Sociedade Brasileira de Infectologia

The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Letter to the Editor

Can the data of microbiome be used to predict the presence of *Burkholderia* spp in pulmonary microbiota of cystic fibrosis patients?

Fabiana Caroline Zempulski Volpato [a,b,*], *Otávio von Ameln Lovison* [b,c,d], *Daiana de Lima-Morales* [b], *Andreza Francisco Martins* [b,c,d], *Paulo José Cauduro Maróstica* [e], *Afonso Luís Barth* [a,b,c]

[a] *Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências Médicas, Porto Alegre, RS, Brazil*
[b] *Hospital de Clínicas de Porto Alegre, Laboratório de Pesquisa em Resistência Bacteriana (LABRESIS), Porto Alegre, RS, Brazil*
[c] *Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências Farmacêuticas, Porto Alegre, RS, Brazil*
[d] *Hospital de Clínicas de Porto Alegre, Bioinformatics Core, Porto Alegre, RS, Brazil*
[e] *Universidade Federal do Rio Grande do Sul, Programa de Pós-graduação em Saúde da Criança e do Adolescente, Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Received 9 February 2023
Accepted 22 April 2023
Available online 5 May 2023

Dear editor,

There are several species of the genus *Burkholderia*, being the members of *Burkholderia cepacia* complex (Bcc) the main pathogens related to clinical infections in patients with cystic fibrosis (CF).[1] Infection/colonization by Bcc in the airway is a cause of concern in CF as it may lead to a necrotizing pneumonia and clinical deterioration of individuals.[1,2] Bcc species are often transmitted among CF patients therefore, the detection of these pathogens in the airways is relevant information to be considered for segregation of patients.[1] The detection of microbial pathogens in the CF airways is traditionally performed by bacteriological culture[3] but this method requires a quantity of viable bacterial cells in the clinical specimen. In contrast, culture independent methods, such as microbiome analysis which is based on Nucleic Acid Sequencing (16S rRNA), allows the identification of bacterial communities without the need of conventional culture.[3]

As a part of a more comprehensive study of microbiome analysis of sputum from CF patients; we conducted this study in order to compare the detection of the genus *Burkholderia* in the CF sputum by microbiome analysis and by the bacteriological culture.

A total of 22 sputa collected between July 2019 to March 2020 for routine bacteriological culture of 9 patients (5–18 years old) from a CF reference hospital in southern Brazil, were submitted to microbiome sequencing. Patients had

* *Corresponding author.*
E-mail address: fabiana_volpato@yahoo.com.br (F.C.Z. Volpato).

https://doi.org/10.1016/j.bjid.2023.102777

Table 1 – Comparison between the results of the bacteriological culture and microbiome analysis of 27 sputa from 9 CF patients considering the genus *Burkholderia*.

Patient	Age[a]	Specimen identification	Date of sputum collection	Genus *Burkholderia* positive by microbiome	Bcc detected by bacteriological culture	Date of last culture positive for Bcc	Number of bacteriological cultures realized between 2014–2022
A	14	T7CF	August/2019	Yes	Yes	February/2020	39
		T23CF	October/2019	No	No		
		T39CF	February/2020	Yes	Yes		
B	17	T3CF	July/2019	Yes	No	November/2017	109
		T4CF	August/2019	Yes	No		
		T17CF	October/2019	No	No		
C	5	T6CF	August/2019	No	Yes	October/2019	59
		T22CF	October/2019	Yes	No		
D	18	T43CF	February/2020	No	No	*	61
		T44CF	March/2020	Yes	No		
		T47CF	March/2020	No	No		
E	14	T8CF	August/2019	Yes	No	October/2018	47
		T25CF	October/2019	Yes	No		
F	18	T19CF	October/2019	Yes	No	*	129
		T38CF	February/2020	No	No		
G	14	T29CF	November/2019	Yes	No	*	33
		T37CF	February/2020	No	No		
H	13	T12CF	September/2019	No	No	March/2014	60
		T20CF	October/2019	Yes	No		
		T33CF	January/2020	Yes	No		
I	13	T1CF	June/2019	Yes	Yes	March/2022	63
		T9CF	August/2019	Yes	No		

Bcc, *Burkholderia cepacia* complex.

[a] The age of the patients was calculated based on the data of sputum collection for microbiome analysis.
* CF patients without Bcc positive in bacteriological culture.

confirmed diagnosis of CF by the identification of the CFTR gene mutation. Bacteriological culture used selective media for Bcc and colonies with characteristics of *Burkdolderia* spp were submitted to Matrix-Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) for final identification. For microbiome analysis, the DNA was extracted and was performed by 16S rRNA sequencing (V3V4 region) in an Illumina MiSeq (Illumina, San Diego, US). The bioinformatic analysis were performed with Bioconductor Workflow for Microbiome Data Analysis.[4] DADA2 R package v1.16 algorithm and the SILVA version 138.1 were used for the determination of Amplicon Sequence Variants (ASV).

According to the microbiome analysis, it was possible to detect the genus *Burkholderia* in 14 specimens (63.6%) with at least one sputum specimen positive for all nine patients. Conversely, the bacteriological culture was capable to detect Bcc in only 4 sputa (18.2%) from 3 different patients. There was only one case (Patient "C") of the same sputum ("T6CF" collected in August/2019) with bacteriological culture positive for Bcc and microbiome negative for *Burkholderia* spp. However, another sputum collected from Patient "C" two months later, presented the genus *Burkholderia* in the microbiome but no Bcc in the culture (Table 1). Noteworthy, three patients (D, F and G) never had Bcc identified by bacteriological culture, although, it was possible to detect the *Burkholderia* genus by microbiome. Analysis of previous data of bacteriological culture indicated that six of the nine CF patients included in this study presented Bcc in the sputum. The time lapse between the results of the culture positive for Bcc and the detection of *Burkholderia* spp by microbiome varied from 2 months to more than 5 years.

The data of this study indicated a high prevalence of the genus *Burkholderia* in sputum of CF patients according to microbiome when compared with the same specimens analyzed by bacteriological culture. Whether the identification of *Burkholderia* spp by microbiome analysis can predict the presence of Bcc in the airway of CF patients, it could be used to anticipate the treatment with antibiotics to prevent the increase of growth of the viable cells of the bacteria in the respiratory tract. It is important to avoid the growth of *Burkholderia* spp in the airway of CF for two main reasons: 1) To protect the individual patient from exacerbation of the respiratory disease associated with Bcc and 2) To decrease the transmission/circulation of Bcc among CF patients.

The eradication of Bcc was commonly accepted when the bacterium was not recovered by bacteriological culture over a follow-up period of at least 1 year with 3 sputum cultures negative.[5] We found that, in three CF patients, Bcc was not

identified by culture after more than one year, but their airway microbiome presented *Burkholderia* spp. This suggests that the concept of eradication of Bcc should be revised considering the information of 16S rRNA sequencing.

It is important to mention that the microbiome detected *Burkholderia* spp by 16S rRNA which may not guarantee the presence of viable bacteria in the specimen. Moreover, it is possible that the genus of *Burkholderia* as identified by microbiome does not include the species of Bcc related to the exacerbation of the respiratory disease in CF. However, one would be reckless to not consider the presence of the *Burkholderia* genus as an important information to be considered for the management of the CF respiratory disease. We consider that the data of both the bacteriological culture and microbiome analysis are complementary and should be considered in conjunction to contribute to the best clinical management of CF airway infections.

Funding

This work has been funded by INPRA – Instituto Nacional de Pesquisa em Resistência Antimicrobiana – Brazil (INCT/CNPq: 465718/2014-0 and INCT/FAPERGS: 17/2551-0000514-7) and by Fundo de Incentivo à Pesquisa e Eventos do Hospital de Clínicas de Porto Alegre (FIPE/HCPA) (Project n 2019-0659).

Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval

This work has been approved by the Ethics Committees from "Hospital de Clínicas de Porto Alegre" (CAAE: 23417319.0.0000.5327).

Acknowledgments

We thank the staff of "Serviço de Diagnóstico Laboratorial – Microbiologia" of the Hospital de Clínicas de Porto Alegre for providing the specimens and data used in this study.

REFERENCES

1. Scoffone VC, Chiarelli LR, Trespidi G, Mentasti M, Riccardi G, Buroni S. *Burkholderia cenocepacia* infections in cystic fibrosis patients: drug resistance and therapeutic approaches. Front Microbiol. 2017;8:1592.
2. Cipolla L, Rocca F, Martinez C, Aguerre L, Barrios R, Prieto M. Prevalence of *Burkholderia cepacia* complex species in cystic fibrosis patients in Argentina during the period 2011-2015. Enferm Infecc Microbiol Clin. 2018;36:431–4.
3. Mahboubi MA, Carmody LA, Foster BK, Kalikin LM, VanDevanter DR, LiPuma JJ. Culture-based and culture-independent bacteriologic analysis of cystic fibrosis respiratory specimens. J Clin Microbiol. 2016;54:613–9.
4. Callahan BJ, Sankaran K, Fukuyama JA, McMurdie PJ, Holmes SP. Bioconductor workflow for microbiome data analysis: from raw reads to community analyses [version 1; referees: 3 approved]. F1000Res. 2016;5:1492.
5. Garcia BA, Carden JL, Goodwin DL, et al. Implementation of a successful eradication protocol for *Burkholderia cepacia* complex in cystic fibrosis patients. BMC Pulm Med. 2018;18:35.