

Outcomes of Adults with Cystic Fibrosis Infected with Antibiotic-Resistant *Pseudomonas aeruginosa*

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Key Words

Antibiotic resistance · Cystic fibrosis · Outcomes · *Pseudomonas aeruginosa* · Pulmonary function

Abstract

Background: Although *Pseudomonas aeruginosa* is the most common bacterial infection in adults with cystic fibrosis and frequently develops resistance to multiple classes of antibiotics, it has not been determined whether patients with multiple antibiotic-resistant *Pseudomonas aeruginosa* have worse clinical outcomes than patients with more susceptible strains. **Objectives:** This study assessed the impact of multiply-resistant *P. aeruginosa* on lung function, hospitalizations, antibiotic use, lung transplantation and survival in adults with cystic fibrosis. **Methods:** In a cohort study at a university-based adult cystic fibrosis program, 75 consecutive adult cystic fibrosis patients who had *P. aeruginosa* isolated from sputum cultures were studied over a 4-year period. Outcomes included decline in FEV₁, clinic visits, hospitalizations, courses and days of intravenous antibiotics, and lung transplantation. Multiple linear and Poisson regres-

sion for repeated measures were used to assess the outcomes. **Results:** In comparison to patients with susceptible strains, patients with resistant *P. aeruginosa* had more severe baseline lung disease, more rapid decline in FEV₁ (160 ml/year, $p = 0.003$) and were significantly more likely to undergo lung transplantation (17.6 vs. 0%, $p = 0.005$). **Conclusions:** Infection with multiple-antibiotic-resistant *P. aeruginosa* is associated with accelerated progression of cystic fibrosis, and has important implications for infection control strategies, antibiotic use and lung transplantation.

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Introduction

Chronic bacterial pulmonary infections are responsible for much of the morbidity and mortality in cystic fibrosis (CF) [1, 2]. *Pseudomonas aeruginosa* is the most common organism isolated from the sputum of adults with CF [3], with 80% of patients being infected by 18 years of age [4, 5]. At the time of initial infection, the majority of *Pseudomonas* isolates are susceptible to commonly used antibiotics. Unfortunately, as patients are exposed to repeated courses of antimicrobial therapies, drug resistance often develops [6]. As the median survival in CF has increased from less than 10 years in 1960 to more

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than 33 years in 2001 [7] and the adult CF population has grown [8], infection with resistant *P. aeruginosa* has emerged as an increasingly important concern.

Infection with highly resistant strains of *P. aeruginosa* is much more common in CF than infection with other resistant organisms [7]. Many clinical decisions including implementation of infection control measures [9], timing of lung transplantation [10, 11], and treatment of pulmonary exacerbations are based on the presumed deleterious impact of multiple-antibiotic-resistant *P. aeruginosa* (MARPA). However, there are no published data regarding outcomes in CF patients with MARPA who have not undergone lung transplantation, and the optimum evidence-based approach has not been defined. Therefore, this study was designed to evaluate the effect of MARPA on lung function, survival and other clinical outcomes in adults with CF.

Patients and Methods

Study Design and Population

This was a nonconcurrent cohort study of all patients with CF followed at the Johns Hopkins Hospital Adult CF Program between January 1998 and September 2001. The hospital's institutional review board approved this study. Data were collected retrospectively from the hospital's microbiology database, pulmonary function database, and patient charts.

All patients were at least 18 years of age and had been diagnosed with CF according to accepted criteria [12]. Patients were excluded from analysis if they did not have *P. aeruginosa* isolated from a sputum or throat culture during the study period. Patient outcomes were not analyzed beyond the date of lung transplantation.

MARPA was defined based on the 1994 CF Foundation guidelines [11] as resistant to all antibiotics tested in at least two of the three following classes: fluoroquinolones, β -lactams [13], and aminoglycosides [11]. Once an individual with CF was infected with MARPA, they remained in the multiple-antibiotic-resistant group for the remainder of the study period.

Microbiology

Antibiotic susceptibility was performed in the Johns Hopkins Clinical Microbiology laboratory using the reference agar dilution method [14]. There were no changes in susceptibility testing techniques during the study period.

Diagnosis of Cystic Fibrosis

Patients all met standard diagnosis criteria of typical phenotype with an abnormal sweat chloride (>60 mg/dl), two known CF mutations, or nasal potential difference measurement documenting chloride channel dysfunction.

General Management

Patients at this center receive care according to the CF Foundation's adult care consensus conference guidelines [15]. This includes routine quarterly visits for a history and physical examina-

tion, spirometry, sputum culture, and assessment by a multi-disciplinary team. Individuals are also screened annually for allergic bronchopulmonary aspergillosis, atypical mycobacteria, and diabetes. Patients use dornase α and inhaled antibiotics on a regular basis, and receive oral and intravenous antibiotics only in the setting of documented pulmonary exacerbations. Antibiotic selection is based on sputum microbiology data from the hospital laboratory and a cystic fibrosis reference laboratory.

The infection control policy of this center during the study period required that hospital inpatients were admitted to private rooms and did not share common areas such as lounges with other CF patients. Patients with *Burkholderia cepacia* infection were seen on separate clinic days from other patients. Outpatients were discouraged from having close contact with other CF patients.

Analyses

The outcomes of interest were: decline in FEV₁, hospitalizations per year, courses of intravenous antibiotics per year, days on intravenous antibiotics per year, number of clinic visits, and progression to lung transplantation or death.

Bivariate (or unadjusted) analyses compared patients who were never infected with MARPA (MARPA-Never) to patients who had new infection with MARPA during the study period (MARPA-Intermittent) to patients who had MARPA the entire study period (MARPA-Always). The slope of FEV₁ was calculated using the method of least squares. To compare continuous variables, t tests were applied, and Fisher's exact test was used for categorical variables.

Multivariable models using generalized estimating equations were developed to analyze the effect of MARPA on FEV₁ while adjusting for baseline FEV₁ and other potentially important factors. These potentially important covariates included time in quarters of the year, multiple-antibiotic-resistance status, age, gender, race, pancreatic insufficiency, presence of *B. cepacia*, presence of methicillin-resistant *Staphylococcus aureus*, and an interaction term between time and MARPA. The latter indicates the effect of MARPA on FEV₁ over time. This is shown in table 3 as 'MARPA*Time' and represents the change in FEV₁ per quarter of a year after adjusting for the other variables in the model. Collinearity, a high degree of correlation between predictor variables, of the covariates was tested using variance inflation factors [16]. Forward stepwise selection was used for model development in which variables were included in the model if their unadjusted regression coefficient p value was less than 0.2. Time series Poisson regression was used to assess the relationship between MARPA status and hospitalizations, intravenous antibiotic courses, days of intravenous antibiotic use, and clinic visits. Models included FEV₁, age, and pancreatic insufficiency status.

In the bivariate analyses, patients were divided into three groups: MARPA-Always, MARPA-Never, and MARPA-Intermittent. The results in table 2 reflect these categories. In the multivariable analyses, all patients' data were included and each patient contributed information based on his/her MARPA status in any given quarter. Therefore, results for multivariable analyses shown in table 3 do not contain the same groupings as the bivariate analyses.

A survival analysis was performed using a combined endpoint of death or transplantation. Nonparametric analysis using the Kaplan-Meier method was performed using log-rank testing and multivariable analyses were performed using Cox's proportional haz-

Table 1. Baseline characteristics

Characteristics	Total population (n = 75)	MARPA- Never (n = 41)	MARPA- Intermittent (n = 21)	MARPA- Always (n = 13)
Age, years	28.4 ± 8.14	28.9 ± 8.9	28.5 ± 7.9	26.8 ± 6.2
Caucasian (%)	70 (93.3)	39 (95.1)	20 (95.2)	11 (84.6)
Male (%)	33 (44.0)	17 (41.5)	10 (47.6)	6 (46.2)
Pancreatic insufficiency (%)	66 (88.0)	35 (85.4)	18 (85.7)	13 (100.0)
FEV ₁ , liters	1.88 ± 0.94	2.14 ± 0.97	1.46 ± 0.86 ^a	1.60 ± 0.54 ^b
FEV ₁ , % predicted	53.3 ± 26.0	60.2 ± 28.4	40.9 ± 21.9 ^{c, d}	48.4 ± 16.9

^a p = 0.007, ^b p = 0.016, vs. MARPA-Never; ^c p = 0.005 vs. MARPA-Never; ^d p = 0.034 vs. the total population. Groups were not statistically significant with respect to age, race, gender, or pancreatic insufficiency status.

Table 2. Bivariate outcomes

Outcome measure	Total population	MARPA- Never	MARPA- Intermittent	MARPA- Always
Slope of FEV ₁ , %/quarter	-0.46 ± 2.5	0.17 ± 2.5	-0.03 ± 1.98	-1.37 ± 2.7
Slope of FEV ₁ , liters	-0.02 ± 0.09	0.004 ± 0.1	-0.006 ± 0.06	-0.045 ± 0.11
Hospitalizations	0.29 ± 0.57	0.18 ± 0.44	0.49 ± 0.72	0.33 ± 0.62
IV antibiotic courses	0.40 ± 0.65	0.16 ± 0.36	0.75 ± 0.80 ^a	0.63 ± 0.77 ^b
Days of IV antibiotics	6.1 ± 10.0	2.4 ± 5.48	11.6 ± 12.97 ^c	9.42 ± 12.11
Days of IV antibiotics if not zero	27.4 ± 18.3	21.4 ± 9.5	31.0 ± 22.98	27.1 ± 15.4
Clinic visits	2.10 ± 2.09	1.55 ± 1.62	3.18 ± 2.40 ^d	2.26 ± 2.38
Transplant (%)	6 (8.8)	0	5 (23.8) ^e	1 (7.7)
Death (%)	2 (2.7)	1 (2.4)	0	1 (7.7)

IV = Intravenous; days of IV antibiotics if not zero = the days on IV antibiotics per year if a patient received IV antibiotics. Slopes represent the change in FEV₁ per quarter of a year. Hospitalization rates, IV course, days of IV antibiotics, and clinic visits are per year. ^a p = 0.004 vs. MARPA-Never; ^b p = 0.05 vs. MARPA-Never; ^c p = 0.003 vs. MARPA-Never; ^d p = 0.009 vs. MARPA-Never; ^e p = 0.003 vs. MARPA-Never.

ards models, which were used in order to adjust for differences in FEV₁, pancreatic insufficiency, age and gender.

p < 0.05 was considered statistically significant for all analyses. Results are presented as means ± SD unless otherwise specified. Analyses were performed using STATA Statistical Software [17].

Results

Seventy-five adults with CF were studied between January 1998 and September 2001. Baseline characteristics of the study population are shown in table 1. Forty-one patients were never infected with MARPA during the study period (MARPA-Never), 21 patients developed MARPA during the study period (MARPA-Intermit-

tent), and 13 patients were infected with MARPA throughout the study (MARPA-Always). The median follow-up was 1.25 years (interquartile range = 0.75–1.75 years). There was no difference in duration of follow-up for MARPA and non-MARPA patients. The average age was 28 years, over 90% were Caucasian, slightly more than half the group was female and over 85% had pancreatic insufficiency. Overall, both groups of patients with MARPA (MARPA-Intermittent and MARPA-Always) were similar in demographics to the susceptible patients (MARPA-Never), except that the resistant groups had significantly lower pulmonary function (p < 0.05).

In bivariate analyses, both the patients who developed MARPA during the study (MARPA-Intermittent) and those with MARPA throughout the study (MARPA-

Fig. 1. Kaplan-Meier plot comparing combined outcome of death or lung transplantation in MARPA-positive vs. MARPA-negative individuals. $p < 0.006$ by log-rank test.

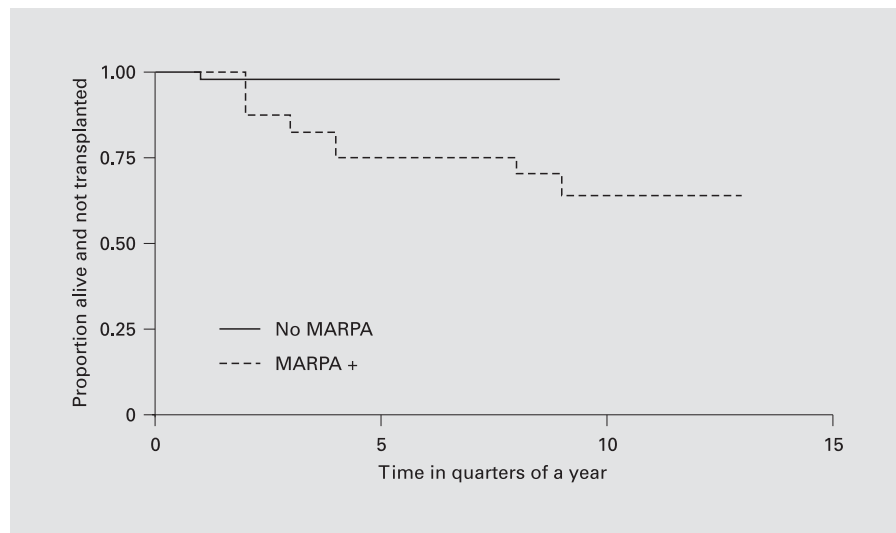


Table 3. Multivariable predictors of change in FEV₁ (liters) over time

Factor	Coefficient	95% confidence interval	p value
<i>MARPA*time</i>	-0.04	-0.06 to -0.01	<0.003
<i>FEV₁</i>	-0.95	0.86-1.03	<0.001
<i>MARPA</i>	-6.01	2.03-9.98	<0.003
Time	0.006	-0.01-0.02	0.55
Age	-0.002	-0.01-0.01	0.75
Male gender	-0.02	-0.17-0.13	0.80
Non-white race	-0.02	-0.24-0.20	0.90
Cepacia	-0.01	-0.43-0.40	0.95

A negative coefficient indicates the presence of the predictor variable is associated with a lower FEV₁. *MARPA*time* is an interaction term between the presence of MARPA and time in quarters of a year. The coefficient indicates the change in FEV₁ over time when MARPA is present compared to when it is not present. Time is in quarters of a year. Age is in years. Statistically significant covariates are in italics.

Always) had worse outcomes than the susceptible group. MARPA patients had significantly more courses of intravenous antibiotics per year, more total days of intravenous antibiotics, and more clinic visits (table 2). This was most pronounced in those with new isolation of MARPA. Within the group that acquired MARPA during the study period (MARPA-Intermittent), rates per year of intravenous antibiotic courses (1.06 ± 0.27 vs. 0.28 ± 0.13) and clinic visits (4.1 ± 0.77 vs. 1.02 ± 0.44) were higher during

the period prior to the first isolation of MARPA ($p = 0.02$ and $p = 0.002$ for intravenous courses and clinic visits, respectively). MARPA-Always patients also had a trend toward more rapid decline in FEV₁ than the patients without MARPA ($p = 0.08$). Of the 6 patients undergoing lung transplantation, all came from the MARPA-infected group ($p = 0.003$). There were 2 deaths, 1 in the MARPA-Always group and 1 in the MARPA-Never group.

In multivariable analyses controlling for potential confounding factors (table 3), the presence of MARPA was associated with a more rapid decline in FEV₁ per quarter compared to patients without MARPA ($p = 0.003$). The decline in FEV₁ over time is indicated by the interaction term between MARPA and time. The coefficient of -0.04 indicates that the FEV₁ declines by 40 ml per quarter faster in cases in whom MARPA is present compared to non-MARPA cases. This model adjusted for the fact that MARPA patients had lower baseline FEV₁ as well as other potential confounding variables.

MARPA infection was significantly associated with the combined endpoint of death and lung transplantation in both Kaplan-Meier analysis (fig. 1) and Cox's proportional hazards models. Cox's proportional hazards model controlled for FEV₁, pancreatic insufficiency, age, and gender (table 4). The presence of MARPA infection resulted in a 14-fold increase in the risk of death or lung transplantation ($p = 0.02$). The results of the multivariable analysis in table 4 indicate that even after accounting for the fact that MARPA patients had lower FEV₁, presence of MARPA was still significantly associated with progression to lung transplantation and death.

Table 4. Cox's proportional hazard model for death and transplant

Variable	Hazard ratio	95% confidence interval	p value
MARPA	13.9	1.4–135.3	0.02
FEV ₁ in liters	0.9	0.3–2.4	0.77
Pancreatic insufficiency	0.4	0.0–3.9	0.40
Age in years	0.9	0.8–1.1	0.36
Male sex	1.1	0.2–4.8	0.9

Discussion

The results of the present study clearly demonstrate MARPA to be associated with more severe lung disease, more rapid decline in FEV₁, and progression to end-stage lung disease as evidenced by lung transplantation. Additionally, patients infected with MARPA require more treatment with intravenous antibiotics and more clinic visits. This is the largest study to date that demonstrates an association between antibiotic-resistant *P. aeruginosa* and poor outcomes in patients with CF prior to lung transplantation. The observation that the presence of MARPA is associated with accelerated physiologic decline and the need for lung transplantation has particular relevance for future approaches to infection control, transplantation, and antibiotic therapy in adults with CF.

While the association between MARPA and poor outcomes in CF may seem almost intuitive, there are several reasons that infection with resistant bacteria has been proposed as potentially less detrimental than expected. Bacteria may become less virulent as they develop greater antibiotic resistance due to loss of virulence factors, alterations in biofilm formation, and inhibition of cell-cell signaling [18–21]. Smith et al. [22] have also recently demonstrated a lack of correlation between results of antibiotic susceptibility testing for *P. aeruginosa* and clinical response to parenteral antibiotics.

But continued apprehension about the potential risk of resistant organisms in CF has prompted the Cystic Fibrosis Foundation to publish extensive guidelines on infection control [23]. While this topic has generated concern among healthcare professionals and patients alike, with the exception of *B. cepacia* [24–26] there has been relatively little objective evidence that infection with resistant organisms in CF is detrimental. Goss et al. [27] have recently demonstrated in a large cohort study that the presence of *Stenotrophomonas maltophilia* did not

result in decreased survival. Similarly, individuals with CF and methicillin-resistant *S. aureus* or nontuberculous mycobacteria do not consistently demonstrate accelerated progression of disease [28, 29]. In one of the few reports examining the effect of resistant *P. aeruginosa* in CF prior to transplant, 12 patients infected with a single strain of resistant, epidemic *P. aeruginosa* demonstrated poor outcomes [30]. Similar to our findings, these patients had accelerated decline in FEV₁ and had a trend toward increased hospitalizations and antibiotic use.

Since the time *B. cepacia* was first identified as being transmissible [31], our clinic has made extensive efforts to decrease patient-to-patient transmission of resistant bacteria. There were only 2 patients with *B. cepacia* complex in our cohort. One patient had genomovar II and 1 patient had genomovar III. There have been no new cases of *B. cepacia* in our clinic. Our results support current CF Foundation consensus recommendations that all patients with MARPA be given special consideration with respect to infection control [23]. However, this study did not utilize molecular typing of MARPA strains and it is therefore impossible to say if isolates represent epidemic clones. Furthermore, we cannot implicate person-to-person spread as a risk factor for the development of MARPA in this population without further study.

The need for lung transplantation is one of the strongest markers for progression to end-stage lung disease. That only patients with MARPA required transplantation again suggests an association of MARPA with more severe CF lung disease. One could argue that decisions regarding lung transplantation could be influenced by the presence of MARPA and this could affect our findings. However, transplant centers are generally more reluctant to transplant patients with resistant bacteria than those with more susceptible strains. This is the case at our institution, though no patient has been denied transplantation due to resistant *Pseudomonas*. Coupled with the more rapid decline in FEV₁ observed in the MARPA group, this result suggests that resistant bacteria may contribute to more rapid progression to end-stage lung disease. We did not assess the impact of MARPA on outcomes after transplantation. Aris et al. [10] have shown that CF patients with MARPA have comparable survival and complication rates to patients with susceptible strains. This contrasts with an earlier study showing increased mortality in transplant patients with resistant *P. aeruginosa* infection [32]. Concern about outcomes in these patients has caused many transplant centers to view resistant *P. aeruginosa* infection as a relative or absolute contraindication to transplantation [10]. While this study

shows that MARPA has deleterious effects before transplantation, our findings cannot be extrapolated to the post-transplant period.

It is noteworthy that the acquisition of MARPA was not associated with an immediate dramatic decline in pulmonary function, but was associated with increased rates of antibiotic use and clinic visits. Investigators have questioned whether CF exacerbations are associated with isolation of new strains of *P. aeruginosa* [33]. While recent evidence suggests this is not the case [34], our findings raise the question of whether there is a mechanistic link between recurrent pulmonary exacerbations and new acquisition of MARPA. A recent study by Aaron et al. [34] demonstrated that CF exacerbations are not associated with isolation of new strains of *P. aeruginosa* at the time of exacerbation or 14 days after treatment. Our finding of increased rates of intravenous antibiotic use in the MARPA-Intermittent patients just prior to having MARPA detected suggests that either early undetected MARPA infection leads to significant worsening of symptoms requiring more aggressive therapy, or antibiotic exposure at the time of exacerbations is partially responsible for the development of MARPA. The precise relationship between antibiotic use and the development of MARPA deserves more extensive investigation in the future.

In this study, patients were considered to be in the 'MARPA group' for the remainder of the study after MARPA was first isolated. This reflected our goal of attempting to understand the clinical implications of a patient being identified for the first time as infected with resistant *P. aeruginosa*. Though patients with CF often also have antibiotic-susceptible strains isolated simultaneously and subsequently to the isolation of MARPA [9], we felt it would be most useful to determine the effect on outcomes after a patient first has MARPA isolated. Further studies will be needed to understand if there is a difference between patients who have intermittently positive MARPA cultures and patients with persistently positive MARPA cultures.

One aspect of MARPA infection that this study will not be able to fully address is whether MARPA primarily precipitates a decline in lung function and increased exacerbation rates, or represents a marker, which develops in sick CF patients who have frequent exacerbations and receive repeated courses of antibiotics. While this study makes it clear that MARPA infection is associated with worse clinical outcomes in CF, a larger investigation with longer follow-up will be required to fully assess the effects of MARPA infection.

Several limitations of our study merit consideration. While we were able to demonstrate a clear deleterious ef-

fect of MARPA in adults with CF, the study group was a relatively small sample of patients from a single adult CF referral center and did not include pediatric CF patients. Being a referral center may have resulted in our patients having characteristics different from the typical adult CF population. To determine if our findings can be projected to the pediatric and adult CF population as a whole, a similar analysis will be required utilizing information from multiple centers. Another potential limitation to the study is the possibility of surveillance bias. Culture data may have been obtained more frequently during exacerbations and intravenous antibiotic use, making the detection of MARPA more likely. This could contribute to the high rates of antibiotic use in the MARPA-Intermittent group prior to the isolation of MARPA. A final potential limitation of our study is the possibility of misclassification. At their entry into the study, patients were assigned to either the resistant or the susceptible group based on their most recent sputum culture results. We did not evaluate multiple previous sputum cultures prior to their entry into the study. Therefore, an individual who had cultured MARPA in the past could have been labeled erroneously as susceptible at the time of study entry. Because the effect of this would be to potentially include MARPA-positive patients in the susceptible group and minimize the measurable detrimental effect of MARPA, the actual difference between MARPA-positive and -negative patients may be greater than we found.

Despite these potential limitations, however, this study provides evidence of a detrimental effect of MARPA on clinical outcomes in CF. Infection with MARPA in CF is associated with more severe lung disease and lung transplantation, and results in more rapid decline in FEV₁, increased use of intravenous antibiotics, and increased frequency of clinic visits. MARPA was associated with more rapid decline in FEV₁ and more lung transplantation even after accounting for FEV₁. Because the results of this study suggest that MARPA is associated with accelerated progression to end-stage lung disease, strategies for antibiotic use and timing of lung transplantation should continue to be reexamined. The study results also highlight the importance of continued infection control efforts to prevent the spread of resistant *P. aeruginosa*.

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