JOURNAL OF BASIC PHARMACOLOGY AND TOXICOLOGY

Original Research Article

Genetic relatedness between human and animal *Candida albicans* isolates recovered from Southeastern Nigeria using random amplification of polymorphic DNA-PCR

Ifeanyi Elibe Mba, Emeka Innocent Nweze*

Department of Microbiology, University of Nigeria, Nsukka, Nigeria

Abstract

Candida species is currently recognized as the major cause of a variety of human and animal fungal infections globally. Since the world has become a global village, the knowledge of local epidemiology of *Candida albicans* and the evaluation of its diversity is important and will help in understanding and controlling their transmission globally. The present study was conducted to evaluate the genetic relatedness between human and animal *Candida albicans* isolates in Nigeria. The *C. albicans* isolates (n=15) were typed by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) using three primers (P4, OPA-03 and OPE-18) after identification, antifungal susceptibility and virulence profile screening using standardized methods. A high degree of separation of human and animal *C. albicans* isolates into distinct genotypes was clearly evident. Some degree of genotypic relatedness was seen but at varying distance of separation. The highest level of genetic similarities (84%) was observed between *C. albicans* recovered from cow faeces and that recovered from human high vaginal swab, suggesting that they were related but not completely identical. Moreover, no clonal relationship was found. Though this is a preliminary study, we observed different banding patterns suggesting a high degree of discriminatory power for the three primers used. It further confirms the usefulness of RAPD-PCR in molecular typing of *Candida* isolates. Although no clonal relationship was seen and considering the sample size in this study, the possibility of transfer cannot be ruled out when humans are exposed to animals that harbor virulent and resistant strains or when such animals are eaten.

Keywords: *Candida albicans*, humans, animals, genotyping, epidemiology, RAPD PCR

1 Introduction

Candida is a human and animal commensal [1] and has previously been isolated from several animals like rheas, dogs, horses, goats and sheep [2, 3]. Candidiasis (the infection caused by *Candida* spp) is an important health problem in both humans and animals and poses a huge public health problem globally. Animals can act as sources of *Candida* infection to humans or animals of the same or different species [4]. Importantly, human infections caused by *Candida* have rapidly increased over the last few years and the increasing rate of candidiasis adds extra burden to the healthcare system.

Candida albicans happens to be the most frequently documented *Candida* pathogen responsible for majority of the infections [5]. However, recent studies have shown an increase in infections caused by non-albicans *Candida* species. Even with the use of antifungal agents, disseminated candidiasis is accompanied with a high mortality rate (about 40-60%). Poor diagnosis, inappropriate disease management and the general critical condition of the patients also contribute to the increase in mortality. Risk factors for most *Candida* infection include the use of antibiotics, immunosuppressive agents and intravenous catheters. Age, nutritional status and some diseases can also facilitate candidiasis. Moreover, the extracellular hydrolytic enzymes are one of the major virulence factors in *C. albicans* [6].

Reports of candidiasis in animals and its link to human infections are scanty. Moreover, information on the possible role of animals as reservoirs of pathogenic and drug resistant *Candida* spp. is scarce [7]. There is also paucity of data about the identities and origins of infecting strains. Importantly, the genetic relatedness between human and animal *Candida* isolates and the likelihood of transmission from animals to humans have not been well studied in many regions, including our study area. To elucidate the molecular epidemiology of *Candida* species in Nigeria, molecular typing studies are clearly needed. Studies of this nature are important from an epidemiological perspective and will be useful in the global management and control of candidiasis.

Several techniques have previously been used in molecular typing experiments: multilocus sequence typing, microsatellite analysis, restriction endonuclease method, pulsed-field gel electrophoresis, and RAPD-PCR. RAPD-PCR technique has frequently been used to characterize

*Corresponding author: Email: emeka.nweze@unn.edu.ng

Candida isolates in medical and epidemiological studies [8]. RAPD-PCR relies on the application of short arbitrary primers that anneal to multiple genomic sites and can detect strain polymorphisms without prior knowledge of species-specific sequences [9]. It is a very useful method in epidemiological studies for genotyping *Candida* species.
This study though a preliminary study, evaluated the genetic relatedness between human and animal *Candida albicans* species using RAPD-PCR.

2 Materials and methods

2.1 Sample collection

Clinical human isolates were obtained from patients in three different hospitals (Bishop Shanaham Hospital, University of Nigeria Medical Center and Enugu Ezike General Hospital). The *Candida* species were recovered from high vaginal swab (HVS) and urine samples. Animal isolates were sourced from sheep, cattle, pig and chicken. The sample types include rectal swab, blood and faeces. All the samples were sourced within Enugu state, Nigeria.
A total of 112 *Candida albicans* isolates were obtained. However, a representative sample total of 15 *Candida albicans* isolates were genotyped in this study because of cost restraints. It was assumed that similarity in enzymatic and antifungal susceptibility profile between human and animal *C. albicans* isolates could have a profound influence in their genetic similarity. Therefore, animal and human *C. albicans* isolates that showed similar enzymatic and antifungal resistance profile were specifically considered for RAPD-PCR analysis. Moreover, the isolates were also selected because of their strong enzymatic activities in addition to being resistant to the azole antifungal drugs tested in this study. All guidelines in experiments involving the study of humans and animals were followed in the study. Ethical clearance for the study was obtained from Bishop Shanahan Hospital.

2.2 Identification, enzymatic activity and antifungal susceptibility testing of Candida albicans

The samples were cultured on Sabouraud dextrose agar (SDA) (HiMedia, Mumbai, India) supplemented with 1% chloramphenicol (0.05g/L) and then incubated at 37 ºC for 24-48 hr. Identification of *Candida albicans* was based on colony morphology, germ tube test, and growth characteristics on CHROMagar *Candida* (Oxoid, Thermo Fisher Scientific, Basingstoke, United Kingdom) [10].
The virulence profile (enzymatic activity) for proteinase, phospholipase, lipase and haemolysin were accessed using plate methods and the precipitation zone (Pz) values were determined as described by Price et al [11].
Susceptible testing was done via disk diffusion method. The inhibition zone diameter (IZD) was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) approved protocol, CLSI document M44-A2 [12]. The minimum inhibitory concentration (MIC) was determined using broth microdilution method. The MIC values for fluconazole and itraconazole were compared to the Clinical and Laboratory Standard Institute (CLSI) interpretative guidelines on susceptibility testing [13].

2.3 DNA typing for genotypic relatedness using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR)

Genomic DNA of the isolates was extracted using Quick-DNA fungal/bacterial miniprep kit (Zymo Research Corporation, Irvine, California, USA). The extraction was performed according to the manufacturer's instructions. The DNA samples were amplified using oligonucleotide primers. The reactions were carried out in a DNA thermal cycler (Bio-Rad, Foster city, California, USA)[9]. The DNA sequences of the primers used for genotyping were as follows: OPE-18 5′ - GGACTGCAGA - 3′ [14], OPA-03 5′ - AGTCAGCCAC - 3′ [15] and P4 5′ - AAGAGCCCGT - 3′ [16]. The amplification reaction was performed in a final reaction volume of 25 µL containing 1 µL of genomic DNA, 1.25 Units of Taq DNA polymerase, 0.3 mM of each four deoxynucleoside triphosphate, 1.5 mM of $MgCl_2$, 0.4 µM of each individual primer and 2.5 µL of 10x PCR buffer. The reaction conditions for the amplification were as follows: 4 min of initial denaturation at 94 ºC followed by 32 cycles of 94 ºC for 1 min, 42 ºC for 30 s and 72 ºC for 90 s and final extension at 72 ºC for 10 min.
The amplified products were visualized on 1.5% agarose gel run in tris-borate-EDTA (TBE) buffer (0.09 M Tris, 0.09 M boric acid, and 20 mM EDTA; pH 8.3) for 60 min at 110 V and compared with Gene Ruler 100 bp or 1 kb ladder (Thermo Scientific, London, UK). The products were stained with 0.5 µg/mL of ethidium bromide. The stained gel was photographed using ultraviolet photography. Polymorphism was detected after ethidium bromide staining, as the presence or absence of bands of particular sizes and intensity. RAPD-PCR DNA banding patterns represent the DNA fingerprint. The amplicon arrays were analyzed and dendrograms was constructed using a matrix generated by UPGMA (Unweighted Pair Group Method with Arithmetic Means) using NCSS statistical package (2019).
Isolates with 100% similarity were determined to be identical. Isolates with values ranging from 80 to 99% were considered related but not identical and may suggest microevolution of a single strain while those with values less than 80% were considered unrelated [17]. For obtaining reliable results by RAPD method, using more than one primer and comparative analysis of these primers are appropriate. Thus, the dendrogram of the fingerprint generated was constructed using a combination of the three primers.

3 Results

All the representative (15) isolates were positive for one or more of the tested enzymes (virulence factors). The isolates showed very strong enzymatic activities indicating that they

are highly pathogenic strains. The isolates also showed high resistance to the azole antifungal drugs. The inhibition zone diameter (IZD) and MIC values for all the tested isolates, in addition to the enzyme profiles of the isolates are shown in Table 1.

The three primers used in this study produced appropriate multi-shape bands encoded within a range from 100bp-1300bp. Regardless of isolate sources, several DNA bands with different sizes and variable electrophoretic patterns were observed in this study. All the primers presented some degree of polymorphism indicating the high efficiency of the primers in discriminating *Candida albicans* species (Figures 1-3). Among the 15 *Candida albicans* isolates, 15 RAPD profiles were distinguished. Eight isolates were allocated into two clusters while the remaining 7 genotypes were represented by a single isolate only. The profiles generated from our result showed differences between the isolates of all the *C. albicans.* Some human and animal *C. albicans* isolates had homologies ranging from 80-84%, suggesting that they are related but not identical. However, no clonal relationship was seen.

Table 1: Description of 15 representative isolates of *Candida albicans* from human and animal isolates showing the isolate source, site of isolation, enzymatic activity and susceptibility profile

				Enzymatic Pz values (mm)				IZD (mm)			MIC (µg/ml)	
S. No	**Sample reference number**	**Sample type**	**Sample source**	**PRO**	**PHL**	**LIP**	**HAE**	**FLU**	**ITR**	**NYS**	**FLU**	**ITR**
1	HVS 30	Vagina swab	Human	0.60	0.74	0.53	0.72	0.0	0.0	16.0	32	1
2	U 27	Urine	Human	0.54	0.71	0.69	0.40	0.0	0.0	0.0	>64	>1
3	HVS 4b	Vagina swab	Human	0.43	0.65	0.45	0.57	0.0	0.0	12.0	64	>1
4	HVS 8b	Vagina swab	Human	0.79	0.72	0.33	0.51	0.0	0.0	11.6	>64	>1
5	HVS 2a	Vagina swab	Human	0.38	0.68	0.35	0.39	0.0	0.0	13.3	>64	>1
6	U 81a	Urine	Human	0.43	0.89	0.46	0.60	0.0	0.0	10	32	1
7	HVS 16a	Vagina swab	Human	0.42	0.69	1	0.65	10.0	0.0	17.3	64	1
8	AB 83b	Blood	Pig	0.47	0.53	0.40	0.56	10.3	11.6	12.7	>64	0.5
9	AB 88b	Blood	Goat	0.35	0.85	0.58	0.48	12.0	0.0	16.3	>64	1
10	A 30	Feces	Cow	0.43	0.70	0.43	0.42	0.0	0.0	21.6	>64	1
11	A 27	Feces	Cow	0.39	-	0.89	1	0.0	0.0	14.3	>64	>1
12	AB 87b	Blood	Goat	0.41	0.64	0.58	0.43	0.0	0.0	9.5	>64	>1
13	A 43a	Rectal swab	Pig	0.70	1	0.65	0.72	0.0	0.0	15.3	64	>1
14	A 34b	Rectal swab	Sheep	0.53	0.60	0.40	0.49	0.0	0.0	18.3	>64	0.5
15	AB 81a	Blood	Pig	0.34	0.89	0.54	0.53	0.0	0.0	0.0	32	1

Key: PRO: proteinase; PHL: phospholipase; LIP: lipase; HAE: haemolysin; IZD: inhibition zone diameter (mm); FLU: fluconazole (25 µg); ITR: itraconazole (30 µg**);** NYS: nystatin (50 IU); MIC: minimum inhibitory concentration (µg/ml). The enzymatic activities were expressed as Pz values (obtained by measuring the colony diameter and the diameter of colony plus the precipitation zone) as described by Price and co-workers [11].

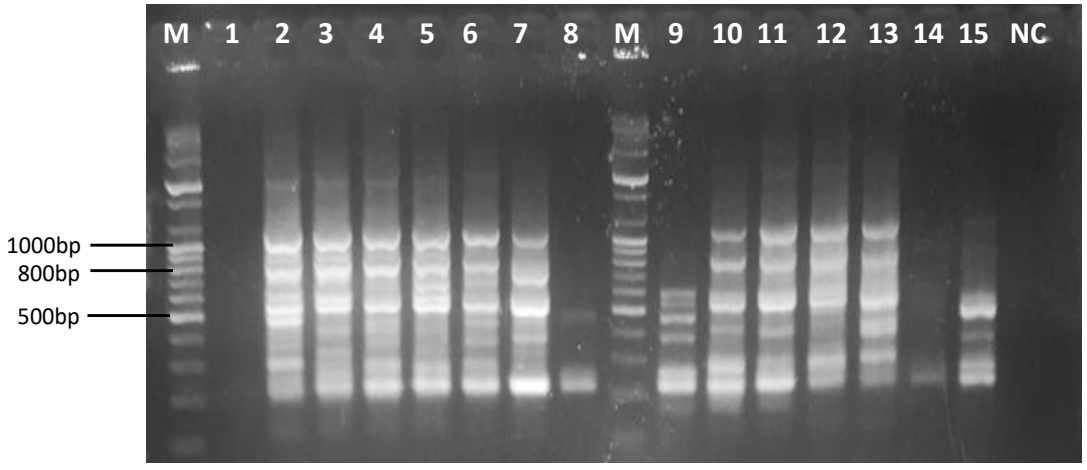

Figure 1. Genetic variation among different *Candida albicans isolates* determined by RAPD analysis using primer P4; lane M, molecular marker (100bp ladder); lanes 1-7, human *Candida albicans* isolates; lanes 8-15, animal *Candida albicans* isolates; NC, negative control

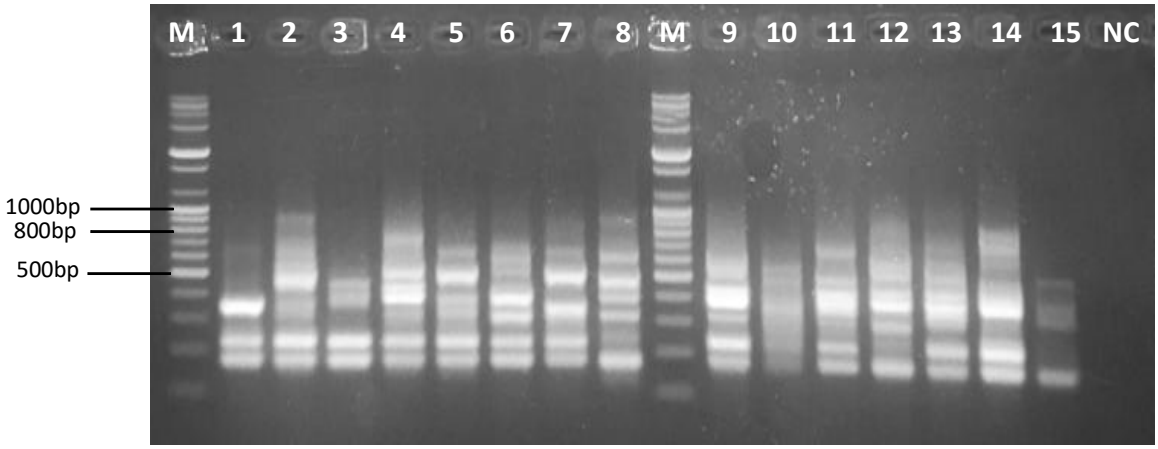

Figure 2. Genetic variation among different *Candida albicans* isolates determined by RAPD analysis using primer OPE 18; lane M, molecular marker (100bp ladder); lanes 1-7, human *Candida albicans* isolates; lanes 8-15, animal *Candida albicans* isolates; NC, negative control

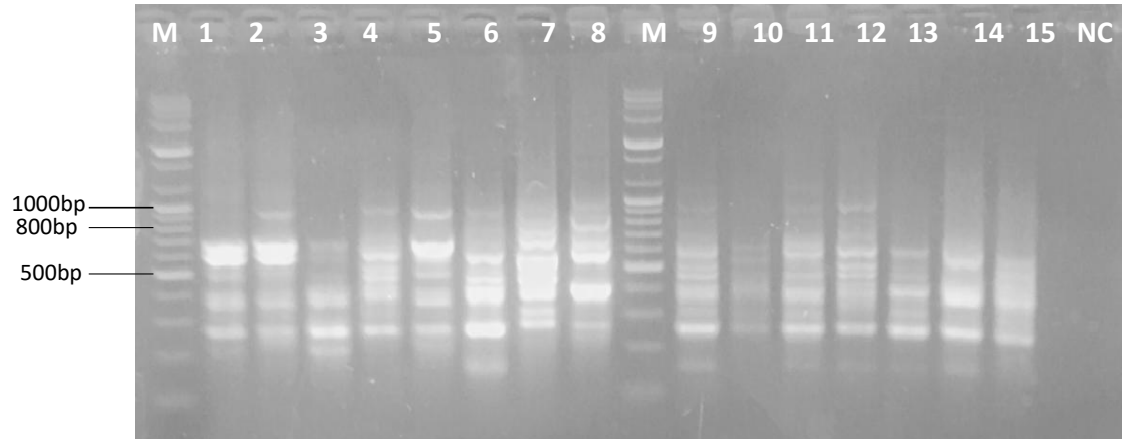

Figure 3. Genetic variation among different *Candida albicans isolates* determined by RAPD analysis using primer OPA 03; lane M, molecular marker (100bp ladder); lanes 1-7, human *Candida albicans* isolates; lanes 8-15, animal *Candida albicans* isolates; NC, negative control

As expected, the tree illustrates the similarity of RAPD patterns seen on the gels. None of the *C. albicans* isolates in our study revealed the same RAPD profile when results from all primers were combined to generate the dendrogram (Figure 4). The highest level of genetic similarities was observed between *Candida albicans* from cow feces (A 27) and *Candida albicans* isolate from high vaginal swab (HVS 8b) with 84% homology. AB 87b, HVS 2a, A 43a and AB 88b also showed some degree of similarity among themselves and with A 27 and HVS 8b although at different level as shown in the distance of separation. Some of the isolates (HVS 30, A 30, AB 81a, HVS16a, HVS 4b) appear to be highly genetically different from the rest of the isolates as indicated by the high distance of separation. Thus, we observed that the RAPD-PCR was able to point out clearly the genomic variability within *C. albicans* population.

4 Discussion

Different banding patterns were mostly seen in majority of the isolates, suggesting high discriminatory power for the three primers in genotyping. Generally, our RAPD analysis showed high degree of heterogeneity. Most of our isolates showed less than 80% similarity suggesting the presence of independent strains and confirming the results of Zanni et al [18], who reported high level of heterogeneity among human *Candida* isolates using RAPD method. Jianchai et al [19] in a study that isolated seventy-two *C. albicans* isolates from poultry birds in China suggested that *C. albicans* poultry isolates were relatively independent but not completely separated from human isolates. Edelmann et al [20] in previous studies on the genetic relationship between human and animal isolates of *Candida albicans* reported no evidence of species-specific lineages, even though a certain degree of separation between human and animal isolates was found.

According to the dendrogram obtained in this study, the highest level of genetic similarity (84%) was observed among *Candida albicans* from Cow feces (A 27) and *Candida albicans* isolate from high vaginal swab (HVS 8b), suggesting that they were related based on dice coefficient but not completely identical. One of the isolates from the animal blood (AB 87b) showed 80% similarity with A27 and HVS 8b. However, none of our isolates revealed the same RAPD profile when results from all primers were

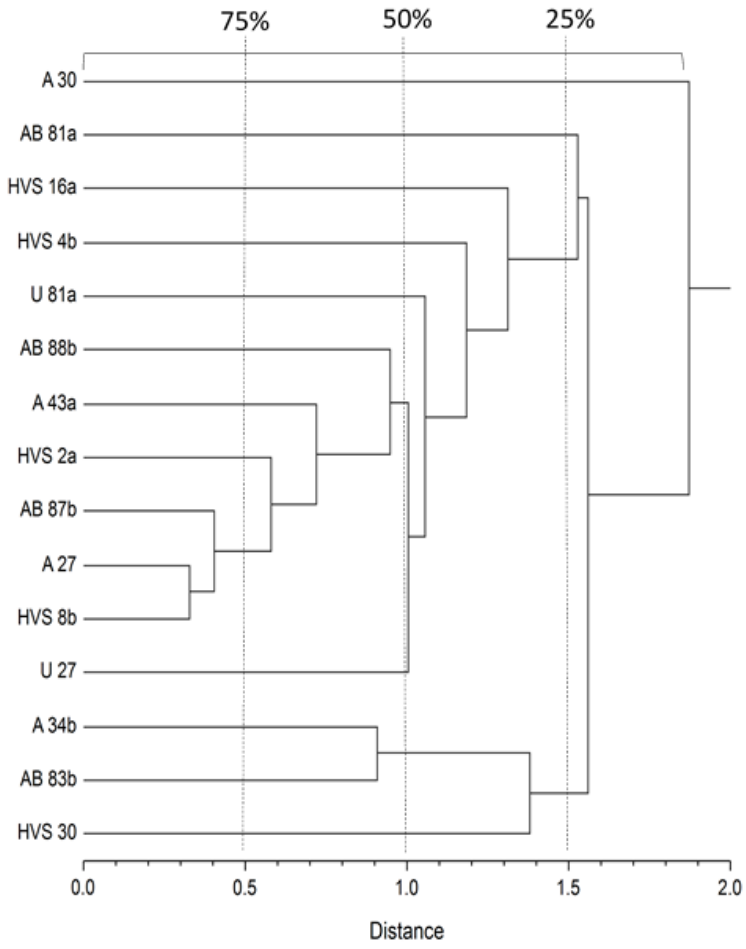

Figure 4. Dendrogram of genetic relatedness constructed using a combination of the three primer (P4, OPA-03, OPE-18); the vertical lines divide the strains according to various similarity level

combined to generate the dendrogram. These findings were in accordance with results obtained by Paluchowska et al [17] who studied the genetic homology of *C. albicans* and *C. glabrata* using five arbitrary primers. Several studies have reported a higher degree of genetic diversity among *Candida albicans* strains. Previously, Marol and Yucesoy [21] reported that four *Candida albicans* cultured from various specimens from the same patients were genetically different while Costa et al. [22] also reported a high level of genetic diversity among 15 *C. albicans* strains using five primers. It is worthy to note that some of the primers used in the reported studies are the same with the ones used in this study.

The three primers, P4, OPA-03, OPE-18 were able to detect intraspecific polymorphism in *C. albicans* species. All presented some degree of polymorphism indicating the high efficiency of the primers in discriminating *Candida albicans* species. These primers were chosen because of their previous usefulness in genotyping *Candida* species [14-17]. As reported by Fahami et al [23], ability to effectively discriminate between Candida strains is a major consideration in selecting primers. It has been reported by some authors that the strains classified as identical by one primer are not always classified as belonging to the same cluster when analysed by another primer [16]. Thus, it becomes appropriate that the data obtained with a number of oligonucleotides should be combined to generate a higher discriminatory power (DP) [24]. RAPD is not only reliable but also fast and simple to perform. They are reliable methods that have been used for studying pathogenic fungi. It has proven to be useful in genotypic profiling, which allow intra-specific identification of isolates as well as detection of small differences in nucleic acid content of species and ancestries of the same species. It has also proven useful in the determination of the genetic relatedness and to assess the transmission routes of fungi [25].

It was previously suggested by Pfaller et al [26] that the use of a combination of different molecular typing procedures may help to distinguish certain isolates from one another. According to Merseguel et al [26], for inferences on the natural history of hematogenous infection caused by the rapidly emerging *Candida* species, molecular studies aimed at identifying closely related and emerging species is appropriate. Thus, we justify that the use of molecular markers, such as RAPD-PCR is very helpful in discriminating the genetic diversity of *Candida* species. RAPD-PCR is an accurate and simple to administer technique with several advantages. The known and important drawback of RAPD-PCR technique is its poor interlaboratory reproducibility, hence highly standardized experimental procedures are needed because of their sensitivity to the reaction conditions [28, 29]. RAPD PCR

requires strict standardization of PCR conditions because variations in different DNA polymerase concentrations, DNA template, primer ratios or annealing temperatures can lead to differences in the final results [30]. These draw backs could affect the transference or comparison of our results with those obtained from other researchers working on the genetic variability among *Candida* species.

In summary, the dendrogram from our study revealed a high degree of separation of human and animal isolates. It was observed that some human and animal *C. albicans* isolates had homologies ranging from 80-84% (suggesting that they are related but not identical). Microevolutionary changes of a single isolate that might have occurred during adaptation to fluctuating environmental conditions [16] and the possibility that the unique strains in our dendrogram belonged to a different *Candida* species (non-albicans *Candida* species) might have been the reason for such variation in our genotyping experiments. The observed genetic variations can also be attributed to the barriers to transmission (low frequency of contact) considering that the exact location where the animal samples were sourced was different from that of humans.

Our study further suggest that animals are potential reservoirs of highly pathogenic and drug resistant *Candida* species and might pose a potential threat to humans. This is supported by the findings from Cordeiro et al. [3] who reported that *Candida* isolates from animals showed high rate of resistance to azoles, expressed virulence factors and may represent a potential threat to human and animal health. Recently, Osman et al [31] highlighted the public health importance of bovines as potential sources of *C. albicans* zoonotic transmission to humans in an urban-rural community. It is also important to emphasize that for strain transfer to occur between humans and animals, the recipient must come in contact with a *C. albicans*-colonized animal or animal-derived products. From our initial hypothesis in addition to previously published studies, the directionality of *Candida* transfer is considered from a human perspective (animals act as reservoirs of human *Candida* spp). This perspective is understandable and justifiable considering the availability of few published information regarding the frequency of *Candida* isolation from animals and the emphasis on human candidiasis. Direct encounter with animals and their products is a relatively common event in the lives of most humans. Goat, sheep, pig, cow and chicken are commonly found in our immediate environment. Given that our samples were sourced from these animals, questions could further be raised on the role of these animals in the dissemination of candidiasis.

Although our sample size may not be fully representative of the genetic diversity within the study area, our findings on the genetic relatedness of Candida species is consistent with previous studies [17, 20, 32-34]. Moreover, our isolates showed similarity in enzyme and antifungal pattern.

5 Conclusion

It was observed that RAPD-PCR was suitable for fingerprinting analysis, generated reproducible fingerprints in addition to yielding well-resolved banding patterns. The study revealed high degree of separation of human and animal *Candida* isolates as different genotypes were clearly evident. No clonal relationship was found, however, considering the sample size investigated, the possibility of transfer cannot be ruled out when humans are exposed (come in contact with animals and/or their products) to animals that harbor virulent and resistant *Candida* strains or when such animals are eaten. We suggest that analyzing a larger number of isolates with more sensitive molecular techniques and better *C. albicans* identification method is warranted; this will efficiently permit a greater discernment between the genotypic relatedness and the epidemiological pattern of human and animal *Candida* species.

Conflict of interest

The authors declare that they have no competing interests.

References

1. Neville BA, d'Enfert C, Bongnoux ME. *Candida albicans* commensalism in the gastrointestinal tract. FEMS yeast Res. 2015;15:7.
2. Brilhante RSN, Alencar LP, Cordeiro AR, Collares DS, Branco MC, Cordeiro CE, et al. Detection of *Candida* spp. resistant to azoles in the microbiota of rheas (R*hea americana*): Possible implications for human and animal health. J Med Microbiol. 2013;62:889–95.
3. Cordeiro RA, Oliveira JS, Castelo-Branco DSCM, Teixeira CEC, Marques FJF, Bittencourt PV, et al. *Candida tropicalis* isolates obtained from veterinary sources show resistance to azoles and produce virulence factors. Med Mycol. 2015;53:145-52.
4. Rozanski P, Pluta M, Rozanska D. Mycological profile of the integumentary system in felin ponies. Ann Anim Sci. 2017;17(4):1019-28.
5. Nweze EI, Ogbonnaya UL. Oral *Candida* isolates among HIV-infected subjects in Nigeria. J Microbiol Immunol Infect. 2011;44(3):172-7.
6. Mba IE, Nweze EI. Mechanism of *Candida* pathogenesis: revisiting the vital drivers. Eur J Clin Microbiol Infect Dis. 2020;39(10):1797-1819.
7. Subramanya SH, Sharan NK, Bara BP, Hama D, Nayak N, Prakash PY, et al. Diversity, *in-vitro* virulence traits and antifungal susceptibility pattern of gastrointestinal yeast flora of healthy poultry, *Gallus domesticus*. BMC Microbiol. 2017;17:113.
8. Samaka HMA. RAPD-PCR based genetic variation of *Candida albicans* of animal and human origin. AL-Qadisiya J Vet Med Sci. 2015;14(1):2015-45.
9. Avinash M, Anurag KS, Gaur RK. Molecular markers: tool for genetic analysis. Animal Biotechnology, Academic press, Elsevier. 2014;289-305.
10. Raju SB. and Rajappa S. Isolation and identification of *Candida* from the oral cavity. ISRN Dent 2011;7.
11. Price MF, Wilkinson ID, Gentry LO. Plate method for detection of phospholipase activity in *Candida albicans*. Sabouraudia: J Med Vet Mycol. 1982;20:7-14.
12. Method for antifungal disk diffusion susceptibility testing of yeast; approved guidelines-second edition vol.29 No.17, Aug-2009 CLSI document M44-A2.
13. Clinical and Laboratory Standards Institute (CLSI).: Reference method for broth dilution antifungal susceptibility testing of

yeasts, 3rd ed. Clinical and Laboratory Standards Institute, Wayne, PA (M27-A3) 2008.
14. Pires EG, de FFreita EM, Bonan PRF, Nobre SAM. Agreement between RAPD, API20C AUX, CHROMagar *Candida* and microculture on oral *Candida* identification. Braz J Oral Sci. 2015;14:2.
15. Valério HM, Weikert-Oliveira RCB, deResende MAR. Differentiation of *Candida* species obtained from nosocomial candidemia using RAPD-PCR technique. Rev Soc Bras Med Trop. 2006;39(2):174-8.
16. Bonfim-Mendonça PS, Fiorini A, Shinobu-Mesquita CS, Baeza LC, Fernandez MA, Svidzinski TIE. Molecular typing of *Candida albicans* isolates from hospitalized patients. Rev Inst Med Tropl Sao Paulo. 2013;55(6):385-91
17. Paluchowska P, Tokarczyk M, Bogusz B, Skiba I, Budak A. Molecular epidemiology of *Candida albicans* and *Candida* glabrata strains isolated from intensive care unit patients in Poland. Mem Oswaldo Cruz. 2014;109(4):436-41
18. Zanni PCMD, Bonfim-Mendonca PS, Negri M, Nakamura SS, Donatti L, Svidzinski TIE, et al. Vurulence factors and genetic variability of vaginal *Candida albicans* isolates from HIV-infected women in the post-highly active antiretroviral era. Rev Inst Med Trop Sao Paulo. 2017;59:e44
19. Jianchai L, Huanzhang L, Jinkun Y, Na L, Heping Z, Chengrui Z, et al. Molecular typing and genetic relatedness of 72 clinical *Candida albicans* isolates from poultry. Vet Microbiol. 2017;214:36-43
20. Edelmann A, Kruger M, Chmid J. Genetic relationship between human and animal isolates of *Candida albicans*. J Clin Microbiol. 2005;43(12):6164-6.
21. Marol S, Yucesoy M. Molecular epidemiology of *Candida* species isolated from clinical specimens of intensive care unit patients. Mycoses. 2008;51(1):40-9.
22. Costa KR, Ferreira JC, Lavrador MA, Baruffi MD, Candido RC. Virulence attributes and genetic variability of oral *Candida albicans* and *Candida* tropicalis isolates. Mycoses. 2012;55:e97-e105
23. Fahami S, Kordbacheh P, Moazeni M, Mahmoodi M, Mirhendi H. Species identification and strain typing of *Candida* isolates by PCR-RFLP and RAPD-PCR analysis for determining the probable sources of nosocomial infections. Ira Red Crescent Med J. 2010;12:539-47.
24. Soll DR. The ins and outs of DNA fingerprinting the infectious fungi. Clin Microbiol Rev. 2000;13(2):332-70.
25. Hanafy AM. Molecular characterization of azole-susceptible and resistant *Candida albicans* isolated from cancer patients. Egypt Soc Exp Biol. 2013;9(1):97-103.
26. Pfaller MA. Epidemiology and control of fungal infections. Clin Infect Dis. 1994;19:S8-13.
27. Merseguel KB, Nishikaku AS, Rodrigues AM, Padovan AC, e Ferreira RC, de Azevedo Melo AS, et al. Genetic diversity of medically important and emerging *Candida* species causing invasive infection. BMC Infect Dis. 2015;15:57.
28. Hadrys H, Balick M, Schierwater B. Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol Ecol. 1992;1:55-63.
29. Saghrouni F, Ben AJ, Abdeljelil J, Boukadida J, Ben SM. Molecular methods for strain typing of *Candida albicans*: a review. J Appl Microbiol. 2013;114:1559-74.
30. Thangaraj M, Vishruth P, Ramesh T, Lipton AP. RAPD fingerprinting and demonstration of genetic variation in three pathogens isolated from mangrove environment. Asian J Biotechnol. 2011;3(3):269-74.
31. Osman K, Abdeen EE, Mousa WS, Elmonir W, El-Diasty EM, Elbehiry A. Genetic diversity among *Candida albicans* isolated from humans and cattle with respiratory distress in Egypt. Vector Borne Zoo Dis. 2019;19(3):199-206.
32. Shadi S, Katiraee F, Farshi Y, Fakhri O. Genetic diversity among Iranian *Candida albicans* isolated from nails and skins. 2nd Iranian Congress on Medical Mycology 2013. Doi:10.5812/jjm.10724
33. Szweda P, Gucwa K, Naumiuk L, Romanowska E, Dzierzanowska-Fangrat K. Evaluation of possibilities of genotyping of *Candida* glabrata clinical isolates with RAPD-PCR method and microsatellite analysis. Sci J Microbiol. 2013;2:194-200.
34. Wrobel L, Whittington JJ, Pujol C, Oh SH, Ruiz MO, Pfaller MA, et al. Molecular phylogenetic analysis of a geographically and temporally matched set of *Candida albicans* isolates from humans and nonmigratory wildlife in central Illinois. Eukaryot Cell. 2008;7(9):1475-86.