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ORIGINAL ARTICLE

Drug susceptibility of *Candida albicans* and non-*albicans Candida* species isolated from ornamental birds

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ABSTRACT

Background and Objectives: The prevalence of *Candida* infections, especially by non-*albicans Candida* species, has led to excessive use of antifungal drugs, resulting in the transfer of resistance and increased minimum inhibitory concentration (MIC) among *Candida* isolates. This study aimed to investigate the susceptibility of clinical *Candida* isolates of ornamental birds to three antifungal drugs: amphotericin B, caspofungin, and itraconazole.

Materials and Methods: Totally 126 samples were analyzed, from which 116 distinct colonies were cultured. Of these, 26 were identified as *Candida* spp., comprising 12 *C. albicans* (46.1%), 8 *C. tropicalis* (30%), 1 *C. glabrata* (3%), 1 *C. krusei* (3%), and 4 isolates (15%) of other *Candida* species. The present study aimed to determine the susceptibility and resistance levels of these *Candida* isolates to three antifungal drugs: amphotericin B, caspofungin, and itraconazole.

Results: According to the CLSI M44 recommended method, by the disk diffusion method, itraconazole (100%) and amphotericin B (86.46%) showed the best susceptibility pattern, compared to caspofungin (0%).

Conclusion: Given that the isolates showed the highest in vitro susceptibility to itraconazole and amphotericin B and the lowest to caspofungin, these findings suggest that itraconazole and amphotericin B could be considered potential first-line agents for treating avian candidiasis.

Keywords: *Candida albicans*; Drug resistance; Fungal; Antimicrobial susceptibility tests; Antifungal agents; Birds

INTRODUCTION

Candidiasis is traditionally considered an opportunistic fungal disease in humans; however, increasing evidence indicates that it also represents a clinically important condition in ornamental birds, particularly psittacine species maintained under captive conditions. The close interaction between humans and companion birds, together with the zoonotic potential of *Candida* spp., underscores the importance of identifying and managing *Candida* infections in avian hosts to minimize the risk of interspecies transmission (1).

Unlike primary infectious diseases acquired from external sources, candidiasis typically develops following disturbances in host-related factors, including alterations in the normal microbiota or immune function (2). Conditions such as advanced age, underlying systemic diseases, and immunesuppression are known to increase susceptibility to *Candida* overgrowth. In birds, prolonged or inappropriate antibiotic use is considered a major predisposing factor, as it disrupts the normal gastrointestinal microbiome. Consequently, oral candidiasis analogous to

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oral thrush in humans, is frequently observed in avian species (2). Additional systemic risk factors, including long-term corticosteroid administration and immunosuppressive conditions, further contribute to disease development (3).

Candida infections have been documented in a broad range of avian species, encompassing both ornamental and commercial birds (1). *Candida albicans* remains the predominant etiological agent of candidiasis and is responsible for the majority of infections in both adult and pediatric human populations. Systemic candidiasis continues to be associated with considerable mortality in humans, highlighting the clinical significance of this pathogen (4). The increasing prevalence of immunocompromised populations, such as organ transplant recipients, patients receiving immunosuppressive therapy, individuals with HIV/AIDS, and patients with malignancies has contributed to a marked rise in opportunistic fungal infections, with candidiasis remaining the most frequently reported (5).

Although *C. albicans* accounts for most cases of candidiasis, shifts in species distribution have increasingly been reported. Non-albicans species, including *C. glabrata* and *C. krusei*, have emerged with greater frequency and often exhibit reduced susceptibility to commonly used antifungal agents. In addition, *C. tropicalis* has become widely distributed in natural environments and commonly colonizes human skin, oral mucosa, and the gastrointestinal tract. This species is recognized as an important opportunistic pathogen and is frequently implicated in nosocomial infections, ranking second only to *C. albicans* in isolation frequency (6).

Despite the continued use of amphotericin B and azole antifungals in the management of candidiasis, increasing reports of reduced antifungal susceptibility and therapeutic failure have raised concerns regarding their long-term effectiveness (7-10). In response to these limitations, echinocandins such as caspofungin have been introduced as alternative agents that inhibit fungal cell wall synthesis and demonstrate activity against both azole-susceptible and azole-resistant *Candida* isolates (11, 12).

In light of the growing clinical importance of antifungal resistance, agar-based susceptibility assays, such as disk diffusion and Etest, are increasingly utilized because of their simplicity, reproducibility, and limited equipment requirements (13).

Within this framework, the present study assessed the in vitro efficacy of three antifungal agents with different mechanisms of action caspofungin, amphotericin B, and itraconazole—against clinical *Candida* isolates, with the aim of defining susceptibility profiles relevant to the treatment of avian candidiasis.

MATERIALS AND METHODS

Sample collection. A prior investigation conducted at the Veterinary Clinic, Faculty of Veterinary Medicine, University of Tehran, involved the collection of 126 fecal samples from ornamental birds during November–December 2020 and January–February 2021 (14). Sampling encompassed three avian families—Psittaciformes, Passeriformes, and Columbiformes—and included ten bird species, namely lovebirds, cockatoos, Dutch canaries, cockatiels, green-cheeked conures, Indian ringnecks, mynahs, canaries, and King pigeons (Table 1).

Culturing on chromogenic media. Chromogenic culture media were employed as an additional tool for yeast identification. Previously grown colonies were subcultured, and 24-hour-old yeast isolates were inoculated in a linear pattern onto CHROMagar™ *Candida* (CHROMagar, Paris, France). The plates were incubated at 35°C for 48 h, after which colony pigmentation was assessed. On this medium, *C. albicans* produced green colonies, *C. tropicalis* blue colonies, and *C. krusei* pink colonies. In contrast, other *Candida* species typically exhibited purple hues, whereas non-*Candida* yeasts formed white colonies (Table 2).

Disk diffusion method. Antifungal susceptibility of the *Candida* isolates was assessed using the disk diffusion technique in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI document M44) (15).

Disk diffusion assays were carried out with amphotericin B, itraconazole, and caspofungin Neo-Sensitabs disks (Rosco, Taastrup, Denmark) following the CLSI M44-A protocol. Mueller–Hinton agar (LabM, Bury, UK), prepared locally, was enriched with 2% glucose (2g/100 mL) and 0.5 µg/mL methylene blue (MB). The plates were incubated at 35°C for 24 h, after which inhibition zone diameters were measured using a millimeter ruler and evaluated in line with CLSI criteria.

According to the manufacturer's (Neo-Sensitabs)

Table 1. Bird Species Distribution

Bird Species	Scientific Name	Number of Samples	Bird Species	Scientific Name	Number of Samples
Cockatiel	*Nymphicus hollandicus*	59	Alexandrine parakeet	*Psittacula eupatria*	8
Lovebird	*Psittacus swindernianus*	20	Rose-ringed parakeet	*Psittacula krameri*	
African Grey Parrot	*Psittacus erithacus*	12	Domestic Canary	*Serinus canaria domestica*	3
Mynah	*Acridotheres tristis*	7	Sulphur-Crested Cockatoo	*Cacatua sulphurea*	3
Green-cheecked parakeet	*Pyrrhura molinae*	3	Domestic Pigeon	*Columba livia domestica*	3

Table 2. Infection frequency of *Candida* species

Fungi Species	Frequency of Infection
C. albicans	12 (46.15%)
C. tropicalis	8 (30.76%)
C. glabrata	1 (3.84%)
C. krusei	1 (3.84%)
Undetected by the culture method	4 (15.36%)

standard evaluation Table, for caspofungin, strains were interpreted as follows: a zone diameter of ≤20 mm indicated resistance; 21-27 mm indicated dose-dependent susceptibility; and a diameter of ≥28 mm indicated susceptibility. Susceptibility was interpreted according to the following breakpoints: for amphotericin B, a zone diameter of ≤10 mm indicated resistance, 11-14 mm indicated dose-dependent susceptibility, and ≥15 mm indicated susceptibility; for itraconazole, a zone diameter of ≤13 mm indicated resistance, 14-17 mm indicated dose-dependent susceptibility, and ≥18 mm indicated susceptibility. Isolates were classified as follows: resistant if the inhibition zone was ≤8 mm, dose-dependent susceptible if it was 9-15 mm, and susceptible if it was ≥16 mm.

RESULTS

The present study evaluated the in vitro antifungal activity of amphotericin B, caspofungin, and itraconazole against *Candida* isolates and assessed their corresponding susceptibility profiles. Using the CLSI M44 disk diffusion method, antifungal responses were determined by measuring inhibition zone diameters after 24 h of incubation. Based on these measurements, isolates were classified as susceptible, resistant, or dose-dependent.

Disk diffusion analysis of 12 *Candida albicans* isolates demonstrated that 10 isolates were susceptible to amphotericin B, whereas 2 exhibited resistance. In contrast, caspofungin resistance was observed in 8 isolates, with the remaining 4 showing dose-dependent susceptibility. All *C. albicans* isolates were susceptible to itraconazole. Among eight *Candida tropicalis* isolates, uniform susceptibility to amphotericin B was observed. Six isolates were resistant to caspofungin, while two displayed dose-dependent responses. Similar to *C. albicans*, all *C. tropicalis* isolates were susceptible to itraconazole.

Susceptibility testing of a single *Candida glabrata* isolate revealed resistance to amphotericin B, a dose-dependent response to caspofungin, and susceptibility to itraconazole. In the case of *Candida krusei*, the isolate tested was susceptible to amphotericin B and itraconazole but resistant to caspofungin. Evaluation of other *Candida* species demonstrated uniform susceptibility to amphotericin B and itraconazole; however, with respect to caspofungin, one isolate exhibited dose-dependent susceptibility, while three showed resistance.

The overall antifungal susceptibility, dose-dependent sensitivity, and resistance patterns of all *Candida* isolates to amphotericin B, caspofungin, and itraconazole are illustrated in Figs. 1-3, respectively. Among all samples, itraconazole (100%) and amphotericin B (86.46%) showed the best susceptibility, while caspofungin (0%) showed the lowest, as shown in Table 3.

DISCUSSION

Birds, including domestic, wild, and ornamental species, have been recognized as reservoirs of yeasts with pathogenic potential for humans, and an increasing frequency of *Candida*-associated infections has been reported in recent decades (16). While *Candida albicans* remains the most common-

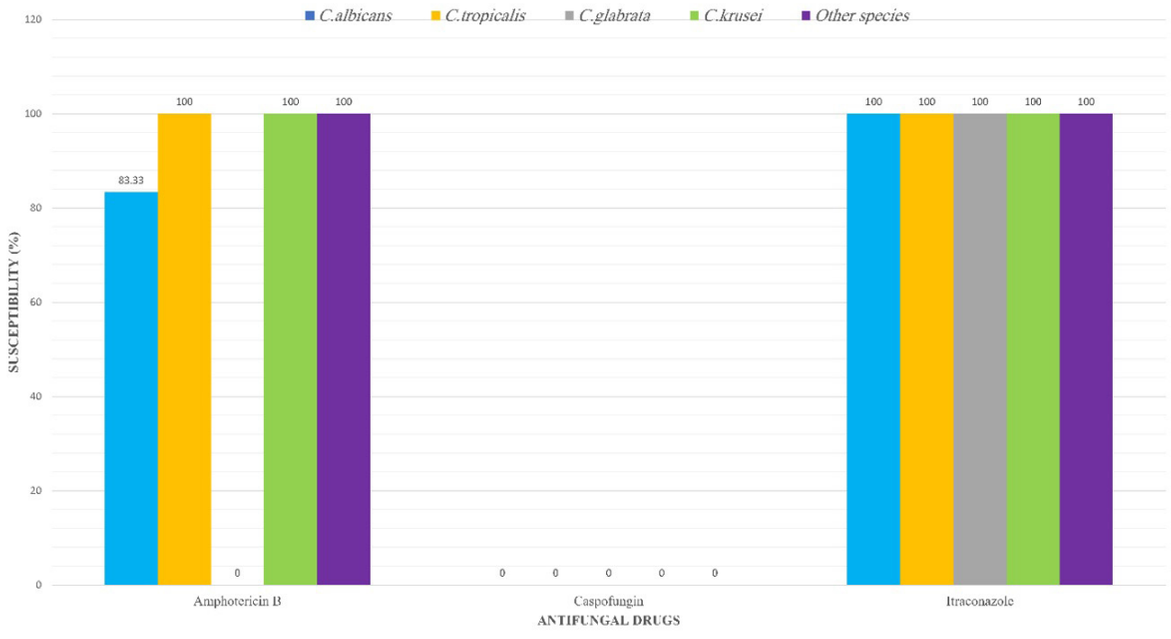

Fig. 1. Antifungal susceptibility profile of *Candida* species – sensitive isolates.
This Figure shows the percentage of *Candida* isolates that are sensitive to amphotericin B, caspofungin, and itraconazole. The results indicate that *C. tropicalis*, *C. krusei*, and other species were fully sensitive (100%) to amphotericin B, while *C. albicans* showed 83.33% sensitivity. All isolates were sensitive to itraconazole, and none responded to caspofungin. These findings highlight the strong efficacy of itraconazole and amphotericin B, particularly against *C. tropicalis* and *C. krusei.*

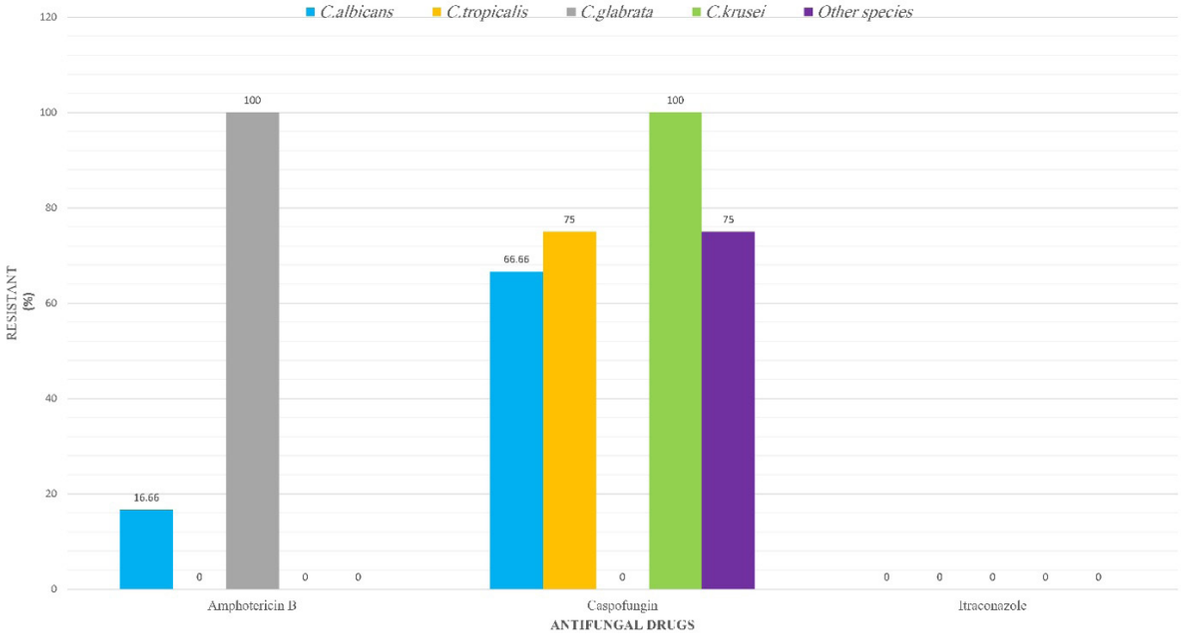

Fig. 2. Antifungal susceptibility profile of *Candida* species – resistant isolates.
This Figure shows the proportion of *Candida* isolates resistant to amphotericin B, caspofungin, and itraconazole. Resistance to amphotericin B was observed in *C. glabrata* (100%) and *C. albicans* (16.66%). Marked resistance to caspofungin was recorded in *C. albicans*, *C. tropicalis*, and *C. krusei* (66.66-100%). No isolate exhibited resistance to itraconazole. These results demonstrate notable resistance to caspofungin among certain *Candida* species.

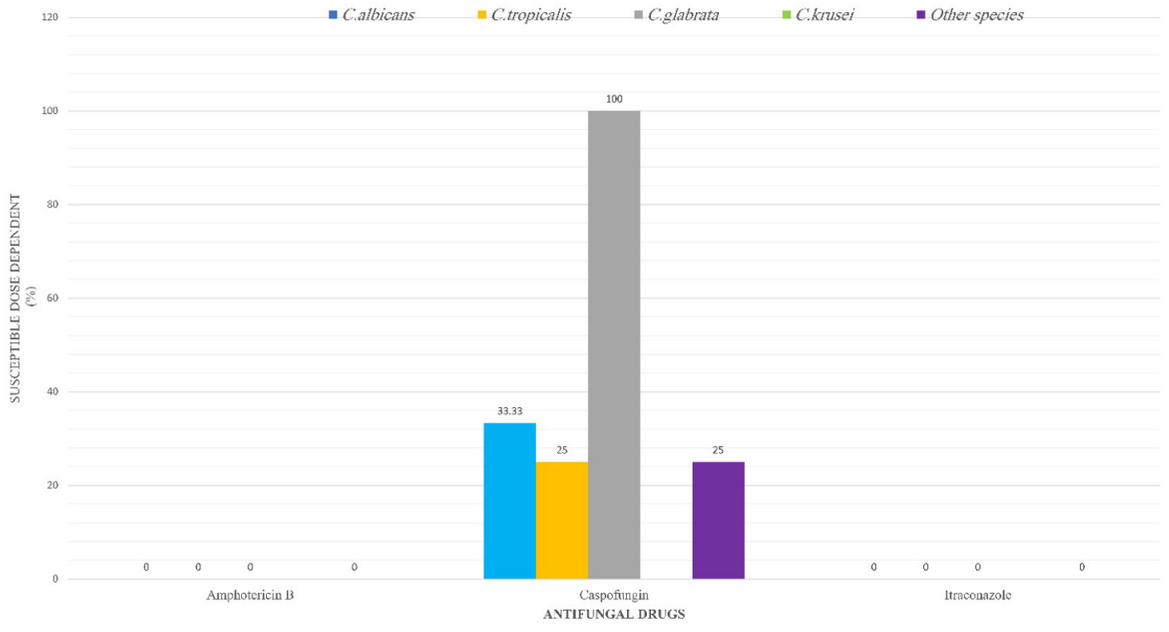

Fig. 3. Antifungal susceptibility profile of *Candida* species – susceptible dose-dependent (SDD) isolates.
This Figure illustrates the percentage of isolates exhibiting a susceptible dose-dependent (SDD) response to amphotericin B, caspofungin, and itraconazole. No SDD response was detected for amphotericin B and itraconazole. caspofungin displayed variable SDD responses, with the highest rate in *C. glabrata* (100%), followed by *C. albicans* (33.33%), *C. tropicalis* (25%), and other species (25%). These findings suggest that caspofungin efficacy in some *Candida* isolates may depend on dosage adjustment.

ly isolated species, accumulating evidence indicates a growing contribution of non-*albicans Candida* to these infections (17). A relatively limited group of *Candida* species including *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. auris*, and *C. krusei* is responsible for most documented infections (18). In addition, treatment choices remain restricted, particularly when infections involve antifungal-resistant strains (19).

The increasing prevalence of systemic fungal infections, together with the growing range of systemically administered antifungal agents, has led to wider adoption of antifungal susceptibility testing. At present, such testing is regarded as an essential tool for optimizing antifungal treatment decisions (15). Among available techniques, the disk diffusion assay remains one of the most accessible approaches for evaluating yeast susceptibility in routine microbiology laboratories (20).

In the present investigation, *Candida albicans* was the most frequently isolated species, with *C. tropicalis* ranking second, a distribution consistent with findings reported in previous studies (21). The frequency of yeast isolation from birds observed in this study is consistent with previous findings, indicating that birds can serve as important reservoirs of opportunistic yeasts, particularly *Candida* species. In the present study, *C. albicans* was the most frequently isolated species, followed by *C. tropicalis* and *C. glabrata*. Similarly, Sidrim et al. (2010) reported that *C. albicans* was the predominant yeast isolated from the gastrointestinal tract of cockatiels, accounting for 39 of 59 isolates, followed by *C. tropicalis*, which accounted for 12 of 59 isolates (22). Our findings agree with those of Cafarchia et al. (2006), who reported *C. albicans* as one of the most frequently isolated yeasts from the cloacae and digestive tracts of birds of prey. This further supports the hypothesis that various bird species, including ornamental birds, may serve as reservoirs and environmental disseminators of potentially pathogenic yeasts such as *Candida* spp. (23). Glushakova and Kachalkin (2024) similarly reported that *Candida albicans* and *C. tropicalis* were the dominant species among yeast isolates obtained from wild and domestic birds (24). Taken together, these results indicate that *Candida albicans* continues to be the predominant yeast species in avian hosts, while reports of non-*albicans Candida* are becoming

Table 3. In vitro antifungal susceptibility patterns of clinical yeast isolates against itraconazole, amphotericin B, and caspofungin

Organism/Antifungal	Mean diameter of growth inhibition zone (mm)	Susceptible n (%)	Susceptible Dose Dependent n (%)	Resistant n (%)
Candida albicans (n:12)				
Amphotericin B	23.55	10 (83.33)	0	2 (16.66)
Caspofungin	19.66	0	4 (33.33)	8 (66.66)
Itraconazole	25.11	12 (100)	0	0
Candida tropicalis (n: 8)				
Amphotericin B	24.5	8	0	0
Caspofungin	12.25	0	2 (25)	6 (75)
Itraconazole	23	8	0	0
Candida glabrata (n:1)				
Amphotericin B	17	0	0	1
Caspofungin	22	0	1	0
Itraconazole	20	1	0	0
Candida krusei (n:1)				
Amphotericin B	27	1	0	0
Caspofungin	18	0	0	1
Itraconazole	16	1	0	0
other species of *Candida* (n:4)				
Amphotericin B	25	4	0	0
Caspofungin	13.25	0	1 (25)	3 (75)
Itraconazole	24	4	0	0
Total (n:26)				
Amphotericin B		23 (88.46)	0	3 (11.54)
Caspofungin		0	8 (30.76)	18 (69.23)
Itraconazole		26 (100)	0	0

more frequent, potentially due to shifts in environmental conditions and host-related factors.

Variations in isolation frequency among studies may be attributed to differences in bird species, habitat, sampling sites (e.g., cloaca, oropharynx, feathers), geographic region, and laboratory methodologies employed.

By disk diffusion, amphotericin B and caspofungin showed resistance rates of 16.66% and 66.66%, respectively. All tested *C. albicans* isolates were susceptible to itraconazole. In contrast, Sidrim et al. (2010) identified itraconazole-resistant *C. albicans* in 35.89% of isolates obtained from cockatiel gastrointestinal samples (22).

All *C. tropicalis* isolates in our study were fully susceptible (100%) to amphotericin B and itraconazole. This high susceptibility to amphotericin B aligns with findings by Glushakova and Kachalkin (2024), who reported a very low resistance rate (3.7%) for this species (24). In contrast, caspofungin showed a high resistance rate of 75%.

For the small number of *C. glabrata* strains tested in this study, susceptibility profiles were as follows: 100% resistance to amphotericin B, 100% susceptibility to itraconazole, and dose-dependent susceptibility to caspofungin. The finding of full itraconazole susceptibility is notable, as the increased use of azoles has led to the emergence of resistant *C. glabrata* strains (25).

In this study, itraconazole exhibited complete susceptibility (100%), followed by amphotericin B with a high susceptibility rate (86.46%), whereas no antifungal activity was observed for caspofungin. These findings differ from reports from Spain (26), where caspofungin showed strong activity against *C. albicans* as well as against *C. glabrata* and *C. krusei* isolates known to display intrinsic resistance or reduced susceptibility to itraconazole (27). In partial

agreement with our results, Talazade et al. (2022) found that 78.5% of *Candida* isolates were susceptible to amphotericin B; however, in their study none of the isolates were susceptible to itraconazole, and resistance exceeded 50% (1).

CONCLUSION

In summary, antifungal susceptibility testing of *Candida albicans* and non-*albicans Candida* isolates obtained from ornamental birds indicated that itraconazole and amphotericin B exhibited the highest levels of antifungal activity, highlighting their potential clinical relevance in the management of avian candidiasis. Caspofungin's poor performance warrants cautious use and underscores the need for ongoing surveillance. Expanding antifungal susceptibility profiling across bird species could support more effective treatment protocols and minimize resistance development.

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