


# *Trichosporon asahii* superinfections in critically ill COVID-19 patients overexposed to antimicrobials and corticosteroids

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## Abstract

**Objectives:** To investigate the occurrence of *Trichosporon asahii* fungemia among critically ill COVID-19 patients.

**Methods:** From 1 July to 30 September 2020, cases of *T asahii* fungemia (TAF) in a Brazilian COVID-19 referral centre were investigated. The epidemiology and clinical courses were detailed, along with a mycological investigation that included molecular species identification, haplotype diversity analysis and antifungal susceptibility testing.

**Results:** Five critically ill COVID-19 patients developed TAF in the period. All five patients had common risk conditions for TAF: central venous catheter at fungemia, previous exposure to broad-spectrum antibiotics, prior echinocandin therapy and previous prolonged corticosteroid therapy. The average time of intensive care unit hospitalisation previous to the TAF episode was 23 days. All but one patient had voriconazole therapy, and TAF 30-day mortality was 80%. The five *T asahii* strains from the COVID-19 patients belonged to 4 different haplotypes, mitigating the possibility of skin origin and cross-transmission linking the 5 reported episodes. The antifungal susceptibility testing revealed low minimal inhibitory concentrations for azole derivatives.

**Conclusions:** Judicious prescription of antibiotics, corticosteroids and antifungals needs to be discussed in critically ill COVID-19 patients to prevent infections by hard-to-treat fungi like *T asahii*.

## KEYWORDS

Brazil, COVID-19, fungemia, *Trichosporon asahii*

## 1 | INTRODUCTION

Recent investigations have pointed out that hospitalised patients with COVID-19 are at risk to develop fungemia.<sup>1</sup> Indeed, classical fungemia risk factors such as prolonged intensive care unit (ICU) stay, central venous catheters (CVC) and exposure to broad-spectrum antibiotics have been reported in such patients.<sup>1</sup>

*Trichosporon asahii* is a yeast-like opportunistic pathogen that was initially associated with fungemia in neutropenic patients and more recently has been increasingly reported as a cause of sepsis in critically ill patients exposed to invasive devices, antibiotics, steroids and antifungals.<sup>2,3</sup> Unlike *Candida* spp., *T asahii* is resistant to the echinocandins, and fungemia episodes are frequently associated with pneumonia and have mortality rates usually above

TABLE 1 Primers developed for the multilocus analysis

Locus	Primer name	Primer sequence	Melting temp.	Product size (bp)
B-1-tubulin	BTUB-F	5'-GCCCCGACAACCTTTGTCTTT-3'	55°C	636
	BTUB-R	5'-TCTTGGCGTCGAACATTTGC-3'	56°C	
Copper-exporting ATPase	ATP-F	5'-CTTCCATCGCAATGCTGGTT-3'	55°C	551
	ATP-R	5'-TGATGTCATCGCCTTCGAGT-3'	55°C	
Phosphate carrier protein	PHCP-F	5'-CAGCAATCATGTCCGACAGA-3'	54°C	664
	PHCP-R	5'-CGAACTTGGCCATGGTGTA-3'	54°C	
Topoisomerase-1	TOP1-F	5'-CGCACTTCTCAAGGCTGGTAAT-3'	57°C	380
	TOP1-R	5'-GGACGTCAAGCCGAATGTCA-3'	57°C	

50%.<sup>3</sup> In this scenario, the occurrence of *T asahii* fungemia (TAF) in COVID-19 hospitalised patients is worrisome and may contribute to increasing the mortality in this population. We report the epidemiology, clinical course and microbiological findings of *T asahii* fungemia episodes in COVID-19 patients hospitalised in an ICU from Salvador, Brazil.

## 2 | MATERIAL AND METHODS

### 2.1 | Epidemiological and clinical investigation

The number of TAF and candidemia episodes per/1.000 ICU admissions was collected, as well as information regarding demographics underlying conditions, body mass index (BMI), previous antimicrobial exposure, previous antifungal exposure, antifungal breakthrough infection as defined by Cornely et al,<sup>4</sup> use of mechanical ventilation, use of renal replacement therapy, laboratory tests on admission and at fungemia, presence of invasive devices, previous bacterial or fungal infections and Pitt bacteremia score at fungemia for each TAF episode.<sup>5,6</sup> Pitt bacteremia score was initially described for as a severity of acute illness in patients with bacteremia and also been applied as a prognostic evaluation in candidemia<sup>6</sup> and, recently, fungemia by *Trichosporon* spp.<sup>7</sup> Clinical management and 30-day outcome were also collected. The concomitance of COVID-19 pneumonia with TAF precluded any further analysis of lung involvement by the fungal pathogen. This study was approved by the Institutional Review Board, protocol number: 44674415.0.1001.5505.

### 2.2 | COVID-19 and *Trichosporon* fungemia diagnosis

Patients had SARS-CoV-2 detected in respiratory samples by RT-PCR after 24–48 h of admission (Allplex™ 2019-nCoV Assay).

Bactec (Becton and Dickinson) blood cultures and MALDI-TOF mass spectrometry (Vitek MS, bioMérieux) were used for the aetiologic diagnosis of sepsis. Molecular identification of *Trichosporon* spp. was confirmed by sequencing the IGS1 region from the rDNA.<sup>8</sup>

### 2.3 | Mycological investigation

To investigate the possibility of *T asahii* horizontal transmission among COVID-19 critically ill patients, we conducted a molecular investigation by multilocus DNA sequence analysis including the five *T asahii* epidemiologic-related clinical isolates and nine controls (2 reference strains and 7 unrelated isolates from the same hospital). Housekeeping genes from *T asahii* genomes (CBS2479 and CBS8904) available in GenBank (<https://www.ncbi.nlm.nih.gov/>) were aligned, and in silico analyses were carried out to design and evaluate the new primers. After PCR reactions and DNA sequence analysis, four loci with higher haplotypic diversity were selected for the multilocus phylogenetic analysis along with the IGS1 region from the rDNA: topoisomerase 1 (A1Q1\_08199), phosphate carrier protein (A1Q1\_08315), beta-1-tubulin (A1Q1\_04313) and copper-exporting ATPase (A1Q1\_05365). Primers and PCR conditions are provided in Table 1. DNA sequences from the five investigated loci were concatenated, and haplotype distribution was constructed by phylogenetic analysis using software MEGA X.<sup>9</sup> The five COVID-19-associated *T asahii* isolates had antifungal susceptibility testing (AST) of fluconazole (FLC), voriconazole (VRC), posaconazole (POS) and amphotericin B (AMB, Sigma) performed by using the CLSI broth microdilution method.<sup>10</sup> The minimal inhibitory concentration (MIC) values were determined after 48 h of incubation.<sup>11</sup>

## 3 | RESULTS

From 1 July to 30 September 2020, a total of 183 patients were admitted to the COVID-19 ICU from the São Rafael Hospital, Salvador, Bahia State, Brazil. In that lapse of time, five patients had TAF (27.3/1.000 ICU admissions) and 18 had candidemia (98.3/1.000 ICU admissions). The TAF patients had a mean age of 71 years (range 57–75), and three (60%) of them were severely obese (body mass index  $\geq 35$ ). Two (40%) had diabetes mellitus and were under insulin therapy. All five patients had common risk conditions for TAF: CVC at fungemia, previous exposure to broad-spectrum antibiotics, prior echinocandin therapy (anidulafungin, average 8 days) and previous corticosteroid therapy (average 22 days). Three among the five TAF episodes were anidulafungin breakthrough infections.

Corticosteroid therapy consisted of either methylprednisolone 1 mg/kg/day or prednisone 40 mg q12h. All five patients received piperacillin-tazobactam before the TAF episode. The average time of ICU hospitalisation previous to the TAF episode was 23 days (range 11–31 days). All patients were under mechanical ventilation (11–30 days before TF), and four of them need renal replacement therapy (9–16 days before TF). Candidemia was diagnosed in three patients before the TAF, two by *Candida parapsilosis* and one by *Candida tropicalis*. One of the patients had concomitant TAF and *C parapsilosis* candidemia. All *Candida* isolates were susceptible to AMB, FLC, VRC and echinocandins (data not shown). Four patients developed diarrhoea during hospitalisation, and all four had *Clostridioides difficile* A/B toxin and glutamate dehydrogenase antigen tests negative in faeces samples (*C DIFF QUICK CHECK COMPLETE*, Abbott). Three of them had diarrhoea that persisted until fungemia was diagnosed. One patient had *Enterococcus faecalis* bacteremia before the TAF diagnosis, and another one, without diarrhoea, had concomitant bloodstream infection by *T asahii* and *E faecalis*. Concordant *T asahii* blood and catheter tip cultures were diagnosed in one patient.

*Trichosporon* deep-seated infections were investigated, including eye fundoscopy, heart echocardiogram and abdominal ultrasound, but all cases were considered isolated fungemia. None of the patients had neutropenia ( $<500$  cells/mm<sup>3</sup>) before or at the TAF episode. One patient had a low blood lymphocyte count when TAF was diagnosed (300 cells/mm<sup>3</sup>). The average Pitt bacteremia score at fungemia diagnosis was 11 (range 7–14). All but one patient had VRC therapy, either in monotherapy ( $n = 1$ ) or combined with liposomal AMB ( $n = 3$ ). One patient died before receiving antifungal therapy for the TAF episode. The overall 30-day mortality was 80%. The clinical course of the COVID-19 patients that developed TAF is summarised in Figure 1 and Table 2.

The five *T asahii* strains from the COVID-19 patients belonged to 4 different haplotypes, mitigating the possibility of skin origin and cross-transmission linking the 5 reported episodes (Figure 2). GenBank accession numbers are available as appendix data (Table S1). The antifungal susceptibility testing revealed low MICs for azole derivatives, that is 1–2 mcg/mL for FLC, 0.125 mcg/mL for POS and 0.03 mcg/mL for VRC, and high MICs for AMB (1–16 mcg/mL).

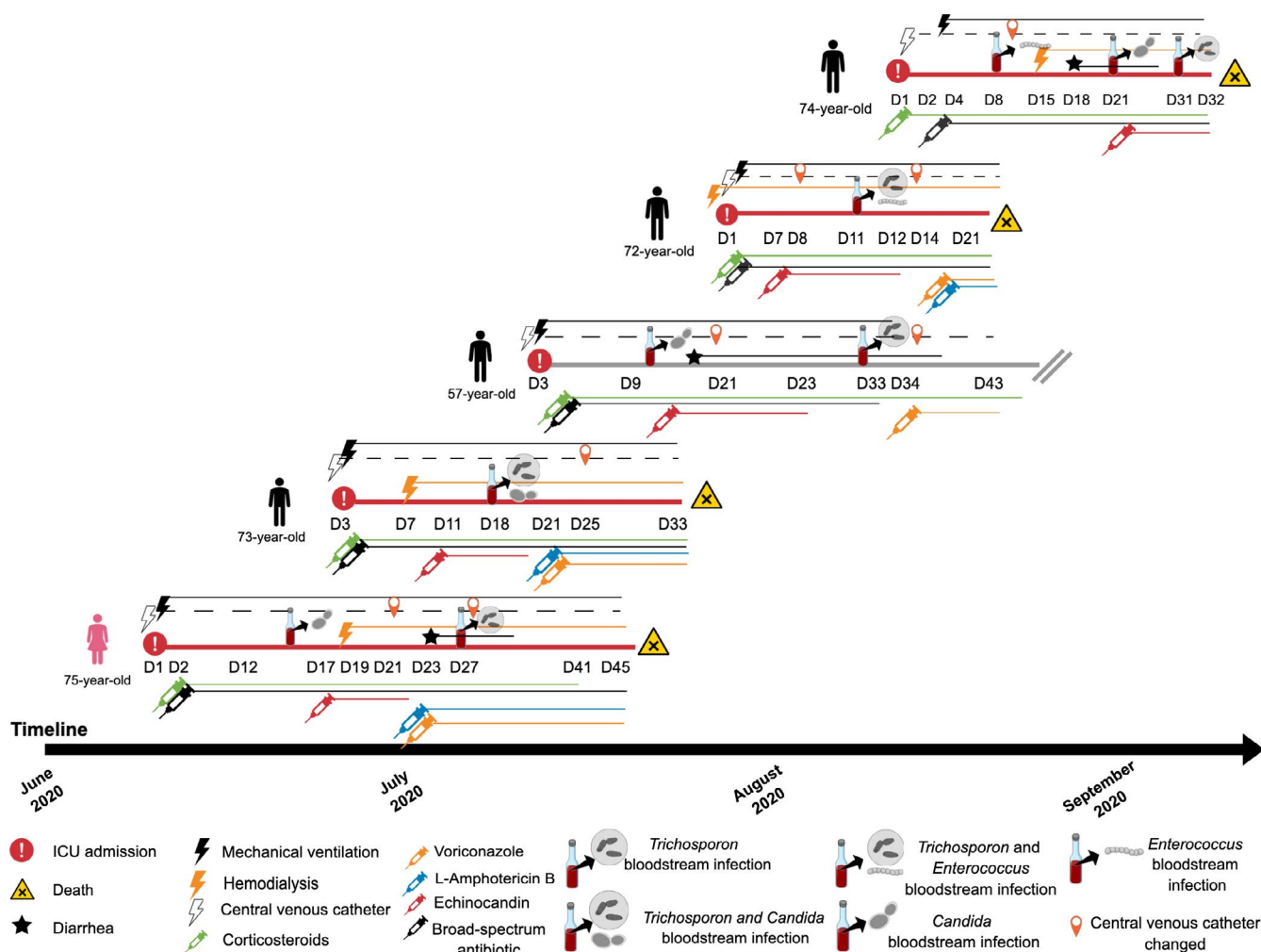


FIGURE 1 Clinical course of the five patients with severe COVID-19 that develop *Trichosporon* fungemia, with red horizontal lines representing the patients that deceased and grey horizontal line representing the patient that survived

Condition	Patient Data				
Age	57	74	75	73	72
Sex	Male	Male	Female	Male	Male
Body Mass Index $\geq 35$	No	No	Yes	Yes	Yes
Diabetes mellitus	Yes	No	No	Yes	No
ICU length of stay before fungemia (days) <sup>a</sup>	30	31	27	15	11
Mechanical ventilation duration before fungemia (days)	30	27	27	15	11
Renal replacement therapy duration before fungemia (days)	0	16	9	11	11
Broad-spectrum antibiotic therapy duration with anti-anaerobe activity before <i>Trichosporon</i> fungemia (days)	30	30	26	15	10
Diarrhoea duration before <i>Trichosporon</i> fungemia (days)	16	12	4	0	0
Corticosteroid therapy duration before fungemia (days)	30	30	26	15	10
Corticosteroid exposure during fungemia	Yes	Yes	Yes	Yes	Yes
<i>Enterococcus</i> spp. bacteremia before or at <i>Trichosporon</i> fungemia	No	Yes	No	No	Yes
Candidemia before <i>Trichosporon</i> fungemia	Yes	Yes	Yes	No	No
Echinocandin exposure before <i>Trichosporon</i> fungemia (days)	13	9	6	9	5
Echinocandin breakthrough infection	No	Yes	No	Yes	Yes
CVC at fungemia <sup>b</sup>	Yes	Yes	Yes	Yes	Yes
Pitt bacteremia score at fungemia	7	14	10	10	14
Blood neutrophils count at fungemia (cells/mm <sup>3</sup> )	7200	2750	18,200	1260	970
Blood Lymphocyte count at fungemia (cells/mm <sup>3</sup> )	1440	300	1500	950	550
Voriconazole therapy	Yes	No	Yes	Yes	Yes
CVC removal after <i>Trichosporon</i> fungemia	Yes	No	Yes	Yes	Yes
7-day outcome	Alive	Dead	Alive	Alive	Alive
14-day outcome	Alive	Dead	Alive	Dead	Dead
30-day outcome	Alive	Dead	Dead	Dead	Dead

<sup>a</sup>ICU, Intensive care unit.

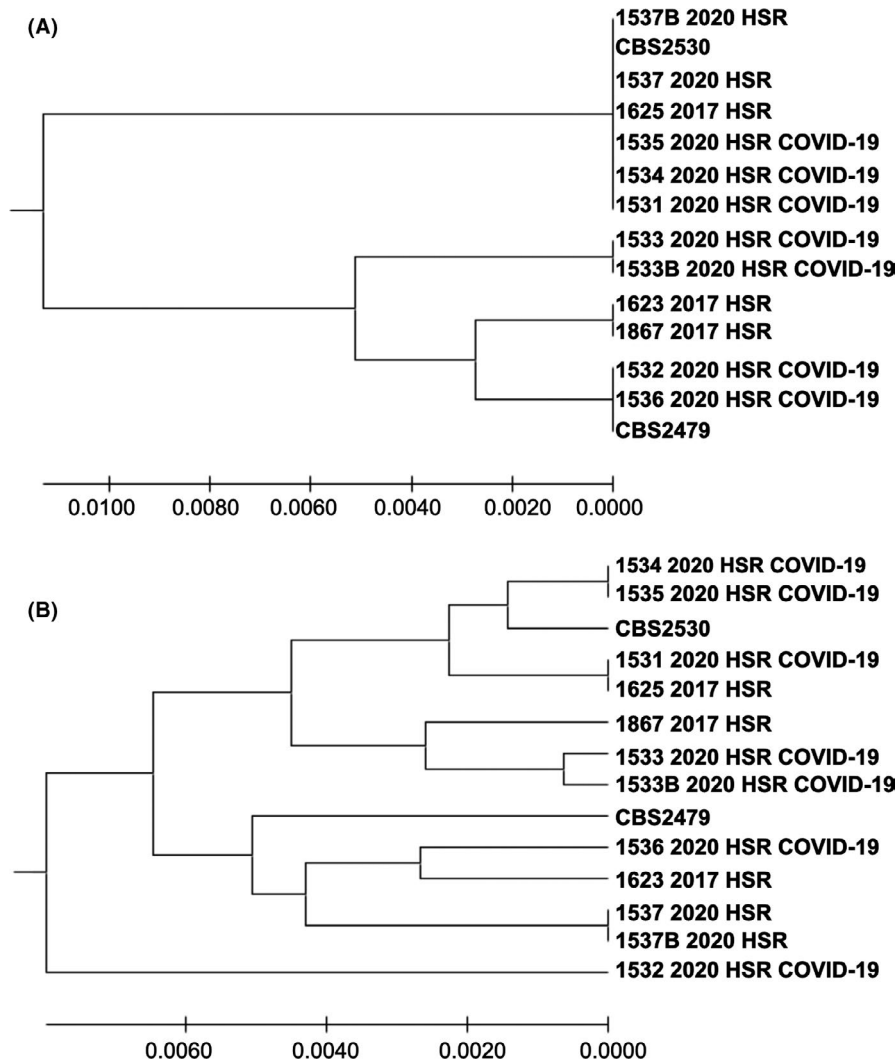
<sup>b</sup>CVC, central venous catheter.

**TABLE 2** Clinical and Epidemiological data of the five patients with severe COVID-19 that developed *Trichosporon asahii* fungemia

## 4 | DISCUSSION

*T. asahii* may be part of the human skin and digestive tract microbiome, and the role of gut and skin colonisation as the portal of entrance for TAF is controversial.<sup>12</sup> In the present cohort, all patients were under prolonged antimicrobial selective pressure, which certainly contributed to the overgrowth of this species in the skin and the digestive tract of the SARS-CoV-2-infected patients. All patients had a CVC in place, which may have been the source of the candidemia and TAF episodes. However, despite catheter removal allied to voriconazole seen in four patients, three died in the first 14 days after the fungemia diagnosis. The prolonged corticosteroid-induced immunosuppression, the intense dysbiosis caused by long periods of antimicrobial therapy associated with

the impairment of the intestinal barrier and the multi-organic damage characteristic of severe COVID-19 may have contributed to the poor outcomes. Corticosteroid therapy has shown to be beneficial for the treatment of severe COVID-19. However, prolonged corticosteroid treatment may be a double-edged sword and detrimental for these patients, facilitating thrombotic events,<sup>13</sup> and also superinfections by opportunistic pathogens, including *Candida* and *Trichosporon*.<sup>14</sup> Translocation from the digestive tract has been pointed out as a relevant source for bacteremia and fungemia in patients with severe COVID-19.<sup>1</sup> Of note, one interesting report from Greece described two patients with COVID-19 that developed *Saccharomyces cerevisiae* fungemia after 4–6 days of probiotic treatment for diarrhoea.<sup>15</sup> Recently, an experimental model of SARS-CoV-2 infection with Syrian hamsters demonstrated



**FIGURE 2** (A) UPGMA phylogenetic tree showing the evolutionary relationship among 14 IGS1 nucleotide sequences, from two reference strains (CBS2479 and CBS2530) and 12 clinical strains from 10 patients from the same hospital. The strains 1533 2020 HSR and 1533B 2020 HSR were recovered from the same patient, as well as the strains 1537 2020 HSR and 1537B 2020 HSR. There were a total of 562 nucleotides positions in the final data set. The final tree with the sum of branch length = 0.0304 is shown. Three strains from different COVID-19 patients had identical sequences (1531 2020 HSR, 1534 2020 HSR, 1535 2020 HSR); (B) UPGMA phylogenetic tree showing the evolutionary relationship among 14 concatenated (five loci) nucleotide sequences, from two reference strains (CBS2479 and CBS2530) and 12 clinical strains from 10 patients from the same hospital. The strains 1533 2020 HSR and 1533B 2020 HSR were recovered from the same patient, as well as the strains 1537 2020 HSR and 1537B 2020 HSR. There were a total of 2467 nucleotides positions in the final data set. The final tree with the sum of branch length = 0.456 is shown. The strains recovered from the patients hospitalised at the COVID-19 ICU showed that only two patients had phylogenetic closely related strains (1534 2020 HSR and 1535 2020 HSR strains belong to the same haplotype)

enterocytes infection and apoptosis by the virus.<sup>16</sup> Thus, one has to keep in mind that direct enterocytes lesions by the SARS-CoV-2 may facilitate bacterial and fungal translocation from the digestive tract of some of these severely infected patients.

Despite the previous reports associating *T. asahii* with horizontal transmission,<sup>17</sup> this event unlikely happened in this cohort. Some key elements against the hypothesis of horizontal transmission can be delineated as follows: patients had facilitating conditions for gut translocation and were exposed to echinocandins, which might have led to *Trichosporon* selection in the digestive tract; the molecular

analysis showed a high haplotype diversity among the clinical isolates. The haplotype diversity translates genetic differences among the isolates, meaning that clonal dissemination which is suggestive of horizontal transmission did not occur in this cohort. These important observations raise the concern that critically ill COVID-19 patients may have a higher risk to develop *Trichosporon* fungemia and not only candidemia. Indeed, the incidence of *Trichosporon* fungemia in this study was at least six times higher than we previously reported in Brazilian centres.<sup>18</sup> For every 3.6 episodes of candidemia, one episode of TAF occurred in this cohort.

## 5 | CONCLUSIONS

We added evidence to the emerging issue regarding superinfections in severe COVID-19 patients. Judicious prescription of broad-spectrum antibiotics, corticosteroids and antifungals needs to be discussed in critically ill COVID-19 patients to prevent infections not only by multidrug-resistant bacteria but also by hard-to-treat fungi like *T asahii*.

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### CONFLICTS OF INTEREST

ALC has received honoraria from Pfizer, United Medical, Eurofarma, MSD, TEVA. All other authors have no conflicts to declare.

### AUTHOR CONTRIBUTION

**Joao Nobrega de Almeida Júnior:** Conceptualization (equal); Methodology (lead); Visualization (lead); Writing-original draft (equal); Writing-review & editing (equal). **Lis Moreno:** Resources (equal); Writing-original draft (equal); Writing-review & editing (equal). **Elaine Cristina Francisco:** Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Gabriela Noronha Marques:** Resources (equal); Writing-original draft (equal); Writing-review & editing (equal). **Ana Verena Mendes:** Resources (equal); Writing-original draft (equal); Writing-review & editing (equal). **Maria Goreth M. de Andrade Barberino:** Resources (equal); Writing-original draft (equal); Writing-review & editing (equal). **Arnaldo Lopes Colombo:** Conceptualization (equal); Funding acquisition (equal); Methodology (supporting); Writing-original draft (equal); Writing-review & editing (equal).

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### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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