Eradication of Burkholderia cepacia complex in cystic fibrosis patients with inhalation of amiloride and tobramycin combined with oral cotrimoxazole

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To the Editor:

The primary cause of morbidity and mortality among patients with cystic fibrosis is respiratory failure resulting from chronic pulmonary inflammation and infection. The *Burkholderia cepacia* complex (BCC) is the collective name for a group of at least 21 genetically related bacteria that can cause pulmonary infection in cystic fibrosis [1]. Between 85% and 97% of BCC infections in cystic fibrpsos patients are caused by the genomovars *Burkholderia multivorans* and *Burkholderia cenocepacia* [1]. Although BCC causes infections in only about 2–4% of cystic fibrosis patients worldwide, they are of particular concern as they are often highly antibiotic resistant and notoriously difficult to treat [1].

BCC is associated with a heterogeneous clinical course, ranging from transient infection to rapidly progressive pneumonia with fulminant respiratory failure known as "cepacia syndrome" [2]. Most infected cystic fibrosis patients will develop chronic BCC infection, which is associated with a more rapid decline in lung function, leading to higher morbidity and mortality [3, 4]. Hypothetically, as with *Pseudomonas aeruginosa*, timely eradication of BCC may therefore prevent lung function decline and improve quality of life.

No consensus exists on the best treatment regimen for eradicating BCC infection. A 2019 Cochrane systematic review found no single randomised trial investigating BCC eradication, only case series and case reports [5]. The most often used treatment regimens include a combination of nebulised, oral and intravenous antibiotics requiring hospital admission [6, 7]. In 2005, a treatment regimen consisting of inhaled amiloride, a sodium-channel blocker, followed by tobramycin inhalation was reported by Middleton *et al.* [8] to be successful in three out of four adult cystic fibrosis patients. However, there is no confirmation if this well-tolerated treatment regimen is successful at eradicating BCC infections in children with cystic fibrosis.

The present retrospective study describes a case series of first or new BCC isolations from 2000 to 2020 in cystic fibrosis patients from the cystic fibrosis centre of the University Medical Center Groningen (UMCG) in the Netherlands. The previously mentioned eradication strategy was used from 2005, consisting of nebulised amiloride followed by tobramycin inhalation, but now with addition of oral trimethoprim/sulfamethoxazole. Despite *Burkholderia* spp. being resistant to tobramycin *in vitro*, local concentrations of tobramycin upon inhalation may well exceed the minimum inhibitory concentration, which may be enhanced by a possible synergistic effect with amiloride [9]. The rationale for adding oral cotrimoxazole is to also give systemic antibiotics to which the BCC is sensitive, providing antimicrobial action in areas where nebulisation does not penetrate well.

Inclusion and exclusion criteria are reported in table 1. As the focus was on incident BCC cases, patients were included more than once if they had more than one BCC infection during the study period and if they were declared free from BCC, whereby all sputum cultures in the 6 months after isolation had to be negative. After at least one positive sputum culture for BCC, treatment with inhaled amiloride and tobramycin was started combined with oral cotrimoxazole, all given for 1 month. All sputum cultures after

Shareable abstract (@ERSpublications)
This case series suggests that successful eradication therapy of BCC in cystic fibrosis can be done with a combination of inhaled and oral medication, which in many cases may eliminate the need for intensive treatment with intravenous antibiotics https://bit.ly/40oOMIn

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TABLE 1 Clinical characteristics

	Burkholderia isolation[#]
Gender	
Female	3 (42.9)
Male	4 (57.1)
Age, years	11.7 (9.0–19.6)
BMI, kg·m^{-2}	17.1 (15.5–20.1)
***CFTR* mutation**	
Homozygote Phe508del	4 (57.1)
Heterozygote Phe508del	3 (42.9)
Other	0
Comorbidities	
Cystic fibrosis-related diabetes	0
Cystic fibrosis-related liver disease	4 (57.1)
Pancreatic insufficiency	7 (100.0)
Osteoporosis	0
Forced expiratory volume in 1 s, % pred	82.7±20.7 (58–107)
Forced expiratory volume in 1 s, L	2.0±0.6 (1.2–3.1)
BCC infection initial/new[¶]	6/1
Exacerbation during first isolation	5
Follow-up after eradication, years, median (range)	1.5 (0.6–7.2)
Coinfection with pathogens	
Staphylococcus aureus	7 (100.0)
Haemophilus influenzae	5 (71.4)
Streptococcus pneumoniae	1 (14.3)
Aspergillus spp.	4 (57.1)
Pseudomonas aeruginosa	1 (14.3)
Acinetobacter spp.	0
Stenotrophomonas maltophilia	0
Nontuberculosis mycobacteria	0
BCC antibiotic resistance	
Tobramycin	7
Trimethoprim/sulfamethoxazole	0
Comedication in year after isolation	
CFTR-modulator therapy	0
Extra course of trimethoprim/sulfamethoxazole during exacerbation in the 6 months after BCC isolation	2
Tobramycin nebulisation for 1 month for eradication therapy for *P. aeruginosa*	1
Sputum samples	
Positive for BCC	10
Total sputum samples	60

Data are presented as n (%), mean (range), n or mean±SD (range), unless otherwise stated. Inclusion criteria: patients diagnosed with cystic fibrosis with clinical signs consistent with cystic fibrosis and sweat chloride >60 mEq·L^{-1} and/or two cystic fibrosis-causing mutations identified; an initial or new *Burkholderia cepacia* complex (BCC) isolation from sputum cultures (or pharyngeal swabs and bronchoalveolar lavage) during the study period, treated with inhalation therapy with amiloride and tobramycin, combined with oral cotrimoxazole; and multiple sputum cultures after the end of treatment. Treatment regimen consisted of nebulised amiloride (produced in the hospital pharmacy, with 1 mL amiloride 0.45% solution dissolved in 5 mL 0.9% saline, given thrice daily) followed by tobramycin inhalation (300 mg twice daily) but now with addition of oral trimethoprim/sulfamethoxazole (cotrimoxazole; 12/60 mg·kg^{-1}·day^{-1}). Exclusion criteria: chronic BCC infection; lung transplantation before BCC isolation; incomplete exposure and/or outcome data. During the study period, a total of 18 cystic fibrosis patients had at least one positive culture for BCC, of which 12 patients did not meet the inclusion criteria for this case series: six were already chronically infected (three with *Burkholderia multocida*, two with *Burkholderia multivorans* and one not otherwise specified); two received treatment other than the study therapy (both *B. multivorans*; both developed chronic BCC infection); and two were not treated at the patient's wish due to pregnancy (one with *B. multocida* and one with *B. multivorans*; both developed chronic BCC infection). Two patients were not treated because they had only one positive culture, were in good clinical condition and had repeat cultures that were negative (one with *Burkholderia cenocepacia* and one not otherwise specified). BMI: body mass index; CFTR: cystic fibrosis transmembrane conductance regulator. [#]: n=7. [¶]: defined as an initial BCC isolation when the patient had never been infected with BCC prior to this BCC isolation; for a new BCC isolation, patients had to be free of BCC, defined as a BCC isolation in the past with all negative sputum cultures in the last 6 months.

treatment were evaluated. The primary outcome was BCC eradication, defined as no re-isolation of BCC in the 6 months after eradication (minimum three sputum cultures). The Medical Ethics Committee of the UMCG granted a waiver (METc2021/185), as they concluded that this study was not subject to the Medical Research Involving Human Subjects Act (WMO). All patients/caregivers gave written informed consent.

In total, seven initial or new BCC isolations were found in six patients (five children and one adult). Determination of these isolations showed four with *B. multivorans*, two with *Burkholderia anthina* and one with *Burkholderia vietnamiensis*. Table 1 shows the clinical characteristics. In four isolations, treatment was started after only one positive culture. The other three isolations consisted of two positive cultures (taken 1–2 months apart). Six (85.7%) out of seven BCC isolations were successfully eradicated according to our predetermined definition. For one patient, eradication failed (*B. multivorans*), also after a second eradication attempt with this same treatment regimen, only now given for 2 months. No intravenous eradication attempt was made; this patient developed chronic BCC infection. One patient had a new BCC isolation 10 months after what had been indicated as a successful eradication. DNA fingerprinting later revealed that it was the same strain of *B. anthina*. This patient was re-treated with the same regimen. Hereafter, eradication was achieved and for 7 years at the time of writing, sputum cultures have been negative for BCC. For the other four patients, the sputum cultures did not show a re-infection until the end of the study, with a median follow-up of 1.5 years after eradication.

From all patients with successful eradication, sputum cultures were available at least 1, 2, 3 and 6 months after treatment, with minimally eight cultures in the year following treatment. In two patients who could not produce sputum samples spontaneously, a bronchoscopy was performed (both 4 months after treatment).

The results of our study are in line with the results of the study by Middleton *et al.* [8] in which this treatment strategy resulted in BCC eradication in three out of four cystic fibrosis patients. Thus, eradication of BCC can be achieved using a combination of inhaled and oral medication, without intravenous antibiotics. This is of relevance as intravenous tobramycin can lead to systemic toxicity, such as ototoxicity and nephrotoxicity, which is uncommon in inhalation therapy. In a 6-month trial of inhaled tobramycin and amiloride in cystic fibrosis patients, no adverse effects were found [10]. In this case series, no treatment-related adverse events were recorded either.

Amiloride is a potassium-sparing diuretic, acting through blockage of sodium channels. Although initially proposed to be a mucolytic [11, 12], amiloride may also have antimicrobial activity, at least *in vitro*. Furthermore, there is antimicrobial synergy with tobramycin, which is still not completely understood. Cohn and Aronoff [13] demonstrated that extracellular sodium inhibits the antibacterial effect of tobramycin against BCC but that this was reversed with amiloride, suggesting an effect of amiloride on the protective mechanism of the BCC.

Our study has some strengths and limitations. This is the largest case series and the first independent (modified) replication of the results reported by Middleton *et al.* [8]. Using our microbiology registry, we could track all BCC infections of the previous 20 years. However, the number of isolations is small. Furthermore, a control group is lacking. This is of importance since there is no consensus about the definitions of colonisation and eradication of BCC. Two retrospective studies found that around 20–39% of newly acquired BCC infections were transient, defining colonisation as one positive sputum culture and eradication as three consecutive negative sputum cultures over $\geqslant$1 year [3, 14]. Within these BCC isolations, they also reported differences in the incidence of transient infection between the different strains of BCC, with 6–19% spontaneous resolution with *B. cenocepacia* but 50–55% spontaneous resolution with *B. multivorans*. Since we used the definition of colonisation with only one positive sputum culture, it may be argued that infection could have resolved spontaneously in at least the patients who were treated after only one positive sputum sample. Nonetheless, our eradication percentages are higher than was reported for spontaneous resolution of BCC species. Furthermore, none of the treated cases had *B. cenocepacia*, which is regarded as the most virulent and challenging BCC species.

In conclusion, this case series underlines that eradication therapy of BCC does not always have to consist of intensive treatment with intravenous antibiotics, but that treatment with a combination of inhaled amiloride and tobramycin, with addition of oral trimethroprim/sulfamethoxazole, can also be used. Although this current study adds to the available evidence, there is still an urgent need for a randomised controlled trial to investigate the optimal therapy for the eradication of BCC.

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References

1 Drevinek P, Mahenthiralingam E. *Burkholderia cenocepacia* in cystic fibrosis: epidemiology and molecular mechanisms of virulence. *Clin Microbiol Infect* 2010; 16: 821–830.
2 Courtney JM, Dunbar KE, McDowell A, *et al.* Clinical outcome of *Burkholderia cepacia* complex infection in cystic fibrosis adults. *J Cyst Fibros* 2004; 3: 93–98.
3 Jones AM, Dodd ME, Govan JRW, *et al. Burkholderia cenocepacia* and *Burkholderia multivorans*: influence on survival in cystic fibrosis. *Thorax* 2004; 59: 948–951.
4 Ledson MJ, Gallagher MJ, Jackson M, *et al.* Outcome of *Burkholderia cepacia* colonisation in an adult cystic fibrosis centre. *Thorax* 2002; 57: 142–145.
5 Regan KH, Bhatt J. Eradication therapy for *Burkholderia cepacia* complex in people with cystic fibrosis. *Cochrane Database Syst Rev* 2019; 4: CD009876.
6 Garcia BA, Carden JL, Goodwin DL, *et al.* Implementation of a successful eradication protocol for *Burkholderia cepacia* complex in cystic fibrosis patients. *Pulm Med* 2018; 18: 35.
7 Kitt H, Lenney W, Gilchrist FJ. Two case reports of the successful eradication of new isolated of *Burkholderia cepacia* complex in children with cystic fibrosis. *Pharm Tox* 2016; 17: 14.
8 Middleton PG, Kidd TJ, Williams B. Combination aerosol therapy to treat *Burkholderia cepacia* complex. *Eur Respir J* 2005; 26: 305–308.
9 Cohn RC, Rudzienski L, Putnam RW. *In vitro* antimicrobial activity of amiloride analogs against *Pseudomonas*. *Chemotherapy* 1992; 38: 232–237.
10 Uluer AZ, Waltz DA, Kalish LA, *et al.* Inhaled amiloride and tobramycin solutions fail to eradicate *Burkholderia dolosa* in patients with cystic fibrosis. *J Cyst Fibros* 2013; 12: 54–59.
11 App EM, Kind M, Helfesrieder R, *et al.* Acute and long-term amiloride inhalation in cystic fibrosis lung disease. A rational approach to cystic fibrosis therapy. *AmRev Respir Dis* 1990; 141: 605–612.
12 Pons G, Marchand MC, d'Athis P. French multicenter randomized double-blind placebo-controlled trial on nebulized amiloride in cystic fibrosis patients. The Amiloride-AFLM Collaborative Study Group. *Pediatr Pulmonol* 2000; 30: 25–31.
13 Cohn RC, Aronoff SC. The effect of sodium on amiloride-tobramycin synergy in *Pseudomonas cepacian*. *J Lab Clin Med* 1989; 114: 724–727.
14 Zlosnik JEA, Zhou G, Brant R, *et al. Burkholderia* species infections in patients with cystic fibrosis in British Columbia, Canada. 30 years' experience. *Ann Am Thorac Soc* 2015; 12: 70–78.