Factors Affecting the Incidence of Stenotrophomonas maltophilia Isolation in Cystic Fibrosis*

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Study objectives: To identify factors predisposing cystic fibrosis (CF) patients to Stenotrophomonas maltophilia infection and to determine whether coinfection with S maltophilia affects the clinical response to therapy with tobramycin solution for inhalation (TSI), 300 mg bid.

Design: Retrospective review of data collected from two identical, 6-month, randomized, placebo-controlled trials.

Setting: Sixty-nine CF centers in the United States.

Interventions: Active drug administration of 300 mg TSI.

Patients: Five hundred twenty CF patients with chronic Pseudomonas aeruginosa endobronchial infections.

Measurements and results: A logistic regression analysis identified factors contributing to increased S maltophilia isolation frequency. In this multivariate analysis, the only significant predictors of S maltophilia isolation during the last month of the trial were the concomitant use of oral quinolones (primarily ciprofloxacin; p = 0.0015) and S maltophilia isolation prior to treatment (p < 0.0001). Treatment group, gender, age, use of systemic or inhaled steroids, use of oral sulfonamide, IV cephalosporins, or penicillin antibiotics, baseline FEV1 percent predicted, and pretreatment Aspergillus isolation were not significant predictors of subsequent S maltophilia infection. In addition, S maltophilia-positive culture frequency was compared to the change in pulmonary function. Patients who either never had culture results positive for S maltophilia or who were positive at <25% of observations had greater clinical response to TSI at the final study visit compared to patients who were positive at ≥ 25% of observations.

Conclusions: TSI therapy did not result in a greater risk for isolation of S maltophilia than standard care alone. In contrast, oral quinolone antibiotic use during the trial was associated with a 2.7-fold increased risk of having a culture positive for S maltophilia (p = 0.0015). The use of TSI to suppress P aeruginosa resulted in improved lung function, regardless of S maltophilia culture frequency. However, improvement was not as great among patients who were persistently coinfected with S maltophilia.

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Key words: cystic fibrosis; quinolone; Stenotrophomonas maltophilia; steroids; tobramycin

Abbreviations: CF = cystic fibrosis; PCR = polymerase chain reaction; TSI = tobramycin solution for inhalation

Cystic fibrosis (CF) is characterized by the presence of a chronic endobronchial infection that leads to progressive suppurative obstructive lung disease, which is the primary cause of death in > 90% of patients.1 Pseudomonas aeruginosa is the most common bacterial pathogen isolated from the CF respiratory tract.2–4 However, the microbiologic flora of CF lung disease is evolving. Increases in the frequency of Stenotrophomonas (previously Pseudomonas then Xanthomonas) maltophilia isolation from CF respiratory tract secretions have been reported.3,5–7 Baseline data from the clinical trials of tobramycin solution for inhalation (TSI) found 28 different Gram-negative organisms in cultures made...
from specimens from the respiratory tracts of CF patients, in which _S. maltophilia_ was second only to _P. aeruginosa_ in frequency of isolation. In that study, the prevalence of _S. maltophilia_ was 28.6% when three screening cultures were examined. Over 6 months of treatment, 99 of 520 patients acquired the organism, for an incidence of 19%. Previously, a high frequency of isolation was reported by Ballestero et al, who found that 31.0% of their patients had at least one positive culture over 5 years. However, incidence and prevalence rates were not reported in that study.

Unlike the pathogenic roles of _P. aeruginosa_ and _Burkholderia cepacia_ in CF, that of _S. maltophilia_, and thus the implications for acquiring the organism, is uncertain. The study by Ballestero et al associated _S. maltophilia_ infection with an overall worse course in CF; however, this has not been demonstrated in subsequent epidemiologic studies in CF patients. Nonetheless, the problematic antimicrobial resistance patterns of _S. maltophilia_ and the pathogenic role of the organism in non-CF disease make the increasing frequency of _S. maltophilia_ isolation in CF patients a cause for concern.

The factors underlying the changing microbial flora in the CF population are not clear, but it has been hypothesized that this evolution is at least partly caused by selective pressure exerted on bacterial populations by the use of new antipseudomonal therapies, including oral quinolones and aerosolized aminoglycosides. Among non-CF patients, the use of carbapenem antibiotics also has been associated with an increased risk for infection with _S. maltophilia_. Last, oral steroid use has been identified as a possible contributing factor to the increased frequency of isolation of _S. maltophilia_. In order to identify factors that may predispose CF patients to infection with _S. maltophilia_ and to evaluate the effect of _S. maltophilia_ on clinical responses to TSI, we conducted a retrospective study utilizing data from the TSI trials.

**Materials and Methods**

**Study Design**

Two identical, randomized, placebo-controlled trials of TSI were conducted. Patients were randomized to treatment either with 300 mg bid TSI (TOBI; Chiron; Emeryville, CA) or a taste-masked placebo administered bid. The study drug was administered in a series of cycles consisting of 28 days receiving the drug followed by 28 days not receiving the drug. The study design, study drug formulation, and drug delivery have been described previously. During the trials, patients could receive any standard therapy for CF, with the exception of inhaled antibiotics other than the study drug.

Rausey et al previously have described the selection criteria. However, it is important to note that this population represents a subset of the CF population as a whole, since only _P. aeruginosa_-infected patients who were > 6 years of age with an FEV1 of 25 to 75% of predicted and had cultures negative for _B. cepacia_ were eligible.

There were a total of 11 scheduled visits during the studies. During the screening period and the first treatment cycle (ie, study visits 1 to 5), visits occurred every 2 weeks. During the second and third treatment cycles (ie, study visits 6 to 11), visits occurred every 4 weeks. Microbiological and pulmonary function data were collected at each visit. Spirometry testing was performed according to American Thoracic Society standards. The FEV1 was expressed as a percentage of the value predicted based on age and height according to the methods of Knudson et al. The relative change in FEV1 percent predicted was calculated as described by Ramsey et al.

**Microbiology**

Sputum specimens were refrigerated and shipped on ice within 48 h to the microbiology laboratory at Children's Hospital and Regional Medical Center, Seattle, WA, for culturing. Quantitative cultures of sputum were performed as previously described using liquefaction of samples in dithiothreitol, serial dilutions in phosphate-buffered saline solution (pH 7.0) with 0.1% added gelatin, and plating on selective agar. The quantitation of all Gram-negative bacilli was performed on sputum specimens at all time points. Oropharyngeal swabs were collected from patients who could not expectorate sputum. Oropharyngeal swabs were cultured only for _P. aeruginosa, B. cepacia, S. maltophilia, and Achromobacter_ (previously _Alcaligenes_ _xylosoxidans_). All Gram-negative isolates from sputum and oropharyngeal cultures that were obtained during these trials were identified using standard techniques, including the use of a biochemical panel for the identification of non- _P. aeruginosa_, _Gram-negative, nonlactose fermenting organisms._

**Data Analysis and Statistical Methods**

All analyses were performed using only data from patients who completed 6 months of treatment. This population was chosen in order to achieve a realistic assessment of the percentage of visits at which patients had cultures that were positive for _S. maltophilia_. The frequency of treatment-emergent _S. maltophilia_ and the use of therapy with concomitant oral quinolones or corticosteroids (systemic and/or inhaled) were compared between treatment groups using a chi square test. Patients were categorized according to the frequency with which they had _S. maltophilia_-positive cultures (ie, 0% of visits, <25% of visits [intermittent], or ≥25% of visits [persistent]). The frequency of culture positivity was compared with a mean relative change in the FEV1 percent predicted, as well as with baseline _S. maltophilia_ sputum density (in terms of colony-forming units per gram of sputum), and the use of oral quinolone antibiotics or steroids (ie, systemic and/or inhaled) was compared by means of a chi square test. A multiple logistic regression was used to determine whether variables including age, gender, baseline culture status (ie, yes/no) for _S. maltophilia_ and _Aspergillus_ spp, treatment group, disease severity (ie, relative FEV1 percent predicted), and the use of concomitant oral quinolone antibiotics, other antibiotics (ie, cephalosporins, penicillins, and sulfonamides), or steroids (ie, systemic and/or inhaled) could be used to predict subsequent _S. maltophilia_ isolation. For the purposes of the analyses reported here, patients were considered negative for _S. maltophilia_ and _Aspergillus_ if they had produced no positive cultures at visits 1, 2, or 3 (ie, the pretreatment visits). Significance was determined at the p < 0.05 level.

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Results

Demographic Data

A total of 520 patients were enrolled in the trials (TSI group, 258 patients; placebo group, 262 patients). Of these, 464 (89.2%) completed 6 months of treatment (TSI group, 232 patients [89.9%]; placebo group, 232 patients [88.6%]). There were no significant differences in the baseline characteristics of the two treatment groups (Table 1). When evaluated by *S maltophilia* culture status (i.e., yes/no), demographic data revealed proportionally more pediatric patients and fewer adult patients among those who had at least one positive culture during the trial (p = 0.027) [Table 2]. There were no other significant differences between *S maltophilia* culture status groups at baseline. A comparison of demographic characteristics of the intent-to-treat and completer populations revealed no meaningful differences.

Correlation of Positive Culture Frequency and Sputum Density

The majority of patients in both treatment arms who completed the trial tested negative for *S maltophilia* at all observations during 6 months of treatment. A total of 58 TSI patients (25.0%) and 81 placebo patients (34.9%) tested positive for *S maltophilia* at least once, for an overall prevalence of 30%. Among those patients from whom *S maltophilia* was isolated at some point during the trial, the majority of patients in both arms of the trial (TSI group, 36 [62.1%]; placebo group, 54 patients [66.6%]) intermittently produced positive cultures. Figure 1 shows the distribution of *S maltophilia* isolation frequency. An evaluation of the mean *S maltophilia* sputum density showed significantly higher density among persistently colonized patients (p = 0.003).

Factors Associated With Positive *S maltophilia* Culture

For patients who had cultures that were negative for *S maltophilia* during the screening period, the incidence of isolation at subsequent visits was greater among those randomized to placebo treatment than to TSI treatment (p = 0.038) [Fig 2]. Significantly more placebo patients (68.1%) than TSI patients (54.7%) used oral quinolones during the treatment period (p = 0.003). Furthermore, oral quinolone use following the screening period was significantly correlated with categories of *S maltophilia*-positive cultures (p = 0.0005 [χ² test]) [Fig 3]. Ciprofloxacin represented >90% of all quinolone use during the trial, with the remainder of quinolone use being in the form of ofloxacin. Among patients who were

| Table 1—Baseline Demographics of Patients Who Completed 6 Months of Treatment, Listed by Treatment Group |
| Variable                          | TSI (n = 232) | Placebo (n = 232) |
| Male gender*                     | 132 (56.9)    | 116 (50.0)        |
| Age,† yr                         | 20.8 (9.4)    | 20.6 (9.6)        |
| Age group*                       |               |                  |
| < 13 yr                          | 54 (23.3)     | 58 (25.0)         |
| 13–17 yr                         | 57 (24.6)     | 63 (27.2)         |
| ≥ 18 yr                          | 121 (52.2)    | 111 (47.8)        |
| FEV1,‡ % predicted               | 50.2 (15.1)   | 52.4 (16.4)       |

*Values given as No. (%).
†Values given as mean (SD).

| Table 2—Baseline Demographics of Patients Who Completed 6 Months of Treatment, Listed by *S maltophilia* Culture Status |
| Variable                          | Positive* (n = 139) | Negative (n = 325) | p Value |
| Male gender†                     | 66 (47.5)           | 182 (56.0)         | 0.14    |
| Age,† yr                         | 19.6 (9.6)          | 21.0 (9.5)         | 0.79    |
| Age group†                       |                    |                   |         |
| < 13 yr                          | 43 (30.9)           | 69 (21.2)          |         |
| 13–17 yr                         | 35 (25.2)           | 83 (26.2)          | 0.03    |
| ≥ 18 yr                          | 61 (43.9)           | 171 (52.6)         |         |
| FEV1,‡ % predicted               | 53.4 (17.1)         | 50.4 (15.2)        | 0.14    |

*Patients were considered to be culture-positive for *S maltophilia* if the organism was isolated at any visit.
†Values given as No. (%).
‡Values given as mean (SD).

**Figure 1.** The frequency of isolation of *S maltophilia*. The majority of patient specimens never produced a culture that was positive for *S maltophilia*. In most patients with *S maltophilia*, the frequency of isolation was < 25%.
intermittently culture-positive, 61 of 90 patients (67.8%) received concomitant oral quinolones. Of those who had persistently positive results, 41 of 49 patients (83.7%) received concomitant oral quinolones. The baseline isolation of *S. maltophilia* was also significantly correlated with use of therapy with oral quinolones during the study period (*p* < 0.018).

The percentage of patients receiving IV cephalosporins differed significantly between treatment groups (TSI group, 48.6%; placebo group, 61.5%; *p* < 0.01). Despite this difference, IV cephalosporin use was not significantly associated with *S. maltophilia* isolation by univariate analysis. The use of IV penicillins and also of oral sulfonamide antibiotics (primarily trimethoprim-sulfamethoxazole) did not differ significantly between treatment arms and was not associated with *S. maltophilia* isolation by univariate analyses.

No difference in the percentage of patients who received therapy with systemic or inhaled steroids was observed between treatment groups (TSI group, 18.1% and 55.1%, respectively; placebo group, 20.3% and 49.6%, respectively), although > 50% of the patients received inhaled steroids during the trial. A univariate evaluation of steroid use by route of administration (ie, systemic, inhaled, or both) showed a significant relationship between presence of *S. maltophilia* (ie, yes/no) and the use of systemic steroids (*p* = 0.03 [χ² test]). Inhaled steroid use was not associated with *S. maltophilia* isolation.

A multiple regression analysis demonstrated that of the nine factors evaluated (ie, treatment group, gender, age, disease severity, baseline isolation of Aspergillus spp or *S. maltophilia*, concomitant use of oral quinolones, use IV antibiotics [ie, cephalosporins or penicillins], or use of systemic and/or inhaled steroids), only baseline *S. maltophilia* isolation and the use of oral quinolones at any time during the trial were significant predictors of isolation during the last month of the trial (ie, visits 10 and 11) [Table 3]. Patients from whom *S. maltophilia* was isolated during the screening period were nearly eight times more likely to have positive culture results at visits 10 and 11 (*p* < 0.0001). Similarly, the isolation of *S. maltophilia* was nearly three times as likely among patients who received oral quinolones at some point during the trials (*p* = 0.0015).

**Relationship Between Positive Culture Frequency and Improvement in Pulmonary Function**

Over the 6-month period of treatment, FEV₁ percent predicted improved among TSI-treated patients and decreased among those treated with pla-
cebo, regardless of the frequency of an \textit{S maltophilia}-positive culture. Improvements in pulmonary function were smaller among persistently culture-positive patients in the TSI arm of the study. However, there did not appear to be any correlation between isolation frequency and change in pulmonary function in either treatment group (Fig 4).

\begin{table}
\centering
\caption{Predictors of \textit{S maltophilia}-Positive Culture at Visits 10 and 11$^*$}
\begin{tabular}{llll}
\hline
Variable & OR & 95\% CI & \textit{p} Value \\
\hline
Treatment group & 1.02 & 0.6-1.7 & 0.95 \\
Gender & 1.3 & 0.8-2.2 & 0.28 \\
Age & 0.97 & 0.94-1.0 & 0.06 \\
Disease severity & 0.99 & 0.98-1.0 & 0.55 \\
Baseline Aspergillus & 0.94 & 0.5-1.8 & 0.85 \\
Systemic steroid use & 1.14 & 0.6-2.1 & 0.68 \\
Oral quinolone use & 2.7 & 1.5-5.1 & 0.0015 \\
IV antibiotic use$^\dagger$ & 0.97 & 0.56-1.7 & 0.91 \\
Pretreatment \textit{S maltophilia} isolation & 8.8 & 4.4-17.1 & <0.0001 \\
\hline
\end{tabular}
\label{tab:s-maltophilia-predictors}
\end{table}

\*OR = odds ratio, CI = confidence interval.
\daggerIncludes cephalosporins and penicillins.

\textit{S maltophilia} is an opportunistic pathogen that commonly infects the respiratory tract of patients with CF. The role that \textit{S maltophilia} plays in the pathophysiology of CF lung disease is uncertain, but in light of the resistance to the antibiotic agents commonly used to manage CF patients, the reported increase in the isolation of \textit{S maltophilia} from CF sputum$^{3,5-7}$ has become a cause for concern within the CF community. The current study was undertaken to determine whether inhaled tobramycin might play a role in the increased frequency of isolation of \textit{S maltophilia}.

During 6 months of treatment, the majority of specimens from patients in the TSI trials never produced a culture that was positive for \textit{S maltophilia}. Among those who had an \textit{S maltophilia}-positive culture at some point, most had positive cultures only intermittently; persistent infection was rare. Thus, the pattern of isolation shown in this study is similar to that described in previous reports.$^{4,5,10,16}$ The reported isolation rate from this study implies that the frequency of \textit{S maltophilia} isolation frequency.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{The mean relative change in FEV\textsubscript{1} percent predicted by \textit{S maltophilia} isolation frequency. Lung function improved in patients treated with TSI regardless of the frequency of \textit{S maltophilia}-positive cultures.}
\end{figure}
infection is increasing in the CF population. However, an analysis of the baseline data from the TSI trials suggested that under-reporting of this organism from CF respiratory samples may occur because of inadequate culture techniques. In addition, we observed that the frequency of S maltophilia isolation directly correlated with its density in sputum. This result is consistent with a recently published longitudinal analysis of S maltophilia epidemiology from a CF center in Madrid, Spain. A polymerase chain reaction (PCR) test with sensitivity and specificity approaching 100% has been developed for the detection of S maltophilia. We hypothesize that if this technology is applied to a larger population of CF patients, the reported prevalence might increase dramatically. However, whether this will be clinically relevant remains to be determined.

Previous reports have implicated the use of nebulized aminoglycosides, parenteral antipseudomonal antibiotics, oral quinolones, and systemic steroids as risk factors for subsequent S maltophilia isolation. In our regression analysis, however, neither TSI nor steroid use was associated with an increased risk of isolation, whereas patients who received oral quinolones during the study were nearly three times as likely to have S maltophilia isolated from their sputum. This is consistent with our finding that placebo patients, who received oral quinolones significantly more frequently (p = 0.003), also had a significantly greater incidence of S maltophilia isolation (p = 0.038). The underlying cause of this correlation is not apparent from the current analysis. It has been suggested that patients with more advanced lung disease are more susceptible to S maltophilia infection, and it is possible that therapy with oral ciprofloxacin is a surrogate marker for more advanced lung disease. However, our analyses failed to show that baseline lung function was a predictor of subsequent S maltophilia isolation and that fewer patients who were randomized to TSI received oral quinolones, despite similar degrees of lung disease. It is also possible that oral ciprofloxacin use selects for quinolone-resistant organisms, although the data presented here are not sufficient to determine causality.

Although a correlation between baseline S maltophilia isolation and the subsequent use of oral quinolones was observed, the relationship of these two variables is not obvious. For instance, it is unlikely that the patients in this study were quinolone-naïve at enrollment, and it is not clear to what extent prior quinolone use may have contributed to positive S maltophilia culture at baseline. Following randomization, differences in oral quinolone use may have led to the observed differences between treatment groups in emergent S maltophilia isolation at the end of the study.

In contrast to the findings for quinolone antibiotics, we found that two other broad classes of antibiotics (ie, cephalosporins and penicillins) did not increase the risk of S maltophilia isolation. Unfortunately, the small number of patients using carbapenem antibiotics in these trials precluded statistical analysis. Thus, the determination of the role of these agents in predisposing patients to S maltophilia infection awaits study in a larger group of patients. Perhaps the CF registry data might provide sufficient numbers to allow for such a statistical analysis. One question that arises when considering the large proportion of intermittently culture-positive patients is whether this pattern of isolation is due to eradication followed by reinfection or to methodological factors whereby the organism is present continually but remains undetected. Newer and more sensitive methods of detection, such as PCR, may help to answer this question. As part of an independent analysis, the genotypes of S maltophilia that were isolated from nine patients with a culture frequency of ≤ 25% were determined using randomly amplified polymorphic DNA (RAPD). That analysis demonstrated that specimens from five of the nine intermittently culture-positive patients cultured S maltophilia with different genotypes at different times, while specimens from four patients cultured organisms with the same genotype at separate visits. Of the latter four patients, a distinction between reinfection with an isolate of the same genotype from an environmental source and continuous low level infection with the same isolate cannot be made. However, it appears that a substantial portion of intermittently culture-positive patients in the trial were transiently reinfected with different isolates at different visits and, thus, were not continuously colonized with S maltophilia. It is also possible that the culture-positive patients were being infected with multiple genotypes of S maltophilia and that we isolate different genotypes as a result of our culturing techniques. Again, results from this multicenter cross-sectional analysis are strikingly similar to recently published longitudinal data from a Madrid CF center, in which 5 of 11 patients who had experienced repeated episodes of S maltophilia isolation showed genotypic variation in isolates over time. As a result, it is unclear whether any of the factors identified as risks for S maltophilia isolation do, in fact, predispose patients to colonization/infection, or whether colonization is a common, transient, and recurring phenomenon among CF patients.

Our finding that the clinical response to TSI therapy was reduced in patients who were persistently colonized with S maltophilia supports the
findings of Ballestero et al. Nonetheless, in the placebo-controlled trials, there was still a mean improvement in pulmonary function for the TSI-treated group compared to the placebo group, regardless of the frequency of S maltophilia isolation. Interestingly, the greatest improvement in FEV₁ occurred in patients in the TSI-treated group who were intermittently infected. Although the mean FEV₁ improvement among persistently infected patients was small, it was still greater among TSI patients than among patients in the placebo group. Whether this is an effect on bacterial flora (P aeruginosa and/or S maltophilia) in the CF lung remains to be determined.

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REFERENCES