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Relevance of multidrug-resistant *Pseudomonas aeruginosa* infections in cystic fibrosis

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ABSTRACT

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* is an important issue for physicians who take care of patients with cystic fibrosis (CF). Here, we review the latest research on how *P. aeruginosa* infection causes lung function to decline and how several factors contribute to the emergence of antibiotic resistance in *P. aeruginosa* strains and influence the course of the infection course. However, many aspects of the practical management of patients with CF infected with MDR *P. aeruginosa* are still to be established. Less is known about the exact role of susceptibility testing in clinical strategies for dealing with resistant infections, and there is an urgent need to find a tool to assist in choosing the best therapeutic strategy for MDR *P. aeruginosa* infection. One current perception is that the selection of antibiotic therapy according to antibiogram results is an important component of the decision-making process, but other patient factors, such as previous infection history and antibiotic courses, also need to be evaluated. On the basis of the known issues and the best current data on respiratory infections caused by MDR *P. aeruginosa*, this review provides practical suggestions to optimize the diagnostic and therapeutic management of patients with CF who are infected with these pathogens.

1. Introduction

Lung infections are responsible for most of the morbidity and mortality in patients with cystic fibrosis (CF). The pathogens involved, mainly bacteria, are becoming challenging in terms of resistance and acquisition of virulence, rendering the management of these patients more difficult. In recent decades, new antibiotic therapies (Narasimhan and Cohen, 2011; Jain and Smyth, 2012; Ryan et al., 2011; Sawicki et al., 2012; Zobell et al., 2013; Waters and Smyth, 2015) have emerged to treat respiratory infections that result in decline of lung function, respiratory failure and premature death of patients with cf. The quality of life and the length of survival largely depend on the success of antibiotic therapy in eradicating the initial infection (Mayer-Hamblett et al., 2015 Sep 1), suppression of chronic infections, and treatment of pulmonary exacerbations. Despite recent progress in the treatment of CF (Wainwright et al., 2015), antibiotics are one of the most important components of CF management and have been responsible for an increase in median life expectancy to almost 40 years (Cystic Fibrosis Foundation, 2014; Döring et al., 2012). In recent years, the microbial community resident in CF lungs has changed considerably, mainly as a result of intensive antibiotic pressure and alterations in antibiotic regimens, with the consequence that bacteria with newly acquired resistance traits or new pathogens are appearing (Nguyen and Singh, 2006; Bittar et al., 2008).

One of the most important issues for physicians who take care of patients with CF is the increasing problem of multidrug-resistant (MDR) isolates of *Pseudomonas aeruginosa* (Lund-Palau et al., 2016). MDR *P. aeruginosa* is defined as being resistant to all antibiotics routinely

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evaluated in two or more of the following groups: aminoglycosides (tobramycin, gentamicin and amikacin), fluoroquinolones (ciprofloxacin), and β-lactams (ceftazidime, meropenem, imipenem, piperacillin, piperacillin-tazobactam, ticarcillin-clavulanate and aztreonam) (Saiman et al., 1994). These MDR strains result from inherent antibiotic resistance of the bacteria themselves and by their ability to acquire a plethora of adaptive mutations and diverse resistance determinants (Winstanley et al., 2016), and they are associated with worse clinical outcomes (Conway et al., 2003).

These considerations well describe the current situation. We have, nevertheless, to stress that at the moment a uniform definition of multidrug-resistance (MDR) is lacking and would be desirable. Important initiatives in this area are being made by scientific societies but attempt to obtain a homogeneous definition in CF and non-CF contexts have not yet created a result in daily clinical practice. *The attempt to find homogeneous definitions is not the objective of this work and would be premature and not based on objective data* This review is based on definitions of MDRs exclusively used in the CF field and therefore uses the same definition of multiresistance in use in the North American CF Registry (www.cff.org).

Recent data from more than 28,000 individuals with CF included in the most recent Cystic Fibrosis Foundation Patient Registry annual report (2014) (Cystic Fibrosis Foundation, 2014) show that the percentage of patients with MDR *P. aeruginosa* has reached about 18.1%. Rates of MDR *P. aeruginosa* infection have increased substantially in older patients with cf. These findings likely reflect cumulative exposure to antibiotics.

In addition, epidemic *P. aeruginosa* strains, which have an increased propensity to transmission among patients, display enhanced virulence and antimicrobial resistance, becoming an important nosocomial pathogen (Jones et al., 2001; Salunkhe et al., 2005). In the setting of CF, a recent study has pointed out the interplay between key microbiological aspects of chronic respiratory infection by *P. aeruginosa* in CF: (1) the occurrence of transmissible strains and persistent strains; (2) the emergence of variants with enhanced mutation rates (Fischera et al., 2017); and (3) the evolution of antibiotic resistance (López-Causapé et al., 2013). In consideration of the increasing rate of MDR *P. aeruginosa*, this review provides advice to support the management of patients infected with MDR *P. aeruginosa* and discusses whether susceptibility testing is still needed to guide clinical strategies and tackle resistant infection.

2. *P. aeruginosa* adaptation mechanisms

The microbiology of pulmonary infections in patients with CF is often different from similar infections in individuals who do not have CF, and the phenotypes of CF bacteria are frequently atypical (Folkesson et al., 2012). Microbiological and clinical studies have demonstrated that the infectious process in CF airways is a very complex phenomenon and several factors can influence the infection course. The bacterial species with established clinical relevance in CF lung disease are relatively few, but the potential pathogenicity of a large number of species that are frequently isolated remains undefined.

The role of *P. aeruginosa* infection in the decline of lung function has been well explained. *P. aeruginosa* enters the lower airways by inhalation; some patients with CF (10–50%) are able to clear the pathogen spontaneously or become culture-negative in subsequent specimens (Burns et al., 2001a; Van Ewijk et al., 2006). However, the pathogen can persist or recur and eventually may contribute to transforming transient colonization into a chronic infection. *P. aeruginosa* infection is defined as chronic when it is detected in more than 50% of the cultures performed over a time span of 12 months (Lee et al., 2003) (at least four airway cultures are required in different months spread throughout the year).

It is well known that the long-term persistence of *P. aeruginosa* in CF airways is associated with sophisticated mechanisms of adaptation (Folkesson et al., 2012; Hogardt and Heesemann, 2013; Sousa and Pereira, 2014). In patients with CF, *P. aeruginosa* isolates display significant phenotypic variations, such as development of a mucoid phenotype and highly adherent small-colony variants (SCVs), the absence of cell motility, development of variants resistant to macrophage phagocytosis and acquisition of resistance to multiple antibiotics (Martin et al., 1993; Govan and Deretic, 1996; Häussler et al., 1999; Déziel et al., 2001; Häussler et al., 2003; Webb et al., 2004; Oliver, 2010; Oliver and Mena, 2010; Mulcahy et al., 2010; Wei et al., 2011; Kirisits et al., 2005).

In particular, SCVs exhibit different properties from the wildtype (WT) parent strains, including a slow growth rate, superior adherence, reduced motility, hyperpiliation (extra hairlike appendages), increased hydrophobicity, increased biofilm formation, reduced pyoverdin and pyocyanin production and increased resistance to antibiotics (Déziel et al., 2001; Kirisits et al., 2005; Schneider et al., 2008; Starkey et al., 2009; Nelson et al., 2010).

An interesting property, peculiar to *P. aeruginosa* strains isolated from CF lung, is the unusual hypermutability (Oliver et al., 2000). They possess the ability to react promptly to their environment not only by switching genes on or off but also by increasing the frequency of mutation events within the genome. In a cross-sectional study, Ciofu et al. (Ciofu et al., 2005) detected mutator strains in 54% of Danish patients with CF who were chronically colonized with *P. aeruginosa*. In this study, a longitudinal (up to 25 years) evaluation of the prevalence of *P. aeruginosa* mutator strains in patients with CF highlighted that the proportion of hypermutable isolates increased from 0% at onset/early colonization to 65% after 20 years of chronic colonization, suggesting that the hypermutable phenotype is associated with mutations that confer adaptation of the bacteria in the lung and persistence of the infection. In a recent genomic analysis of *P. aeruginosa* isolated from Italian patients with CF, Marving et al. (Marvig et al., 2015a) correlated mutations with changes in CF-relevant phenotypes such as antibiotic resistance. In another recent work (Marvig et al., 2015b) based on isolates of *P. aeruginosa* from the lungs of 34 children affected by CF, 52 genes were identified as more frequently mutated than what would be expected under genetic drift. These were all candidate pathoadaptive genes, where mutation optimized pathogen fitness conferring those adaptation traits already mentioned, such as antibiotic resistance (β-lactams, quinolones, chloramphenicol, macrolides, penicillin, aminoglycosides).

Other authors have documented the prevalence of hypermutable *P. aeruginosa* isolates of approximately 5–10% at onset/early colonization in patients with cf. (Kenna et al., 2007; Mena et al., 2008) One of such mutation event triggers conversion to a mucoid phenotype, which is almost pathognomonic for increased severity of infection (Pedersen, 1992; Schurr et al., 1993). In response to environmental triggers such as nutritional stress or hypoxia found within a CF mucus plug, exopolysaccharide (alginate)-producing mucoid mutants are selected.

Another highly successful survival strategy involves the production of biofilms. This process is regulated by a “quorum sensing” system comprising networks of genes and regulators able to modulate the entire life of the microorganism (Singh et al., 2000). When these QS systems are activated, the bacterium can produce molecules such as acyl homoserine lactones that diffuse freely in and out across the bacterial membrane. As a result of this free diffusibility, the concentration within the organism reflects the concentration outside, which enables the bacteria to “sense” other bacteria in the vicinity. Once a critical mass has been achieved, the QS molecules induce expression of the genes responsible for adhesion and biofilm production. In this state, microcolonies of bacteria are surrounded by a dense matrix, which protects them against phagocytosis and prevents the penetration of antibiotic agents. A recent work (Fischera et al., 2017), involving 54 children and adolescents with CF, and aiming to the identification of subclonal variants of *P. aeruginosa,* has identified six non-synonymous SNPs in the LasR gene, a key transcriptional regulator of QS. The