**Pseudomonas aeruginosa** antibiotic susceptibility during long-term use of aztreonam for inhalation solution (AZLI)

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**Objectives:** Aztreonam for inhalation solution (AZLI) is an inhaled antibiotic for patients with cystic fibrosis (CF) and *Pseudomonas aeruginosa* airway infection. The risk of selecting for *P. aeruginosa* isolates with reduced susceptibility to antibiotics is inherent to their use, but is of particular concern following repeated exposure and when complete eradication of lung pathogens is difficult to obtain. We investigated whether repeated treatment courses of AZLI led to decreases in *P. aeruginosa* susceptibility to aztreonam or other antibiotics.

**Methods:** Serial sputum specimens were collected and processed for isolation and quantification of all *P. aeruginosa* isolates in a Phase 3 open-label, 18 month study (NCT00128492) including 274 CF patients receiving up to nine courses of AZLI twice daily (AZLI2) or thrice daily (AZLI3) (28 days on/28 days off). *P. aeruginosa* antibiotic susceptibility testing was conducted.

**Results:** No changes were observed in the aztreonam MIC50 for all *P. aeruginosa* isolates collected from AZLI3 patients, while intermittent increases were observed in the aztreonam MIC90. Approximately 70% of the *P. aeruginosa* isolates with the highest aztreonam MIC from each patient receiving AZLI3 remained unchanged or decreased relative to that patient’s equivalent isolate at baseline; 30% experienced an increase in MIC. Few decreases in *P. aeruginosa* susceptibility to other antibiotics were observed in AZLI3 patients, while increases in *P. aeruginosa* susceptibility to tobramycin were observed.

**Conclusions:** Few decreases in aztreonam susceptibility were reported in patients receiving AZLI3. Increases in tobramycin susceptibility were observed, suggesting that novel treatment paradigms may be able to prolong antibiotic susceptibility in CF patients.

**Keywords:** cystic fibrosis, chronic infection, resistance

**Introduction**

The majority of patients with cystic fibrosis (CF) die of respiratory failure (CF Foundation Patient Registry 2008). This is the end result of a vicious cycle of airway obstruction, inflammation, and infection leading to bronchiectasis, parenchymal destruction and loss of pulmonary function.1 Of the many pathogens that establish chronic sinopulmonary infection in patients with CF, *Pseudomonas aeruginosa* is most closely linked to increased morbidity and earlier mortality.2,3 In order to establish a chronic infection, *P. aeruginosa*, presumably acquired from the environment, adapts within the microbiome of the CF airway to avoid eradication by the host immune system and antibiotic therapy.4,5 Over the last 10–15 years, antibiotic therapy for patients with CF has included chronic suppressive therapy directed primarily against *P. aeruginosa*, usually in the form of an inhaled antibiotic, and may in part be responsible for the increased median predicted survival from 31.7 to 37.4 years (1998–2008).6

Chronic suppressive therapy, the proactive use of an antibiotic regimen rather than the reactive use of antibiotics following patient deterioration, has become the standard of care over the last decade.7 Inhaled antibiotics are frequently used in chronic suppressive regimens, and have the advantage of targeting the site of infection and achieving higher antibiotic sputum concentrations within the airway surface liquid than intravenous antibiotics. Approved inhaled antibiotic therapy consists of...
28 days on therapy followed by ≥28 days off therapy, ostensibly to lower the risks of developing antibiotic-resistant bacteria. However, a trend of decreasing susceptibility of *P. aeruginosa* to antibiotics since 1995 has been identified. Specifically, a decrease in *P. aeruginosa* susceptibility to aminoglycosides has been recognized in the USA, and is attributed to the common use of tobramycin and amikacin in both intravenous and inhaled treatment regimens. Ultimately, the long-term microbiological effects of chronic suppressive therapy on *P. aeruginosa* antibiotic susceptibility are of crucial importance to clinicians treating individuals with CF.

Aztreonam for inhalation solution (AZLI; Cayston®) is a new inhaled antipseudomonal antibiotic. Two pivotal studies, AIR-CF19 and AIR-CF210, showed that the use of AZLI improved respiratory symptoms, increased lung function and delayed the need for intravenous or inhaled antibiotics. The open-label extension trial AIR-CF3 (NCT00128492), which included serial, monthly lower respiratory tract cultures from up to 274 subjects, showed that the improvement in respiratory symptoms and lung function persisted over nine 28 day on–off courses of AZLI and that the improvement in respiratory symptoms, increased lung function and delayed the need for intravenous or inhaled antibiotics. The open-label extension trial AIR-CF3 (NCT00128492), which included serial, monthly lower respiratory tract cultures from up to 274 subjects, showed that the improvement in respiratory symptoms and lung function persisted over nine 28 day on–off courses of AZLI and that the antibacterial effect was sustained. The serial sputum specimens collected during AIR-CF3 were used herein to describe changes in the antibiotic susceptibility of *P. aeruginosa* over an 18 month period.

**Methods**

**Study design**

The study design for this open-label trial has previously been described. Briefly, subjects who participated in either AIR-CF19 or AIR-CF210 were eligible to enrol in this open-label, follow-on study. All subjects had CF and were infected with *P. aeruginosa*. Subjects received up to nine courses of AZLI (28 days of AZLI followed by 28 days off therapy) in addition to usual care, including other antibiotics, as prescribed by each subject’s treating physician. Subjects attended up to 20 scheduled visits, and received the first and last dose of each treatment course in the clinic; there was also a follow-up visit 28 days after completing the last course of AZLI. Subjects were dosed with 75 mg of AZLI three times daily (AZLI3) or twice daily (AZLI2) via an investigational nebulizer (PARI eFlow® Electronic Nebulizer, PARI Innovative Manufacturers, Midlothian, VA, USA). Each subject’s regimen (AZLI2 or AZLI3) was determined by the dosing schedule assigned in either AIR-CF1 or AIR-CF2. Subjects were instructed to use an inhaled bronchodilator prior to each dose of AZLI. Subjects were also instructed to take the doses of AZLI ≥4 h apart. All research was conducted in accordance with the Declaration of Helsinki, and national and institutional standards. Approval was obtained from institutional ethics committees and all subjects provided written informed consent.

**Culture and susceptibility testing**

Sputum specimens were collected at all visits for qualitative and quantitative culture for *P. aeruginosa*, and were processed at a central laboratory according to guidelines set forth by the CF Foundation consensus conference on CF Microbiology. If a patient was unable to produce sputum, an oropharyngeal swab was collected for qualitative culture only. Sputum and swab specimens were collected prior to the in-clinic administration of AZLI and ≥4 h after an at-home AZLI dose. Sputum *P. aeruginosa* density in colony forming units per gram of sputum (cfu/g) was determined using sputum dilutions plated onto MacConkey agar. The MIC of six antibiotics for *P. aeruginosa* isolated from subject specimens was determined using a microbroth dilution technique. The following antibiotics and ranges were tested: aztreonam, 1–2048 mg/L; ceftazidime, 0.5–64 mg/L; ciprofloxacin, 0.12–16 mg/L; meropenem, 0.12–32 mg/L; piperacillin, 0.5–256 mg/L; and tobramycin, 0.12–1024 mg/L. A ≥4-fold change in the MIC was considered an increase or decrease in the MIC.

**Results**

Patient demographics were similar between the AZLI2 and AZLI3 treatment groups (Table 1). Fewer patients received AZLI2 therapy (n=85) compared with AZLI3 (n=189); only AIR-CF2 tested AZLI2 therapy and was exclusively conducted in the USA. At baseline, the mean age was 28.5 years, 55% of the patients were male and the mean FEV1% value was 55.6% of predicted. One or more mucoid *P. aeruginosa* isolate was observed in 85% of subjects.

In the AZLI2 treatment group, the mean *P. aeruginosa* density in expectorated sputum (log10 cfu/g) at baseline was 5.71, after the first treatment it was 5.50 and after the last treatment it was 5.39. In the AZLI3 treatment group, the mean *P. aeruginosa* density in expectorated sputum (log10 cfu/g) at baseline was 6.16, after the first treatment it was 5.41 and after the last treatment it was 5.58. Over nine treatment courses of AZLI, continuous suppression of *P. aeruginosa* in expectorated sputum was observed; sputum concentrations persisted below baseline values over the 18 month study (Figure 1). *P. aeruginosa* sputum density at the end of all nine treatments in the AZLI2 and AZLI3 groups was consistently lower than at the beginning of each treatment period.

The aztreonam MIC50 and MIC90 values for all *P. aeruginosa* isolates collected at baseline in both treatment groups (AZLI2 and AZLI3) were 4 and 128 mg/L, respectively (Table 2). In AZLI2 patients, the MIC50 increased 4-fold to 16 mg/L at eight visits over the course of the study. Notably, at the end of the study, the MIC50 was 25 mg/L.

**Table 1.** Patient demographics and baseline characteristics

<table>
<thead>
<tr>
<th>Country, n (%)</th>
<th>AZLI2 (N=85)</th>
<th>AZLI3 (N=189)</th>
<th>Total (N=274)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA and Canada</td>
<td>85 (100.0)</td>
<td>165 (87.3)</td>
<td>250 (91.2)</td>
</tr>
<tr>
<td>Australia and New Zealand</td>
<td>0</td>
<td>24 (12.7)</td>
<td>24 (8.8)</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>27.3 (11.4)</td>
<td>29.0 (13.0)</td>
<td>28.5 (12.5)</td>
</tr>
<tr>
<td>Age ≥18 years, n (%)</td>
<td>66 (77.6)</td>
<td>153 (81.0)</td>
<td>219 (79.9)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>51 (60.0)</td>
<td>100 (52.9)</td>
<td>151 (55.1)</td>
</tr>
<tr>
<td>Weight (kg), mean (SD)</td>
<td>59.5 (13.3)</td>
<td>59.3 (15.8)</td>
<td>59.4 (15.1)</td>
</tr>
<tr>
<td>Body mass index (kg/m²), mean (SD)</td>
<td>21.3 (3.3)</td>
<td>21.4 (4.0)</td>
<td>21.4 (3.8)</td>
</tr>
<tr>
<td>FEV1% of predicted value, mean (SD)</td>
<td>56.7 (17.5)</td>
<td>55.1 (15.4)</td>
<td>55.6 (16.1)</td>
</tr>
<tr>
<td>Subjects with ≥1 mucoid <em>P. aeruginosa</em>, n (%)</td>
<td>74 (89.2)</td>
<td>153 (82.7)</td>
<td>227 (84.7)</td>
</tr>
</tbody>
</table>

#At baseline (day 0, month 0).

#If baseline isolates were not available, the next available post-baseline isolates were used.
The MIC50 returned to the baseline value of 4 mg/L. In AZLI3 patients, the MIC50 never demonstrated a significant (≥4 fold change) change from baseline. In AZLI2 patients, the MIC90 increased 4-fold from baseline to a concentration of 512 mg/L at three visits over the course of the study. Notably, at the end of the study (month 18) the MIC90 returned to the baseline value of 128 mg/L. In AZLI3 patients, the MIC90 increased 4-fold from baseline to a concentration of 512 mg/L at three visits over the course of the study and otherwise remained unchanged (±2-fold change) from the baseline value. The initial study design of AIR-CF3 had only three treatment courses, ending at month 6. Due to positive clinical responses, the study design was extended to nine treatment courses. However, a large number of patients completed their participation in the study prior to the protocol revision. The number of isolates contributing to the MIC50 and MIC90 data at month 6 compared with at month 7 is reflective of this decrease in the patient population size.

From months 1 to 18, each patient's *P. aeruginosa* isolate with the highest aztreonam MIC was compared with that patient's *P. aeruginosa* isolate at baseline. Over the first three AZLI2 treatment courses, ~60% of patients experienced no change in the MIC of aztreonam, while ~20% experienced an increase and ~20% experienced a decrease in the MIC of aztreonam (Figure 2a). Following this period, AZLI2 patient-specific changes in aztreonam susceptibility were more variable: 41%–76% of patients experienced no change in the MIC of aztreonam, while 19%–46% experienced an increase and 2%–22% experienced a decrease in the MIC of aztreonam. In contrast, throughout all nine AZLI3 courses, ~50% of patients experienced no change from baseline in the MIC of aztreonam, while ~30% and ~20% of patients experienced an

Table 2. MIC50 and MIC90 of aztreonam (mg/L) for all *P. aeruginosa* isolates collected from patients during nine courses of AZLI [twice daily (AZLI2) and thrice daily (AZLI3)]

<table>
<thead>
<tr>
<th>Month</th>
<th>AZLI2</th>
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<th>AZLI3</th>
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<tr>
<td></td>
<td>subjects (n)</td>
<td><em>P. aeruginosa</em> isolates (n)</td>
<td>MIC50</td>
<td>MIC90</td>
</tr>
<tr>
<td>Baseline</td>
<td>76</td>
<td>131</td>
<td>4</td>
<td>128</td>
</tr>
<tr>
<td>1°</td>
<td>76</td>
<td>120</td>
<td>4</td>
<td>256</td>
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<td>2</td>
<td>76</td>
<td>124</td>
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<td>128</td>
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<td>18</td>
<td>40</td>
<td>64</td>
<td>4</td>
<td>128</td>
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Numbers in bold identify ≥4-fold increases in MIC50 or MIC90.  
°MIC data collected at the end of 28 days of AZLI therapy.
increase or decrease from baseline in the MIC of aztreonam, respectively (Figure 2b). The percentages of patients undergoing increases, decreases or no change in the P. aeruginosa isolate MIC remained relatively stable in AZLI3 patients throughout the study compared with AZLI2 patients at month 6 and thereafter. Notably, directional changes in the percentage of patients who experienced an increase or decrease in the MIC of aztreonam do not appear to consistently coincide with AZLI treatment (AZLI2 and AZLI3) and off-treatment periods; no distinct pattern is apparent.

In addition to aztreonam, the susceptibility of every P. aeruginosa morphotype cultured from expectorated sputum and oropharyngeal swabs was determined for tobramycin, ciprofloxacin, piperacillin, meropenem and ceftazidime. The MIC$_{50}$ and MIC$_{90}$ of these antibiotics for all P. aeruginosa morphotypes were calculated for all visits (Figure 3). In addition, the percentage of patients in whom a P. aeruginosa isolate had an MIC greater than the concentration considered susceptible to antibiotic therapy (according to CLSI criteria) was determined for each antibiotic (Figure 4). With regard to breakpoints established by the CLSI, decreases in P. aeruginosa susceptibility to antibiotics are not predictive of clinical efficacy in CF patients.$^{14}$

![Week](image-url)
The tobramycin MIC50 for all P. aeruginosa isolates collected at baseline in both treatment groups (AZLI2 and AZLI3) was 2 mg/L, while the MIC90 in the AZLI2 and AZLI3 groups was 128 and 64 mg/L, respectively. Decreases in the tobramycin MIC50 of ≥4 fold occurred within both the AZLI2 and AZLI3 treatment groups, while no changes (+2-fold change) in the tobramycin MIC90 occurred. Notably, the decreases did not lower the MIC90 value below the parenteral susceptibility breakpoint of 4 mg/L. At the end of the study, the tobramycin MIC50 for P. aeruginosa isolates collected from patients at month 18 was 32 mg/L for AZLI2 and 16 mg/L for AZLI3. At baseline, 42% and 40% of AZLI2 and AZLI3 patients, respectively, were infected by a P. aeruginosa isolate considered resistant to tobramycin (MIC of >4 mg/L), according to the parenteral breakpoint. Throughout the treatment courses, fewer AZLI3 patients (26.9%–38.5%) and generally fewer AZLI2 patients (27.3%–42.1%) were infected by a tobramycin-resistant P. aeruginosa isolate. 

Throughout the study, the MIC50 and MIC90 for all P. aeruginosa isolates collected from AZLI2 patients remained unchanged (+2-fold change) for ciprofloxacin, piperacillin, ceftazidime and meropenem, except for two intermittent increases from baseline (month 0) in the MIC50 of ceftazidime. Notably, the ceftazidime and piperacillin MIC90 values at baseline were the highest concentrations tested in the AZLI2 and AZLI3 groups. In P. aeruginosa isolates collected from AZLI2 patients, persistent increases in the MIC50 of meropenem were observed, while four and five intermittent increases were observed in the MIC50 of ceftazidime and piperacillin, respectively. One 4-fold increase in the ciprofloxacin MIC90 in the AZLI2 treatment group was observed (Figure 3).

At baseline in patients treated with AZLI3, 54% of patients were infected by a P. aeruginosa isolate considered ciprofloxacin resistant (MIC of >1 mg/L), while 27%, 32% and 25% were infected by a P. aeruginosa isolate considered resistant to piperacillin (MIC of >64 mg/L), ceftazidime (MIC of >8 mg/L) and meropenem (MIC of >4 mg/L), respectively. Similar percentages were observed at baseline in patients treated with AZLI2. The long-term use of AZLI did not progressively increase the percentage of patients with isolates ‘resistant’ to β-lactams or quinolones. Rather, an initial increase in the ‘resistant’ population size was observed at month 1 and was generally maintained throughout the treatment period (Figure 4).

Discussion

Several factors are likely to have contributed to the trend of decreased antibiotic susceptibility in P. aeruginosa isolates collected from CF patients over the last 10–15 years. The most important factor may be the underlying nature of chronic, as opposed to acute, infection. The paradigm for the treatment of acute infection is the eradication of pathogens through intensive short-term antimicrobial therapy. Conversely, the paradigm for the treatment of CF lung disease is the suppression of pathogens through the use of long-term therapies. As demonstrated here, the lowest mean P. aeruginosa sputum density observed was ~5 log10 cfu/g. In most infections, anything less than eradication is typically considered treatment failure and can lead to antibiotic resistance. The clinical significance of decreases in antibiotic susceptibility in patients with CF over the last 10–15 years is yet to be defined. However, during the period in which P. aeruginosa antibiotic susceptibility decreased, life expectancy has increased. Further, in a centre-based analysis, the upper quartile treatment centres with the highest median value for the FEV1 % of predicted had more intravenous and inhaled antibiotic use as well as a higher incidence of P. aeruginosa considered multidrug resistant as compared with lower quartile treatment centres. Accordingly, chronic suppressive therapy, typically in the form of aninhaled antipseudomonal antibiotic, in addition to the treatment of acute exacerbations with parenteral and oral antibiotics is perhaps responsible for both increases in life expectancy and decreases in antibiotic susceptibility.

When considering the repeated antimicrobial effect observed in AIR-CF3 (Figure 1), one would expect that P. aeruginosa isolates that have become less susceptible to aztreonam through mutations would replicate or persist under selective pressure more readily than ‘wild-type’ P. aeruginosa isolates. Unexpectedly, changes in antibiotic susceptibility during chronic, intermittent use were favourable for the patients treated with AZLI3. Further, patient-specific changes in aztreonam susceptibility were in agreement with P. aeruginosa population changes in aztreonam susceptibility. For example, at any one visit, ~50% of the P. aeruginosa isolates with the highest MIC of aztreonam for patients receiving AZLI3 remained unchanged in relationship to that patient’s equivalent morphotype at baseline. Furthermore, decreases in aztreonam susceptibility occurred as frequently as increases in susceptibility in the AZLI3 patient population. Concurrently, ≥4-fold changes in the aztreonam MIC50 and MIC90 were uncommon in the AZLI3 treatment group. Arguably, then, chronic suppressive therapy with AZLI3 did not result in a continuous decrease in aztreonam susceptibility and efficacy was maintained throughout the study.

While only intermittent decreases in susceptibility (≥4-fold increases in MIC50) to aztreonam were observed with repeated courses of AZLI3, patients who received AZLI2 therapy demonstrated prolonged decreases in susceptibility (≥4-fold increases in MIC50 and MIC90) to aztreonam. The differences between the changes in susceptibility between the two treatment regimens may be informative and support a thrice-daily treatment regimen. It is plausible that AZLI3 therapy is likely to achieve more effective pharmacokinetic parameters (sputum aztreonam concentrations) for a time-dependent antibiotic than AZLI2 therapy. Or, these differences, which are apparent from month 7 onward, may be random and an artefact of the differences in sample size (from months 7 to 18 the AZLI2 sample size drops to <100 for all P. aeruginosa isolates and to <50 for the least susceptible P. aeruginosa isolate).

Tobramycin was the most commonly used non-study antibiotic in this study. In spite of the concomitant use of tobramycin, decreases in the tobramycin MIC90 occurred and a trend of
greater susceptibility occurred in the MIC₅₀. While fitness costs associated with mechanisms of resistance may help to suppress the development of antibiotic resistance, alternating between different classes of antibiotics may also suppress the development of antibiotic resistance. More specifically, alternating between antibiotics for which the mechanisms of resistance employed by P. aeruginosa do not confer cross-resistance may suppress development.

Several chromosomally encoded efflux systems play an important role in P. aeruginosa antibiotic resistance. The MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM systems extrude a wide variety of antibiotics, including aminoglycosides and β-lactams. Most β-lactams (e.g. penicillins, cepham and carbapenems) are pump substrates for all of these efflux systems. In contrast, aztreonam can only be extruded by the MexAB-OprM pump system, while the main mechanism of resistance to aminoglycosides in P. aeruginosa isolates collected from CF patients involves up-regulation of the MexXY-OprM efflux pump. Similarly, high-level constitutive production of chromosomal AmpC as a result of chromosomal mutation(s) contributes to decreased susceptibility to aztreonam as well as cephalosporins, but not to aminoglycosides. Accordingly, it is unlikely that aztreonam and tobramycin confer cross-resistance to each other. In support of the theory that alternating between antibiotics that do not confer cross-resistance may suppress the development of resistance, increases in tobramycin susceptibility were observed and changes in aztreonam susceptibility were lower than expected in this study.

Repeated courses of AZLI over 18 months did not progressively increase the percentage of patients with isolates ‘resistant’ to β-lactams or quinolones. Rather, an initial increase in the ‘resistant’ population size was observed at month 1 and was generally maintained throughout the treatment period. Because the increase occurred following the first AZLI course, it is unlikely that the concurrent use of antibiotics, which did occur during the study, contributed to this initial increase.Rather, cross-resistance between aztreonam, ciprofloxacin and these β-lactams is most likely responsible, as a decrease was observed in the tobramycin data.

In summary, chronic suppressive therapy with AZLI is effective against P. aeruginosa. While decreases in antibiotic susceptibility are ultimately expected as a consequence of chronic AZLI therapy, surprisingly few, if any, decreases in aztreonam susceptibility were observed in patients receiving AZLI3 for up to 18 months. The antibiotic profile of AZLI3 patients appears superior to that of AZLI2 patients and this reinforces the approval of the AZLI3 regimen. Due to the chronic nature of P. aeruginosa infection in patients with CF, decreases in antibiotic susceptibility are perhaps inevitable and clinicians may need to devise novel treatment paradigms to preserve antibiotic efficacy in their patients. The increases in tobramycin susceptibility as well as the few, if any, decreases in aztreonam susceptibility suggest that (ciprofloxacin) and three β-lactams (an extended-spectrum penicillin (piperacillin), a carbapenem (meropenem) and a cephalosporin (ceftazidime)). Each AZLI treatment period is designated with a grey box separated by an off-treatment period. Both AZLI2 patient data and AZLI3 patient data are presented. BL, baseline.
novel treatment paradigms that maintain efficacy are feasible. Studies that directly test the continuous inhaled use of the same antibiotic or alternating antibiotic classes may be informative.

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Author contributions
All authors contributed to: conception and design, acquisition of data, or analysis and interpretation of data; drafting the article and/or revising it critically for important intellectual content; and final approval of the version to be published. C. M. O. and A. B. M. (Gilead Sciences, Inc.) assure that this manuscript is free of bias.

References