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Are Brazilian laboratories capable of identifying *Candidozyma auris*? Results obtained from an external quality control program

¿Son los laboratorios brasileños capaces de identificar *Candidozyma auris*? Resultados obtenidos de un programa externo de control de calidad

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Abstract

Background: *Candidozyma auris* (formerly *Candida auris)* has emerged as a global health threat due to its rapid transmission, multidrug resistance, and high mortality rates, and has become increasingly relevant in Brazil since the first confirmed case in 2020. Laboratory capacity to promptly identify this pathogen is critical for effective clinical management and outbreak control.

Aims: This study evaluated the proficiency of Brazilian laboratories in correctly identifying *C. auris* within an external quality control program.

Methods: An analysis was conducted using a 2023 batch containing an isolate of *C. auris* distributed to laboratories participating in the mycology specialty program.

Results: Proficiency outcomes revealed that only 25.9% of the laboratories correctly identified *C. auris*, and 62.1% reached only the genus level. An incorrect identification of the species within the genus *Candida* was 10.3%, and an incorrect identification of the genus level occurred in 1.7% of the laboratories.

Conclusions: These findings highlight major gaps in the laboratory participants' diagnostic capacity, underscoring the urgent need for improved infrastructure.

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Keywords: *Candida auris*; Candidemia; Laboratories; Diagnostic tests; Quality control; Brazil

Resumen

Antecedentes: *Candidozyma auris* (antes *Candida auris*) ha emergido como una amenaza para la salud global debido a su rápida transmisión, resistencia a múltiples fármacos y altas tasas de mortalidad, y se ha vuelto cada vez más relevante en Brasil desde el primer caso confirmado en 2020. La capacidad de los laboratorios para identificar oportunamente este patógeno es fundamental para una gestión clínica eficaz y el control de brotes.

Objetivos: Este estudio evaluó la competencia de los laboratorios brasileños para identificar *C. auris* en un programa externo de control de calidad.

Métodos: Se realizó un análisis de un lote de 2023 que contenía un aislamiento de *C. auris* y que fue distribuido a los laboratorios participantes del programa de especialidad en micología.

Resultados: Los resultados de competencia revelaron que sólo el 25,9% de los laboratorios identificaron correctamente *C. auris*, mientras que el 62,1% llego únicamente a la identificación del género. El 10,3% emitió una identificación incorrecta de la especie dentro del género *Candida*, mientras que en el 1,7% de los laboratorios la identificación del género fue errónea.

Conclusiones: Estos hallazgos ponen de relieve importantes deficiencias en la capacidad diagnóstica de los laboratorios participantes y subrayan la urgente necesidad de mejorar la infraestructura.

Palabras clave: *Candida auris*; Candidemia; Laboratorios; Pruebas diagnósticas; Control de calidad; Brasil

TEXT

Since its first isolation in 2009, *Candidozyma auris* (previously *Candida auris*) has become a globally distributed nosocomial pathogen, gaining special attention during the COVID-19 pandemic [10, 24]. In 2022, the World Health Organization (WHO) classified it as a critical priority pathogen [26]. In Brazil, the National Sanitary Surveillance Agency (*Agência Nacional de Vigilância Sanitária* - ANVISA) has been warning about the global emergence of *C. auris* since 2017, with the first case confirmed by the end of 2020 [10] and outbreaks reported in 2021 and 2022 [2].

Some characteristics of this species are associated with its emergence and spread. For example, *C. auris* can persist for long periods in hospital environments, causing outbreaks in hospital wards [4], and there are also reports of multidrug-resistant strains (15, 17). Hence, *C. auris* infection is associated with prolonged hospital stays and high mortality rates, which can vary from 29 to 62% [18].

Laboratory readiness to provide correct and early identification is essential for infection control [2, 4, 18]. However, correct identification of this species remains a challenge [11]. The reference identification methods are matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) and polymerase chain reaction (PCR) [20].

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However, access to these technologies is also a limitation, especially in low-income countries [8, 14]. In addition, having well-trained laboratory personnel is crucial for ensuring high-quality results. According to the Ministry of Health and the ANVISA, it is mandatory for clinical laboratories to participate in external quality control programs [23]. The National Quality Control Program (PNCQ), coordinated by the Brazilian Society of Clinical Analysis, is one of the traditional and available programs in Brazil [25]. In 2025, the program enrolled 6333 clinical laboratories from 31 countries, the majority of which were from various regions of Brazil. The program offers external quality control services across various laboratory areas and at different levels of complexity, organized into programs ranging from basic to advanced. Mycological identification proficiency is included only in the advanced program, so it is not performed at all laboratories attending PNCQ.

The global scenario of invasive fungal infections underscores the need for Brazilian clinical laboratories to accurately identify fungi, particularly those designated as critical by the WHO. The study aimed to assess the proficiency of the participating Brazilian laboratories, specifically in identifying *C. auris*, given its worldwide emergence.

Methods

Samples for mycological evaluation were sent to laboratories across Brazil. The biological material supplied by the PNCQ is accompanied by clear and standardized instructions for handling, manipulation, and laboratory processing, developed in compliance with the biosafety best practices established by current legislation. All stages of isolation, identification, and microbiological validation are documented in specific forms and archived within the quality management system, in compliance with General requirements for the competence of reference material producers ABNT NBR ISO/IEC 17034 [5] and Conformity assessment - General requirements for the competence of proficiency testing providers ABNT NBR ISO/IEC 17043 [6]. In mycology, the program evaluates the correct identification (to the genus and/or species level) of the fungal specimen sent [25]. The PNCQ analysis focuses exclusively on the technical quality of the submitted responses. No information that could identify the participating laboratorios was used or considered in the analysis.

In July 2023, a batch containing a *C. auris* (ATCC MYA-5000) sample, accompanied by a clinical case, was sent to participant laboratories. The clinical case briefly describes the patient's clinical history and the type of sample as follows: “Male, 82 years old, ICU patient presenting with persistent fever even with antibiotic therapy; blood culture was positive for yeasts.”

We performed a retrospective analysis of the results submitted by the enrolled laboratories for *C. auris* identification. Despite the recent taxonomic revisions within the *Candida* genus (e.g., *Candidozyma auris,* formerly *Candida auris*; *Nakaseomyces glabratus,* formerly *Candida glabrata*; *Pichia kudriavzevii*, formerly *Candida krusei*; and *Meyerozyma guilliermondii,* formerly *Candida guilliermondii*), and considering that all these organisms are considered as causal agents of candidiasis, and that the updated nomenclature is not yet widely adopted, this external quality assessment program used the former species names and considered all these organisms as belonging to the *Candida* genus.

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This study uses only data about the technical performance of the different laboratories. At no time was there any disclosure or handling of information capable of directly or indirectly identifying any laboratory, technical supervisor, or affiliated institution. We assessed proficiency results by identification level (species, genus) and by laboratory type (public vs. private) across Brazilian regions. Differences between groups were assessed using Fisher's exact test, followed by Cramér's V analysis to evaluate the strength of associations. Additionally, a multivariable binary logistic regression model was performed, including the funding source and the geographic region as predictors. The level of fungal identification of the strain was stratified, being considered complete (correct species identification) or not. Effect sizes were expressed as odds ratios (OR) with 95% confidence intervals. All analyses were conducted using IBM SPSS Statistics version 32.0.

Results

From 116 responses, 30 participants (25.9%) correctly identified *C. auris*; 72 participants (62.1%) correctly identified the genus *Candida* spp., without species identification; and 12 participants (10.3%) correctly identified the genus *Candida* but misidentified the species (table 1). Misidentifications occurred with *C. glabrata* (n=5), *C. albicans* (n=2), *C. krusei* (n=2), *C. parapsilosis* (n=2), and *C. guilliermondii* (n=1). Additionally, two participants (1.7%) misidentified both the genus and species, reporting *Cryptococcus neoformans* (1) and *Cryptococcus gattii* (1).

The participant laboratories were distributed regionally as follows: 5.2% from the North, 7.8% from the Northeast, 9.5% from the Midwest, 34.5% from the Southeast, and 43.1% from the South. The Brazilian states with the highest number of participants were Rio Grande do Sul (n=21), Paraná (n=18), São Paulo (n=18), Minas Gerais (n=11), and Santa Catarina (n=11) (Figure 1). Despite regional variations, no statistically significant differences were observed (p-value = 0.569; 95% CI: 0.559–0.579) (Table 1). The Cramér's V indicated a weak association effect size (V = 0.168, p = 0.629; 95% CI: 0.619–0.638). Concerning the region, no significant association with the fungal identification reached was observed. All regional categories presented non-significant odds ratios with wide confidence intervals, indicating there was no regional influence on classification outcomes ($p > 0.05$).

Private laboratories accounted for 81% of participants, while public laboratories accounted for 19%. Despite the uneven proportions, private laboratories had more partial identifications, while public laboratories demonstrated higher correct species identification rates (p-value = 0.018). The strength of this association was moderate based on Cramér's V (V = 0.290, exact p = 0.030), suggesting that the funding source had a relevant influence on laboratory identification accuracy. A binary logistic regression showed that public laboratories were significantly associated with higher odds of complete fungal identification compared with private laboratories (OR = 4.021, 95% CI: 1.473–10.981, p = 0.007). Table 2 presents the identification levels according to public and private funding.

Although public laboratories demonstrated overall performance higher than that of private institutions in complete and correct species identification, this was not consistent across all regions. These findings need to be interpreted with caution, as some subgroup analyses produced sparse cell counts and wide confidence intervals, indicating limited precision of the estimates.

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Discussion

Since the first reported case of *C. auris* in Brazil in 2020, ANVISA has reported 13 outbreaks and 84 cases [1, 12]. The rapid spread of *C. auris* underscores the urgent need for proficient laboratory identification. Patients infected with *C. auris* undergo a colonization phase preceding infection, which is essential for early diagnosis and infection prevention [10, 24]. This study assessed the capacity of Brazilian clinical laboratories to correctly identify *C. auris* from the perspective of a quality control program.

In our study, only about a quarter of the laboratories participating in the PNCQ proficiency test completely and correctly identified *C. auris*. Similar results were found in a study with laboratory participants of a quality external control from Argentina, where they noticed a good performance of the laboratories to identify common yeasts, such as *Candida albicans* (95%), but markedly decreased when the species was the emerging yeast, like *C. auris* (13%) [22]. In contrast, a study from the Netherlands reported that 35 of 47 participants (74.5%) in an external quality assessment correctly identified *C. auris* [8]. Likewise, Dewaele et al. [13] found that 82 out of 142 Belgian laboratories (57.7%) correctly identified *C. auris*. In both European studies, most participants used MALDI-TOF and identified more than one strain [8, 13]. One limitation of our study is the lack of information regarding the methodologies used by the participating laboratories for yeasts' identification. However, our results are similar to those reported in the Argentinian study. We hypothesize that these findings may reflect the limited diagnostic capabilities of the laboratories participating in this quality control program. This hypothesis is supported by the study by Falci and Pasqualotto [14], which conducted a survey among laboratories in Central and Latin America and highlighted participants' limited diagnostic capabilities. Of the 124 respondents, the majority were from Brazil (n=96), and only 19% reported access to MALDI-TOF technology and 17% to DNA sequencing. In contrast, 74% of the laboratories used biochemical tests (71%) or automated tests (e.g., Vitek®) in their routine. In the same study, only 14% of respondents identified yeasts at the species level. An epidemiologic study on *C. auris* in South America also emphasizes the need to improve laboratories' capacity to identify this speciess [19].

MALDI-TOF is one of the preferred identification methods, but it may lead to the misidentification of *C. auris* if its database is not updated with the specific profile of this species [8, 16]. The same applies to Vitek®, which can identify *C. auris* only if its database has been updated to include this species. Molecular methods are also recommended; PCR with species-specific primers [9, 16, 21] is more suitable due to its lower cost, shorter processing time than sequencing, and high specificity, or a gold-standard strategy using panfungal primers followed by sequencing for identification confirmation. On the other hand, biochemical and phenotypic assays are not very effective because *C. auris* is closely related to other species, such as those in the *C. haemulonii* complex [4], and cannot be distinguished from them using these methods. While culture can assist in species identification, it often fails to differentiate *C. auris* from more common yeast species unless a specifically modified culture medium is used [8, 9, 16]. Even in these cases, culture media provide presumptive identification that must be confirmed by gold-standard methods.

Despite emphasizing the importance of achieving an accurate species identification, the majority of the reports identified the isolate solely at the genus level (i.e. as *Candida*).

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This incomplete identification, or any incorrect species identification, poses significant clinical challenges. The primary risk factors for *C. auris* colonization include contact with infected patients or contaminated environments [16]. Misidentification may lead to a lack of control measures and the appearance of an outbreak in the hospital setting. Additionally, the majority of this species' lineages show resistance to antifungal agents commonly used empirically, such as triazoles and amphotericin B [16], which may expose patients to unnecessary toxicity without effectively treating the pathogen and can lead to treatment failure.

The laboratories that failed to correctly identify *Candida* genus submitted their responses as *Cryptococcus* species, which was considered the most critical error. Infections caused by *Candida* and *Cryptococcus* species have different clinical presentations, and the protocols for identifying the species differ as well [23, 28]. No statistically significance was found among Brazilian regions in terms of the correct identification rate. Another hypothesis for misidentification in our study is about the training of the laboratory staff. An analysis of the results of continuing education tests on mycology among PNCQ participants between 1999 and 2009 revealed that only 63.7% of laboratories.scored 8-10 correct answers out of ten questions [26]. This data suggests that there is still room for improvement, particularly in laboratory management, which should prioritize enhanced training of its personnel.

In our study, public laboratories demonstrated higher accuracy in identifying *C. auris.* Nevertheless, the imbalance between the public and private groups is a limitation in this study. Public laboratories, as reference centers, should play a critical role in accurate yeast identification and in epidemiological surveillance and outbreak reporting [3, 25, 22]. According to ANVISA, any *C. auris* identification must be confirmed in a reference laboratory and, in Brazil, almost all of them are public. This could explain the best performance of these centers in species-level identification. However, the reference laboratories are based in state capitals and sometimes far from the original laboratory where the isolate was collected [2], which can delay proper identification and outbreak prevention. The best results in the public service can also be explained by the best training conditions and greater accessibility to them for personnel working in the public service, in contrast to the private sector.

Most participants in our study were from the south and southeast. According to official data, those are the richest regions in our country. However, we did not find statistical significance between the level of fungal identification and the location of the laboratorios. Furthermore, most laboratories in the richest regions identify yeast isolates at the genus level only [7]. As mentioned before, the lack of appropriate methods in the labs is a significant barrier to reach an accurate species identification. Another suggestion is the low level of awareness of fungal diseases in laboratories not located in hospital contexts.

Although the major limitation of this study is the lack of information on the methods used by each laboratory enrolled in this round of the proficiency program, the results have shown that the majority of Brazilian laboratories are not yet prepared to accurately identify *C. auris*. The misidentification of this species has major public health implications, as it may lead to inappropriate antifungal therapy, delayed implementation of infection control measures, continued hospital transmission, and underestimation of its true epidemiological burden. Hence, it is imperative for these laboratories to enhance their mycologic diagnostic capabilities to ensure precise results in any specific clinical background. The present study aims to raise awareness of this urgent situation and intends to alert

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the clinical community to the need for mitigation measures. Enhancing mycology diagnostic capacity will not only support outbreak control, but also elevate the standard of care for fungal infections in Brazil.

Conclusion

Based on this proficiency testing round, the laboratory participants are not yet adequately prepared to accurately identify *C. auris* . The uneven distribution of diagnostic capabilities across the country highlights the need for investments in technology, training, and regional reference networks. Strengthening mycology diagnostic capacity is essential not only to contain outbreaks, but also to improve the overall quality of care for fungal infections in Brazil.

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Declarations

Author contributions: LBR: conceptualization, writing, data analysis, and interpretation; CBS and HS: conceptualization, editing, reviewing, and final approval for submission; SCW, SBL, LR, PA, and PMN: reviewing and final approval for submission.

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Table 1. Identification of *C. auris* among the laboratories participating (n= 116) in the National Program of Quality Control by regions (Brazil).

Region	Species identification	Genus identification only	Species misidentification	Misidentification	Total of responses
North	16.7% (1/6)	66.7% (4/6)	16.7% (⅙)	- (0/6)	6
Northeast	33.3% (3/9)	44.4% (4/9)	11.1% (1/9)	11.1% (1/9)	9
Midwest	36.4% (4/11)	45.4%(5/11)	18.2%(2/11)	- (0/11)	11
Southeast	30% (12/40)	62.5% (25/40)	37.5% (3/40)	- (0/40)	40
South	20% (10/50)	68% (34/50)	10% (5/50)	2% (1/50)	50
Total (n)	25.9% (30/116)	62.1% (72/116)	10.3% (12/116)	1.7% (2/116)	116

Species misidentification: Correct genus and incorrect species; **Misidentification:** Other microorganisms than *Candida* spp.

Table 2. Identification of *C. auris* among the laboratories participating (n=116) in the National Program of Quality Control (Brazil) according to laboratories'funding.

Funding source	Species identification	Genus identification only	Species misidentification	Misidentification	Total of responses
Private	20.1% (19/94)	68.1% (64/94)	9.6% (9/94)	2.1% (2/94)	94
Public	50% (11/22)	36.4% (8/22)	313.6% (3/22)	- (0/22)	22

Species misidentification: Correct genus and incorrect species; **Misidentification:** Other microorganisms than *Candida* spp.