Distribution, antifungal susceptibility pattern and intra-\textit{Candida albicans} species complex prevalence of \textit{Candida africana}: A systematic review and meta-analysis

Sanaz Aghaei Gharehbolagh\textsuperscript{1}, Bahareh Fallah\textsuperscript{2}, Alireza Izadi\textsuperscript{3}, Zeinab Sadeghi Ardestani\textsuperscript{2}, Pooneh Malekifar\textsuperscript{3}, Andrew M. Borman\textsuperscript{4}, Shahram Mahmoudi\textsuperscript{5}\textsuperscript{*}

\textsuperscript{1} Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, \textsuperscript{2} Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, \textsuperscript{3} Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, \textsuperscript{4} Public Health England UK National Mycology Reference Laboratory, Southmead Hospital Bristol, Medical Research Council Centre for Medical Mycology (MRC CMM), University of Exeter, Exeter, United Kingdom, \textsuperscript{5} Department of Medical Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

* Mahmoudi.sh@iums.ac.ir, sh.mahmoudi93@gmail.com

\section*{Abstract}

\textit{Candida africana} is a pathogenic species within the \textit{Candida albicans} species complex. Due to the limited knowledge concerning its prevalence and antifungal susceptibility profiles, a comprehensive study is overdue. Accordingly, we performed a search of the electronic databases for literature published in the English language between 1 January 2001 and 21 March 2020. Citations were screened, relevant articles were identified, and data were extracted to determine overall intra-\textit{C. albicans} complex prevalence, geographical distribution, and antifungal susceptibility profiles for \textit{C. africana}. From a total of 366 articles, 41 were eligible for inclusion in this study. Our results showed that \textit{C. africana} has a worldwide distribution. The pooled intra-\textit{C. albicans} complex prevalence of \textit{C. africana} was 1.67\% (95\% CI 0.98–2.49). Prevalence data were available for 11 countries from 4 continents. Iran (3.02\%, 95\%CI 1.51–4.92) and Honduras (3.03\%, 95\% CI 0.83–10.39) had the highest values and Malaysia (0\%) had the lowest prevalence. Vaginal specimens were the most common source of \textit{C. africana} (92.81\%; 155 out of 167 isolates with available data). However, this species has also been isolated from cases of balanitis, from patients with oral lesions, and from respiratory, urine, and cutaneous samples. Data concerning the susceptibility of \textit{C. africana} to 16 antifungal drugs were available in the literature. Generally, the minimum inhibitory concentrations of antifungal drugs against this species were low.

In conclusion, \textit{C. africana} demonstrates geographical variation in prevalence and high susceptibility to antifungal drugs. However, due to the relative scarcity of existing data concerning this species, further studies will be required to establish more firm conclusions.
Introduction

The medically important polyphyletic genus *Candida* contains more than 300 different yeast species, around 20 of which are regularly reported from human infections ranging in spectrum from superficial mycoses to deep-seated and disseminated infections [1–3]. *Candida albicans* is widely accepted as the most virulent species in the genus, and is the etiological agent in approximately 50%, 95%, and 80–90% of cases of nosocomial bloodstream *Candida* infections, oropharyngeal and vulvovaginal candidiasis, respectively [4–7].

*C. albicans* is a complex of three closely-related species, *C. albicans sensu stricto*, *C. dubliniensis*, and *C. africana* [6, 8]. *C. africana*, which was first isolated in Africa in 1995, was proposed as a new species within the *C. albicans* complex in 2001 [9, 10]. With a worldwide distribution, *C. africana* has been isolated from diverse clinical specimens (mucous membranes, cutaneous samples, specimens from the urinary and respiratory tracts, blood) and has been reported to cause a wide variety of human infections including vulvovaginal candidiasis, oral thrush, and blood stream infections [11–15].

Unlike the other members of *C. albicans* complex, *C. africana* is unable to form chlamydo-spores and cannot assimilate glucosamine, N-acetylglucosamine, trehalose, or DL-lactate. However, in common with *C. albicans* and *C. dubliniensis* it has retained the capacity to produce germ-tubes. Moreover, molecular studies have demonstrated high levels of genetic relatedness between *C. africana* and *C. albicans* [16–18]. Thus, differentiation of *C. africana* from the other members of *C. albicans* complex using conventional identification techniques is difficult [19, 20].

Given these issues, molecular methods such as an end point PCR based on size polymorphism of the *hwp1* gene (*C. albicans*: 941bp, *C. dubliniensis*: 569 bp, and *C. africana*: 700 bp) have been designed to discriminate between *C. albicans*, *C. dubliniensis*, and *C. africana* [21]. Using such approaches, the prevalence of *C. africana* within the *C. albicans* complex has been reported to vary significantly from 0 to 8.4% depending on the geographic regions in which analyses were performed [11, 19, 22–24]. Furthermore, while some studies have suggested that the susceptibility profiles of *C. africana* to antifungal drugs are similar to those of *C. albicans* [25], others have reported different antifungal susceptibility patterns for these species [8, 26]. In light of the above discrepancies concerning *C. africana* prevalence and antifungal susceptibility, the present review and meta-analysis was designed to summarize all of the available data concerning this recent addition to the *C. albicans* species complex.

Methods

Search strategy

Two independent researchers conducted bibliographic search in PubMed, Scopus, and Web of Science databases as well as in Google Scholar using keywords or phrases “*Candida africana*”, “*C. africana*”, “*Candida albicans* complex”, “*Candida albicans sibling species*”, and “*Candida albicans cryptic species*” and their combinations. Since *Candida africana* was first described as a novel species in 2001 [10], our search covered the literature published in the English language from 2001 to 21st March 2020.

Study selection

Citations were included into EndNote software version X8, duplicates were deleted and the title and abstract of remaining citations were reviewed to exclude irrelevant articles. For the remaining citations, full texts were downloaded and evaluated. All English language articles with available full texts that reported data on antifungal susceptibility patterns of *Candida*
africana and/or prevalence of Candida africana within the Candida albicans species complex using molecular methods met the inclusion criteria. Conference abstracts, review articles, and articles reporting data other than the susceptibility pattern and/or prevalence of Candida africana were excluded. The quality of the selected studies was checked using the STROBE checklist [27]. References cited in the eligible articles were also screened to guarantee the inclusion of all relevant studies.

Data extraction

Data including the name of the first author, publication year, country, number of Candida albicans complex isolates, number of identified Candida africana isolates, the source of Candida africana isolates, and the minimum inhibitory concentration (MIC) values of various drugs against Candida africana isolates were extracted into a pre-prepared excel file by two independent researchers. Corresponding authors of studies reporting only the summary data of antifungal susceptibility pattern such as MIC range, geometric mean (GM), and MIC₅₀ were contacted via email for the raw data. In the case of no response, the summarized data of antifungal susceptibility patterns were excluded from the final analysis.

Data analysis

The pooled estimated prevalence of C. africana within the C. albicans complex was calculated using Stata software version 14. Variances and their confidence intervals were calculated using exact method. The pooled estimate was between 0 to 1. For studies reporting a prevalence of 0%, Freeman-Tukey double arcsine transformation was used to stabilize variances. Heterogeneity was determined using the I² statistic which was calculated using the DerSimonian-Laird method. For quantification of heterogeneity, Cochrane Q test was used. In the presence of heterogeneity, random effect model provides better estimates [28, 29], accordingly, we used this model in calculations when heterogeneity was proved to exist. Subgroup analysis was done to define the prevalence of C. africana within the C. albicans complex in different countries and continents. The presence of publication bias was checked by using the funnel plot and the Begg’s test. In the case of asymmetric funnel plot, Trim and Fill method was used to define the number of missing studies and the imputed estimated prevalence. To check for changes in prevalence over time, meta regression was conducted where the year of publication was set as the independent variable. In all calculations p-values <0.05 were considered to be significant.

Results

A summary of the results of the search strategy is depicted in Fig 1. The original bibliographic search identified 363 articles. An additional 3 articles were identified though examination of all of the literature cited in the retained articles (other sources, Fig 1). After de-duplication and exclusion of irrelevant citations based on the title and abstract, 73 articles were retained for full text evaluation. At this stage, 32 articles were excluded on the basis of the criteria listed in Fig 1 and 41 articles were eligible to be included in the present study (Table 1). Due to the presence of heterogeneity (I² = 66.02%, 95% CI 44–77, p<0.001), random-effect model was used. The pooled prevalence of C. africana within the C. albicans complex was 1.67% (95% CI 0.98–2.49) (Fig 2). Data on prevalence were available for 11 countries from 4 continents. Iran (3.02%, 95%CI 1.51–4.92) and Honduras (3.03%, 95% CI 0.83–10.39) had the highest values and Malaysia (0%) had the lowest reported prevalence. (Table 2, S1 Fig and S2 Fig).

As shown in Fig 3, the funnel plot was broadly symmetrical, suggesting the absence of publication bias. This finding was confirmed using Begg’s test (Z = 1.26, p = 0.215). In meta-
regression analysis, no evidence for significant change in the prevalence of *C. africana* over time was found (Coefficient = -0.0013, SE = 0.0052, \( p = 0.802 \)) (S3 Fig).

Information on isolation source was available for a total of 167 isolates. Although the vast majority of isolates were from the female genital tract (vagina; \( n = 155, 92.81\% \)), there were also isolates from patients with balanitis (\( n = 5, 2.99\% \)), oral lesions (\( n = 4, 2.39\% \)), and isolates from respiratory, urine, and skin samples (1 isolate each, 0.6%).

MIC values were available for *C. africana* isolates and 16 antifungal drugs including azoles, echinocandins, polyenes, allylamine, and 5-flucytosine. As shown in Table 3, the MIC ranges, MIC\(_{50}\) and MIC\(_{90}\) and geometric mean values were generally low.

**Discussion**

*C. africana*, a member of *C. albicans* species complex, is genetically and phenotypically closely related to *C. albicans*. The pathogenicity of *C. africana* and its impact on the health of humans is poorly understood. Moreover, the global prevalence and antifungal susceptibility profiles of this species are not clearly defined [16, 18, 30]. In this study we tried to provide an overview of the available data published to date on both of these aspects of *C. africana* epidemiology/biology.

*C. africana* appears to be globally distributed, with an intra-*C. albicans* complex prevalence that varies in different regions and countries [16, 31, 43]. To date, data concerning prevalence are available for 11 countries from 4 continents (Africa, America, Asia, and Europe), with a pooled intra-complex prevalence of 1.67% (95% CI 0.98–2.49). Based on the available
Table 1. Characteristics of 41 studies reporting data on intra-*Candida albicans* complex prevalence and/or antifungal susceptibility pattern of *Candida africana* which were eligible to be included in the current systematic review and meta-analysis.

| Reference            | Year | Country      | No. of *C. albicans* complex/C. africana | Source of isolates (N) | Data of antifungal drugs                                                                 |
|----------------------|------|--------------|-----------------------------------------|------------------------|------------------------------------------------------------------------------------------|
| Alonso-Vargas et al. | 2008 | Spain        | NA/1                                     | Vagina (1)             | Flu, ITR, VRC, KTC, AmB, FLC                                                            |
| Borman et al. [17]   | 2013 | United Kingdom | 826/15                                  | Vagina (15)            | Flu, ITR, MCN, KTC, CLT, ECN, AmB, NYS                                                   |
| Dieng et al. [31]    | 2012 | Senegal      | 112/3                                    | Vagina (3)             | NA                                                                                       |
| Fakhim et al. [32]   | 2020 | Iran         | 114/3                                    | Vagina (3)             | Flu, ITR, VRC, AmB, FLC, CSP, ANF, MCF                                                   |
| Farahyar et al. [33] | 2020 | Iran         | 100/3                                    | Vagina (3)             | Flu, ITR                                                                                 |
| Feng et al. [22]     | 2015 | China        | 49/0                                     | -                      | NA                                                                                       |
| Fontechea et al. [6] | 2019 | Honduras     | 66/2                                     | Vagina (1), Urine (1)  | NA                                                                                       |
| Gil-Alonso et al. [8] | 2015 | Spain        | NA/2                                     | Vagina (1), Reference strain (1) | MCF                                                                                     |
| Gil-Alonso et al. [34] | 2015 | Spain        | NA/2                                     | Vagina (1), Reference strain (1) | CSP, ANF, MCF                                                                 |
| Gil-Alonso et al. [26] | 2016 | Spain        | NA/2                                     | Vagina (1), Reference strain (1) | CSP                                                                                     |
| Gil-Alonso et al. [35] | 2019 | Spain        | NA/2                                     | Vagina (1), Reference strain (1) | ANF                                                                                     |
| Gumral et al. [23]   | 2011 | Turkey       | 195/0                                    | -                      | NA                                                                                       |
| Guzel et al. [36]    | 2013 | Turkey       | 58/0                                     | -                      | NA                                                                                       |
| Hashemi et al. [37]  | 2019 | Iran         | 44/2                                     | Vagina (2)             | NA                                                                                       |
| Hazirokana et al. [25] | 2017 | Turkey       | 376/3                                    | Vagina (3)             | Flu, VRC, KTC, AmB, ANF, MCF                                                            |
| Hu et al. [38]       | 2015 | China        | 129/5                                    | Balanitis (5)          | Flu, ITR, VRC, PSC, AmB, FLC, CSP, MCF                                                   |
| Kardos et al. [39]   | 2017 | Hungary      | NA/2                                     | Vagina (1), Reference strain (1) | MCF                                                                                     |
| Khedri et al. [13]   | 2018 | Iran         | 74/4                                     | Oral lesions (4)       | Flu, ITR, VRC, AmB, CSP                                                                 |
| Kova’cs et al. [39]  | 2017 | Hungary      | NA/2                                     | Vagina (1), Reference strain (1) | MCF                                                                                     |
| Lortholary et al. [40] | 2007 | France       | NA/3                                     | -                      | NA                                                                                       |
| Majdabadi et al. [41] | 2018 | Iran         | 40/2                                     | Vagina (2)             | Flu, ITR, AmB                                                                            |
| Mucci et al. [5]     | 2017 | Argentina    | 57/0                                     | -                      | NA                                                                                       |
| Naemiri et al. [19]  | 2018 | Iran         | 119/10                                   | Vagina (10)            | Flu                                                                                      |
| Ngouana et al. [20]  | 2014 | Cameroon     | 115/2                                    | Vagina (2)             | Flu, ITR, KTC, AmB                                                                      |
| Ngouana et al. [42]  | 2019 | Cameroon     | 115/2                                    | Vagina (2)             | NA                                                                                       |
| Nnadi et al. [43]    | 2012 | Italy        | 84/2                                     | Vagina (2)             | Flu, VRC, PSC, AmB, CSP, KTC, ITR, FLC                                                   |
| Pakshir et al. [44]  | 2017 | Iran         | 110/0                                    | -                      | NA                                                                                       |
| Rezazadeh et al. [45] | 2016 | Iran         | 67/4                                     | Vagina (4)             | NA                                                                                       |
| Rezazadeh et al. [45] | 2016 | Iran         | NA/4                                     | NA                     | Flu, ITR, VRC, PSC, AmB, CSP                                                            |
| Romeo et al. [46]    | 2009 | Italy        | 376/27                                   | Vagina (27)            | NA                                                                                       |
| Romeo et al. [46]    | 2009 | Italy        | 134/1                                    | Vagina (1)             | NA                                                                                       |
| Scordino et al. [1]  | 2019 | Italy        | 21/0                                     | -                      | NA                                                                                       |
| Shan et al. [3]      | 2014 | China        | 1014/15                                  | Vagina (15)            | Flu, ITR, NYS, MCN, CLT                                                                  |
| Sharifynia et al. [14] | 2015 | Iran         | 83/1                                     | Lung (1)               | Flu, ITR, AmB, CSP                                                                       |
| Sharma et al. [16]   | 2014 | India        | 283/4                                    | Vagina (4)             | Flu, ITR, MCN, VRC, KTC, CLT, PSC, IS, AmB, FLC, CSP, ANF, MCF, TRB                      |
| Shokohi et al. [47]  | 2018 | Iran         | 47/1                                     | Skin (1)               | NA                                                                                       |
| Solimani et al. [24] | 2014 | Iran         | 35/0                                     | -                      | NA                                                                                       |
| Theill et al. [2]    | 2016 | Argentina    | 287/1                                    | Vagina (1)             | Flu, ITR, VRC, CLT, AmB, TRB, NYS                                                        |

(Continued)
literature, Malaysia (0.0%; 95% CI 0.0–3.77) has the lowest prevalence. Iran (3.02%; 95% CI 1.51–4.92) and Honduras (3.03%; 95% CI 0.83–10.39) have the highest prevalence. However, since prevalence in Iran was drawn from 12 different studies, it is likely to be a more reliable estimate than the prevalence reported for Honduras, which was based on a single study. Variation in prevalence could be seen between and across continents. For instance, although Iran has the highest prevalence, the prevalence of *C. africana* in a neighboring country, Turkey, is dramatically lower (0.22%; 95% CI 0.0–0.91). It is unclear whether this difference in relative prevalence is the result of an insufficient number of studies in Turkey, genuine local geographical variation, or a combination of both. It is also worth mentioning that the prevalence values reported in the current study are estimated with limited numbers of studies from each country. Data are also lacking for the majority of countries. Thus, the present view might change if there were more studies internationally that addressed the prevalence of *C. africana*.

The intra-complex prevalence of *C. africana* appears to be constant over time. In recent decades, the prevalence of non- *albicans* Candida species has increased [49, 50] and there are reports describing species other than *C. albicans* as being the most common etiologic agents of infection locally [51–53]. However, it seems that a similar scenario has not been occurring within the *C. albicans* species complex since the meta-regression analysis of our data indicates that there is no significant change in the intra-complex prevalence of *C. africana* with the passage of time. However, once again there are caveats to this suggestion. First, it is based on data from a limited number of countries. Moreover, the power of meta-regression analyses is low especially when the number of studies included is low, which is the case in the present study.

Female genital specimens are the most common source of isolation of *C. africana*. Of 167 *C. africana* isolates with available data, the majority (n = 155, 92.81%) were from the vagina. Vaginal candidiasis due to *C. africana* has been reported in various countries [32]. This species was also isolated from cases of balanitis (n = 5, 2.99%) and oral lesions (n = 4, 2.39%), and from respiratory, urine, and skin samples (each 1 isolate, 0.6%), all of which could conceivably become contaminated with vaginal flora or pathogens. The apparent preponderance of *C. africana* for the female genital tract highlights the need for appropriate methods for discrimination of *C. africana* from *C. albicans* complex isolates, especially for vaginal specimens.

Data on antifungal susceptibility of *C. africana* to 16 antifungal drugs are available in the published literature (Table 3). It should be highlighted that the data presented in Table 3 are limited to articles in which detailed results of antifungal susceptibility testing are provided. Other articles that have reported their results as the number of resistant/susceptible isolates or as geometric mean and MIC range (and not the raw MICs) could not be included in Table 3. Similar patterns of susceptibility to various antifungal drugs has been reported for *C. africana* and *C. albicans* [25], while other studies have noted that *C. africana* exhibits a different

| Reference          | Year | Country | No. of *C. albicans* complex/*C. africana* | Source of isolates (N) | Data of antifungal drugs |
|--------------------|------|---------|------------------------------------------|------------------------|--------------------------|
| Yazdanpanah et al. [11] | 2014 | Malaysia | 98/0                                     | -                      | NA                       |
| Yazdanparast et al. [48] | 2015 | Iran    | 114/5                                    | Vagina (5)             | Flu, ITR, VRC, PSC, AmB, CSP, ANF, MCF |
| Zhu et al. [12]     | 2019 | China   | NA/43                                    | Vagina (43)            | Flu, ITR, MCN, VRC, CLT, BTC, TRC |

Abbreviations: NA: not available, Flu: fluconazole, ITR: itraconazole, VRC: voriconazole, KTC: ketoconazole, AmB: amphotericin B, FLC: 5-fluorocytosine, MCN: miconazole, CLT: clotrimazole, ECN: econazole, NYS: nystatin, CSP: caspofungin, ANF: anidulafungin, MCF: micafungin, PSC: posaconazole, TRB: terbinafine, ISC: isavuconazole, BTC: butoconazole, TRC: terconazole

https://doi.org/10.1371/journal.pone.0237046.t001
susceptibility pattern to \textit{C. albicans} [8, 26]. Since there are no specified clinical breakpoints or epidemiological cut-off values (ECVs) for antifungal drugs against \textit{C. africana}, the interpretation of MICs as susceptible/resistant or wild-type/non wild-type is potentially controversial. However, there are reports in which isolates of \textit{C. albicans} have been categorized as resistant to itraconazole, 5-flucytosine, terbinafine, fluconazole, and clotrimazole [28, 33, 54]. By applying the clinical breakpoints for \textit{C. albicans} (CLSI M60) [55], the species most closely related to \textit{C. africana}, it could be inferred that almost all isolates of \textit{C. africana} with available MICs for fluconazole, voriconazole, anidulafungin, caspofungin, and micafungin are susceptible to these antifungal drugs. For itraconazole, in contrast to CLSI (M60 supplement) [55] which no longer proposes breakpoints for \textit{Candida} species, the European Committee on Antimicrobial
Table 2. The pooled intra-\textit{Candida albicans} complex prevalence of \textit{Candida africana} in different countries and continents based on the reported studies between 2001 to March 2020.

| Continent | Country     | Prevalence (%) (95% confidence interval) |
|-----------|-------------|-----------------------------------------|
| Africa    | Cameroon    | 1.74 (0.65–4.54)                        |
|           | Senegal     | 2.68 (0.87–7.98)                        |
|           | Overall     | 2.09 (1–4.32)                           |
| America   | Argentina   | 0.11 (0.00–1.04)                        |
|           | Honduras    | 3.03 (0.83–10.39)                       |
|           | Overall     | 0.51 (0.00–2.46)                        |
| Asia      | China       | 1.50 (0.22–3.57)                        |
|           | India       | 1.41 (0.55–3.58)                        |
|           | Iran        | 3.02 (1.51–4.92)                        |
|           | Malaysia    | 0.00 (0.00–3.77)                        |
|           | Turkey      | 0.22 (0.00–0.91)                        |
|           | Overall     | 1.66 (0.81–2.73)                        |
| Europe    | Italy       | 2.33 (0.04–6.79)                        |
|           | United Kingdom | 1.82 (1.10–2.97)                    |
|           | Overall     | 2.17 (0.29–5.25)                        |

https://doi.org/10.1371/journal.pone.0237046.t002

Fig 3. The funnel plot of available studies reporting data on intra-\textit{Candida albicans} complex prevalence of \textit{Candida africana} between 2001 to March 2020 (each circle is representative of one study).

https://doi.org/10.1371/journal.pone.0237046.g003
Susceptibility Testing recently published new breakpoints for itraconazole against *C. albicans* and *C. dubliniensis* [29]. Using those breakpoints (>0.06 μg/mL = resistance) 12 out of 43 (27.91%) *C. africana* isolates with available data would be itraconazole-resistant. Further studies will be required to generate MIC data for sufficient numbers of isolates of *C. africana* to allow the establishment of robust species-specific ECVs and clinical breakpoints for this species.

### Conclusion

*C. africana* is a minor species within the *C. albicans* complex with a pooled prevalence of 1.67%. Reports of this species are available from a limited number of countries and further investigations are required internationally to fully address its global distribution. The vagina is the most common human source of *C. africana* and based on clinical breakpoints established for the related *C. albicans*, this species can be inferred to be generally susceptible to most currently available antifungal drugs.

### Supporting information

S1 Fig. The forest plot of intra-*Candida albicans* complex prevalence of *Candida africana* in different countries based on the reported articles between 2001 to 2020 (size of squares is representative of the relative weight of studies).

(TIF)

S2 Fig. The forest plot of intra-*Candida albicans* complex prevalence of *Candida africana* in different continents based on the reported articles between 2001 to 2020 (size of squares is representative of the relative weight of studies).

(TIF)

S3 Fig. The meta-regression of intra-*Candida albicans* complex prevalence of *Candida africana* with the time (size of circles is representative of the relative weight of studies;
studies with a prevalence of 0% are not shown).

(TIF)

S1 Appendix. The search strategy used in PubMed database to find relevant literature.

(CSV)

S2 Appendix. The completed PRISMA checklist for systematic reviews.

(DOC)

Author Contributions

Conceptualization: Shahram Mahmoudi.

Data curation: Sanaz Aghaei Gharehbolagh, Bahareh Fallah, Alireza Izadi, Zeinab Sadeghi Ardestani, Pooneh Malekifar, Andrew M. Borman, Shahram Mahmoudi.

Formal analysis: Pooneh Malekifar, Shahram Mahmoudi.

Investigation: Sanaz Aghaei Gharehbolagh, Bahareh Fallah, Alireza Izadi, Zeinab Sadeghi Ardestani.

Methodology: Sanaz Aghaei Gharehbolagh, Shahram Mahmoudi.

Project administration: Shahram Mahmoudi.

Software: Pooneh Malekifar.

Supervision: Shahram Mahmoudi.

Writing – original draft: Sanaz Aghaei Gharehbolagh, Bahareh Fallah, Alireza Izadi, Shahram Mahmoudi.

Writing – review & editing: Andrew M. Borman, Shahram Mahmoudi.

References

1. Scordino F, Giuffre L, Felice MR, Orlando MG, Medici MA, Merlo FM, et al. Genetic diversity of Candida albicans isolates recovered from hospital environments and patients with severe acquired brain injuries. Infect Genet Evol. 2019; 76: 7.

2. Theil L, Dudiuk C, Morano S, Gamarna S, Nardin ME, Méndez E, et al. Prevalence and antifungal susceptibility of Candida albicans and its related species Candida dubliniensis and Candida africana isolated from vulvovaginal samples in a hospital of Argentina. Rev Argent Microbiol. 2016; 48(1): 43–49. https://doi.org/10.1016/j.ram.2015.10.003 PMID: 26922471

3. Shan Y, Fan S, Liu X, Li J. Prevalence of Candida albicans-closely related yeasts, Candida africana and Candida dubliniensis, in vulvovaginal candidiasis. Med Mycol. 2014; 52(6): 636–640. https://doi.org/10.1093/mmy/myu003 PMID: 25023482

4. Bitar I, Khalaf RA, Harastani H, Tokajian S. Identification, typing, antifungal resistance profile, and biofilm formation of Candida albicans isolates from Lebanese hospital patients. BioMed research international. 2014; 2014: 931372. https://doi.org/10.1155/2014/931372 PMID: 24982915

5. Mucci MJ, Cuestas ML, Landanburu MF, Mujica MT. Prevalence of Candida albicans, Candida dubliniensis and Candida africana in pregnant women suffering from vulvovaginal candidiasis in Argentina. Rev Iberoam Micol. 2017; 34(2): 72–76. https://doi.org/10.1016/j.riam.2016.09.001 PMID: 28385421

6. Fontecha G, Montes K, Ortiz B, Galindo C, Braham S. Identification of Cryptic Species of Four Candida Complexes in a Culture Collection. J Fungi (Basel). 2019; 5(4): 12.

7. Vila T, Sultán AS, Montelongo-Jauregui D, Jabra-Rizk MA. Oral Candidiasis: A Disease of Opportunity. J Fungi (Basel). 2020; 6(1): 15.

8. Gil-Alonso S, Jauregizar N, Eraso E, Quindos G. Postantifungal Effect of Micafungin against the Species Complexes of Candida albicans and Candida parapsilosis. PloS one. 2015; 10(7): e0132730. https://doi.org/10.1371/journal.pone.0132730 PMID: 26168269
Prevalence and susceptibility pattern of *Candida africana*

9. Tietz H, Küssner A, Thanos M, De Andrade MP, Presber W, Schönian G. Phenotypic and genotypic characterization of unusual vaginal isolates of *Candida albicans* from Africa. J Clin Microbiol. 1995; 33(9): 2462–2465. https://doi.org/10.1128/JCM.33.9.2462-2465.1995 PMID: 7494047

10. Tietz HJ, Hopp M, Schmalreck A, Sterry W, Czaika V. *Candida africana* sp. nov., a new human pathogen or a variant of *Candida albicans*? Mycoses. 2001; 44(11–12): 437–445. https://doi.org/10.1046/j.1439-0507.2001.00707.x PMID: 11820255

11. Yazdianpanah A, Khaltir TMN. Issues in identifying germ tube positive yeasts by conventional methods. J Clin Lab Anal. 2014; 28(1): 1–9. https://doi.org/10.1002/jcla.21635 PMID: 24375729

12. Zhu YX, Shi Y, Fan SR, Liu XP, Yang J, Zhong SL. Multilocus sequence typing analysis of *Candida africana* from vulvovaginal candidiasis. BMC Infect Dis. 2019; 19(1).

13. Khedri S, Santos ALS, Roudbary M, Hadighi R, Falahati M, Farahyari S, et al. Iranian HIV/AIDS patients with oropharyngeal candidiasis: identification, prevalence and antifungal susceptibility of *Candida* species. Lett Appl Microbiol. 2018; 67(4): 392–399. https://doi.org/10.1111/lam.13052 PMID: 30019443

14. Sharifynia S, Badali H, Sorkherizi MS, Shidfar MR, Hadian A, Shahrokhi S, et al. In vitro antifungal susceptibility profiles of *Candida albicans* complex isolated from patients with respiratory infections. Acta Med Iran. 2016; 54(6): 376–381. PMID: 27306344

15. Odds FC, Bougnoux M-E, Shaw DJ, Bain JM, Davidson AD, Diogo D, et al. Molecular phylogenetics of *Candida albicans*. Eukaryotic cell. 2007; 6(6): 1041–1052. https://doi.org/10.1128/EC.00041-07 PMID: 17416899

16. Sharma C, Muralidhar S, Xu J, Meis JF, Chowdhary A. Multilocus sequence typing of *Candida africana* from patients with vulvovaginal candidiasis in New Delhi, India. Mycoses. 2014; 57(9): 544–552. https://doi.org/10.1111/myc.12193 PMID: 24697839

17. Borman AM, Szekely A, Linton CJ, Palmer MD, Brown P, Johnson EM. Epidemiology, antifungal susceptibility, and pathogenicity of *Candida africana* isolates from the United Kingdom. J Clin Microbiol. 2013; 51(3): 967–972. https://doi.org/10.1128/JCM.02816-12 PMID: 23303503

18. Felice MR, Gulati M, Guffre L, Giosa D, Di Bella LM, Criseo G, et al. Molecular Characterization of the N-Acetylgalactosamine Catabolic Genes in *Candida africana*, a Natural N-Acetylgalactosamine Kinase (HXK1) Mutant. PloS one. 2016; 11(1): e0147902. https://doi.org/10.1371/journal.pone.0147902 PMID: 26808192

19. Naeini B, Mirhendi H, Khamsipour G, Sadeghzadeh F, Ahmadi B. *Candida africana* in recurrent vulvovaginal candidiasis (RVVC) patients: Frequency and phenotypic and genotypic characteristics. J Med Microbiol. 2018; 67(11): 1601–1607. https://doi.org/10.1099/jmm.0.00834 PMID: 30248002

20. Ngouana TK, Krasteva D, Drakulovski P, Toghue RK, Kouanfack C, Ambe A, et al. Investigation of minor species *Candida africana*, *Candida stellatoides* and *Candida dubliniensis* in the *Candida albicans* complex among Yaoundé (Cameroon) HIV-infected patients. Mycoses. 2015; 58(1): 33–39. https://doi.org/10.1111/myc.12266 PMID: 25289589

21. Romeo O, Criseo G. First molecular method for discriminating between *Candida africana*, *Candida albicans*, and *Candida dubliniensis* by using hwp1 gene. Diagn Microbiol Infect Dis. 2008; 62(2): 230–233. https://doi.org/10.1016/j.diagmicrobio.2008.05.014 PMID: 18640803

22. Feng X, Ling B, Yang X, Liao W, Pan W, Yao Z. Molecular identification of *Candida* species isolated from onychomycosis in Shanghai, China. Mycopathologia. 2015; 180(5–6): 365–371. https://doi.org/10.1007/s11046-015-9927-9 PMID: 26227864

23. Gumrak R, Sancak B, Guzel AB, Saraci MA, Ilkt M. Lack of *Candida africana* and *Candida dubliniensis* in Vaginal *Candida albicans* Isolates in Turkey Using HWP1 Gene Polymorphisms. Mycopathologia. 2011; 172(1): 73–76. https://doi.org/10.1007/s11046-011-9401-2 PMID: 21380767

24. Solimani P, Salari S, Khalizadeh S, Hassanraz P, Abastabar M, et al. Use of PCR-RFLP and PCR-HWP1 for identification of *Candida* species isolated from cystic fibrosis patients. Res Mol Med. 2014; 2(3): 23–27.

25. Hazrironi G, Altun HU, Gumrak R, Gursoy NC, Oltu B, Sancak B. Prevalence of *Candida africana* and *Candida dubliniensis*, in vulvovaginal candidiasis: First Turkish *Candida africana* isolates from vulvovaginal candidiasis. J Mycol Med. 2017; 27(3): 376–381. https://doi.org/10.1016/j.jmycmed.2017.04.106 PMID: 28641919

26. Gil-Alonso S, Jauregizar N, Eraso E, Quindós G. Postantifungal effect of caspofungin against the *Candida albicans* and *Candida parapsilosis* clades. Diagn Microbiol Infect Dis. 2016; 86(2): 172–177. https://doi.org/10.1016/j.diagmicrobio.2016.07.011 PMID: 27492134

27. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandebroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Annals of internal medicine. 2007; 147(8): 573–577. https://doi.org/10.7326/0003-4819-147-8-200710160-00010 PMID: 17938396
28. Kontopantelis E, Reeves D. Performance of statistical methods for meta-analysis when true study effects are non-normally distributed: A simulation study. Statistical methods in medical research. 2012; 21(4): 409–426. https://doi.org/10.1177/0962280210392008 PMID: 21148194

29. Brockwell SE, Gordon IR. A comparison of statistical methods for meta-analysis. Statistics in medicine. 2001; 20(6): 825–840. https://doi.org/10.1002/sim.650 PMID: 11252006

30. Alonso-Vargas R, Eldoruy L, Eraso E, Cano JF, Guarro J, Ponton J, et al. Isolation of Candida africana, probable atypical strains of Candida albicans, from a patient with vaginitis. Med Mycol. 2008; 46(2): 167–170. https://doi.org/10.1080/13693780701633101 PMID: 17885960

31. Dieng Y, Sow D, Ndiaye M, Guichet E, Faye B, Tine R, et al. Isolation of Candida africana, probable atypical strains of Candida albicans, from a patient with vaginitis. Med Mycol. 2008; 46(2): 167–170. https://doi.org/10.1080/13693780701633101 PMID: 21148194

32. Fakhim H, Vaezi A, Javidnia J, Nasri E, Mahdi D, Diba K, et al. Candida africana Vulvovaginitis: Prevalence and Geographical Distribution. J Mycol Med. 2020: 100966.

33. Farahyar S, Izadi S, Razmjou E, Falahati M, Roudbar Y, Ashrafi-Khoozani M, et al. Low prevalence of antifungal resistant Candida africana, in the C. albicans complex causing vulvovaginal candidiasis. Heliyon. 2020; 6(3): e03619.

34. Gil-Alonso S, Jauregizar N, Cánón E, Eraso E, Quindós G. Comparison of the in vitro activity of echinocandins against Candida albicans, Candida dubliniensis, and Candida africana by time–kill curves. Diagn Microbiol Infect Dis. 2015; 82(1): 57–61. https://doi.org/10.1016/j.diagmicrobio.2015.01.010 PMID: 25703894

35. Gil-Alonso S, Quindós G, Eraso E, Jauregizar N. Postantifungal effect of anidulafungin against Candida albicans, Candida dubliniensis, Candida africana, Candida parapsilosis, Candida metapsilosis and Candida orthopsilosis. Rev Esp Quimioter. 2019; 32(2): 183–188. PMID: 30847462

36. Guzel AB, Kucukgoz-Gulec U, Aydin M, Gumral R, Kalkanci A, Ilkit M. Candida vaginitis during contraceptive use: the influence of methods, antifungal susceptibility and virulence patterns. Journal of obstetrics and gynaecology: the journal of the Institute of Obstetrics and Gynaecology. 2013; 33(8): 850–856.

37. Hashemi SE, Shokohi T, Abastabar M, Aslani N, Ghadamzadeh M, Haghani I. Species distribution and susceptibility profiles of Candida species isolated from vulvovaginal candidiasis, emergence of C. lusitaniae. Current medical mycology. 2019; 5(4): 26–34. https://doi.org/10.18502/cmm.5.4.2062 PMID: 32104741

38. Hu Y, Yu A, Chen X, Wang G, Feng X. Molecular Characterization of Candida africana in Genital Specimens in Shanghai, China. BioMed research international. 2015; 2015.

39. Kovacs R, Saleh Q, Bozo A, Toth Z, Gesztesy L, Kardos T, et al. Killing Activity of Micafungin Against Candida albicans, Candida dubliniensis, Candida africana, Candida parapsilosis, Candida metapsilosis and Candida orthopsilosis. Rev Esp Quimioter. 2019; 32(2): 183–188. PMID: 30847462

40. Lortholary O, Dannaoui E, Raoux D, Hoinard D, Datry A, Paugam A, et al. In vitro susceptibility to posaconazole of 1,903 yeast isolates recovered in France from 2003 to 2006 and tested by the method of the European committee on antimicrobial susceptibility testing. Antimicrob Agents Chemother. 2007; 51(9): 3378–3380. https://doi.org/10.1128/AAC.00496-07 PMID: 17576839

41. Majdabadi N, Falahati M, Heidarie-Kohan F, Farahyar S, Rahimi-Moghaddam P, Ashrafi-Khoozani M, et al. Effect of 2-Phenylethanol as Antifungal Agent and Common Antifungals (Amphotericin B, Fluconazole, and Itraconazole) on Candida Species Isolated from Chronic and Recurrent Cases of Candidal Vulvovaginitis. Assay Drug Dev Technol. 2018; 16(3): 141–149. https://doi.org/10.1089/adt.2017.837 PMID: 29658789

42. Ngouana TK, Toghuoe RMK, Kenfack IF, Lachaud L, Nana AK, Tadjou L, et al. Characterization of Candida africana and susceptibility testing of non-albicans Candida species colonizing mucosal of HIV-infected patients in Yaoundé (Cameroon). J Mycol Med. 2019; 29(3): 233–238. https://doi.org/10.1016/j.mycmed.2019.06.003 PMID: 31204235

43. Nnadi NE, Ayanbipm GE, Soordino F, Okolo MO, Enweani IB, Criso G, et al. Isolation and molecular characterization of Candida africana from Jos, Nigeria. Med Mycol. 2012; 50(7): 765–767. https://doi.org/10.3109/13693786.2012.662598 PMID: 22380533

44. Pakshir K, Bordbar M, Zomorodian K, Nouraei H, Khodadadi H. Evaluation of CAMP-like effect, biofilm formation, and discrimination of Candida africana from vaginal Candida albicans species. J Pathog. 2017; 2017.

45. Rezagazadeh E, Sabokbar A, Moazeni M, Rezaei MS, Badali H. Microdilution in vitro antifungal susceptibility patterns of Candida species, from mild cutaneous to bloodstream infections. Jundishapur J Microbiol. 2016; 9(7).
46. Romeo O, Criseo G. Molecular epidemiology of Candida albicans and its closely related yeasts Candida dubliniensis and Candida africana. J Clin Microbiol. 2009; 47(1): 212–214. https://doi.org/10.1128/JCM.01540-08 PMID: 18987171

47. Shokohi T, Moradi N, Badram L, Badali H, Atoollahi MR, Afsarian MH. Molecular Identification of Clinically Common and Uncommon Yeast Species. Jundishapur J Microbiol. 2018; 11(10): 6.

48. Yazdanparast SA, Khodavaisy S, Fakhir H, Shokohi T, Haghani I, Nabili M, et al. Molecular Characterization of Highly Susceptible Candida africana from Vulvovaginal Candidiasis. Mycopathologia. 2015; 180(5–6): 317–323. https://doi.org/10.1007/s11046-015-9924-z PMID: 26183965

49. Ben-Ami R. Treatment of invasive candidiasis: A narrative review. J Fungi (Basel). 2018; 4(3): 97.

50. Makanjula O, Bongomin F, Fayemiwo SA. An update on the roles of non-albicans Candida species in vulvovaginitis. J Fungi (Basel). 2018; 4(4): 121.

51. Juyal D, Sharma M, Pal S, Rathaur VK, Sharma N. Emergence of non-albicans Candida species in neonatal candidemia. N Am J Med Sci. 2013; 5(9): 541. https://doi.org/10.4103/1947-2714.118919 PMID: 24251272

52. Rishi S, Jain B. Prevalence of Albicans and Non-Albicans Candiduria in a Tertiary Care Hospital of Jai- pur, India. Galore International Journal of Health Sciences and Research. 2020; 5(1): 1–5.

53. Sun M, Chen C, Xiao W, Chang Y, Liu C, Xu Q. Increase in Candida parapsilosis candidemia in cancer patients. Mediterr J Hematol Infect Dis. 2019; 11(1).

54. Al-Hedaily SS, Fotedar R. Recovery and studies on chlamydospore-negative Candida albicans isolated from clinical specimens. Med Mycol. 2002; 40(3): 301–306. https://doi.org/10.1080/mmy.40.3.301.306 PMID: 12146780

55. CLSI performance Standards for Antifungal Susceptibility Testing of Yeasts. 1st ed. CLSI supplement M60. Wayne, PA: CLinical and Laboratory Standards Institute; 2017.