. (*Lautropia mirabilis*) and *Pandoraea* spp.(*Pandoraea aptisa, Pandoraea pneomenusa, Pandoraea pulmonicola, Pandoraea sputorum* and *Pandoraea norimbergensis*), which all belong to this family have been isolated from the CF lung [4,5,15,65,77,81,87,88,96,101].

8.7 *Moraxellalaceae*

Moraxella cattarhalis is one of the better known pathogens of this family and has been known to cause severe lung disease [102,103]. This pathogen as well as *Moraxella osloensis* have been isolated from the CF lung along with members of the *Acinetobacter* genus [4,5,65,78,81,97,104]. *Acinetobacter baumanii* as well *as Acinetobacter calcoaceticus, Acinetobacter haemolyticus, Acinetobacter johnsonii, Acinetobacter junii* and *Acinetobacter ursingii* have been isolated from the CF lung [4,65,81,100].

8.8 *Pseudomonadaceae*

Pseudomonas aeruginosa is not the only *Pseudomonas* spp. to be isolated from the CF lung [5,79,81,88]. *Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas alcaligens, Pseudomonas brassicacearum, Pseduomonas mendocina, Pseudomonas saccharophilia, Pseudomonas huttiensis, Pseudomonas synxantha* and *Pseudomonas stutzeri* have been isolated from the CF lung [65,79,81,105]. Additionally another member of the *Pseudomonadaceae* family, *Chryseomonas* spp., has been isolated from the CF lung [5,93–95].

8.9 *Neisseriaceae*

Neisseria spp., the type genus for this family, is often found as part of the normal flora in the upper airways of healthy individuals [106,107]. However, *Neisseria* spp. such as *Neisseria cinera, Neisseria subflava, Neisseria flava* and *Neisseria mucosa* have been isolated from the sputum of CF patients [5,65,78,81,83,108–112]. Other members of the family such as *Chromobacterium* spp. (*Chromobacterium violaceum*), *Eikenella* spp. (*Eikenella corrodens*) and *Kingella* spp. (*Kingella denitrificans* and *Kingella oralis*) have also been isolated from the CF patients [5,65,81]/

8.10 *Comamonadaceae*, *Rhizobiacea* and *Oxalobacteraceae*

Several members of the *Comamonadaceae* family have been isolated from the CF lung including: *Comamonas* spp. (*Comamonas testeroni*), *Pelomonas* spp., *Variovax* spp., *Diaphororbacter* spp. and *Acidovorax* spp. [65,78,81,87,88,97,104,113]. Additionally family members of the *Rhizobiaceae* (*Rhizobium radiobacter* and *Agrobacterium radiobacter*) and *Oxalobacteraceae* (*Herbaspirillium* spp.-*Herbispirillum frisingense, Herbaspirillium putei, Herbaspirillium seropedicae* and *Herbaspirillium huttiense)* have been recovered from CF patients [4,15,65,81,88,97,104].

8.11 *Flavobacteriaceae*, *Sphingomonadaceae*, *Sphingobacteriaceae*, *Rhodobacteraceae* and *Cytophagaceae*

Several species belonging to the *Flavobacteriaceae,* such as *Flavobacterium* spp., *Chryseobacterium* spp. (*Chryseobacterium indologenes* and *Chryseobaterium gleum*), *Capnocytophaga* spp (Capnocytophaga infantium), *Cloacibacterium* spp., *Bergeyella* spp. (*Bergeyella zoohelcum*) and *Elizabethkingia* spp. (*Elizabethkingia meningoseptica*, formerly known as *Chryseobacterium meningoseptica*) have been isolated from the CF lung [5,65,79,81,83,87,88,97,100,104,113–115]. The *Shingomonadaceae* [*Novosphingomonas* spp. and *Sphingomonas* spp. (*Shingomonas paucimobilis*)], *Rhodobacteraceae* (*Paracoccus halodenitrificans*), *Cytophagaceae* (*Arcicella* spp.) and *Sphinobacteriaceae* (*Spingobacterium* spp.) families all have species which have been isolated (rarely) from the CF lung [5,15,65,81,93–95,104,113].

8.12 *Brucelleaceae*, *Coxiellaceae* and *Porphyromonadaceae*

Several unusual bacteria belonging to the *Brucelleaceae* and *Porphyromonas* families have been isolated from the CF lung including *Ochrobacterum anthropi* (*Brucelleaceae* family), *Porphyromonas* spp. (*Porphyromonadaceae*) and *Tannerella forsythensis* (*Porphyromonadaceae*) [5,15,65,78,81]. Additionally members of the *Coxiellaceae* family have been recovered from the CF lung as well [15,65].

8.13 *Bacteroidaceae*, *Caulobacteraceae* and *Prevotellaceae*

There has been a recent emergence in anaerobic bacteria isolated from CF patients, in part due to culture-independent methods [4,35]. Several of these Gram-negative anaerobes belong to either *Bacteroides* spp. (*Bacteroidaceae*) or *Prevotella* spp. (*Prevotellaceae*) [4,5,116]. The source of some of these species such as *Bacteroides fragilis* and *Prevotella oris* can be found in the gut or oral cavity [117]. Other *Prevotella* spp. including *Prevotella salivae, Prevotella pallens, Prevotella melaninogenica, Prevotella disiens, Prevotella denticola, Prevotella corporis* and *Prevotella histicola* have been recovered from the CF lung [81,89,117]. Furthermore, *Brevundimonas diminuta*, a colistin resistant pathogen represents the *Caulobacteraceae* family in CF infection [15,65,81,100].

8.14 *Fusobacteriaceae*, *Bradyrhizobiaceae*, *Campylobacteraceae*, *Leptotrichiaceae* and *Alteromonadaceae*

In addition to *Prevotella* spp. and *Bacteroides* spp., other anaerobic species belonging to the *Fusobacteriaceae, Campylobacteraceae* and *Leptrotrichiaceae* families such as *Fusobacterium* spp. (*Fusobacterium gonidaformans* and *Fusobacterium nucleatum*), *Camplyobacter* spp. (*Camplyobacter concisus*) and *Leptotrichia* spp. (*Leptotrichia wadei*) have been isolated from the CF lung [5,81,118]. Additionally *Microbulbifer* spp. (belonging to the *Alteromonadaceae* family) and *Bradyrhizobium* spp. (*Bradyrhizobiaceae* family), a nitrogen-fixing species have been found in the CF lung [5,113,119].

8.15 *Hyphomicrobiaceae*, *Methylobacteriaceae*, *Bdellovibrionaceae* and *Acetobacteraceae*

Besides, *Bradyrhizobium* spp., other nitrogen-fixing bacteria such as *Devosia* spp. (*Hypomicrobiaceae*) and *Methylobacterium* spp. (*Methylobacteriaceae*) have been isolated from CF patients [5,81,93–95,119]. Additionally members of *Ricketsiales* as well as the *Bdellovibrionaceae* (*Bdellovibrio* spp.) and *Acetobacteraceae* (*Acetobacter indonensis*) families have been isolated from the CF lung [15,65,81,93–95].

9. Conclusion

The environment of the CF lung is ideal for colonisation by bacterial pathogens. These bacteria exist in a polymicrobial community with some microorganism predominating. Examples of predominating bacteria include *B. cepacia* complex and *P. aeruginosa,* whose presence in the CF lung is associated with a decrease in lung function. In some instances however, it is harder to identify the association between disease and bacteria and its impact on lung function such as with *S. maltophilia* and *A. xylosoxidans*. Advances in molecular techniques have allowed for a clear picture of the microbial community in the CF lung to elucidated, with an ever expanding list of bacteria previously unheard of in the CF context having been identified. However, the role of these bacteria (and other pathogens) in the disease progression of CF is still unclear.

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