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Epidemiology, Antifungal Susceptibility, and Pathogenicity of *Candida africana* Isolates from the United Kingdom

Andrew M. Borman, Adrien Szekely, Chistopher J. Linton, Michael D. Palmer, Phillipa Brown, Elizabeth M. Johnson

UK National Mycology Reference Laboratory (MRL), Health Protection Agency South-West, Bristol, United Kingdom

***Candida africana* was previously proposed as a new species within the *Candida albicans* species complex, together with *C. albicans* and *C. dubliniensis*, although further phylogenetic analyses better support its status as an unusual variant within *C. albicans*. Here we show that *C. africana* can be distinguished from *C. albicans* and *C. dubliniensis* by pyrosequencing of a short region of ITS2, and we have evaluated its occurrence in clinical samples by pyrosequencing all presumptive isolates of *C. albicans* submitted to the Mycology Reference Laboratory over a 9-month period. The *C. albicans* complex constituted 826/1,839 (44.9%) of yeast isolates received over the study period and included 783 isolates of *C. albicans*, 28 isolates of *C. dubliniensis*, and 15 isolates of *C. africana*. In agreement with previous reports, *C. africana* was isolated exclusively from genital specimens, in women in the 18-to-35-year age group. Indeed, *C. africana* constituted 15/251 (6%) of "*C. albicans*" isolates from female genital specimens during the study period. *C. africana* isolates were germ tube positive, grew significantly more slowly than *C. albicans* and *C. dubliniensis* on conventional mycological media, could be distinguished from the other members of the *C. albicans* complex by appearance on chromogenic agar, and were incapable of forming chlamydospores. Here we present the detailed evaluation of epidemiological, phenotypic, and clinical features and antifungal susceptibility profiles of United Kingdom isolates of *C. africana*. Furthermore, we demonstrate that *C. africana* is significantly less pathogenic than *C. albicans* and *C. dubliniensis* in the *Galleria mellonella* insect systemic infection model.**

The incidence of invasive fungal infections caused by unusual *Candida* spp. continues to rise, in part due to increased populations of immunocompromised patients and those undergoing invasive procedures (1–8). Nevertheless, *Candida albicans* remains the most frequently isolated *Candida* species in the clinical setting, is the principal agent of nosocomial yeast infections (1, 4–6), and is widely accepted as being the most pathogenic *Candida* species (reviewed in reference 9). In addition to *C. albicans sensu stricto*, two closely related species have been described and added to the *C. albicans* species complex. In 1995, *Candida dubliniensis*, predominantly associated with cases of oral candidiasis in human immunodeficiency virus-infected patients, was described (10). While *C. dubliniensis* is phylogenetically closely related to *C. albicans* and shares the ability to produce germ tubes, true hyphae, and chlamydospores, epidemiological analyses have revealed that *C. dubliniensis* is far less prevalent than *C. albicans* (11–14), is comparatively rarely associated with systemic infection (11, 14), and is less pathogenic than *C. albicans* in a variety of infection models (13–16).

Similarly, *Candida africana* was initially described as an atypical, chlamydospore-negative variant of *C. albicans* (17, 18) but was proposed as a new species on the basis of morphological, biochemical, and physiological differences (18, 19). While subsequent molecular analyses supported a varietal distinction (*C. albicans* var. *africana*) (20), the taxonomic status of *C. africana* remains controversial. Although they are germ tube positive like *C. albicans* and *C. dubliniensis*, *C. africana* isolates studied to date have reportedly grown and produced hyphae more slowly than either *C. albicans* or *C. dubliniensis* and could be distinguished from both by an inability to assimilate several sugars or to produce chlamydospores and by appearance on chromogenic agars (18, 19, 21–23). Moreover, despite an almost worldwide distribution, the overwhelming majority of *C. africana* isolates have been recovered from female genital specimens (reviewed in reference 16). Epidemiological analyses of *C. africana* have been hampered by the failure of the commonly employed commercially available identification methods to distinguish it from *C. albicans* (reviewed in references 21 and 16). However, studies employing PCR amplicon length analyses of the Hwp-1 gene (24) revealed that *C. africana* constituted 7.2% of *C. albicans* complex isolates in three different hospitals in southern Italy, a prevalence 3 times that of *C. dubliniensis* in samples from principally nonsterile sites from the same cohort of hospitalized patients (25).

Here we have shown that *C. africana* can be distinguished from *C. albicans* and *C. dubliniensis* by pyrosequencing of a short fragment of the internal transcribed spacer region 2 (ITS2) and have subsequently used pyrosequencing to assess the prevalence of *C. africana* among *Candida* isolates from clinical specimens submitted to the UK National Mycology Reference Laboratory (MRL) over a 9-month period spanning 2011 to 2012. Isolates identified as *C. africana* by pyrosequencing were subjected to phenotypic and biochemical analyses, and their antifungal susceptibility profiles were established. Finally, we compared the pathogenicity of *C. africana*, *C. albicans*, and *C. dubliniensis* clinical isolates *in vivo* in the *Galleria mellonella* systemic infection model.

MATERIALS AND METHODS

Antifungal agents. Antifungal drugs were obtained from their respective manufacturers as standard powders. Amphotericin B, nystatin, and clo-

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Address correspondence to Andrew M. Borman, Andy.Borman@uhBristol.nhs.uk.

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TABLE 1 Pyrosequencing signature sequences of members of the *C. albicans* species complex[a]

Species/sequence variant	Pyrosequencing signature sequence[b]	Identity[c]	Percentage[d]
Candida albicans 1	**GTCAA AGTTT GAAGA TATAC GTGGT GGACG TTACC**	35	65.3
Candida albicans 2	**GTCAA AGTTT GAAGA TATAC GTGGT** a**GACG TTACC**	34	24.9
Candida albicans 3	**GTCAA AGTTT GAAGA TATAC GTGGT** a**GACG** c**TACC**	33	7.0
Candida albicans 4	**GTCAA AGTTT GAAGA TATAC GTGGT** a**GACG TT**g**CC**	33	2.8
Candida stellatoidea (CBS1905)	**GTCAA AGTTT GAAGA TATAC GTGGT GGACG TTACC**	35	ND
Candida africana	**GTCAA AGTTT GAAGA TATAC GT**a**GT** a**GACG TTACC**	33	NA
Candida dubliniensis 1	**GTCAA AGTTT GAAGA** ata**A**a a**TGG**g c**GACG** cc**A**ga	24	88.0
Candida dubliniensis 2	**GTCAA AGTTT GAAGA** ata**A**a a**TGG**c –**GACG** cc**A**ga	24	12.0

[a] Sequences are aligned against the 35-bp signature of the most common *C. albicans* variant (*C. albicans* 1). A total of 213 isolates (*C. albicans*), 25 isolates (*C. dubliniensis*), and 15 isolates (*C. africana*) were subjected to pyrosequencing.
[b] Bold type indicates totally conserved nucleotides. Lowercase letters indicate positions in which the sequence differs from that of *C. albicans* 1.
[c] Identity is given as the number of identical bases compared to the signature sequence of *C. albicans* 1.
[d] Percentage of isolates of a given species with that particular signature sequence. ND, not determined; NA, not applicable.

trimazole (all from Sigma Chemical Co, St. Louis, MO) were dissolved in dimethyl sulfoxide (DMSO) (amphotericin and nystatin) and polyethylene glycol (PEG) 400 with heating to 70°C (clotrimazole). Itraconazole, miconazole, econazole, and ketoconazole (all from Janssen Research Foundation, Beerse, Belgium) were prepared in PEG 400 with heating to 70°C (itraconazole and miconazole) or in DMSO (econazole and ketoconazole). Fluconazole (Pfizer Central Research, Sandwich, United Kingdom) was resuspended in sterile water. Serial 2-fold dilutions of the various drugs were prepared in RPMI 1640 medium (with L-glutamine, without bicarbonate; Sigma Chemical Co, St. Louis, MO), and buffered to pH 7.0 using a 0.165 M solution of MOPS (morpholinepropanesulfonic acid) (Sigma Chemical Co, St. Louis, MO).

Yeast isolates and conventional phenotypic identification approaches. A total of 1,839 clinical isolates of yeast were included in the current study. Isolates had been submitted to the UK National Mycology Reference Laboratory (MRL) for identification, susceptibility testing, or both. Isolates were from both sterile and nonsterile sites (female genitals, $n = 381$; male genitals, $n = 10$; blood and line tips, $n = 600$; sputum and bronchoalveolar lavage [BAL] fluid, $n = 199$; feces, $n = 16$; drain fluids, $n = 144$; tissue, $n = 136$; eyes, $n = 37$; cerebrospinal fluid [CSF], $n = 14$; urine, $n = 71$; ear, $n = 12$; and site not stated, $n = 219$). All isolates were subcultured on plates of Oxoid Sabouraud dextrose agar containing 0.5% (wt/vol) chloramphenicol (Unipath Limited, Basingstoke, England). Cultures were incubated for 24 h at 35°C prior to testing. Isolates of *C. africana* have been stored in sterile water in a metabolically inactive state in the National Collection of Pathogenic Fungi (NCPF).

The clinical isolates included in this study were all subjected to initial germ tube testing (inoculation into sterile horse serum and incubation at 37°C for 3 h). Isolates were also cultured on Dalmau plates (Oxoid cornmeal agar, supplemented with 1% Tween 80, with a sterile coverslip over a single streak of the organism to establish the additional morphological characteristics required for complete AUXACOLOR2 profiles) and MAST-ID CHROMagar Candida plates to establish the purity of isolates. Strains formally identified as *Candida africana* by molecular approaches were also subjected to the AUXACOLOR2 identification kit (Bio-Rad Laboratories Ltd., Hemel Hempstead, United Kingdom) exactly as described previously (26).

Molecular identification. All 1,839 test isolates were subjected to pyrosequencing analysis of a portion of ITS2 exactly as described previously (27–29). For isolates identified by pyrosequencing as *C. africana*, identity was confirmed by PCR amplification and sequencing of a region of the large subunit gene (LSU) and the internal transcribed spacer 1 (ITS1) region using the primers described previously (30, 31) and direct inoculation of PCR products with yeast suspensions as described in reference 32. Organisms were identified using BLAST searches against fungal sequences in existing public DNA databases (EMBL) and multiple sequence alignments using ClustalW and a database of formally identified organisms compiled by the MRL.

Broth microdilution determination of yeast MICs. MICs were determined according to CLSI methodologies (CLSI M27-A3 [33]) in round-bottomed 96-well plates with yeast blastospore suspensions prepared in saline and then diluted into RPMI 1640 and adjusted to final concentrations of 2.5×10^3 CFU/ml. Inoculated plates were incubated for 24 to 48 h at 35°C. MICs were read at 24 and 48 h as the concentration of drug that elicited 100% inhibition of growth (amphotericin B, nystatin) or significant (approximately 50%) inhibition of growth compared with a drug-free control (itraconazole, fluconazole, miconazole, ketoconazole, econazole, and clotrimazole).

***Galleria mellonella* virulence assays.** Killing assays were performed in *Galleria mellonella* essentially as described previously (34–37). Briefly, final (sixth) instar larvae weighing approximately 300 mg each (Livefood UK Ltd., Rooks Bridge, Somerset, United Kingdom) that were absent of gray markings were maintained at room temperature in the dark and inoculated within 1 week of receipt. Suspensions of individual *Candida* isolates that had been grown on Sabouraud agar for 24 h at 37°C were harvested by gentle scraping of colony surfaces with sterile plastic loops, washed twice in sterile phosphate-buffered saline (PBS), counted in hemocytometers, and adjusted to 10^5 cells per microliter in sterile PBS. Individual larvae were inoculated in the left rear proleg with 1×10^6 yeast cells in PBS (final inoculum volume, 10 μl) using a 10-μl Hamilton syringe fitted with a 26-gauge blunt needle. Fifteen larvae were inoculated per isolate per experiment (experiments employed 4 independent isolates of each *Candida* test species). Control groups of larvae received 10 μl of sterile PBS in exactly the same manner. Inoculated larvae were incubated at 30°C, 37°C, or 42°C and scored for viability at 8-h intervals as described previously (34–37). Differences in resulting Kaplan-Meier survival plots were evaluated using the Mann-Whitney (two-sample Wilcoxon) test. In experiments designed to assess differences in fungal cell filamentation postinfection, representative larvae from each inoculum group were cut open at 24 h postinfection and the fat body/solid internal structures and hemolymph were collected aseptically as described previously (37) and examined microscopically.

RESULTS

We have previously demonstrated that pyrosequencing of 35 bp of a portion of the internal transcribed spacer region 2 (ITS2) is sufficient to robustly identify the vast majority of pathogenic yeasts (27), including genetically closely related isolates within species complexes (28, 29). This is also the case for the key species within the *C. albicans* species complex. To date, we have identified 4 *C. albicans* sequence variants within the 35-bp ITS2 signature sequence we employ for yeast identification, 2 *C. dubliniensis* variants, and a single signature sequence shared by all *C. africana* isolates encountered to date (Table 1). All *C. dubliniensis* and *C. africana* isolates differ from *C. albicans* at 10 or 11 and 1 or 2

TABLE 2 Strain collection data and MICs for *C. africana* isolates

Strain[a]	Patient site[b]	Age (yr)	Location	MIC (mg/liter)[c]							
				AMB	FLZ	ITR	MIC	NYS	KET	CLO	ECON
NCPF 8945	HVS	22	Wrexham	0.125	<0.125	<0.03	<0.125	2.0	<0.125	<0.125	<0.125
NCPF 8946	Genital	22	Ipswich	0.125	<0.125	<0.03	<0.125	1.0	<0.125	<0.125	<0.125
NCPF 8947	Vaginal swab	25	Bath	0.125	<0.125	<0.03	<0.125	1.0	<0.125	<0.125	<0.125
NCPF 8948	Genital	31	Portsmouth	0.25	0.25	<0.03	<0.125	2.0	<0.125	<0.125	<0.125
NCPF 8949	HVS	22	Crewe	0.125	<0.125	<0.03	<0.125	1.0	<0.125	<0.125	<0.125
NCPF 8950	HVS	28	Crewe	0.125	<0.125	<0.03	<0.125	1.0	<0.125	<0.125	<0.125
NCPF 8951	HVS	26	Bangor	0.125	<0.125	<0.03	<0.125	2.0	<0.125	<0.125	<0.125
NCPF 8952	Vaginal swab	20	Epsom	ND	<0.125	<0.03	<0.125	0.5	<0.125	0.25	ND
NCPF 8953	HVS	25	London	ND	<0.125	<0.03	<0.125	0.5	<0.125	<0.125	ND
NCPF 8954	HVS	22	Coventry	ND	ND	ND	ND	ND	ND	ND	ND
NCPF 8955	HVS	22	Stoke	ND	<0.125	<0.03	<0.125	0.5	ND	ND	ND
NCPF 8956	HVS	31	Bristol	ND	0.25	<0.03	<0.125	1.0	<0.125	<0.125	<0.125
NCPF 8957	LVS	25	Bristol	0.25	<0.125	<0.03	<0.125	0.5	<0.125	<0.125	<0.125
NCPF 8958	HVS	21	Bristol	ND	<0.125	ND	<0.125	1.0	<0.125	<0.125	ND
Isolate F111 (dead)	Vaginal swab	18	Northampton	0.125	0.25	<0.03	<0.125	1.0	<0.125	<0.125	<0.125

[a] NCPF, National Collection of Pathogenic Fungi, UK Mycology Reference Laboratory.
[b] All patients were female. HVS, high vaginal swab; LVS, low vaginal swab.
[c] Drug abbreviations: AMB, amphotericin B; FLZ, fluconazole; ITR, itraconazole; MIC, miconazole; NYS, nystatin; KET, ketoconazole; CLO, clotrimazole; ECON, econazole. ND, not determined.

positions, respectively, within the 35-base pyrosequencing region (Table 1). Conversely, the ITS2 sequence for *C. stellatoidea* (based on published ITS2 sequences for the type strain, isolate CBS 1905; EMBL AJ853768) is indistinguishable from that of *C. albicans* variant 1 (Table 1), which is the most common ITS2 sequence variant encountered in United Kingdom isolates.

In order to evaluate the prevalence and potential clinical significance of *C. africana* in clinical samples in the United Kingdom, between September 2011 and July 2012, the UK National Mycology Reference Laboratory identified 1,839 yeast isolates to species level using pyrosequencing. *C. albicans* species complex constituted 44.9% (826/1,839) of all isolates identified and comprised 783 isolates of *C. albicans* (42.6% of all isolates), 28 isolates of *C. dubliniensis* (1.5%), and 15 isolates of *C. africana* (0.8%). Thus, when considering isolates from all clinical sites, *C. dubliniensis* and *C. africana* represented 3.4% and 1.8%, respectively, of all *C. albicans* complex isolates (Table 2). In agreement with the published reports concerning the occurrence of *C. africana* (22), all 15 isolates identified in this study had been isolated from genital specimens (Table 2) in young female adults (age range, 18 to 31 years; mean age, 24.7 years). Indeed, when only the *C. albicans* complex isolates from female genital specimens were considered (251 isolates in total), *C. africana* (15 isolates) was much more prevalent than *C. dubliniensis* (3 isolates) and represented 6% of *C. albicans* complex isolates from such specimens.

Candida africana isolates had been submitted to the MRL from hospitals across central and southern England and Wales. In agreement with one published evaluation (38), all isolates that were tested were found to be susceptible (with very low MICs) to all antifungal agents that would be appropriate for treating vaginal candidiasis (Table 2). Examination of clinical details submitted with the isolates did not reveal a significant or unusual proportion of recalcitrant or recurrent infections associated with *C. africana*, compared to those associated with isolates of *C. albicans sensu stricto*, although generally the majority of yeast isolates from genital sites received by the MRL are from recurrent or recalcitrant infections. In agreement with previous reports (18, 22, 23), all 15 *C. africana* isolates were capable of producing germ tubes upon incubation in horse serum for 3 h at 37°C (Table 3). Similarly, *C. africana* isolates formed true hyphae in Dalmau cultures, although the quantity of hyphae produced and the speed of hyphal formation were much reduced compared to those for *C. albicans* and *C. dubliniensis* isolates (Table 3; data not shown). All isolates of *C. africana* failed to produce chlamydospores in culture, even after prolonged incubation, were incapable of assimilating trehalose, and lacked *N*-acetyl-galactosaminidase (hexosaminidase) activity

TABLE 3 Phenotypic characteristics of *C. albicans*, *C. dubliniensis*, and *C. africana* isolates[a]

Species	Germ tube result	Dalmau culture result		Growth[b] at:			Trehalose assimilation[c]	NAG act[d]	Color on CHROMagar	Growth on actidione	Growth speed[e]
		Hyphae	Chlamydospores	30°C	37°C	42°C					
Candida albicans	+	Abundant	Present	+++	++++	+++	+	+	Apple green	+	100
Candida dubliniensis	+	Abundant	Present	++	+++	+/−	+	+	Apple green	+	85
Candida africana	+	Scant	Absent	++	++	+	−	−	Turquoise-green	+	50

[a] +, positive; −, negative (except as described in footnote *b*).
[b] Growth: −, undetectable; +/−, barely detectable; +, detectable; ++, good; +++, abundant; ++++, prolific.
[c] Ability to assimilate trehalose.
[d] *N*-acetyl-galactosaminidase (hexosaminidase) activity.
[e] Growth speed expressed as a percentage of that observed with *C. albicans* isolates.

FIG 1 Appearance of *C. albicans* (left panel) and *C. africana* (right panel) after 48 h of incubation at 35°C on CHROMagar.

(18, 22, 23) (Table 3). Thus, *C. africana* could be distinguished from *C. albicans* and *C. dubliniensis* by AUXACOLOR2, although the former yielded unique profiles that are not currently in the databases provided with the kit. *C. africana* could also be distinguished from *C. albicans* on the basis of its appearance on CHROMagar, with the former producing smaller and deeper turquoise-green colonies than *C. albicans* (Fig. 1). However, in contrast to previously published descriptions (22, 39), all 15 United Kingdom isolates of *C. africana* were capable of growth at 30°C, 37°C, and 42°C, although growth was restricted at the latter temperature (Table 3). Importantly, current matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) approaches are apparently unable to robustly distinguish *C. africana* and *C. albicans* (see, for example, Bruker Daltonik TechNote 1.0; Bruker Daltonik GmbH, Bremen, Germany).

Given the reduced formation of hyphae and slower growth of *C. africana* compared to *C. albicans*, as well as previous reports demonstrating that *C. dubliniensis* exhibits reduced pathogenicity compared to *C. albicans* in a variety of infection models (reviewed in references 15 and 14), we compared the virulence of isolates of *C. albicans*, *C. africana*, and *C. dubliniensis* in a systemic infection model using the insect larval model *Galleria mellonella* (34–37). Although recent studies have failed to reveal differences in pathogenicity between mucosal and systemic isolates of key pathogenic yeast species in *Galleria* larvae (40), any such potential bias in the current study was avoided by comparing *C. africana* strains with *C. albicans* and *C. dubliniensis* isolates that had also been recovered from female genital samples. In preliminary experiments, and in agreement with previous reports (34), no significant larval killing was observed with any isolate when larvae were inoculated with 10^5 CFU/larva at any temperature (data not shown). However, when 10^6 CFU per larva were employed, species-specific and temperature-dependent larval killing was observed (Fig. 2). *Candida albicans* was the most virulent species studied, and larval killing increased in a temperature-dependent manner. *C. dubliniensis* isolates exhibited virulence (in terms of mortality rates and time to kill) that was indistinguishable from that of *C. albicans* in larvae maintained at 37°C, but they were significantly less virulent than *C. albicans* in larvae incubated at 42°C ($P < 0.015$), a temperature at which most *C. dubliniensis* isolates show extremely

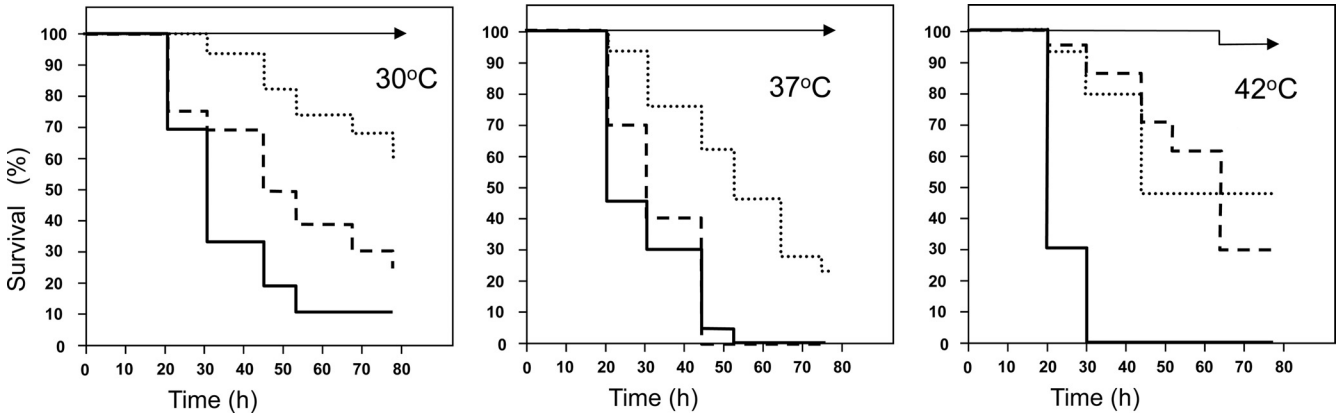

FIG 2 Virulence of *C. albicans*, *C. dubliniensis*, and *C. africana* in *Galleria mellonella* larvae is species specific and temperature dependent. Kaplan-Meier plots of *G. mellonella* survival after injection with 10^6 CFU/larva of *C. albicans* (solid lines), *C. dubliniensis* (dashed lines), or *C. africana* (dotted lines) or an equivalent volume of sterile PBS (arrowed lines) and incubation at 30°C (left panel), 37°C (middle panel), and 42°C (right panel). Four strains were tested per species, with 15 larvae per strain (60 larvae per species). Experiments were performed in duplicate; curves represent the combined (additive) data from all strains and all experiments.

restricted growth *in vitro* (39). Conversely, larval killing by *C. africana* isolates was significantly slower than that observed with *C. albicans* in larvae incubated at all three temperatures ($P < 0.030$, $P < 0.065$, and $P < 0.007$ for 30°C, 37°C, and 42°C, respectively) or *C. dubliniensis* in larvae incubated at 30°C or 37°C ($P < 0.08$ and < 0.015, respectively) (Fig. 2). Finally, analysis of hemolymph from *G. mellonella* larvae inoculated with the various strains and incubated at 37°C for 8 h revealed significant hyphal and pseudohyphal proliferation in larvae inoculated with *C. albicans* and *C. dubliniensis* but not those inoculated with *C. africana* (data not shown). Indeed, no significant filamentation was observed in larvae infected with *C. africana* at any time postinfection.

DISCUSSION

In the current study, we have employed pyrosequencing to evaluate the prevalence of *Candida africana* among isolates of pathogenic *Candida* species submitted to the UK National Mycology Reference Laboratory. In agreement with the existing literature (reviewed in reference 22), *C. africana* was isolated exclusively in this study from female genital specimens. Indeed, *C. africana* constituted 6% of *C. albicans* species complex isolates from female genital specimens and was much more prevalent than the related *C. dubliniensis* in such samples, as has been reported previously from both Europe and the African subcontinent (18, 41). Phenotypically, *C. africana* differed from *C. albicans* and *C. dubliniensis* by the delayed formation of hyphae and absence of chlamydospores in Dalmau cultures, the inability to assimilate trehalose, the lack of *N*-acetyl-galactosaminidase activity, and the development of smaller, more intensely colored turquoise-green colonies on CHROMagar. Despite slower growth and reduced formation of hyphae, all isolates of *C. africana* were found to be germ tube positive under standard incubation conditions (3 h at 37°C). However, in contrast to previous reports (22, 39), the United Kingdom isolates of *C. africana* evaluated here were capable of growth, albeit restricted, at 42°C.

The current study is the first to have evaluated and compared the pathogenicity of *C. africana* isolates with that of its close relatives *C. albicans* and *C. dubliniensis*. As assessed by the ability to kill *Galleria mellonella* larvae in a systemic infection model, *C. africana* isolates were significantly less pathogenic than their *C. albicans* counterparts at 30°C, 37°C, and 42°C and less pathogenic than *C. dubliniensis* isolates at both 30°C and 37°C (Fig. 2). In agreement with many previous studies in a wide range of infection models (reviewed in reference 15), *C. dubliniensis* was less pathogenic than *C. albicans* in *G. mellonella* at all incubation temperatures evaluated, although this difference was not significant in larvae incubated at 37°C. Thus, at least in *Galleria* larvae, the members of the *C. albicans* complex can be ordered *C. albicans* > *C. dubliniensis* > *C. africana* in terms of virulence. Virulence factors have been extensively studied in *C. albicans* and include adhesins, kinases, secreted extracellular enzymes, and, importantly, the ability to alternate between filamentous (hyphal) and budding yeast forms (reviewed in references 15, 42, and 43). Indeed, hyphal formation has been proposed to play pivotal roles in adhesion to host cell surfaces and subsequent biofilm formation, tissue invasion, and immune evasion (44). In this respect, it is noteworthy that mutations in *C. albicans* genes associated with hyphal formation and regulation resulted in reduced virulence in *Galleria* larvae (36). Previously, the reduced virulence of *C. dubliniensis* compared to *C. albicans* has been attributed to lower filamentation rates in the former (45, 46), the loss of several virulence factor genes that appear to be preferentially expressed by the hyphal form (reviewed in reference 15), and a reduced ability to tolerate a wide range of environmental stresses (46).

Here we have reported that *C. africana* grows more slowly than both *C. albicans* and *C. dubliniensis in vitro* and has a much reduced ability to form hyphal filaments on Dalmau culture. This lower filamentation rate was also apparent in *G. mellonella* larvae. In larvae sacrificed 8 h after inoculation with the various *Candida* species, *C. albicans* and to a lesser degree *C. dubliniensis* were present throughout both the insect hemolymph and fat body as a mixture of budding yeasts, true mycelium, and pseudohyphae. In larvae inoculated with *C. africana*, only replicating yeast forms could be detected (data not shown). Thus, it is plausible that this lower filamentation rate contributes to the reduced virulence of *C. africana* in this infection model. For *C. dubliniensis*, it has been proposed that reductive evolution involving the loss of key virulence genes has resulted in the narrowing of environmental conditions conducive to hyphal formation (15), perhaps as an adaptive change to a particular anatomical niche in which filamentation is less required for colonization. In future comparative genomic studies, it will be important to establish whether the genetic repertoire of *C. africana* is similarly reduced and whether the inability of *C. africana* to form hyphae accounts for the apparent restriction of this organism in humans to colonization of the female genital tract. Further studies will be required to compare the abilities of *C. africana*, *C. albicans*, and *C. dubliniensis* to colonize this particular anatomical niche.

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