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# Molecular Detection of Medically Important *Candida* species from Droppings of Pigeons (Columbiformes) and Captive Birds (Passeriformes and Psittaciformes)

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**HIGHLIGHTS**

- All fecal samples from *Columbia livia* and captive birds were positive for yeast.
- A simple boiling method provides DNA to ensure success in specific amplifications.
- Pigeon feces held more potentially pathogenic *Candida* spp. compared to captive birds' stool.
- 80% of the samples isolated from birds' feces were *Candida* non-*albicans*.
- Cleaning urban spaces and captivity can provide fewer risks to individuals.

**Abstract:** Passeriformes and Psittaciformes birds and pigeons (*Columba livia*) are known to be reservoirs of microorganisms, and their stool allows fungi development. Since accumulated avian excreta can interfere with public health, this study aimed to perform a molecular screening of medically important *Candida* species in pigeon droppings in public places and birds raised in captivity. Excreta collected from captive birds (3 residences) and pigeons (4 districts) were inoculated on Sabouraud dextrose agar with chloramphenicol for Gram staining and subculture on Hicrome®*Candida*. Three DNA extraction methods were performed for

comparison (commercial kit, in-house and by boiling) and PCR to screen 6 clinically important *Candida* species among the isolates. The correlation between phenotypic and molecular methods was calculated by kappa/K. Only 6 *C. parapsilosis* (20%) were identified from captive birds' feces among 30 isolates (80% not identified), while pigeons' feces harbored a greater diversity, with the 6 pathogenic species confirmed among 41 isolates: *C. albicans* (31.70%/13), *C. krusei* (14.63%/6), *C. tropicalis* (14.63%/6), *C. parapsilosis* (17.10%/7), *C. glabrata* (14.63%/6) and *C. guilliermondii* (7.31%/3); 100% correlation between tested methods (K = 1) for the first 3 species. Boiling DNA extraction method was fast and efficient to obtain viable DNA from *Candida* spp. for PCR. Our results indicate that pigeon droppings harbor more potentially pathogenic species than birds in residential captivity, which probably have non-*albicans Candida* less frequently isolated in infectious processes. The greater availability of nutrients may have contributed to a diversity of *Candida* spp. in feces from public environments.

**Keywords:** Avian excreta; *Candida* spp.; DNA extraction; PCR; captive birds.

## INTRODUCTION

Psittaciformes and Passeriformes are the most common bird orders found captive in Brazil [1], while pigeons (*Columba livia*/Columbiformes) are considered as urban plagues among the synanthropic birds, acting as pathogenic agent disseminators [2]. Nitrogen is one of the avian excreta's components, and in great quantity, avian stool can promote the development of pathogenic fungi [3] and can even modify fungal communities in the soil [4]. Excreta accumulation is a risk of public health since there is the possibility of transmission of diseases through the inhalation of microorganisms [5].

The composition of the gastrointestinal microbiota of birds is quite varied and depends on the species, being also composed of fungi. *Candida* spp., *Cryptococcus neoformans, Cryptococcus gatti and Trichosporon* sp. were reported on the excreta of captive birds [6,7] and around 60 different pathogens have been detected on pigeons feces such as *Cryptococcus* sp., *Candida* spp., *Trichosporon* sp. and *Rhodotorula* sp. [8–11]. Despite of this, several birds such as specimens of *Melopsittacus undulatus* (Budgerigar; Psittaciformes), *Paroaria dominicana* (Red-cowled cardinal; Passeriformes), *Amazona* sp. (Amazon parrot; Psittaciformes) and pigeons (*Columba livia*; Columbiformes) can be susceptible of fungal diseases, for example, with symptoms that can affect the gastrointestinal tract and cause weakness [12–14].

Even though these birds pose a low risk to the health of the immunocompetent, immunocompromised individuals have a higher risk of acquiring mycosis from bird droppings in comparison to the general population [8]. Opportunistic fungal infections are expanding as individuals go under immunological or hematological disorders, medical treatments with long-term antibiotics, corticosteroids or immunosuppressive drugs, for example [15].

*C. albicans* and non-*albicans Candida* (NAC) can cause candidiasis with wide symptomatic variation, such as superficial mycosis or disseminated candidiasis, mainly affecting individuals with comorbidities and immunocompromised [16,17] . Although it is observed that *C. albicans* is more frequent and virulent, there has been an increase in the frequency of other species such as *C. tropicalis, C; krusei, C. glabrata* and *C. parapsilosis* [17–19].

Thus, the current study aims to detect potentially pathogenic yeasts (*Candida* species) from feces of pigeons' and captive birds in the city of Maceió, located in northeastern Brazil, comparing the frequencies observed in both avian groups, while also comparing the phenotypic and molecular methods for identification.

## MATERIAL AND METHODS

### Sample collection, processing and phenotypic identification

Excreta samples from birds of the species *Melopsittacus undulatus* (Psittaciformes), *Amazona* sp. (Psittaciformes) and *Paroaria dominicana* (Passeriformes) were collected from 3 residencies in different neighborhoods: Serraria (latitude: -9.602239; longitude: -35.723018), Barro Duro (latitude: -9.616528; longitude: -35.721437) and Petrópolis (latitude: -9.604141; longitude: -35.760321) in the city of Maceió (Alagoas, Brazil). Pigeons' feces (*Columba livia*, Columbiformes) were gathered in urban areas from 4 districts with intense flow of people: Downtown (latitude: -9.666677; longitude: -35.737206) and in the neighborhoods Prado (latitude: -9.668246; longitude: - 35.744972), Gruta de Lourdes (latitude: -9.621754; longitude: -35.731682) and Jatiúca (latitude: -9.650810; longitude: -35.708308).

Each species of captive bird was kept in their individual cages; therefore, the samples were individually labeled according to which avian species they were obtained from. Pigeon fecal dry samples were collected in public squares, all kept in individual sterile containers, for laboratory processing.

Fecal samples (1g) were macerated with a mortar, suspended in 25 mL of sterilized saline (0.9%) with chloramphenicol (0.05g/L) (Sigma Aldrich), vortexed (5 min) and decanted (30 min). Subsequently, 100µL of the supernatant were inoculated in triplicate on Sabouraud dextrose agar with chloramphenicol and plates were incubated at 30ºC until 72h [20], with daily inspections. Yeast colonies were assessed for Gram staining and cultivated on Hicrome®*Candida* differential agar (Himedia) (30ºC; 48 h), followed by storage on Brain Heart Infusion (Sigma Aldrich) with glycerol (10%) under -20ºC.

## DNA extraction and molecular identification

Three different methods of DNA extraction were implemented in this study, in order to find a simple and effective way of obtaining the yeast's genomic material, and they were: (1) Wizard® Genomic DNA Purification Kit (Promega); (2) in-house described by Ferrer and coauthors [21] with phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol); (3) and adapted from Mähnß and coauthors [22]: a single yeast colony was suspended in 100 µL of sterile ultra-pure water and kept at 95ºC (10 minutes) for cell lysis. After reaching room temperature, the microtubes were kept under -20ºC until the partial freezing of the debris, then centrifuged at 15.000 rpm (11.340× g) for 5 minutes. 50µl of the supernatant containing genomic material was transferred to sterile microtubes for direct use or stored at -20ºC for following amplifications. Method (1) was applied on four isolates obtained from caged birds, whereas method (2) was used on 26 isolates, also from caged birds. Method (3) was used on all the isolates gathered from pigeons. The three methods were compared among each other through electrophoresis and polymerase chain reactions (PCRs).

In the PCR, the primers indicated in Table 1 were used for the amplification of ITS1-5.8S-28S and ITS2 fragments, according to previous studies [23–28]. The PCR mix (20µl) contained 1.25X GoTaq® Reaction Buffer (PROMEGA), 0.1 mM PCR Nucleotide Mix, 3.75 mM magnesium chloride, 1.25U/µL GoTaq® polymerase and 0.625pmol primers [12–14], with 1µL of DNA in a final volume per 20µL. PCR conditions were: 5 minutes at 96ºC for initial denaturation, followed by 40 cycles of 94ºC for 30 seconds (denaturation), annealing at 58ºC for 30 seconds, extension at 72ºC for 30 seconds and a final step of 72ºC for 15 minutes [24,25].

**Table 1.** Oligonucleotide's primers used for the molecular identification of *Candida* species (Adapted from HSU and coauthors, 2003 – [25]).

| Target species | Primer | Sequence (5' to 3') | Amplicon size (pb) | Genbank accession number/Reference |
|---|---|---|---|---|
| *C. albicans* | CALB 1 | TTTATCAACTTGTCACACCAGA | ≈273 | L47111 [26] |
| | CALB 2 | ATCCCGCCTTACCACTACCG | | L28817 [27] |
| *C. krusei* | CKRU 1 | GCATCGATGAAGAACGCAGC | ≈258 | AX592669 [28] |
| | CKRU 2 | AAAAGTCTAGTTCGCTCGGGCC | | [23] |
| *C. parapsilosis* | CPA 1 | GCCAGAGATTAAACTAACCA | ≈300 | AF287909* |
| | CPA 2 | CCTATCCATTAGTTTATACTCCGC | | L47109 [26] |
| *C. tropicalis* | CTR 1 | CAATCCTACCGCCAGAGGTTAT | ≈357 | AF287910* |
| | CTR 2 | TGGCCACTAGCAAAATAAGCGT | | AF268095* |
| *C. guilliermondii* | CGU 1 | GCATCGATGAAGAACGCAGC | ≈315 | AX592669 [28] |
| | CGU 2 | GTTTGGTTGTTGTAAGGCCGGG | | [23] |
| *C. glabrata* | CGL 1 | TTATCACACGACTCGACACT | ≈423 | AB032177* |
| | CGL 2 | CCCACATACTGATATGGCCTACAA | | AF167993* |

*Unpublished references

Amplicons were electrophoresed in an agarose gel (1%) in Tris-acetate-EDTA (70v), stained with Nancy-450® (Sigma-Aldrich) and using a 100bp DNA Ladder (Promega). For validation of PCR reactions, reference strains of the American Type Culture Collection were used: *C. albicans* (ATCC 90028), *C. tropicalis* (ATCC 13803), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019) and *C. glabrata* (ATCC 90030).

## Map and Statistical analyses

A map was generated through Qgis (v. 3.18) for indication of the neighborhoods from Maceió/AL: Barro duro (1), Petrópolis (2), Serraria (3), Gruta de Lourdes (4), Jatiúca (5), Downtown (6) and Prado (7). Descriptive analyses were made through JASP (v. 0.8.0.6) software, and Cohen's Kappa coefficient was

applied to calculate the level of agreement between phenotypic (Hicrome®*Candida* differential agar) and molecular identification method (PCR). Sensitivity for Hicrome®*Candida* differential agar was calculated through the following equation:

$$\text{Sensitivity } \frac{(\text{true positive})}{(\text{true positive+false negative})}$$

## RESULTS

The droppings' gathering sites were distributed in different neighborhoods of Maceió, northeastern Brazil (Alagoas). Among the captive birds, *Amazona* sp. was found in the Barro Duro (1), *Paroaria dominicana* in Petrópolis (2), *Melopsittacus undulatus* specimens in a house from Serraria (3), neighborhoods located in the central part of Maceió and characterized as residential areas, with a small flow of people around these birds. On Figure 1 it is possible to confirm the localization of each numbered collection point.

Pigeon's samples were collected in a bus station from Gruta de Lourdes (4), where kids played near to pigeons that were eating, and in the neighborhoods Jatiúca (5), Prado (7) and Downtown (6) (Figure 1), places with a high flow of people. The neighborhood Jatiúca has been a source of tourism value for Maceió, since it is close to the seashore, while Prado is a traditional neighborhood in the city, and its closeness to the city's Downtown also attracts many individuals.

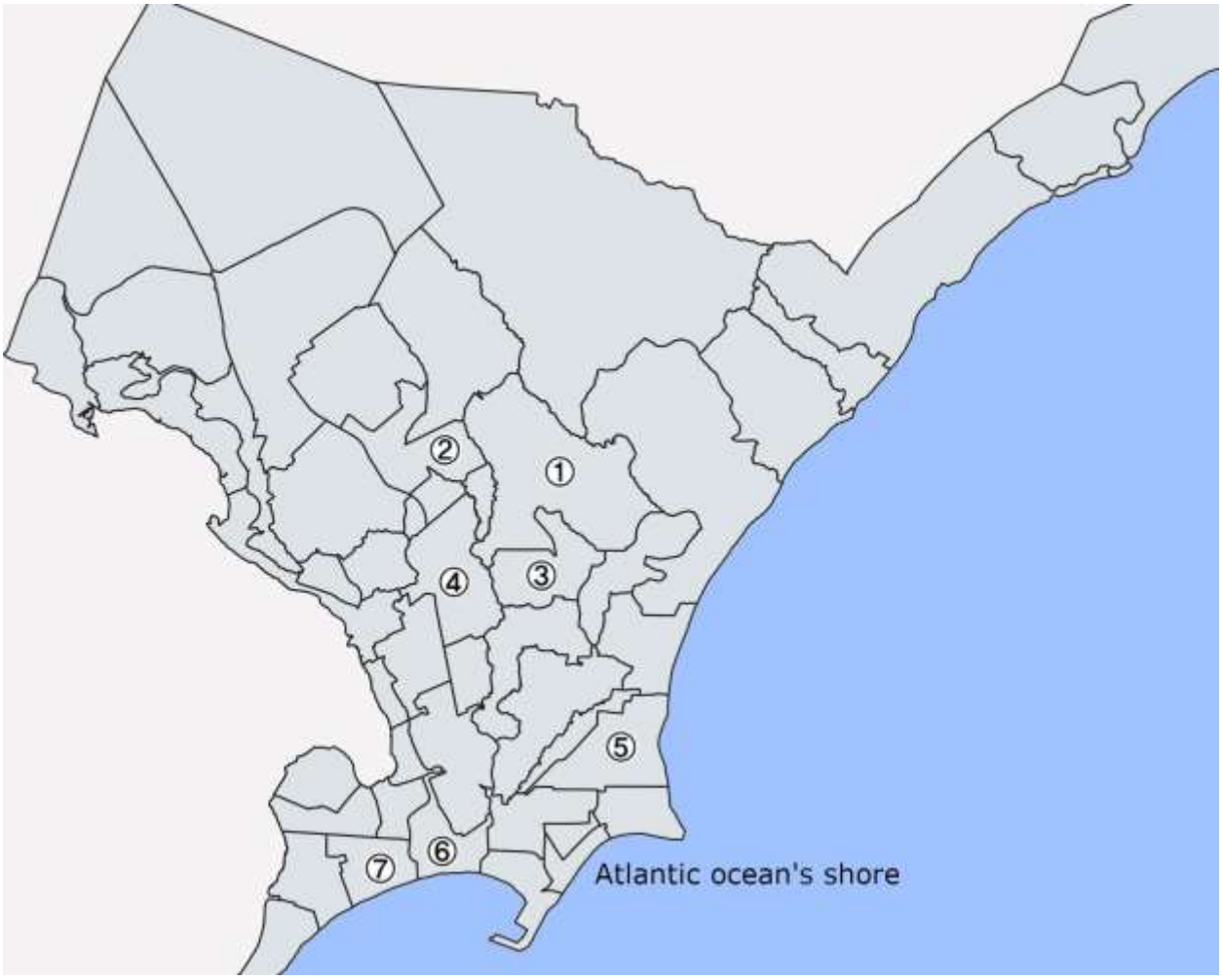


**Figure 1.** Partial map from the city of Maceió (Alagoas, Brazil) with localization of neighborhoods for sampling: Barro duro (1), Petrópolis (2), Serraria (3) for caged birds' stool sampling; Gruta de Lourdes (4), Jatiúca (5), Downtown (6) and Prado (7) for pigeon's samples**.**

After culturing (48h), all fecal samples from *C. livia* and birds held in captivity were positive for yeast growth. Through phenotypic methods (Gram stain and microscopy), we selected 71 strains with the morphology consistent with yeast, where 30 (42.25%) of them came from captive poultry stool and 41 (57.75%) were isolated from pigeons' excreta. We identified on the chromogenic medium three species of

*Candida* from pigeon excreta: *C. albicans* (31.70%; 13/41), *C. tropicalis* (14.63%; 6/41) and *C. krusei*, (14.63%; 6/41), whereas 39.04% were indicated as *Candida* sp., without specific color. All the isolates (30) from captive birds' feces remained as *Candida* sp. in chromogenic media.

As mentioned previously, we aimed to find a simple, fast, and low-cost method for obtained viable DNA for ITS-region amplification, and three distinct DNA extraction protocols were used in this study: (1) Wizard® Genomic kit, (2) in-house protocols with phenol-chloroform-isoamyl alcohol and (3) adapted from Mähnß et al. (2005). As expected, DNA extraction performed with Wizard® Genomic kit (1) and with phenol/chloroform (2) provided cleaner and higher quality DNA, while the method adapted from Mähnß and coauthors [22] carried out by high temperature followed by centrifugation provided genomic material of inferior quality, with impurities ("dirty"), but with sufficient quality to ensure accurate amplifications (PCR) for the identification of different *Candida* species.

Molecular identification by ITS-region (Fig. 2) confirmed *C. albicans* as the most frequent species among the pigeon excreta (31.70%). Regarding non-*albicans* species (NAC), *C. parapsilosis* was isolated in 17.10%, followed by *C. krusei*, *C. tropicalis* and *C. glabrata* with the same prevalence (14.63%, each), while *C. guilliermondii* was less observed in pigeon droppings (7.31%).

From the samples isolated in captive birds' feces, *C. parapsilosis* was the only species identified, present in 20% (6/30) of the isolates. Thus, 80% of the samples (24/30) could not be identified as any of the six species screened with specific primers. We used primers for the identification of six medically important species of *Candida* (*C. albicans, C. tropicalis, C. glabrata, C. krusei*, *C. parapsilosis* and *C. guilliermondii*); therefore, the remaining 24 strains were indicated as probable *Candida* non-albicans, not screened at this point. Total frequencies of *Candida* sp. isolated from the droppings of all avian species analyzed are shown on Table 2.

**Table 2.** Prevalence of *Candida* sp. isolates recovered from droppings of pigeons and captive birds in different locations in the city of Maceió, capital of Alagoas.

| **Fungal species** | **Bird specimens** | | | | **Total** |
|---|---|---|---|---|---|
| | *Melopsittacus undulatus* | *Paroaria dominicana* | *Amazona* sp. | Pigeons (*Columba livia*) | |
| *C. albicans* | 0 | 0 | 0 | 13/41 (31.70%) | 13 |
| *C. parapsilosis* | 2 (6.67%) | 0 | 4(13.33%) | 7/41 (17.10%) | 13 |
| *C. krusei* | 0 | 0 | 0 | 6/41 (14.63%) | 6 |
| *C. tropicalis* | 0 | 0 | 0 | 6/41 (14.63%) | 6 |
| *C. glabrata* | 0 | 0 | 0 | 6/41 (14.63%) | 6 |
| *C. guilliermondii* | 0 | 0 | 0 | 3/41 (7.31%) | 3 |
| *Candida* sp. | 10 (33.33%) | 3 (10%) | 11(36.67%) | 0 | 24 |
| Total | 12/30 (40%) | 3/30 (10%) | 15/30 (50%) | 41 (100%) | 71 |

Species-specific PCR and conventional phenotypic method of species identification presented an interrater correlation for all *C. albicans*, *C. krusei* and *C. tropicalis* isolates (Kappa = 1), when the sensitivity in species identification reached 100% (Table 3). The sensitivity of the Hicrome®*Candida* differential agar for the identification of *C. glabrata*, *C. parapsilosis* and *C. guilliermondii* was not calculated since the colonies can appear very similar on the chromogenic media. In Figure 2 it is possible to observe the specific amplicons for each species identified by the ITS region: *C. guilliermondii* (A), *C. krusei* (B), *C. albicans* (C, D), *C. tropicalis* (C), *C. glabrata* (D) and *C. parapsilosis* (E). All the isolates on Figure 2 were recovered from pigeon's stool, and the DNA extraction method used on them was adapted from Mähnß [22], confirming that even though providing us with lower quality DNA, this protocol is effective for carrying out PCR.

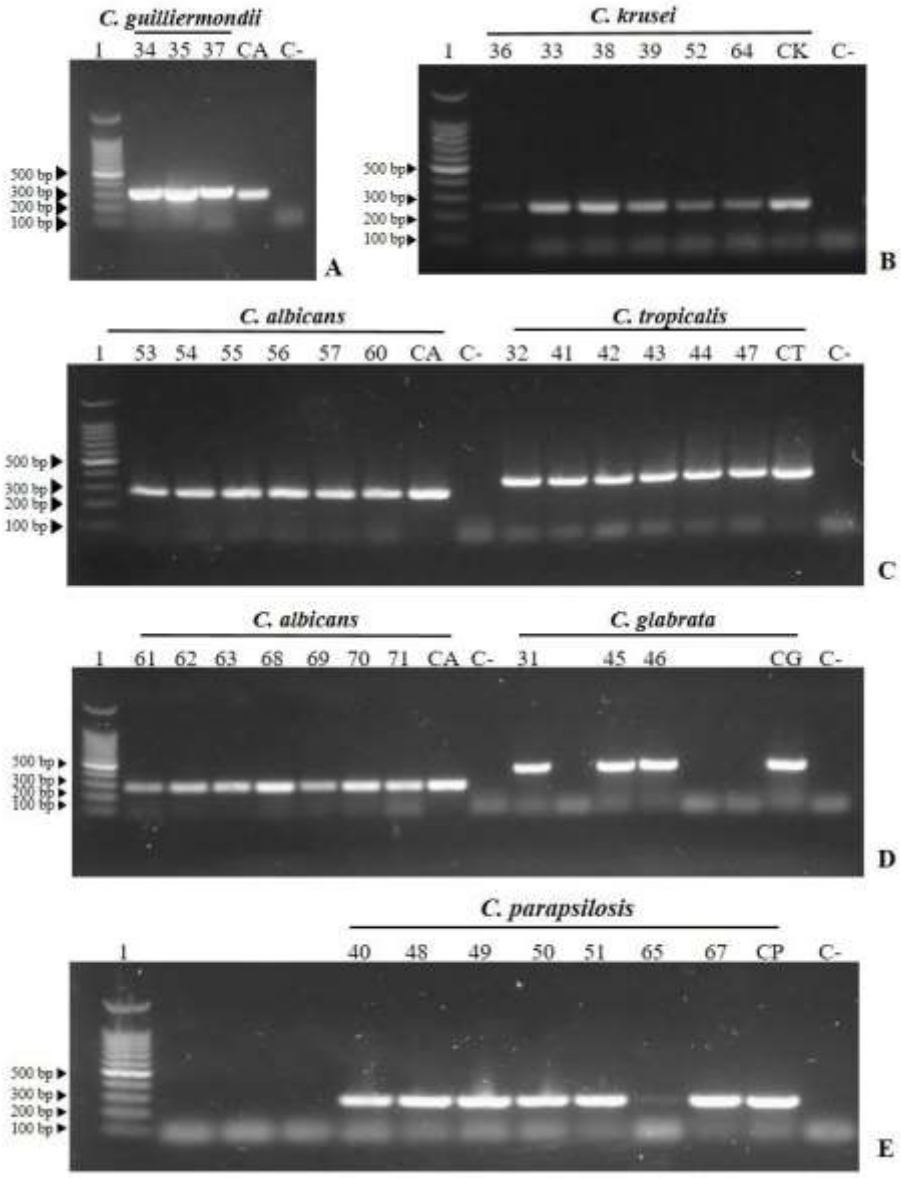


**Figure 2.** Different ITS-profiles for *Candida* species in agarose gel (1%). 1: 100bp DNA Ladder; C-: Negative control; (A) *C. guilliermondii* (≈315pb), CA: positive control of *C. albicans* was used for reaction validation, due to the absence of *C. guilliermondii* ATCC; (B) *C. krusei* (≈258pb), CK: ATCC strain n° 6258 (positive control for *C. krusei*); (C*) C. albicans* (≈273bp): from 53 to 60, CA: ATCC strain n° 90028 (positive control for *C. albicans*); *C. tropicalis* (≈357pb): from 32 to 47, CT: ATCC strain n° 13803 (positive control for *C. tropicalis*); (D) *Candida albicans* (≈273pb): from 61 to 71, CA: ATCC strain n° 90028 (positive control for *C. albicans*); *C. glabrata* (≈423pb): from 31 to 46, CG: strain n° 90030 (positive control for *C. glabrata*); (E) *Candida parapsilosis* (≈300pb), CP: strain n° 22019 (positive control for *C. parapsilosis*).

**Table 3.** *Candida* species obtained from droppings of pigeons and captive birds identified by phenotypic and molecular methods.

| ***Candida* species** | **N° of species identified** | | **Sensitivity** |
|---|---|---|---|
| | **Phenotypic identification** | **Molecular identification** | |
| *C. albicans* | 13 | 13 | 100% |
| *C. krusei* | 6 | 6 | 100% |
| *C. tropicalis* | 6 | 6 | 100% |
| *C. parapsilosis*[1] | 0 | 7 | - |
| *C. glabrata*[1] | 0 | 6 | - |
| *C. guilliermondii*[1] | 0 | 3 | - |
| Not identified | 46 | 24 | - |

[1] for these species (-), sensitivity tests have not been done since their morphologies on Hicrome®*Candida* differential agar can appear relatively similar.

## DISCUSSION

The incidence of the main *Candida* species of medical importance in avian feces was investigated in Maceió (Brazil), using phenotypic methods and PCR-ITS for identification and focusing on areas of great circulation of people and under domestic care. Despite *Candida* spp. being part of the microbiota of healthy humans, many species are involved in nosocomial bloodstream infections (BSI), invasive candidiasis (IC) cases and superficial mycoses in a wide variety of anatomical sites, and the acquisition can be endogenous or exogenous by environmental sources that facilitate contamination [29,30].

Domestic and wild birds are carriers of human pathogenic fungi with zoonotic potential, as demonstrated by Cafarchia and co-workers (2006) after the isolation of *Rhodotorula rubra*, *Cryptococcus albidus*, *Trichosporon cutaneum*, *C. albicans*, *C. guilliermondii* and *C. tropicalis* [31]. The gastrointestinal microbiota of cockatoos (*Nymphicus hollandicus*) from pet shops, houses and breeders was evaluated in Fortaleza/CE (Brazil), identifying *C. tropicalis* and *C. albicans* (43.8%; 6.6%) [32], two species that were not detected in our captive birds droppings samples. In Pelotas/RS (Brazil), Mendes and coauthors [33] also observed species in Passeriformes' excreta that were sought with specific primers, but not confirmed in Maceió (*C. albicans*/31% and *C. guilliermondii*/4%), also isolating *C. famata* (11%), *C. catenulata* (4%), *C. intermedia* (2%), *C. sphaerica* (4%), *C. ciferri* (6%) and *C. globosa* (2%), species that appear less frequently in clinical cases [29,34].

Common species in infectious processes (*C. albicans, C. krusei, C. tropicalis, C. glabrata* and *C. parapsilosis*) have been detected in the feces of crow (*Corvus* sp., Passeriformes) in Malaysia [35], while in Maceió it was only possible to confirm *C. parapsilosis* in Passeriformes droppings. The difference is probably due to the fact that the avian species evaluated in Malaysia is considered synanthropic, whereas the animals we studied held in captivity. Simi and coauthors [7] evaluated birds of prey and the Psittacidae family with serious health problems, kept in public enclosures and quarantined, and found rarer *Candida* species in both groups (e. g., *C. krusei, C. kefyr* and *C. famata*), probably due to the impairment of the immune system. Since in our research only the 6 most frequent species of medical importance were screened, many strains of *Candida* spp. collected from feces of birds from the orders Passeriformes and Psittacidae did not have the species defined and may be rarer species.

In different points of Maceió, the presence of a wide variety of *Candida* species was found in pigeons feces, as well as in towers in Iran, where *C. albicans, C. tropicalis, C. krusei, C. parapsilosis* and *C. guilliermondii* were confirmed [36]. Medina and coauthors [37] detected pathogenic fungi on pigeons in the Canary Islands (Spain) from three different sources: droppings, cloaca and crop. Even though the presence of *C. albicans* and *C. parapsilosis* was also reported, *C. guilliermondii* was the most detected species in all the sites researched, and its frequency in excreta was even higher in comparison to the yeasts found in the cloaca and crop. These different patterns are probably due to climate, geographic and temporal conditions of each city, being *C. guilliermondii* the sixth most frequently isolated *Candida* species at hospitals in Latin America, and considered as an emerging pathogen [38,39].

Considering that the avian cages in our study were cleaned daily and the pigeon excreta were old, and a greater diversity of potentially pathogenic *Candida* species was confirmed in the pigeon excreta compared to the fresh feces of birds in captivity, probably the cleaning actions interfered in the difference in abundance of *Candida* spp. Passeriformes' and Psittaciformes' excreta were not in contact with any organic substrate such as soil, which limits the contamination by diverse fungal species, while older excreta from pigeons were more exposed to the environment and consequently, to a great variety of fungi. Lee and coworkers (2017) stated that geographical, sociological and meteorological factors have a considerable role on the relative abundance of clinically relevant fungal species after evaluating pigeon stool in Seoul (South Korea). In addition, it is likely that many fungal species do not survive the passage through the gastrointestinal tract, and the transmission to the excreta happens more frequently through the air or soil [40].

At pigeon breeding farms in Beijing, China, Wu and coauthors [41] detected a low frequency of species such as *C. glabrata* (1/120), *C. albicans* (1/120) and *C. catenulata* (2/120), once the feces were fresh in a clean environment. The fact that there is no pigeon migration on breeding farms also interferes, as it avoids direct contact of these birds with fungal reproductive structures. Public places with organic matter available usually attract pigeons, and there are indications that the formation of fungal communities indoors (e.g., apartments) is influenced by the fungal composition of outdoors [42]; furthermore, fungi showed signs of dispersal limitation in hundreds of meters, indicating that there is a relation between the distance and dispersal decay in microbial communities. Therefore, many sources indicate that pigeon droppings in public places have a better chance of grouping a variety of fungi, as we have confirmed in different locations in Maceió, distributed from the central region of the city to near the coastal area.

Environmental yeast strains can also show antifungal resistance and produce virulence factors, as demonstrated in *C. albicans* isolates resistant to itraconazole and fluconazole, or phospholipase-producing species [43,44]. Isolates of *Candida* spp. (41%) collected from pigeon droppings around a university hospital showed the ability to adhere to the oral epithelium, in addition to the majority having varying abilities regarding the action of phospholipase and biofilm production, important virulence mechanisms for success in infection [45]. Even though *C. albicans* is still considered the most frequent species in fomites and infections, there has been an increase in the frequency of non-*albicans Candida* (NAC) in patients, asymptomatic carriers and in the environment, additionally, there is confirmation of *Candida* isolates showing intermediate susceptibility to azoles and echinocandin resistance [46,39]. As reported here, NAC have been identified in 45.83% of the isolates from Maceió, and according to Pfaller [47] *C. albicans, C. parapsilosis, C. tropicalis, C. krusei* and *C. glabrata* were responsible for more than 95% of community and hospital-acquired BSI cases in several countries, with a high incidence in North and Latin America [47].

In the Midwest region of Brazil, it was confirmed the incidence of 35.3% of *C. albicans* and 64.7% NAC, besides to indicate the emergence of non-*C. albicans* species azole-resistant [48], while in Alagoas more than 3,000 isolates of *Candida* spp. have been observed in different clinical samples for 8 years; then, it is clear that yeasts of the genus *Candida* continue to be the most involved in superficial and systemic mycoses [30,47]. Yapar (2014) concluded that researchers worldwide pointed to a decrease of 65% to 44% in cases of IC by *C. albicans* between the late 1990s and 2010, followed by an increase of cases assigned to *C. tropicalis* and *C. parapsilosis.* Therefore, since pigeons pose risks to animal and human health, the prevention of infections in immunosuppressed and immunocompetent individuals who live or work in environments with birds must be associated with a daily care, including cleaning and disinfecting urban and captivity spaces (washing and brushing with antimicrobial agents).

In addition to confirming the frequency of species, we also compared different methodologies for identifying *Candida* species, using a common chromogenic medium and gene-specific PCR. Chromogenic medium has been commonly used in clinical analysis laboratories for the identification of the main *Candida* species of medical interest, and the accuracy between this medium and the PCR was confirmed by a previous study, where Daef [50] tested the Hicrome®*Candida* medium in clinical isolates in Egypt and reported 100% sensitivity and 98.9% specificity for *C. albicans* and 100% for *C. tropicalis* and *C. krusei* when compared with semi-Nested PCR results. Our tests showed the same, and we indicated the positive correlation between the results observed in the chromogenic medium used with PCR for the species of *C. albicans, C. tropicalis* and *C. krusei*.

The choice to boil colonies for PCRs from the adapted method described by Mähnß and coauthors [22] provided us DNA with more impurities compared to the other protocols performed in this study (by kit and phenol-chloroform-isoamyl alcohol). However, despite its low quality, the DNA obtained was satisfactory, since our PCR-amplifications worked well. Junqueira and coauthors [51] and Ribeiro and coauthors [52] also adopted the same base-protocol for the molecular identification of clinical samples of *Candida* spp. and their PCR reactions were successful. This protocol is simple, fast, inexpensive, and efficient, being a good alternative for laboratories to use in PCR-based methods.

## CONCLUSION

Based on our findings, pigeon droppings harbored more potentially pathogenic *Candida* species compared to the stool gathered from captive birds, indicating that in more exposed and older bird feces there will be a greater abundance of yeasts of *Candida* genus. This data demonstrates the need to monitor the presence of birds in places where there is a great circulation of people, since dispersed yeasts in their feces may act as opportunistic pathogens. Therefore, the appropriate maintenance of public areas and cages decreases the risks to individuals exposed to bird feces. In addition, we recommend an effective, simple and affordable methodology for identification of *Candida* species by PCR amplification of genomic DNA.

**Conflicts of Interest:** The authors declare no conflict of interest.

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