collaborative research, and increase the visibility of the research performed. Furthermore, it will build a virtual 'corridor' facilitating the production of scientific evidence for developing policy and guidelines.

Data collected and analysed by EU-funded research projects have already been shown to be central to shaping policies that reduced the use of antimicrobials [9]. The new European One Health Action plan against AMR[xi] now provides a framework for more extensive actions, to further strengthen the alliance between research innovation and policy in Europe and beyond.

Resources

[i] https://digitallibrary.un.org/record/842813/files/A_71_L-2-EN.pdf
[ii] http://ec.europa.eu/health/amr/sites/amr/files/communication_amr_2011_748_en.pdf
[iii] www.jpiamr.eu/
[iv] www.gardp.org/
[v] https://carb-x.org/
[vi] www.eib.org/en/products/blending/innovfin/products/infectious-diseases.htm
[vii] www.mhlw.go.jp/seisakunitsuite/bunya/hokabunya/kokusai/g7kobe/KobeCommunique_en.pdf
[viii] www.g20.org/en/g20/previous-summits
[ix] www.edctp.org/
[x] www.jpiamr.eu/activities/jpiamr-virtual-research-institute/
[xi] https://ec.europa.eu/health/amr/sites/amr/files/amr_action_plan_2017_en.pdf

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Special Issue: Antimicrobial Resistance and Novel Therapeutics

Spotlight

The Scourge of Antibiotic-resistant Infections in Cystic Fibrosis

Erin P. Price [1,2,*] and Derek S. Sarovich [1,2]

Bacterial infections are the primary cause of respiratory decline and mortality in cystic fibrosis (CF) patients. In a recent study, Diaz Caballero and colleagues [1] (*PLoS Pathog*. 2018;14:e1007453) catalogued the molecular adaptation of a decade-long *Burkholderia multivorans* infection in a Canadian CF patient, which evolved to become resistant towards multiple classes of antibiotics.

CF is the most common heritable fatal disease in people of European descent. CF pathogenesis is most prominent in the airways, where accumulation of thick, tenacious mucus promotes a vicious cycle of excessive inflammation and infection. Chronic and recurrent infections are a major driver of disease in CF, leading to rapidly declining lung function, and in 80–95% of cases, respiratory failure and death [2]. Airway infections with members of the bacterial genus *Burkholderia* are especially feared because of their pathogenicity (e.g., cepacia syndrome, melioidosis), their impressive ability to intrinsically evade many antibiotics, their propensity for chronic persistence and associated airway decline, and, in the case of *Burkholderia cepacia* complex (Bcc) species, their transmissibility between CF patients [3]. Perhaps the most pressing concern is acquired antimicrobial resistance (AMR), a phenomenon whereby bacteria evolve within their host to evade being targeted by antibiotics, rendering once-potent drugs ineffective. In a recent study, published in *PLoS Pathogens*, Diaz Caballero and colleagues [1] monitored the evolution of the common CF pathogen, the Bcc species *Burkholderia multivorans*, over a 10-year period, during which time the infection became chronically adapted to the airways of a Canadian CF patient and was unable to be eradicated using aggressive and long-term antibiotic therapies.

Whole-genome sequencing (WGS) and RNA sequencing are high-resolution techniques that have yielded invaluable mechanistic insights into *Burkholderia* diversification and adaptation in chronically infected airways [4–9]. In this vein, Diaz Caballero *et al*. [1] used WGS to catalogue genome-wide variation among 111 *B. multivorans* isolates collected from CF airways during three phases of infection over a 10-year period. They performed repeated deep sampling at a single time point between 6 and 7 years after initial diagnosis to identify fine-scale variation and distinct lineages in the CF airways, and 3 years after a bilateral transplant (at year 10) to ascertain the ongoing presence of *B. multivorans* in the airways of this patient. Their study confirmed persistent infection with a single clone despite repeated and long-term antibiotic regimens aimed at *B. multivorans* eradication. Diaz Caballero *et al*. [1] consolidated earlier findings [5,7] showing that *Burkholderia* spp. can persist in CF patients who have undergone a lung transplant, sometimes reinfecting the new lungs within just a couple of weeks [5], indicating unknown external reservoirs of infection within these patients. The adeptness of *Burkholderia* spp. in reinfecting post-transplant lungs highlights the formidability of this genus and the urgent need to identify within-host reinfection sources to maximize longevity of these transplants.

Pathogens must adapt if they are to survive and persist in their mammalian host, with evasion of both the host immune response and clinical interventions (e.g., antibiotics) being paramount. Additionally, CF pathogens must master survival under dynamic and hostile conditions, including nutrient-limiting and low oxygen environments, often in the face of intense competition with other microbes for scarce resources. As illustrated by Diaz Caballero *et al*. [1] and others [4–9], many *Burkholderia* spp. are remarkably adaptable to these challenges. Whilst the exact mechanisms underpinning the transition to chronic infection are complex, there are several common adaptation measures employed by *Burkholderia* spp. that enable these organisms to overcome the many challenges faced in the CF airways. These parallel strategies include enhanced immune evasion, lowered virulence (or in the case of certain *Burkholderia dolosa* strains, increased virulence [7]), altered gene expression and metabolic pathways, increased antibiotic resistance, and improved survival in low-oxygen environments (Table 1).

The most common parallel adaptive mechanism in all chronic CF pathogens is acquired AMR [[1],3–9]. In many cases, AMR towards multiple antibiotics belonging to different classes can arise, a phenomenon known as multidrug resistance (MDR). Both AMR and MDR occur in response to repeated and extended antibiotic administrations typical of current CF treatment regimens. To assess the AMR potential of all 111 *B. multivorans* isolates, Diaz Caballero *et al*. [1] tested their susceptibility against five antibiotics belonging to three antibiotic classes: aminoglycosides, β-lactams, and quinolones. The original infecting isolate was relatively susceptible; however, latter isolates developed resistance towards these important antibiotic classes, consistent with long-term antibiotic administration in this patient. By sampling deeply across lineages from a single collection time point, their study confirmed the contribution of recombination (also known as horizontal gene transfer) in the spread of AMR within the *B. multivorans* population. Evidence of recombination has also been documented in a *Burkholderia pseudomallei* CF infection [5]. Recombination theoretically provides a formidable strategy for rapidly disseminating favourable traits amongst the within-host pathogen population. This strategy, in concert with lineage diversification as infections progress to a chronic phase [1,5–8], probably contributes to the successful persistence of *Burkholderia* in CF airways despite aggressive therapies. This finding is highly concerning given that transmission of

Table 1. Parallel Adaptive Mechanisms in Chronic *Burkholderia* Respiratory Infections

Adaptive mechanism	*B. dolosa*	*B. multivorans*	*B. pseudomallei*
Acquired AMR (including MDR)	Yes [6,7]	Yes [1,8]	Yes [4,5,9]
Altered fatty acid biosynthesis	–	Yes [8]	Yes [4,5]
Altered sigma factors	Yes [7]	Yes [1,8]	Yes [4,5]
Altered transcriptional regulation	Yes [6,7]	Yes [1,8]	Yes [4,5,9]
Decreased motility	–	Yes [8]	Yes [4]
Hypermutation	Yes [6]	Yes [1,8]	Yes [5,9]
Increased biofilm formation/adhesion	–	Yes [1,8]	–
Iron scavenging	Yes [6]	Yes [8]	–
Outer membrane modification/loss	Yes [6,7]	Yes [1,8]	Yes [4,5,9]
Oxygen sensing	Yes [6,7]	Yes [8]	Yes [9]
Reductive evolution	–	–	Yes [4,5,9]
Virulence (attenuation)	–	Yes [1,8]	Yes [4,5,9]
Virulence (gain)	Yes [7]	–	–

AMR or even MDR Bcc strains can occur between or among CF patients, potentially causing untreatable infections in previously naïve patients.

In summary, the recent study by Diaz Caballero *et al*. [1] used WGS to catalogue the adaptive mechanisms employed by *B. multivorans* to survive and persist in its human host for at least a decade. Their study adds to the growing body of literature aimed at unmasking parallel strategies employed by pathogens as they adapt to the CF airways. A consistent finding in these studies is that CF pathogens readily acquire AMR and MDR mechanisms that ensure survival against repeated antibiotic onslaught, which raises questions about the efficacy of current antibiotic treatment strategies. People with CF are critically dependent upon antibiotics to manage their disease, placing them at the greatest risk of acquiring and transmitting AMR and MDR pathogens. These collective findings highlight the need for improving the diagnosis and treatment of respiratory infections and associated airway decline in CF to enhance quality of life and lifespan in this at-risk cohort. The next steps require translation of WGS and RNA sequencing findings to enhance the diagnosis of emerging AMR, to alter current clinical practices to maximize antibiotic stewardship measures and to prevent or delay MDR infections, and to identify targeted alternative treatment strategies (e.g., bacteriophage therapies [10]) to help combat existing AMR and MDR infections.

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Special Issue: Antimicrobial Resistance and Novel Therapeutics

Spotlight

Efflux-Pump Upregulation: From Tolerance to High-level Antibiotic Resistance?

Jakob Frimodt-Møller[1,2,*] and Anders Løbner-Olesen[1,2,*]

A recent study shows that high expression of the efflux-pump AcrAB-TolC, which increases antibiotic tolerance, reduces DNA mismatch repair in *Escherichia coli* to promote spontaneous mutations. Because mutations in target genes can lead to high-level resistance, this highlights how transiently tolerant cells can develop resistance in response to antibiotic treatment.

Antimicrobial resistance is the new challenge of the 21st century [1]. Gram-negative bacteria can adapt to the selective pressure exerted by antimicrobial agents in numerous ways, including (over)expression of efflux pumps. Currently, six families of bacterial efflux pumps that contribute to antibiotic tolerance have been identified, including the resistance-nodulation-cell division (RND) superfamily [2], containing the efflux system AcrAB-TolC. Efflux pumps represent an ancient bacterial defense mechanism to meet and survive the challenge of remaining in environments with toxic compounds and are, for several species, also required for colonization and/or virulence. For instance, lack of AcrAB-TolC renders *Salmonella enterica* serovar Typhimurium nonvirulent in both a mouse infection model and a *Galleria mellonella* infection model [3]. AcrAB-TolC has a diverse set of substrates, including multiple conventional antibiotics, such as β-lactams, chloramphenicol, rifampicin, tetracyclines, and quinolones (for review see [2,4]). The *mar* regulon is the best described regulator of AcrAB-TolC [5]. Here, addition of substrate antibiotics leads to inactivation of the negative regulator MarR, resulting in constitutive expression of the transcriptional activator MarA [5], which, in turn, results in high expression of the AcrA and AcrB proteins and hence a high level of the AcrAB-TolC efflux pump (Figure 1A).

Accumulating evidence, especially from the *E. coli* AcrAB-TolC efflux system,