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ORIGINAL ARTICLE

Effectiveness of a multistep *Pseudomonas aeruginosa* eradication treatment protocol in children with cystic fibrosis in Brazil

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ABSTRACT

Objective: Although various strategies have been proposed for eradicating *Pseudomonas aeruginosa* in patients with cystic fibrosis (CF), only a few employ multistep treatment in children colonized by that pathogen for the first time. The aim of this study was to describe the effectiveness of a three-phase eradication protocol, initiated after the first isolation of *P. aeruginosa*, in children with CF in Brazil. **Methods:** This was a retrospective real-life study in which we reviewed the medical records of pediatric CF patients in whom the eradication protocol was applied between June of 2004 and December of 2012. The three-phase protocol was guided by positive cultures for *P. aeruginosa* in airway secretions, and the treatment consisted of inhaled colistimethate and oral ciprofloxacin. Success rates were assessed after each phase, as well as cumulatively. **Results:** During the study period, 47 episodes of *P. aeruginosa* colonization, in 29 patients, were eligible for eradication. Among the 29 patients, the median age was 2.7 years, 17 (59%) were male, and 19 (65%) had at least one *F508del* allele. All 29 patients completed the first phase of the protocol, whereas only 12 and 6 completed the second and third phases, respectively. Success rates for eradication in the three treatment phases were 58.6% (95% CI: 40.7-74.5), 50.0% (95% CI: 25.4-74.6), and 66.7% (95% CI: 30.0-90.3), respectively. The cumulative success rate was 93.1% (95% CI: 78.0-98.1). Treatment failure in all three phases occurred in only 2 patients. **Conclusions:** In this sample of patients, the multistep eradication protocol was effective and had a high success rate.

Keywords: Cystic fibrosis/therapy; Cystic fibrosis/prevention & control; Pseudomonas aeruginosa; Treatment outcome.

INTRODUCTION

Chronic pulmonary infection with *Pseudomonas aeruginosa* is associated with high morbidity and mortality in patients with cystic fibrosis (CF).[1] *P. aeruginosa* is the most prevalent pathogen causing severe lung disease in patients with CF.[2] *P. aeruginosa* infection can occur very early in life,[1,3-5] the risk of death being 2.6 times higher in CF patients infected with *P. aeruginosa* in the first 5 years of life.[6] Once chronic infection is established, *P. aeruginosa* is virtually impossible to eradicate; therefore, early intervention is mandatory.[1,7]

Several antibiotic strategies for *P. aeruginosa* eradication have been proposed in recent years.[8] Most include inhaled antibiotic therapy in isolation or in combination with systemic antibiotic therapy, and multistep eradication approaches are rarely used. In addition, despite evidence that early eradication of *P. aeruginosa* is beneficial, there are few reports of CF patients receiving eradication treatment upon first isolation of *P. aeruginosa*. Most studies include CF patients with a prior positive airway culture for *P. aeruginosa*.[8] It should be noted that there is currently no standard eradication treatment or evidence that a particular treatment is superior to other treatments.[7]

In 2004, the CF center of the University of São Paulo School of Medicine Hospital das Clínicas Institute for Children, located in the city of São Paulo, Brazil, adopted a multistep *P. aeruginosa* eradication protocol guided by positive cultures for *P. aeruginosa* in airway secretions routinely collected from patients (Figure 1). It is expected that the use of systematic treatment protocols can eradicate initial colonization of the respiratory tract by *P. aeruginosa* in CF patients. A multistep approach is used in order to increase the chance of eradication in cases of new *P. aeruginosa* colonization, through long-term treatment for persistent or early recurrent infection. The objective of the present study was to describe the effectiveness of the multistep eradication protocol used in our institution.

METHODS

Study design

This was a real-life retrospective cohort study with prospective outcome assessment. Data were collected from the medical records of pediatric patients (under 18 years of age) followed at the CF Outpatient Clinic of the University of São Paulo School of Medicine *Hospital das*

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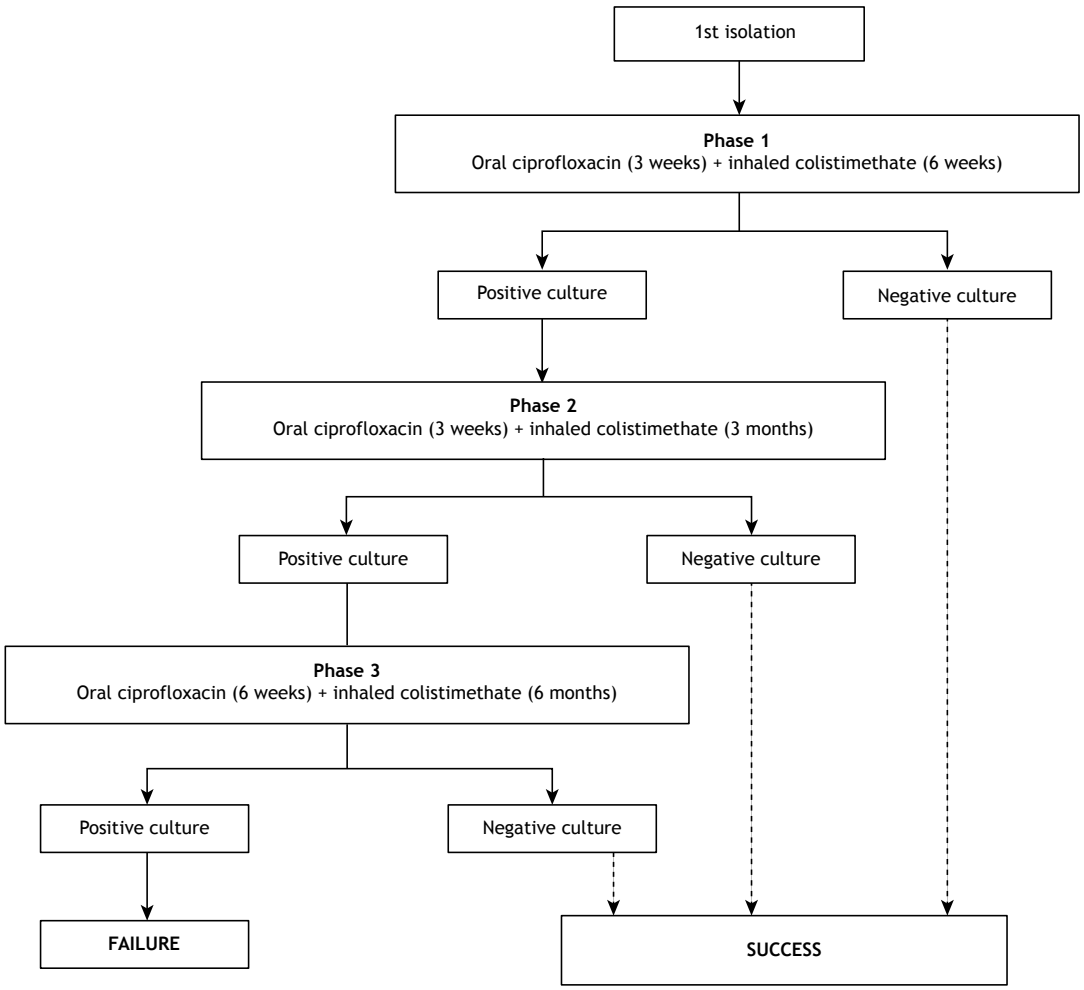

Figure 1. Multistep *Pseudomonas aeruginosa* eradication protocol. Oral ciprofloxacin (25-50 mg/kg/day up to a maximum of 1,500 mg/day, 12/12 h). Inhaled colistimethate (1,000,000 IU, 12/12 h). Patients with pulmonary exacerbations requiring hospitalization received intravenous systemic antibiotics (ceftazidime, 150 mg/kg/day up to a maximum of 9 g/day, 8/8 h, and amikacin, 20 mg/kg/day, 24/24 h), without changes in the inhaled antibiotic regimen.

Clínicas Institute for Children between June of 2004 and December of 2012. The inclusion criteria were as follows: having undergone at least four cultures of airway secretions (oropharyngeal secretions, sputum, or bronchoalveolar lavage fluid) over a 12-month period; having acquired *P. aeruginosa* for the first time; and having undergone our multistep eradication protocol (Figure 2). The study was approved by the Research Ethics Committee of the University of São Paulo School of Medicine *Hospital das Clínicas* (Ruling no. 1175/24/2014).

Multistep eradication protocol

Figure 1 shows a flow chart of our multistep *P. aeruginosa* eradication protocol. Patients with first *P. aeruginosa* isolation were eligible for eradication treatment. Patients were instructed to undergo culture of airway secretions 1-2 weeks after each treatment phase. Patients with a positive culture for *P. aeruginosa* moved on to the second phase of treatment. Those with a positive culture for *P. aeruginosa* after the second phase of treatment moved on to the third. Cases of patients with a negative culture for *P. aeruginosa* after the third phase of treatment were considered to be cases of successful eradication, whereas those of those with a positive culture were considered to be cases of eradication failure.

Variables and outcomes

The efficacy of the eradication protocol was evaluated on the basis of culture results after each phase of treatment, a negative culture for *P. aeruginosa* indicating treatment success and a positive culture for *P. aeruginosa* indicating treatment failure.

Clinical and epidemiological data were collected from medical records during routine patient visits. The body mass index was calculated and converted to a z-score by the WHO Anthro Survey Analyser (World Health Organization, Geneva, Switzerland) for children ≤ 5 years of age or by the WHO AnthroPlus software for those > 5 years of age.[9] The Shwachman-Kulczycki score[10] assesses disease severity by scores for physical

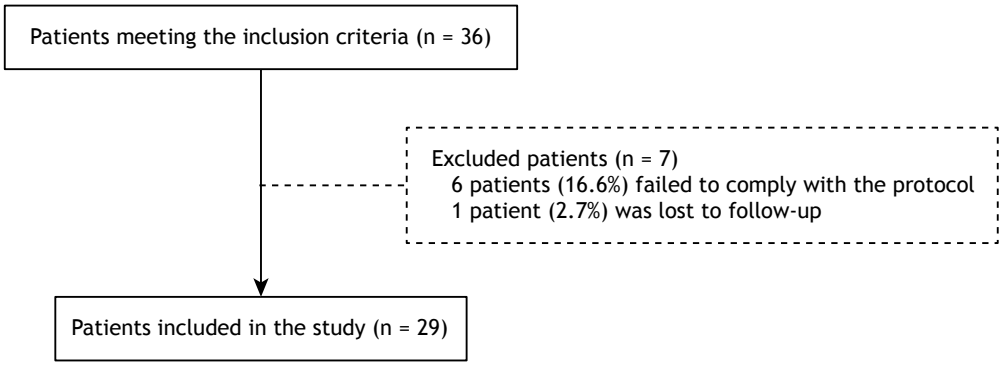

Figure 2. Flow chart of the sample selection process. Pa: *Pseudomonas aeruginosa*.

activity, changes in pulmonary propedeutics, nutritional status, and chest X-ray findings, being measured annually in accordance with the institutional protocol.

Statistical analysis

Success rates were assessed after each phase, as well as cumulatively, by dividing the number of negative cultures by the number of patients treated in each phase. Descriptive statistics were calculated, including measures of central tendency, measures of dispersion (interquartile range—IQR—and 95% CI), and proportions.

RESULTS

Twenty-nine patients were included in the study. Their clinical and epidemiological characteristics are described in Table 1. It is noteworthy that the first colonization by *P. aeruginosa* occurred in preschoolers, most being male.

During the 8.5-year study period, 47 episodes of *P. aeruginosa* colonization, in 29 patients, were eligible for eradication. Of those 47 episodes, 13 (27.7%) were accompanied by pulmonary exacerbations, with hospitalization and intravenous antibiotics being required in only 5.

Of the 47 episodes of *P. aeruginosa* isolation eligible for eradication, 87.2% were episodes of colonization with nonmucoid strains. The mean number of cultures of airway secretions over the course of 12 months was 7 ± 2, and most (75%) of the cultures were cultures of oropharyngeal secretions. The median time elapsed between the end of eradication treatment phase 1 and culture of airway secretions was 16 days (IQR: 6-28 days). The median time elapsed between the end of eradication treatment phase 2 and culture of airway secretions was 35 days (IQR: 14-43 days). The median time elapsed between the end of eradication treatment phase 3 and culture of airway secretions was 43 days (IQR: 31-63 days).

All of the patients included in the present study completed the first phase of the eradication protocol. Eradication was successful in 17, whereas 12 moved on to the second phase of treatment because of a positive culture for *P. aeruginosa* after the first phase. Of those 12 patients, 6 achieved eradication and 6 moved on to the third phase of treatment. Of those 6 patients, 4 achieved eradication and 2 did not (Figure 3). Success rates for eradication in the three treatment phases were 58.6% (95% CI: 40.7-74.5), 50.0% (95% CI: 25.4-74.6), and 66.7% (95% CI: 30.0-90.3), respectively. The cumulative success rate was 93.1% (95% CI: 78.0-98.1).

DISCUSSION

To our knowledge, ours is the first study to evaluate a *P. aeruginosa* eradication protocol in pediatric CF patients in Brazil. Our multistep eradication protocol in pediatric CF patients in Brazil. Our multistep eradication protocol was found to be effective in a real-life setting, with a high (93%) success rate. This finding shows that a multistep approach is a therapeutic strategy that can increase the success rate of eradication. However, the success rate after the first phase of treatment was approximately 60%, which is lower than that recently reported in the literature.[4,5,11] The reason for this is unknown.

In contrast to most eradication protocols, ours was initiated after the first isolation of *P. aeruginosa*. Other studies have included pediatric CF patients previously infected with *P. aeruginosa* and generally older than those in our sample.[1,3,6,7,10-12] A minority received intravenous antibiotic therapy for pulmonary exacerbation at the time of *P. aeruginosa* eradication. We believe that this had no impact on treatment efficacy, given that there is no evidence in the literature that parenteral treatment increases the success of *P. aeruginosa* eradication.[7] The patients included in our study were younger than those in other studies; their median age was 2.7 years, whereas, in other studies, mean ages were 5.5 years[11] and 9 years.[4] This might explain the high success rate in our study, given that structural airway disease is less severe in younger populations. This shows the need for early therapeutic intervention to maximize the chance of success.

Although eradication of *P. aeruginosa* is a well-established treatment, there is insufficient evidence regarding the best approach.[13] There are few studies evaluating the effectiveness of multistep eradication

Table 1. Clinical and laboratory characteristics of patients in each treatment phase.[a]

Parameter	Phase 1 (n = 29)	Phase 2 (n = 12)	Phase 3 (n = 6)
Female sex	12 (41.4)	8 (66.7)	4 (66.7)
Age, years	2.7 (0.9-5.3)	3.6 (1.9-5.8)	3.8 (2.6-4.5)
BMI, z-score	−0.4 (−1.0 to −0.7)	−0.4 (−0.8 to −0.9)	−1.2 (−1.6 to −1.1)
Genotype, %			
Heterozygous *F508del*	13 (44.8)	4 (33.3)	2 (33.3)
Homozygous *F508del*	6 (20.7)	4 (33.3)	1 (16.7)
Other mutations	10 (34.5)	4 (33.3)	3 (50.0)
Shwachman-Kulczycki score	85.0 (85.0-90.0)	77.5 (72.5-90.0)	72.5 (60.0-82.5)
Pulmonary exacerbation of CF	9 (31.0)	4 (33.3)	1 (16.7)
Coinfection			
Staphylococcus aureus	8 (27.6)	5 (41.7)	2 (33.3)
MRSA	1 (3.4)	1 (8.3)	0 (0.0)
Burkholderia cepacia complex	1 (3.4)	2 (16.7)	0 (0.0)
Pancreatic status			
Pancreatic insufficiency	26 (86.2)	11 (91.7)	5 (83.3)
Use of inhaled medications			
3% NaCl	1 (3.4)	0 (0.0)	0 (0.0)
Dornase alfa	3 (10.3)	1 (8.3)	0 (0.0)
Short-acting β_2 agonist	4 (13.8)	1 (8.3)	1 (16.7)

BMI: body mass index; CF: cystic fibrosis; and MRSA: methicillin-resistant *Staphylococcus aureus*. [a]Values expressed as n (%) or median (interquartile range).

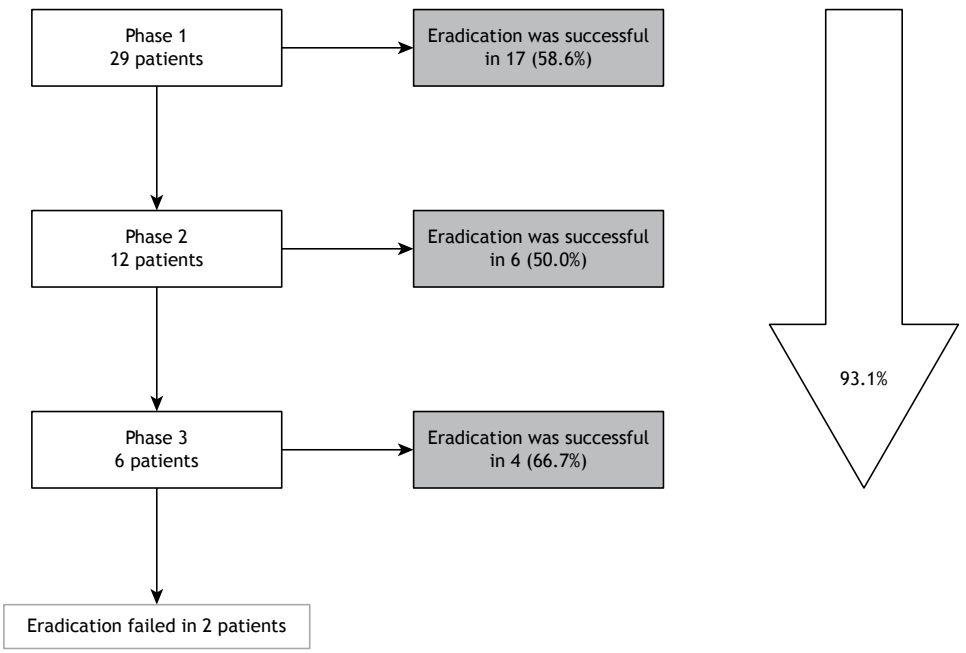

Figure 3. Description of the effectiveness of the eradication protocol (cumulative success rate, 93.1%; 95% CI: 78.0-98.1).

approaches. A CF center in Denmark was the first to publish the results of implementing a multistep protocol for early treatment of *P. aeruginosa* colonization. Patients were treated with nebulized colistimethate and oral ciprofloxacin; drug dose and treatment duration varied across treatment phases, with a maximum duration of 3 months, resulting in a success rate of 78% and improved lung function in the treated patients.[6] In a study conducted in Italy,[14] 173 patients received a multistep eradication treatment with nebulized colistimethate and oral ciprofloxacin for 3 weeks in the first phase. In the second phase, patients received double the dose of colistimethate, which was used for 3 months in those who moved on to the third phase of treatment. The success rate was 81%, with a median time of 18 months between eradication and new *P. aeruginosa* acquisition.[14] In a recently published study conducted at a CF center in

Canada, the effectiveness of a multistep eradication treatment protocol was evaluated.[4] The protocol was based on culture results and the presence of symptoms, with up to three phases of treatment with nebulized tobramycin for 28 days, with or without an intravenous antibiotic. The cumulative success rate was 88%.[4] These results, as well as those of the present study, show that a multistep eradication protocol for *P. aeruginosa* has a high success rate.

Although nebulized colistimethate is commonly used in combination with oral ciprofloxacin for *P. aeruginosa* eradication,[10] the role that nebulized antibiotic alone plays in eradicating *P. aeruginosa* has recently been highlighted, and there is a trend toward reducing the duration of eradication therapy.[7] A large, four-arm randomized clinical trial conducted in the USA was published in 2011,[11] involving 304 CF patients (mean age, 5.5 years) and lasting 18 months. Patients were divided into two groups: one group received cycled therapy every 3 months, regardless of follow-up culture results, and the other received culture-based therapy. The two groups of patients received eradication therapy with nebulized tobramycin for 28 days, with oral ciprofloxacin or oral placebo for 14 days. No difference in the rate of exacerbation or prevalence of *P. aeruginosa* positivity was detected between the two groups. According to the authors, the addition of oral ciprofloxacin produced no benefits and therapy should be guided by culture results.[11] In a study comparing the efficacy and safety of 28 days and 56 days of nebulized tobramycin for the treatment of early onset *P. aeruginosa* infection in CF patients,[5] it was shown that 93% and 92% of the patients were free of *P. aeruginosa* infection 1 month after the end of treatment for 28 days and 56 days, respectively, and that the median time to recurrence of *P. aeruginosa* was similar between the two groups (6-9 months vs. 9-12 months). The authors found that treatment with nebulized tobramycin for 56 days was not superior to treatment with nebulized tobramycin for 28 days.[5] These findings support the use of inhaled antibiotics for 28 days and suggest that the use of an inhaled antibiotic in combination with ciprofloxacin be considered on a case-by-case basis (e.g., in patients with pulmonary exacerbations and first-ever isolation of *P. aeruginosa*).

In European countries, colistimethate is the most commonly used inhaled antibiotic therapy for *P. aeruginosa* eradication,[6,7,13,15] whereas, in North America, tobramycin is.[4,5,11] In a multicenter randomized controlled clinical trial conducted at a CF center in Belgium and including more than 200 patients, eradication therapy with nebulized tobramycin + oral ciprofloxacin was compared with eradication therapy with nebulized colistimethate + oral ciprofloxacin for 28 days, treatment success rates being similar between the two strategies (63% and 65%, respectively).[3] In a study including 105 CF patients and comparing nebulized tobramycin for 28 days with nebulized colistimethate + oral ciprofloxacin for 3 months, the two eradication regimens were shown to be equivalent (eradication rate, 80% vs. 90%).[1] The Brazilian guidelines for the diagnosis and treatment of CF recommend the use of inhaled tobramycin for 28 days as the treatment of first choice, inhaled colistimethate with oral ciprofloxacin for 2-3 weeks being recommended as an alternative treatment option.[16] Therefore, the two inhaled antibiotic therapies are effective in eradicating *P. aeruginosa*, with the choice being based on drug availability and patient tolerance.

The limitations of our study include the retrospective design based on medical record review, which precludes the analysis of data on treatment adherence and correct medication use. However, this probably had little impact on our results, because the eradication rate was high. Another limitation is that lung function was not assessed given the young age of the study population, although it would have shown interesting data. Despite this limitation, the study included patients with first *P. aeruginosa* isolation, thus producing a sample that was more homogeneous and providing important real-life information.

The criterion for successful eradication in the present study was a negative culture for *P. aeruginosa* after the end of each treatment phase. It is known that the primary goal of eradication treatment is to achieve a sustained response (i.e., at least 6 months without *P. aeruginosa* isolation). However, this was not evaluated in our study, meaning that the eradication success rate might have been overestimated.

In conclusion, our multistep *P. aeruginosa* eradication protocol appears to be effective in very young CF patients with first *P. aeruginosa* isolation, with a success rate of 93%. Although many studies have described one-step eradication protocols, the present study demonstrated in a real-life setting that a multistep eradication protocol is effective and sometimes necessary. Larger, long-term multicenter studies with comparator arms evaluating first *P. aeruginosa* colonization are needed in order to identify the best treatment regimen for *P. aeruginosa* eradication and the best intervention in cases of failure.

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