Mycoses in northeastern Brazil: epidemiology and prevalence of fungal species in 8 years of retrospective analysis in Alagoas

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Received: 17 December 2018 / Accepted: 22 May 2019 / Published online: 28 May 2019
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Abstract

Superficial and cutaneous mycoses are common in tropical countries, caused by dermatophytes, yeast, and non-dermatophyte molds in different clinical specimens. In order to define the epidemiology of mycoses and the profile of their etiological agents in Alagoas (northeastern Brazil) between 2009 and 2016, we obtained data of patients from the main laboratories of Alagoas, by examining clinical samples with direct microscopy and culture on Sabouraud dextrose agar and Chromagar[®] *Candida*. A total of 3316 patients were confirmed with mycoses (595 men/2716 women) and 40.25 of average age. Positive samples totaled 3776, mainly vaginal secretion (1593/42.2%), toenails (876/23.2%), and fingernails (589/15.6%). Yeasts were the most isolated (3129/82.9%), including 3012 *Candida* spp. (79.8%), 57 *Malassezia* spp. (1.5%), 42 *Trichosporon* sp. (1.1%), 10 *Geotrichum* spp. (0.3%), and 8 *Rhodotorula* spp. (0.2%). *Candida albicans* was the most frequent species (715/18.9%), followed by *C. krusei* (194/5.1%), *C. tropicalis* (24/0.6%), and 2079 unspecified species (55.1%). Among 17.1% filamentous fungi, 14.8% dermatophytes were distributed as 211 *Trichophyton* sp. (5.6%), 125 *T. rubrum* (3.3%), 106 *T. tonsurans* (2.8%), 72 *T. mentagrophytes* (1.9%), 2 *Microsporum* sp. (0.1%), 15 *M. canis* (0.4%), and 26 *Epidermophyton* sp. (0.7%). Other fungi represented the minority: *Fusarium* sp. and *Aspergillus* sp. These are the first clinical data on the Alagoas population affected by fungi pathogens, confirming a higher incidence of candidiasis (mainly vulvovaginal and onychomycosis) and dermatophytes, providing a better understanding of different mycoses in northeastern Brazil.

Keywords Mycosis · Dermatophytoses · Candidiasis · Opportunistic fungi · Epidemiology · Northeast Brazil

Responsible Editor: Rosana Puccia

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Introduction

Mycoses are common worldwide, especially in tropical countries and its fungal agents cause a wide range of diseases in humans, frequently causing opportunist and chronic infections or systemic forms, mainly in immunosuppressed individuals [1]. Superficial and cutaneous mycoses (ringworms) are mostly frequent and limited on the outermost layer of the skin and its appendages, like hair or nails. Dermatophytes, *Candida* and *Malassezia* species, are the major etiological agents of these mycoses, whereas non-dermatophyte molds (NDM) can cause infections to a lesser extent. The prevalence and distribution of pathogenic fungi can vary according to geographic location, climate, and culture habits of each state or country [2]. Moreover, there have been changes over the last decade in consequence of environmental conditions, presence of pets, lifestyle, and older population and an increase in immunosuppressed therapy, reflecting as changes to clinical patterns [3].

Dermatophytes are classified according to their usual habitat in anthropophilic, zoophilic, and geophilic fungi. Although anthropophilic forms are better suited to infect humans, some zoophilic and geophilic species are adapted to invade human tissue, more frequently involving children and the elderly [2]. *Trichophyton* species are predominant in many countries, represented by *T. rubrum* and *T. tonsurans* as responsible for most of the cases of *tinea corporis* (ringworm) or *tinea unguium* (onychomycosis) and *tinea capitis* (in the scalp), respectively [1, 2, 4]. *Candida* species are associated with mild-to-severe infections in countries with differentiated climate and habits, standing out as the main pathogen of non-dermatophytic onychomycoses; also, they affect millions of women every year and cause vulvovaginal candidiasis (VVC) [4]. Although *C. albicans* is the most prevalent and virulent species confirmed in candidiasis in a variety of anatomic sites, non-*albicans* *Candida* (NAC) isolates are emerging significantly, including *C. glabrata*, *C. krusei*, and *C. parapsilosis* complex, with clinical relevance in opportunistic and disseminated infections (candidemia) in healthcare-associated infections (HAIs), often with an antifungal resistance profile [5, 6].

Another common ringworm is pityriasis versicolor (PV), a benign and non-inflammatory superficial mycosis that occurs worldwide, especially in tropical regions, caused by the commensal yeast *Malassezia* spp. Many species and/or genotypes may cause similar pathologies and be implicated in aggravating seborrheic dermatitis and folliculitis, and some emergent species neglected may have their incorrect diagnosis [7]. Other filamentous fungi known as NDM can be important pathogens in opportunist infections, and although *Aspergillus* spp. and *Fusarium* spp. remain as the most common, other molds such as *Penicillium* spp., *Curvularia* sp., and some dematiaceous species have become prevalent in immunocompromised individuals [8, 9].

Therefore, knowledge about current epidemiological trends in the incidence of superficial fungal infections in different regions is important in the diagnosis, treatment, and prevention of infections by fungi. This study presents the first data about the prevalence of mycoses in different anatomic sites and their etiological agents in patients of Alagoas, (Northeastern Brazil), focusing on positive samples of private laboratories collected during an 8-year period (2009 to 2016), an important advance in the regional and Brazilian vision of medical mycology.

Methods

A retrospective and descriptive study of a quantitative approach was performed in the main tertiary laboratories of reference in Maceió/Alagoas, Brazil (Unilab, Proclínico Laboratory Diagnosis and Dilab Medical Laboratory), for the assessment of the patients' database from several

municipalities of the state. The population of this study consisted of patients with mycoses in different clinical specimens, of both sexes and all ages. This project was approved by the Research Ethics Committee under the no.23065.017665/201164 and ethical principles were safeguarded through ethical secrecy, codifying, and not mentioning the patients' names (SisGen access no. A5B1165). The database was evaluated during about a period of 8 years (from January 2009 to December 2016), and patients with incomplete records (age or genera) were considered.

Collection of clinical specimens and sample processing

Samples from different clinical specimens with suspected mycosis were collected and processed at the microbiology department of each partner laboratory: hair, nail, skin, or scalp scrapings were collected with the aid of a sterile scalpel for storage in sterile Petri dishes; intertriginous lesions were collected with a sterile swab moistened in sterile saline (NaCl 0.9%); in lesions suggestive of pityriasis versicolor, the Jarbas-Porto method was applied along with the scrapping collection; a sterile swab was used to collect vaginal secretion or other secretions; urine, sperm, or sputum were collected in sterile containers. One part of each sample was examined with potassium hydroxide preparation (KOH 20%) or/and lactophenol cotton blue for evaluation under light microscopy (40x; 1000x), and another part was cultured on Sabouraud dextrose agar plate (ASD, Difco). The incubation was at room temperature (28–35 °C) and the cultures were observed daily during 48 h to 2 weeks for interpretation of the results.

Yeast colonies were evaluated using light microscopy and transferred to CHROMagar® *Candida* (Difco Laboratories, USA) for presumptive identification by the production of chromogen pigments (*C. albicans*: green; *C. tropicalis*: metallic blue; and *C. krusei*: pink, fuzzy) based on their morphophysiological patterns [10] and classified as *Candida* sp. when it was not possible to determine the species.

The identification of the dermatophytes was performed as usual in the clinical routine, through morphological observation (culture-based) and identification of reproductive structures and other structures (microscopy), as well as through the in vitro hair perforation test if necessary. Diagnosis of NDM was based on duplicate cultures to confirm the same isolate NDM and positive direct mycological examination after KOH preparation for microscopy. Suspected samples of pityriasis versicolor were evaluated in ASD with olive oil and direct mycological examination. MacConkey agar (MAC) and ASD were used for urine and sperm cultures, while other secretions were cultured on 5% blood agar for further microscopic analysis.

Data analysis

A descriptive analysis of demographical data (age/gender) and clinical characterization of the patients was conducted, and the data was inserted in a spreadsheet for the elaboration of a database in the Microsoft Excel® (Office package) and 23.0 IBM®SPSS software (Statistical Package for the Social Sciences) for Windows, evaluating frequencies of fungi and anatomical sites affected.

Results

We conducted a retrospective study in the three main tertiary centers of fungal diagnosis in Alagoas. Over an 8-year period (2009 to 2016), 3316 patients with mycoses were attended (595 men and 2716 women); male/female 4.56, with 40.25 of average age. However, age data were missing for 40 individuals (1.24%), according to the mycological identification service. Subjects aging less than 20 years represented 384 (11.57%) and 419 (12.63%) of the patients were over 60 years, as set out in Table 1. In addition, fungal infections were most common in individuals with 21–30 years of age (808 patients), followed by patients with 31–40 years (781), then in the elderly with > 80 years old (41).

Positive samples were assessed through direct microscopy plus culture analysis and totaled 3776, since some patients had multiple sites infected by fungi of the same genus/species or different species. As showed in Table 2, vaginal secretion was the most common type for sampling (1593/42.2%). The laboratories diagnosed 38.78% onychomycosis in samples involving mainly toenails (876/23.2%) and fingernails (589/15.6%), besides foot scrapings (301/8%) and hair/scalp scrapings (76/2%), keratinized sites often affected by fungal infections such as candidiasis and dermatophytosis. Other clinical samples positive for fungal pathogens still include urine (11/

0.3%), sputum (4/0.1%), feces (36/1%), oropharyngeal secretion (11/0.3%), ear discharge (20/0.5%), scrotum (1), and sperm (1) and deserve attention and epidemiologic registration. When we compared the first year with the last one (2009/2016), an increase was seen in cases of vulvovaginal candidiasis (121/257; $n = 1593$), onychomycosis (72/156; $n = 1465$), and dermatomycosis in glabrous skin (23/75; $n = 483$) in Alagoas.

Table 3 depicts the distribution of yeast species year-by-year, as well as dermatophytes and other filamentous fungi/NDM. The prevalence of yeasts is significant, present in the most common isolates obtained in our study (82.9%/3119): 3012 *Candida* spp. (79.8%), 57 *Malassezia* spp. (1.5%), 42 *Trichosporon* spp. (1.1%), 10 *Geotrichum* sp. (0.3%), and 8 *Rhodotorula* sp. (0.2%). In such instances, *Candida albicans* was the most frequent species (715), totaling 18.9% among the yeasts in different samples (e.g., nails, skin, urine, and feces), followed by *C. krusei* (194/5.1%) and *C. tropicalis* (24/0.6%), further including 2079 unspecified strains: *Candida* sp. (55.1%). Women were more affected by candidiasis than men were, with 2266 and 302 cases, respectively.

Six hundred and forty-seven filamentous fungi (17.1%) were confirmed, mainly in nails and skin. Infections caused by the three genera of dermatophytes were related in 557 cases (14.8%), with predominance of the anthropophilic genus *Trichophyton* sp. in 211 samples (5.6%). *T. rubrum* was identified in 125 (3.3%), *T. tonsurans* in 106 (2.8%), and *T. mentagrophytes* in 72 (1.9%), followed by 15 *Microsporum canis* (0.4%), 14 *Epidermophyton floccosum* (0.37%), 12 *Epidermophyton* sp. (0.32%), and 2 *Microsporum* sp. (0.1%). Although most of the *Trichophyton* strains have not been identified at the species level, it is remarkable that this genus is predominant (211/5.6%). However, NDM were found and represented the minority (90/2.4%): 56 *Fusarium* sp. (1.5%), 19 *Aspergillus* sp., being 3 *A. niger* (0.08%), in addition to 4 *Curvularia* sp. (0.1%), 3 *Penicillium* sp. (0.1%), and 2 *Verticillium* sp. (0.1%); for *Acremonium* sp., *Neoscytalidium* sp., *N. dimidiatum*, *Phialophora* sp., *Piedra hortae*, and *Mycelia sterilia*, one isolate of each was confirmed (Table 3).

Candida and dermatophytes species represented 94.5% of all fungal pathogens confirmed (Table 4), of which yeasts were the main etiologic agents of onychomycosis in fingernails and toenails (77%), with 42.3% of vulvovaginal candidiasis. *Candida albicans* was the leading causative pathogen in all candidiasis cases with the correct species identification, but a high number of *Candida* strains remained without a proper presumptive identification (55.1% of *Candida* sp.), whereas non-*albicans Candida* (NAC) was responsible for only 5.8%, represented by *C. krusei* ($n = 194$) and *C. tropicalis* ($n = 24$). As indicated, it was not possible to properly determine the NAC and *C. albicans* frequency, probably due to the delay of the laboratories from Alagoas in

Table 1 Age group distribution of patients affected by different mycoses in the State of Alagoas (Brazil) from January 2009 to December 2016 ($n/\%$)

Age in years	n^1	$\%^2$
0–10	91	2.74
11–20	293	8.84
21–30	808	24.37
31–40	781	23.55
41–50	466	14.05
51–60	418	12.61
61–70	253	7.63
71–80	125	3.77
> 80	41	1.24
Without age	40	1.21
Total	3316	100

¹ Total

² Percentage

Table 2 Distribution of different clinical specimens positive for fungi year-by-year in Alagoas

	2009	% ¹	2010	%	2011	%	2012	%	2013	%	2014	%	2015	%	2016	%	Total	%
Toenails	50	5.7	108	12.3	129	14.7	111	12.7	154	17.6	113	12.9	120	13.7	91	10.4	876	23.2
Feet	9	3.0	47	15.6	39	13.0	39	13.0	33	11.0	40	13.3	44	14.6	50	16.6	301	8.0
Fingernails	22	3.7	51	8.7	70	11.9	44	7.5	100	17.0	137	23.3	100	17.0	65	11.0	589	15.6
Hands	3	7.3	8	19.5	4	9.8	9	22.0	4	9.8	3	7.3	4	9.8	6	14.6	41	1.1
Scalp	4	5.3	12	15.8	8	10.5	11	14.5	11	14.5	13	17.1	4	5.3	13	17.1	76	2.0
Trunks	9	15.5	12	20.7	9	15.5	7	12.1	9	15.5	5	8.6	3	5.2	4	6.9	58	1.5
Vaginal	121	7.6	200	12.6	277	17.4	183	11.5	138	8.7	191	12.0	226	14.2	257	16.1	1593	42.2
Lower members	1	2.4	7	16.7	8	19.0	8	19.0	5	11.9	2	4.8	3	7.1	8	19.0	42	1.1
Groin/nates	2	3.8	4	7.5	7	13.2	6	11.3	7	13.2	8	15.1	11	20.8	8	15.1	53	1.4
Upper limbs	1	2.4	6	14.6	5	12.2	9	22.0	5	12.2	4	9.8	4	9.8	7	17.1	41	1.1
Oral	0	0.0	1	9.1	0	0.0	0	0.0	4	36.4	4	36.4	2	18.2	0	0.0	11	0.3
Face	2	9.5	2	9.5	6	28.6	3	14.3	3	14.3	0	0.0	2	9.5	3	14.3	21	0.6
Sputum	0	0.0	2	50.0	1	25.0	1	25.0	0	0.0	0	0.0	0	0.0	0	0.0	4	0.1
Sperm	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1	0.0
Feces	1	2.8	2	5.6	3	8.3	2	5.6	8	22.2	5	13.9	7	19.4	8	22.2	36	1.0
Urine	0	0.0	0	0.0	1	9.1	1	9.1	0	0.0	8	72.7	0	0.0	1	9.1	11	0.3
Scrotum	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.0
Penis	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	1	0.0
Ear	2	10.0	2	10.0	0	0.0	1	5.0	10	50.0	3	15.0	0	0.0	2	10.0	20	0.5
Total	227	—	464	—	567	—	436	—	492	—	537	—	530	—	523	—	3776	100

¹ Percentage

adopting the routine use of chromogenic medium for the identification of the main *Candida* species of medical interest, as reported by the laboratories involved in this research in the first 3 years analyzed.

As it can be observed, still in Table 4, *T. rubrum* was the most common dermatophyte recovered from cases of onychomycosis (6.6%), *tinea pedis* (17%), and *tinea corporis* (18.4%). *T. tonsurans* was the predominant species associated with *tinea capitis* (62.7%), while among *Microsporum* species, 15 were identified as *M. canis*, less than the proportion of *Epidermophyton* sp. isolates. While *Epidermophyton* sp. was concentrated on toenails, fingernails, and in foot scrapings, as expected for this gender, the patients with *M. canis* were affected in the groin (1), face (2), limbs (14), and scalp (17).

Discussion

The epidemiology of superficial mycoses can be altered by climate, historical factors, quality of health services, medical interventions, and social factors, and pathogenic fungi are commonly identified by classical phenotypic methods in clinical laboratories and hospitals [1, 3, 14]. The confirmation of fungal micromorphology associated with examination of the colony in culture is still widely used in the routine of clinical diagnosis of yeasts, filamentous fungi as dermatophytes, and

NDM [2, 9], in spite of problems with the isolates' identification.

France, Italy, and China have stated that men and women are equally affected by superficial mycoses [1–3], while in Iran, a higher incidence in men was confirmed [15]. However, in Chile and South Korea, women are more affected by mycoses [16, 17], as it can also be observed in different Brazilian cities such as São Paulo [18], Rio de Janeiro [19], Manaus [12], Belém [20], Fortaleza [21], Recife [22], Natal [23, 24], and João Pessoa [25]. In fact, there are more women affected in Alagoas due to the high frequency of vaginal secretion for convenience sample. Several factors can determine differences in genus prevalence such as personal hygiene, occupational activity, and exposure to the contamination.

Throughout the world, a higher prevalence of dermatophytosis was observed. As for instance, in France, 57% of 2360 samples positive for dermatophytes was confirmed in 12 years [3], as well in South Korea [15] and for over 10 years in China [1] (84.36% of the 3367 samples), reinforcing the higher prevalence of dermatophytosis in East Asia. A higher frequency of dermatophytes and *Candida* infections has been reported in countries of Latin America, as observed during 13 years in Caracas (Venezuela) with 79.5% of dermatophytes (*T. rubrum* complex, *T. mentagrophytes* complex, and *M. canis*) [26], while 52.09% of dermatophytosis and 30.47% of candidiasis were diagnosed

Table 3 Distribution of yeast, dermatophytes, and other filamentous fungi (year-by-year) confirmed as etiologic agents of mycoses in Alagoas (Brazil) from 2009 to 2016

Etiological agents	Year																	
	2009	% ¹	2010	%	2011	%	2012	%	2013	%	2014	%	2015	%	2016	%	Total	
Yeast																		<i>n</i> ² %
<i>Candida</i> sp.	188	97.4	302	82.7	397	82.2	247	69.2	268	68.5	281	62.0	223	49.3	173	40.7	2079	55.1
<i>Candida albicans</i>	0	0.0	9	2.5	30	6.2	72	20.2	79	20.2	131	28.9	172	38.1	222	52.2	715	18.9
<i>Candida tropicalis</i>	1	0.5	11	3.0	3	0.6	0	0.0	4	1.0	5	1.1	0	0.0	0	0.0	24	0.6
<i>Candida krusei</i>	0	0.0	30	8.2	35	7.2	14	3.9	25	6.4	26	5.7	43	9.5	21	4.9	194	5.1
<i>Malassezia</i> sp.	2	1.0	5	1.4	14	2.9	14	3.9	4	1.0	7	1.5	5	1.1	6	1.4	57	1.5
<i>Trichosporon</i> sp.	1	0.5	8	2.2	4	0.8	7	2.0	9	2.3	2	0.4	8	1.8	3	0.7	42	1.1
<i>Rhodotorula</i> sp.	1	0.5	0	0.0	0	0.0	3	0.8	2	0.5	1	0.2	1	0.2	0	0.0	8	0.2
<i>Geotrichum</i> sp.	1	33.3	3	13.6	1	6.7	0	0.0	5	29.4	0	0.0	0	0.0	0	0.0	10	0.3
Dermatophytes																		
<i>Trichophyton</i> sp.	23	49.0	19	31.2	29	42.2	30	43.6	46	55.5	48	64.7	2	3.0	14	16.2	211	5.6
<i>Trichophyton rubrum</i>	2	4.3	4	6.6	2	2.9	22	31.9	16	19.3	14	18.9	34	50.7	31	35.6	125	3.3
<i>Trichophyton tonsurans</i>	9	19.1	21	34.4	26	37.7	9	13.0	8	9.6	7	9.5	13	19.4	13	14.9	106	2.8
<i>Trichophyton mentagrophytes</i>	1	2.1	15	24.6	9	13.0	3	4.3	6	7.2	3	4.1	13	19.4	22	25.3	72	1.9
<i>Microsporum</i> sp.	0	0.0	1	1.6	1	1.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.1
<i>Microsporum canis</i>	0	0.0	0	0.0	1	1.4	1	1.4	4	4.8	1	1.4	3	4.5	5	5.7	15	0.4
<i>Epidermophyton</i> sp.	12	25.5	1	1.6	1	1.4	4	5.8	3	3.6	1	1.4	2	3.0	2	2.3	26	0.7
Other fungi/molds																		
<i>Aspergillus</i> sp.	1	33.4	9	40.9	7	46.7	0	0.0	1	5.9	1	10.0	0	0.0	0	0.0	19	0.5
<i>Fusarium</i> sp.	0	0.0	6	27.3	3	20.0	6	60.0	11	64.7	8	80.0	11	100.0	11	91.7	56	1.5
<i>Penicillium</i> sp.	0	0.0	0	0.0	0	0.0	2	20.0	0	0.0	1	10.0	0	0.0	0	0.0	3	0.1
<i>Verticillium</i> sp.	0	0.0	2	9.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.1
<i>Curvularia</i> sp.	1	33.3	1	4.5	2	13.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	0.1
<i>Acremonium</i> sp.	0	0.0	0	0.0	1	6.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.0
<i>Mycelia sterilia</i>	0	0.0	0	0.0	1	6.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.0
<i>Phialophora</i> sp.	0	0.0	1	4.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.0
<i>Neoscytalidium</i> sp.	0	0.0	0	0.0	0	0.0	1	10.0	0	0.0	0	0.0	0	0.0	1	8.3	2	0.1
<i>Piedraia hortae</i>	0	0.0	0	0.0	0	0.0	1	10.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.0

¹ The percentage indicated after each annual sampling refers to the annual quantity from each fungus by year, not the total of all identified fungi

² Total

for 2 years in 1004 Chilean patients aged 36–60 years [16]. Similarly, 11,107 dermatophytosis (47%) and 10,830 of mucosal candidiasis (46%) were confirmed in Argentina [27]. Considering only the dermatophytoses, toenails were the most affected anatomic site in Alagoas, as similarly reported in France, Venezuela, and in Brazilian cities (São Paulo and Rio de Janeiro) [2, 18, 19, 26].

The epidemiological pattern of dermatophytoses and the pathogenic spectrum of each species have been examined in several countries, and *Trichophyton* species are known as the most virulent among the dermatophytes [2] and the most frequent agents of dermatophytosis in Alagoas (*Trichophyton* sp. and *T. rubrum*), remaining also in a higher prevalence in others Brazilian cities in the northeast [21, 22]. In Brazil,

T. tonsurans is the most common etiologic agent in the scalp, mostly in children [21], and the frequency of *tinea capitis* and the importance of this dermatophyte in such infections are high in Alagoas (62.7%), also isolated in the lower and upper limbs. The prevalence of *Microsporum* sp. and *M. canis* was the lowest in our study (0.5%), having confirmed more *Epidermophyton* sp. Interestingly, *Microsporum* species were not isolated for 3 years in Fortaleza [21] and *E. floccosum* was the most frequent fungus isolated from the groin (*tinea cruris*) in Iran (75.9%) [15], but frequently, this dermatophyte is the least isolated [23].

In the USA, it is estimated that at least 12% of the prevalence of onychomycosis increases with the age and is the highest among individuals over 65 years [13]. History of

Table 4 Occurrence of dermatophytes and *Candida* species according to the different anatomical sites affected (superficial mycosis) of patients from Alagoas (Brazil) in a period of 8 years

Etiological agents	Feet		Hands		Scalp		V.s. ²		Trunk/ groin		Oral		L./U. ³		Face		n/% ⁴				
	Toenails	% ¹	Skin	%	Fingernails	%	Skin	%	%	%	%	%	%	%	%	%					
<i>Candida</i> sp.	534	68.3	100	37.0	431	77.1	23	59	15	20.0	859	53.9	29	35.8	6	60.0	15	27.8	6	28.6	2018/57.9
<i>C. albicans</i>	14	1.8	3	1.1	22	3.9	0	0.0	0	0.0	665	41.7	1	1.2	4	40.0	0	0.0	0	0.0	709/20.4
<i>C. tropicalis</i>	8	1.0	1	0.4	9	1.6	1	2.6	0	0.0	4	0.3	0	0.0	0	0.0	0	0.0	1	4.8	24/0.7
<i>C. krusei</i>	46	5.9	15	5.6	54	9.7	5	12.8	0	0.0	65	4.1	3	3.7	0	0.0	1	1.9	3	14.3	192/5.5
<i>Trichophyton</i> sp.	96	12.3	43	15.9	31	5.5	2	5.1	4	5.3	0	0.0	21	25.9	0	0.0	11	20.4	1	4.8	211/6.0
<i>T. rubrum</i>	41	5.2	46	17.0	8	1.4	4	10.3	0	0.0	0	0.0	15	18.5	0	0.0	7	13.0	4	19.0	125/3.6
<i>T. tonsurans</i>	15	1.9	12	4.4	1	0.2	4	10.3	47	62.7	0	0.0	10	12.3	0	0.0	14	25.9	3	14.3	106/3.0
<i>T. mentagrophytes</i>	24	3.1	44	16.3	0	0.0	0	0.0	0	0.0	0	0.0	1	1.2	0	0.0	2	3.7	1	4.8	72/2.1
<i>Microsporum</i> sp.	0	0.0	1	0.4	0	0.0	0	0.0	1	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2/0.1
<i>M. canis</i>	0	0.0	0	0.0	0	0.0	0	0.0	8	10.7	0	0.0	1	1.2	0	0.0	4	7.4	2	9.5	15/0.4
<i>Epidermophyton</i> sp.	7	0.5	16	1.9	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	26/0.7

¹ Percentage² Vaginal secretion³ Lower and upper limbs⁴ Total sampling/percentage based on the total of each fungus

cancer, psoriasis, *tinea pedis*, relatives with onychomycosis, regular swimming, or older age (≥ 50 years) are factors associated with more than twice the risk of onychomycosis compared with general the population [28]; in Alagoas people over 50 years old totaled 25.25%. Onychomycosis by dermatophytes and yeasts is observed with a high frequency in our study, as expected, since they are the main fungi of nail infections worldwide. *Tinea unguium* and *tinea pedis* were the most common dermatophytic infection in Grenoble (France) [2], while in Italy, Papini and collaborators [13] identified 76.6% of dermatophytes in nails (17.2% yeasts and 6.3% NDM) with moderate/severe nail involvement in about 74.1% of all cases.

Four-hundred cases of onychomycosis by *Candida* spp. (49%) were confirmed in Rio de Janeiro [19], and focusing on northeastern Brazil, *C. albicans* and *C. tropicalis* were isolated in 30.51% and 22.6%, respectively, from onychomycosis in Ceará [21], while yeasts were more isolated than dermatophytes in Natal/RN and João Pessoa/PB with significant differences [23–25]. Hence, the epidemiological profile of onychomycosis in Alagoas follows the pattern observed in northeastern Brazil, with prevalence of *Candida* spp.

Despite the wide use of the evaluation of aspects of fungal colony and microscopy in the routine procedures for dermatophyte species identification, there are limitations once morphological characteristics can frequently vary. Therefore, species identification is often difficult with classical technique [19] and the results can be released only with the corresponding genus, as for an instance, we then found many reports only indicating, e.g., *Trichophyton* sp. Moreover, a dermatophyte culture can be time-consuming as it requires more than 12–20 days of growth for typical characteristics to be better observed. Molecular techniques for identifying fungi species are faster and more sensitive [29], but are not yet an alternative to be used in routine clinical diagnosis of superficial mycoses, due to the cost and the need for more specialized professionals.

We also received many vaginal samples due to the search of patients for diagnosis, confirming 42.2% of VVC or recurrent vulvovaginal candidiasis (R-VVC), the second most common cause of genital infection worldwide [2, 4]. Although *C. albicans* is still regarded as the most common in VVC, the frequency of NAC species has increased (*C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*), with a dependent distribution of hormonal factors, hygiene habits, sexual activity, and diseases such as diabetes [30, 31].

The high frequency of *Candida* sp. in our study until 2011 could be explained by the use of conventional mycological methods limited to morphocultural and microscopic evaluations aimed to differentiate *C. albicans* from more frequent NAC species by the main laboratories of Alagoas, without presumptive identification of *Candida* species in the routine. The non-adoption of presumptive diagnostic methods such as

medium that differentiates chromogenic phenotypes made it difficult to identify other species besides *C. albicans* [10], despite the limitations of the environment in the differentiation of some species [32]. Jain et al. [33] pointed out inaccuracies of conventional methods and indicated 77 to 100% of sensitivity of identification by chromogenic agar for most commonly isolated *Candida* species, which the sensitivity for diagnosis could be increased with the combined use with Vitek 2 ID.

The chromogenic medium improved the identification of the *Candida* species, which allowed to know *C. krusei* as the most prevalent specie after *C. albicans* in Alagoas over 8 years in the present study. The overall agreement of the semi-nested polymerase chain reaction (sn-PCR) compared with tests with CHROMAgar®*Candida* was already confirmed in 97.5% of 127 *Candida* species and 100% for *C. albicans*, *C. tropicalis*, and *C. krusei* [34], showing the quality of this medium commonly used in the clinical analysis laboratories. Over the past decade, there has been an increased isolation of drug-resistant NAC, and the identification of *Candida* species is undoubtedly necessary in the routine of microbiological laboratories.

Only 57 *Malassezia* spp. were identified by morphologic methods and isolation in culture medium supplemented with lipidic sources. This genus compiles yeasts of the human skin microbiome and agent of pityriasis versicolor (PV), which can cause infections by extension to central venous catheters more in neonates than in adults [35, 36]. PV is very common in northeastern Brazil due to the tropical climate, occurring to a lesser extent in the south and southeast [37], and there is a sub-diagnosis, since not running laboratory tests for the diagnosis can limit the epidemiologic knowledge. The treatment is commonly started without laboratory tests, and the identification of *Malassezia* at the species level does not have much value to define the treatment [38].

Other saprobic yeasts can also parasitize as opportunistic in the nail plate, ear, or upper respiratory tract, and we confirmed a low frequency of *Trichosporon* sp., *Geotrichum* sp., and *Rhodotorula* sp. as etiological agents mainly in toenails. *Trichosporon* spp. is involved in skin and nails infections and disseminated trichosporonosis, when associated with central venous catheters, and vesical and peritoneal catheter-related devices due to the ability to form biofilms [8, 39]. The genus *Geotrichum* presents a wide phenotypic variability and shares morphological characteristics with the filamentous fungi, but the genome phylogeny gives robust support for its inclusion in the subphylum Saccharomycotina as an ascomycetous yeast [40], with *G. candidum* as the most diagnosed in nails [21, 24].

In addition, the predominance of dermatophytes is notable, but filamentous fungi like *Scopulariopsis brevicaulis*, *Fusarium* sp., *Acremonium* sp., *Aspergillus* sp., *Alternaria* spp., *Curvularia* spp., and *Scytalidium* spp. (reclassified as *Neoscytalidium*) can cause onychomycosis, known as NDM

[8, 12, 41], which are commonly found as soil saprophytes and phytopathogens, predominant in tropical and temperate areas [9]. Among the confirmed NDM causing mycoses in our state, we may highlight *Fusarium* spp. and *Aspergillus* spp. in nails, all with worldwide distribution and considered emerging pathogens in humans in several countries [41], but still in low numbers when we consider the 8 years of analysis in Alagoas. Only 29 *Fusarium* spp. were found in almost 3 years in Fortaleza/CE [21], and emerging fungi in Rio de Janeiro/RJ accounted for 4.5% of the total cases of onychomycosis, including *Scytalidium* sp. and *Curvularia* sp. [19]. These species identified in Alagoas are the mostly involved NDM in immunocompromised host infections, and skin structure or nails breakdowns may serve as a gateway to invasive fungal infections (IFIs) [11, 42]. The IFIs rate by NDM in immunosuppressed patients with immune disorders has increased and is an important cause of death among them [42], and onychomycosis in association with, e.g., diabetes mellitus or psoriasis has been diagnosed more frequently [9, 43].

These mycoses are not notifiable diseases, so, many states and countries do not provide accurate data on the extent of individuals affected or the risk of contamination in certain localities. Only the clinical investigation in medical appointments limits the evaluation of many biologic samples that could be properly diagnosed, which hinders an accurate epidemiology in Alagoas. It is important to point out that the culture and microscopy are necessary to identify the etiological agents in routine and to perform antifungal susceptibility tests. However, DNA genotyping methods have been increasingly applied in superficial and opportunistic mycosis agents, as powerful tools for epidemiological studies. Several techniques focusing D1/D2 and ITS regions have already aided in the knowledge of *Malassezia* species, dermatophytes, and cryptic species in *Fusarium* and *Candida* complexes [5, 44, 45]. The identification of the etiological agent is essential to guide the appropriate therapeutics, and it is important to establish the epidemiological situation as close to reality as possible, to determine patterns that can facilitate the use of preventive measures. In addition, to the best of our knowledge, this is the first report on fungic species distribution causing mycoses in a diversity of clinical samples in Alagoas in almost a decade, bringing important information to the medical and research community.

Conclusions

Clinical and laboratory data about dermatologic and pathogenic fungi in Alagoas confirmed the continued prevalence of vaginal candidiasis and onychomycosis, the most common type of fungal infections, and the clinical study on mycosis in our state provides a better understanding about etiological

agents in northeastern Brazil. Our study with a high number of biological samples analyzed in an 8-year long retrospective research helped to establish the epidemiological pattern of mycoses and demonstrated the routine and evolution in the mycological diagnosis in laboratories of Alagoas. Then, this is the first comprehensive survey focusing on superficial mycoses in different clinical settings in our state in northeastern Brazil, with an update of the variety of human fungal pathogens and that can lead to better prevention strategies of fungi infections and the development of new research in medical mycology in Alagoas.

Acknowledgments We thank Alex Lemos and Flávia Soares for the technical support in the tertiary laboratories, and Abilio Borghi for the review of the grammar in this manuscript.

Authors contribution: Maranhão, FCA: Conception and planning of the study; Obtaining, analyzing and interpreting data; Effective participation in research orientation; Statistical analysis; Intellectual participation in the propaedeutic conduction of studied cases; Critical review of the literature; Elaboration and writing of the manuscript; Critical review of the manuscript; Approval of the final version of the manuscript. Oliveira-Júnior, JB: Obtaining, analyzing and interpreting data; Statistical analysis; Critical review of the manuscript. Araújo, MAS: Obtaining, analyzing and interpreting data; Critical review of the manuscript. Silva, DMW: Statistical analysis; Critical review of the manuscript.

Funding information This study was financially supported by the Brazilian research agency CNPq and the Alagoas State agency FAPEAL (20110829-011-0025-0009).

Compliance with ethical standards

This project was approved by the Research Ethics Committee under the no. 23065.017665/201164 and ethical principles were safeguarded through ethical secrecy, codifying, and not mentioning the patients' names (SisGen access no. A5B1165).

Conflict of interest The authors declare that they have no conflicts of interest.

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