Original Research Article

Journal of Biological Research & Biotechnology

Bio-Research Vol. 17 No.1; pp.1044A-1055A (2019). ISSN (print):1596-7409; eISSN (online):9876-5432

Antifungal resistance profile and enzymatic activity of *Candida* species recovered from human and animal samples

Mba IE and Nweze EI

Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

§Corresponding Author: Prof. Nweze Emeka Innocent; emeka.nweze@unn.edu.ng

Abstract

Candida is currently the most implicated pathogenic fungal species recognized as the major cause of a variety of human infections all over the world. This study investigated species distribution, enzymatic activities, and antifungal resistance profiles of human and animal *Candida* species. Clinical *Candida* species (n=220) were isolated from urine, high vaginal swab (HVS) and blood while *Candida* species (n=128) were isolated from rectal swab, ear swab, blood, feces, and milk in animals: goat, sheep, cattle, pig and chicken. The identification of the species was performed using standard methods. Enzymatic activity was screened using plate methods. Susceptibility testing was carried out using disk diffusion and broth microdilution methods. A statistically significant difference (P=0.031) was observed in the distribution of *Candida* spp. recovered from humans and animals. The Pz values of human *Candida* species for proteinase, hemolysin, lipase and phospholipase were 0.65±0.97, 0.61±0.81, 0.59±0.47 and 0.76±0.74 respectively while that of *Candida* species recovered from animal were 0.67±0.13, 0.61±0.95, 0.62±0.67 and 0.69±0.70 respectively. No statistically significant difference (P>0.05) in the *in vitro* enzymatic activity was observed between the two groups. High azole-resistance rate was observed. Resistance was higher among human *Candida* isolates compared to animal isolates although the difference was not considered statistically significant (p = 0.519). Our findings suggest that the enzymatic activity (virulence potential) and resistance patterns are similar in the two groups investigated. This study underscores the importance of animals especially pets and their products as potential sources/reservoirs of pathogenic and multi-azole resistant *Candida* species in Nigeria.

Keywords: *Candida* species, antifungal resistance, virulence factors, human, animal

INTRODUCTION

Candidiasis is now considered the third to fourth most frequent nosocomial infection in the US and worldwide, behind bacterial infection caused by *Clostridium difficile, Neisseria gonorrhea* and Enterobacteriaceae (Lamoth et al., 2018). *Candida* spp., the causative agent of candidiasis is an aerobic, diploid, and dimorphic yeast that belongs to ascomycetes class of fungi. Ascomycetes are commensals of domestic animals and wildlife and have frequently been isolated from rheas, dogs, horses, goats, sheep and sirenians (Brilhante et al., 2013; Cordeiro et al., 2013; Cordeiro et al., 2015). *Candida* is also part of the microbiota of the human body. They colonize various anatomical sites such as the oral cavity, digestive tract, vagina, and skin. Hormansdorfer and Bauer (2000), reported that domestic animals such as horses, cattle, cats, pigs, and dogs as well as birds are susceptible to candidiasis. Therefore, *Candida* may arise as an important health issue in both humans and animals. Besides the environmental impact, animals can serve as sources of infection for humans, and humans can infect animals and vice versa (Rozanski et al., 2017). Recent findings show an increase in the rate of infections associated with *Candida* species (Alkharashi et al., 2019). Furthermore, the resistance of *Candida* species to antifungal drugs especially the azoles are a public health concern. The azoles are widely used because of their few side effects and easy oral bioavailability. They are fungistatic, meaning that they do not kill the fungal cells, rather they merely stop it from growing. The downside to this is that it gives the organism time to develop resistance. Although some *Candida* species have natural resistance even without prior exposure to antifungals agents, it is also possible and common for strains that are initially susceptible to develop resistance. There have also been some insinuations that animals are source of resistant *Candida* species (Brilhante et al., 2013).

It is not surprising that many studies have recently focused on *Candida* pathogenesis, aimed at developing better approaches to the management of candidiasis. The virulent factors have attracted utmost interest. The extracellular hydrolytic enzymes happen to be the major virulence factor necessary for *Candida* infection establishment. The enzymes most commonly implicated in *Candida* pathogenesis process include the proteinase, phospholipase, lipase and hemolysin. Increase in the production and activity of the hydrolytic enzymes highly influence the pathogenic potential of *Candida* species (Maheronnaghsh et al., 2019). There have been some insinuations that animals could serve as vectors for transmission of infectious diseases or as reservoirs of human pathogenic and antifungal resistant *Candida* species and may pose a risk most especially for immunocompromised patients (Brilhante et al., 2013). Over the years, researchers have been paying more attention to human *Candida* infections. While this is understandable, it has created some vacuum in studies related to animals. Few studies, however, have shown that animals harbor potentially pathogenic and antifungal resistant *Candida* species (Cordeiro et al., 2015; Osman et al., 2019).

This study, therefore, assessed and compared *Candida* spp. distribution, extracellular hydrolytic enzyme activity (virulence factors) and antifungal resistant profile among *Candida* isolates recovered from human and animal samples in Enugu State, Nigeria. The key objectives of the study were to ascertain the prevalence and distribution of *Candida* species in humans and animals, ascertain if healthy animals and their droppings harbor potentially pathogenic *Candida* species including antifungal resistant strains and elucidate the extent and similarity in enzyme production and resistance profile between human and animal *Candida* spp.

MATERIALS AND METHODS

Sample collection and identification

Human clinical *Candida* isolates (n=220) were isolated from samples collected from patients who visited three different hospitals (Bishop Shanaham Hospital, University of Nigeria Medical Center and Enugu Ezike General Hospital) during the study period. The *Candida* species were recovered from the following body sites after obtaining informed consent: high vaginal swab (HVS) (n=38), urine (n=129), blood (n=53). *Candida* species were also isolated from animals (n=128) presumed to be healthy. These animals were displayed in an abattoir for slaughtering before selling to the customers. All animal samples were collected before the slaughtering of the animal. Moreover, animal samples were also collected from an animal farm located at the University of Nigeria, Nsukka. The isolates were obtained from sheep, goat, cattle,

pig, and chicken. The sample types include rectal swab (n=76), blood (n=30), feces (n=13), cow milk (n=8) and ear swab (n=1). All samples were sourced within Enugu State, Nigeria. The medical conditions of the sampled subjects for any signs of *Candida* infections (example fungaemia) were not pursued further after samples were collected. All the approved standard procedures for use in human and animal experiments were followed in the study. The samples were cultured on Sabouraud dextrose agar (HiMedia, Mumbai, India) supplemented with 1% chloramphenicol (0.05g/L) and then incubated at 37ºC for 24-48 hr. Identification of *Candida* spp. was based on colony morphology, germ tube test, and growth characteristics on CHROMagar *Candida* (Oxoid, Basingstoke, UK) and the different *Candida* species were differentiated based on the color of the colonies on CHROMagar *Candida* as previously described (Nadeem et al., 2010).

Susceptibility testing using antifungal disks

Antifungal susceptibility of 194 *Candida* isolates recovered from the samples was ascertained by testing the isolates against the following drugs (Himedia, Pennsylvania, USA): fluconazole (25µg), voriconazole (1µg), itraconazole (30µg), ketoconazole (30µg), clotrimazole (10µg) and nystatin (50IU). The disk diffusion technique was performed on Mueller-Hinton Agar (MHA) plates supplemented with 2% glucose with 0.5mcg/ml methylene blue dye medium. The diameter of the zone of inhibition was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) approved protocol, CLSI document M44-A2 (CLSI, 2009).

Minimum inhibitory concentration (MIC)

The MIC was determined using broth microdilution method and according to the CLSI M27- A3 broth microdilution approved standard (CLSI, 2008). The tested drugs for the MIC were fluconazole, itraconazole, and nystatin. The MIC of fluconazole and itraconazole was regarded as the lowest antifungal concentration with substantially lower (50% reduction) turbidity compared to the growth in the drug free tube. The MIC of nystatin was defined as the lowest drug concentration which resulted in complete inhibition of visible *Candida* growth after 48 h of incubation

Enzyme analysis

Phospholipase and lipase activity was determined using the method described by Price et al. (1982). Proteinase activity was determined using bovine serum albumin agar as reported by Junior et al. (2011), while hemolytic activity was determined using the method described by Luo et al. (2001). The precipitation zone (Pz value) for all the enzymes evaluated was calculated and interpreted as already established by Price et al. (1982) using the formula:

$$Pz = \frac{Colony\ diameter}{Colony\ diameter\ + Zone\ of\ Precipitation}$$

Statistical analysis

Chi-square ($\chi 2$) test was carried out on the obtained data to determine whether the differences observed in the prevalence and distribution of *Candida* spp. among the different groups studied were statistically significant. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons post-hoc test was used to assess and compare the differences in enzymatic activity and resistant pattern. Results with $P< 0.05$ was considered significant. The statistical analysis was done using the SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA). The single isolate recovered from animal ear swab was not included in the analysis because it was not representative. All experiment was repeated two times on different days and the average of the values ± standard deviation was calculated.

RESULTS

***Candida* species distribution**

Five *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis*) were recovered and identified from both human and animal samples. Of the entire human isolates (n=220), *C. albicans* accounted for 96 (43.6%) while non-albicans accounted for 124 (56.4%) (Table 1). Among animal isolates (n=128), *C. albicans* account for 41(32%) while non-albicans account for 87(68%). *C. parapsilosis* was the most prevalent, accounting for 25 (19.5%) of the entire animal samples followed by *C. glabrata,* 18 (14.1%) (Table 2). *C. albicans* was the most frequently isolated species in both human and animal samples (Table 3). However, when non-albicans are combined together, they represent a greater percentage than the *C. albicans* recovered from both human and animal isolates. Non- albicans *Candida* were mostly isolated from

animals than from humans where they represent 68% of the entire animal samples compared to 56% recovered from humans. The overall prevalence of *C. albicans* and non-albicans in the study is 39.4% and 60.6% respectively. Moreover, there was a statistically significant difference (P=0.031) in the occurrence of *Candida* spp. between humans and animals.

Enzymatic activities in human and animal *Candida* species

Table 4 illustrates the distribution of human and animal *Candida* species showing different virulence attributes while Figure 1A shows the percentage of different clinical *Candida* isolates producing extracellular enzymes. All the *Candida* species (100%) recovered from human blood were hemolysin and lipase producers. The blood *Candida* isolates also displayed a higher phospholipase activity (71.4%) than the rest of the samples. Overall, the clinical samples showed very strong enzymatic activity. Figure 1B shows the percentage of *Candida* isolates from different animal samples with extracellular enzyme production. *Candida* isolates from cow milk showed 100% lipase activity while the rest of the isolates from rectal swab, blood and feces also displayed very strong enzyme activity as observed in clinical human samples. One notable observation was that over eighty percent of the recovered isolates from animal blood were positive for all the studied enzymes. Comparing the percentage enzymatic activities between human and animal isolates, it was observed that out of 158 human and 98 animal isolates screened for proteinase activity, 84.2% and 83.7% showed positive activity, respectively. 88.2% and 89.6% of human (n=152) and animal (n=96) isolates respectively were positive for hemolysin activity. For lipase activity, 83.2% and 80% of human and animal isolates were respectively positive while 74.3% and 52.3% of human (n=91) and animal (n=65) isolates displayed phospholipase activity respectively (Figure 1C). Majority of the human and animal isolates had very low Pz values indicating strong enzymatic activity except in phospholipase where 52.7% of human and 47.7% of animal had a Pz value of 1.

Resistance profile in human and animal *Candida* species

A high resistance rate was noted in this study. Table 5 shows the *in vitro* antifungal susceptibility profile of the recovered *Candida* species. All the recovered isolates showed higher susceptibility to nystatin than all the azoles as seen in the zones of inhibitions. The minimum inhibitory concentration (MIC) values for some of the recovered *Candida* isolates are shown in Table 6. Figure 2A shows the resistance profile of isolates from clinical samples while Figure 2B shows the resistance profile of isolates from animal samples. Resistance was more prominent among *Candida* isolates recovered from the blood of animals. Out of 14 *Candida* isolates recovered from blood, 13 (92.9%) were resistant to fluconazole only while 12 (85.7%) showed resistance to clotrimazole, voriconazole, itraconazole, and ketoconazole. Figure 2C shows the percentage resistance comparison in human and animal *Candida* species. Overall, resistance was higher in human than in animal isolates. However, the difference was not considered statistically significant (P > 0.05).

Discussion

Candida species are part of the natural microbiota of humans and animals. They are the major cause of fungal infections in humans and animals. A substantial number of the recovered isolates were from urine and blood, which indicate that other than bacteria, pathogenic fungi (*Candida* spp.) are also responsible for a large number of urinary and bloodstream infections frequently reported in hospitals. Findings from our investigation agree with recent epidemiological patterns which suggest a shifting trend from the albicans to the non-albicans. *Candida albicans* was more predominant in human samples. Similar findings have been reported in Nigeria (Nweze and Ogbonnaya, 2011). *C. albicans* was more frequently recovered from the blood of animals (pig and sheep) (43.3%) and were also the most common *Candida* spp. in the whole animal samples. However, when comparing the *C. albicans* and non-albicans recovered from both human and animal samples, the non-albicans *Candida* species were significantly (P<0.05) more than *Candida albicans.* A predominance of non-albicans *Candida* species (68%) was observed in animal samples screened in the study and *C. parapsilosis* was the most frequently occurring. This is contrary to a report by Kemoi et al., (2013) who reported *C. lusitaniae* as the most common *Candida* spp.

Table 1: Distribution of *Candida* species in different human samples

		Candida species						Total
		C. albicans	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C. parapsilosis*	Others	
Human	Urine	47 (36.4%)	28 (21.7%)	14 (10.9%)	16 (12.4%)	10 (7.8%)	14 (10.9%)	129
	HVS	24 (63.2%)	6 (15.8%)	0 (0.0%)	2 (5.3%)	4 (10.5%)	2 (5.3%)	38
	Blood	25 (47.2%)	4 (7.5%)	3 (5.7%)	5 (9.4%)	5 (9.4%)	11 (20.8%)	53
Total		96 (43.6%)	38 (17.3%)	17 (7.7%)	23 (10.5%)	19 (8.6%)	27 (12.3%)	220

Key: HVS-high vaginal swab

Table 2: Distribution of *Candida* species in animal samples

		Candida species						Total
		C. albicans	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C.parapsilosis*	Others	
Animal	(Rectal swab)	25 (32.9%)	10 (13.2%)	11 (14.5%)	6 (7.9%)	15 (19.7%)	9 (11.8%)	76
	(Ear swab)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1
	(Blood)	13 (43.3%)	5 (16.7%)	0 (0.0%)	5 (16.7%)	2 (6.7%)	5 (16.7%)	30
	(Faeces)	3 (23.1%)	1 (7.7%)	4 (30.8%)	0 (0.0%)	3 (23.1%)	2 (15.4%)	13
	(Milk)	0 (0.0%)	1 (12.5%)	0 (0.0%)	2 (25.0%)	5 (62.5%)	0 (0.0%)	8
Total		41 (32.0%)	18 (14.1%)	15 (11.7%)	13 (10.2%)	25 (19.5%)	16 (12.5%)	128

Table 3: Relative distribution of *Candida* species in human and animal samples

		Candida species						Total
		C. albicans	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C. parapsilosis*	*Others*	
Samples	Human	96 (43.6%)	38 (17.3%)	17 (7.7%)	23 (10.5%)	19 (8.6%)	27 (12.3%)	220
	Animal	41 (32.0%)	18 (14.1%)	15 (11.7%)	13 (10.2%)	25 (19.5%)	16 (12.5%)	128
Total		137 (39.4%)	56 (16.1%)	32 (9.2%)	36 (10.3%)	44 (12.6%)	43 (12.4%)	348

Table 4: Distribution of human and animal *Candida* species showing different virulence

Enzyme	*Candida* spp	Human			Animal			
		n	Mean ± SD	Range	n	Mean ± SD	Range	P value
Proteinase	*C. albicans*	73	0.59±0.21	0.31-1	37	0.54±0.18	0.34-1	0.797
	C. glabrata	30	0.72±0.24	0.31-1	18	0.71±0.26	0.39-1	
	C. tropicalis	13	0.51±0.11	0.38-0.73	10	0.90±0.10	0.81-1	
	C. krusei	13	0.64±0.22	0.38-1	9	0.67±0.25	0.37-1	
	C. parapsilosis	14	0.79±0.21	0.46-1	12	0.58±0.23	0.42-1	
	Others	15	0.66±0.21	0.43-1	12	0.60±0.20	0.38-1	
Hemolysin	*C. albicans*	70	0.63±0.18	0.35-1	34	0.61±0.18	0.42-1	0.873
	C. glabrata	29	0.76±0.21	0.51-1	19	0.61±.18	0.43-1	
	C. tropicalis	12	0.61±0.16	0.40-1	10	0.77±0.11	0.65-0.85	
	C. krusei	12	0.52±0.12	0.32-0.69	9	0.48±0.13	0.40-0.71	
	C. parapsilosis	14	0.58±0.10	0.45-0.73	12	0.62±0.15	0.40-0.88	
	Others	15	0.59±0.20	0.41-1	12	0.55±0.16	0.35-0.87	
Lipase	*C. albicans*	71	0.57±0.22	0.33-1	34	0.57±0.19	0.40-1	0.512
	C. glabrata	29	0.66±0.26	0.31-1	17	0.67±0.26	0.41-1	
	C. tropicalis	13	0.58±0.19	0.41-1	11	0.60±0.17	0.50-0.80	
	C. krusei	13	0.53±0.20	0.37-1	9	0.60±0.23	0.39-1	
	C. parapsilosis	14	0.63±.27	0.37-1	12	0.53±0.18	0.34-0.80	
	Others	15	0.59±0.22	0.35-1	12	0.71±0.26	0.39-1	
Phospholipase	*C. albicans*	37	0.76±0.25	0.32-1	24	0.60±0.21	0.38-1	0.117
	C. glabrata	17	0.78±0.20	0.53-1	14	0.70±0.22	0.37-1	
	C. tropicalis	8	0.90±0.21	0.45-1	5	0.76±0.22	0.55-1	
	C. krusei	7	0.76±0.14	0.62-1	7	0.68±0.30	0.41-1	
	C. parapsilosis	11	0.70±0.19	0.45-1	6	0.63±0.15	0.37-0.75	
	Others	11	0.69±0.29	0.38-1	9	0.78±0.29	0.32-1	

Key: n=number of tested *Candida* specie; SD=standard deviation; Pz: precipitation zone (mm); Pz=<0.69, very strong; Pz=0.70-0.79, strong; Pz=0.80-0.89, low; Pz=0.90-0.99, very low; Pz=1, negative; Values with P<0.05 were considered statistically significant

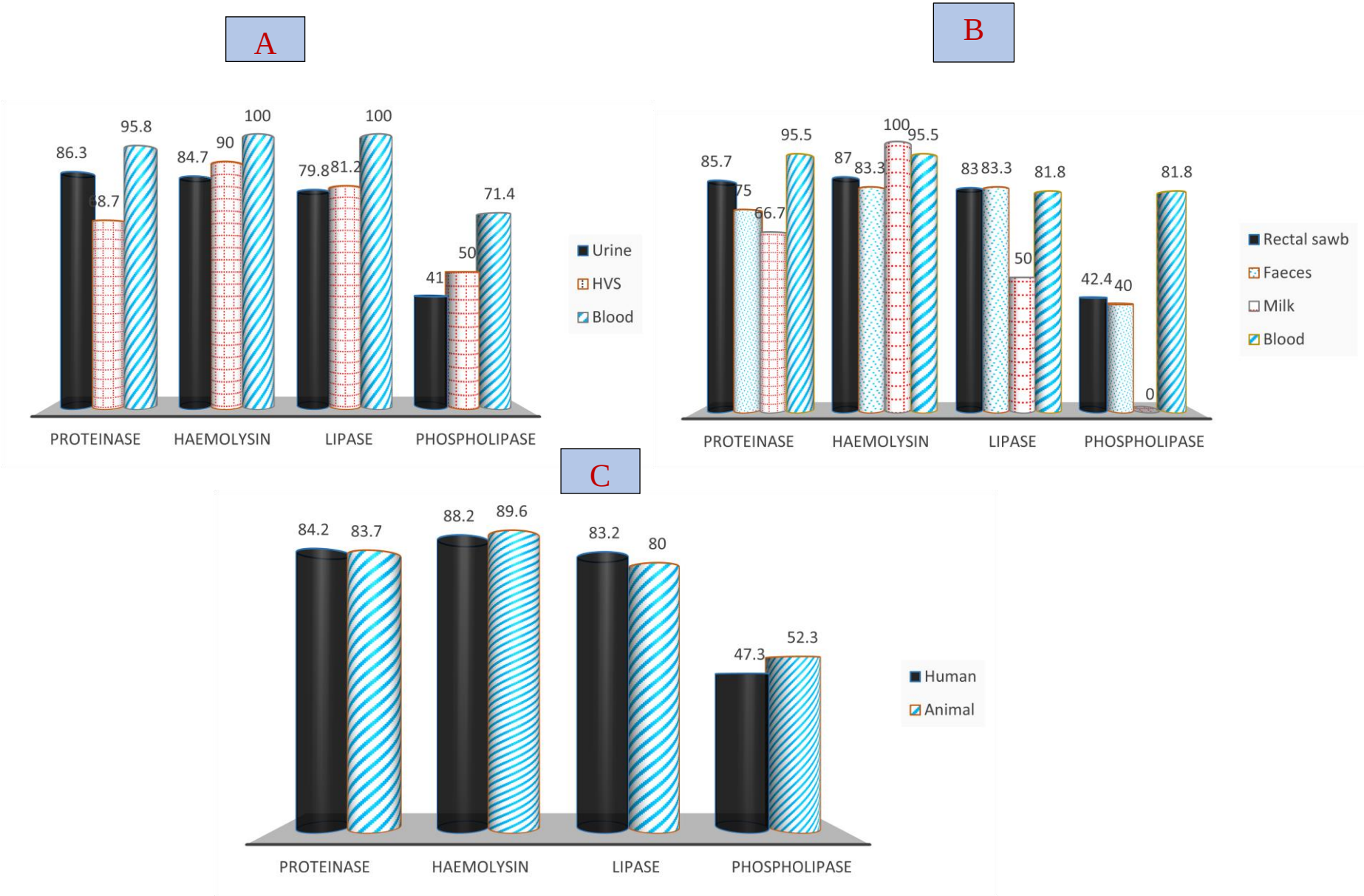

Figure 1: Percentage comparison of the enzyme production. A= Percentage of different human clinical *Candida* isolates with extracellular enzyme production; B= Percentage of different animals *Candida* isolates with extracellular enzyme production; C= Percentage comparison of the enzyme production between human and animal *Candida* isolates

Table 5: *In vitro* antifungal susceptibility profile of the recovered *Candida* species

	Fluconazole (25µg)		Clotrimazole (10µg)		Voriconazole (1µg)		Itraconazole (30µg)		Ketoconazole (30µg)		Nystatin (50IU)	
	R	Mean IZD±SD	R	Mean IZD±SD	R	Mean IZD±SD	R	Mean IZD±SD	R	Mean IZD±SD	R	Mean IZD±SD
C. albicans (n=92)	88	1.22±5.868	86	1.11±4.391	87	1.51±6.367	84	1.66±5.622	88	1.34±5.717	15	12.59±4.328
C. glabrata (n=35)	21	10.74±13.349	27	4.89±9.330	18	12.77±14.128	26	5.34±8.931	26	7.89±13.099	3	16.14±5.600
C. tropicalis (n=12)	9	6.75±12.308	9	4.42±8.447	9	7.50±13.774	9	4.83±8.851	9	6.67±12.287	2	14.33±4.905
C. krusei (n=15)	13	2.33±6.298	14	1.33±5.164	11	6.73±11.949	9	7.60±9.898	14	1.40±5.422	6	10.07±8.722
C. parapsilosis(n=22)	12	11.45±12.701	15	5.36±8.104	12	13.95±16.058	14	7.18±9.272	14	8.77±12.000	5	14.00±5.521
Others (n=18)	16	2.50±7.278	16	1.94±5.724	16	3.50±8.753	13	5.22±8.948	16	3.06±7.368	7	11.11±5.989

KEY: R: Number of resistant *Candida* isolates; IZD: inhibition zone diameter (mm); SD: standard deviation

Table 6: Minimum inhibitory concentration (MIC) of the some recovered *Candida* isolates

		FLU		ITR		NYS	
Sample	*Candida* spp (n)	MIC range	GM	MIC range	GM	MIC range	GM
Human	*C. albicans* (10)	1-64	19.69	NR	NR	0.12-8	0.80
	C. glabrata (3)	32-64	40.32	1-2	1.59	NR	NR
	C. tropicalis (3)	4-64	12.70	0.12-2	1.00	2-4	2.520
	C. krusei (3)	4-64	12.70	0.5-2	1.00	0.5-2	0.79
	C. parapsilosis(3)	0.5-64	10.08	0.12-1	0.49	NR	NR
Animal	*C. albicans* (10)	0.12-64	9.15	0.12-8	0.87	0.12-8	0.80
	C. glabrata (3)	32-64	40.32	1-2	1.26	0.12-0.25	0.15
	C. tropicalis (3)	1-64	4	0.12-2	0.49	2	2
	C. krusei (3)	8-64	25.40	0.25-1	0.63	1	1
	C. parapsilosis(3)	1-4	9.35	0.5-1	0.79	NR	NR

KEY: MIC, minimum inhibitory concentration (µg/ml); GM, geometric mean (µg/ml);
FLU- fluconazole; ITR- itraconazole; NYS- nystatin; NR- no result

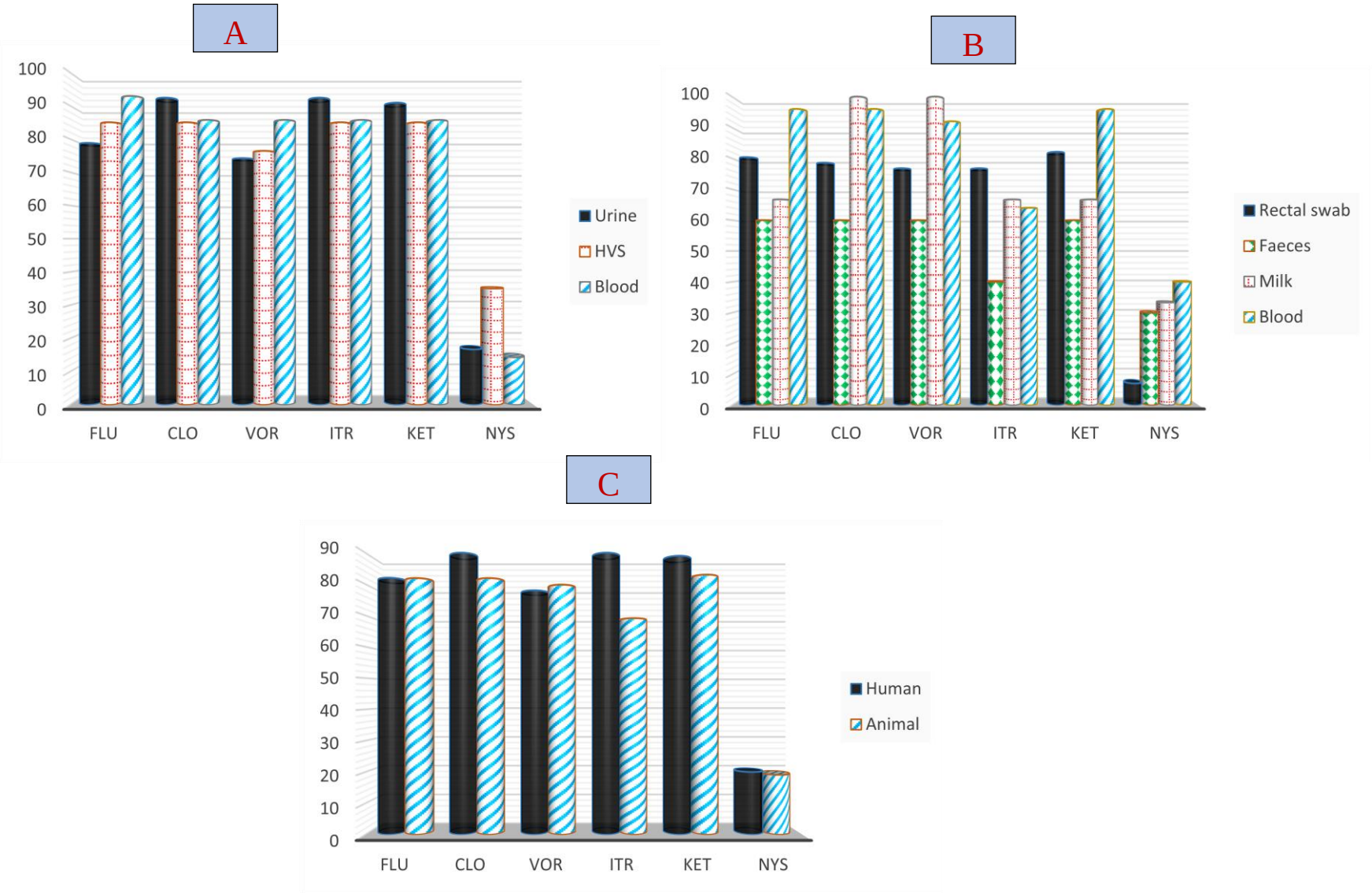

Figure 2: Resistance profiles of *Candida* spp from human and animal samples. FLU (fluconazole, 25µg), CLO (clotrimazole, 10µg), VOR (voriconazole, 1µg), ITR (itraconzaole, 30µg), KET (ketoconazole, 30µg), NYS (nystatin, 50 IU). A=percentage resistance profile of the different human clinical isolates; B=percentage resistance profile of the different animal *Candida* isolates; C=percentage comparison between the resistant profile of human and animal *Candida* isolates

isolated from animal samples. Our results are however similar to the findings by El-Diasty et al. (2017) who isolated and characterized different yeast cells from poultry slaughterhouses and workers. According to the study, *C. albicans* was the most commonly isolated species followed by *C. Lusitaniae, C. parapsilosis,* and *C. tropicalis.* The variation in species distribution with other studies might be due to differences in the sample types screened, geographical location, and the differences in the identification methods used. Although the frequency of occurrence of *Candida* spp. (according to the different localities where the samples were collected) was not reported, it is reasonable to believe that *Candida* spp. is widely distributed in the different localities.

The production of extracellular enzymes is one of the major parameters to distinguish virulent invasive strains from non-invasive strains. These enzymes are crucial for infection establishment (Mba and Nweze, 2020). In the present study, approximately 89% of *C. albicans a*nd 82% of NACs were proteinase producers. Several other researchers have reported similar findings (Pandey et al., 2018; Jasim et al., 2016). Very low Pz values and a greater number of proteinase positive isolates were largely observed from human and animal isolates recovered from blood samples, thus making the blood *Candida* isolates the most potent proteinase producers. The environment (blood) may likely have had a profound influence on the pathogenic potential of the isolated *Candida* species in this study. The high production of proteinase by non-albicans *Candida* isolates recovered from both animals and humans in this study suggest that more attention needs to be given to non-albicans *Candida* species as a result of their emerging clinical relevance.

More than 80% of all our isolates from humans and animals were hemolysin and lipase producers. Other researchers have also documented high hemolysin and lipase production among *Candida* species (Pandey et al., 2018; Akinjogunla et al., 2018). Phospholipase activity was noted in 64% of the *Candida albicans* isolates. This agrees with a study by Butola et al. (2015) who reported phospholipase activity in 60% of *C. albicans* while only 37% of the non-albicans *Candida* spp showed phospholipase activity. Brilhante et al. (2013) and Cordeiro et al. (2015) showed that Can*dida* species isolated from animals show resistance to azoles and also produce extracellular enzymes. In our investigation, *Candida* species recovered from blood samples in humans and animals were the most potent producers of phospholipase. Most non-albicans isolates in our study showed low phospholipase activity. The low phospholipase activity in the majority of the non-albicans *Candida* species in this study may suggest that the enzyme is probably not a significant virulence attribute for these species.

The differences in the enzymatic activity both in human and animal samples suggests that the potential for *Candida* species to produce extracellular enzyme depends on the sample source. Generally, the virulence factors produced by *Candida* spp. may vary depending on the stage, type, site of infection, and even the host immune nature. Also, anatomically distinct sites affect the pathogenic potential of *Candida* spp. Therefore, the variability in enzymatic activity reported here might be due to the biological differences among the isolates recovered from different sample sources/sites. Since the expression and induction of extracellular hydrolytic enzymes correlate with pathogenicity and infection initiation (Mba and Nweze 2020), the presence of these enzymes even among healthy animals, most especially animal blood and cow milk might be a source of worry. Its implication in human health, especially among consumers of these animals and their products and even among those exposed to these animals cannot be overemphasized. Although both the humans and animals were not screened for any systemic fungal infection before sample collection, there is a possibility that some of them that showed positive *Candida* growth (especially in the blood samples) have systemic *Candida* infection. Overall, our data showed that *Candida* isolates from animals and humans exhibit similar and equal virulence attributes.

The high azole resistance rate recorded in our study agrees with previous findings by Owotade et al. (2016) who reported multi-azole resistance in among *Candida* species. The *in-vitro* antifungal testing revealed that susceptibility was higher among *Candida* spp. subjected to the polyene (nystatin) than the azoles. Also, *Candida tropicalis* recovered from animal samples showed a high resistant rate to the azole antifungal drugs. This corroborates the findings of Cordeiro et al. (2015), who reported that *C. tropicalis* isolates from healthy animals showed a high rate of resistance to azoles. Similar reports have also been noted by other researchers (Brilhante et al., 2013). Currently, a lot of antibiotics are used as regular supplements for growth promotion in animals.

This practice exposes a large number of animals, irrespective of their health status to a sub-therapeutic concentration of antimicrobials, thereby increasing the likely occurrence of resistance. Therefore, frequent exposure to antibiotic therapy, immunosuppression, and increased exposure of humans and animals to environmental fungi are some of the predisposing factors. Since the prior treatment history of these animals with antifungals drugs was not investigated, we want to presume that the high resistance to azoles observed in this study may be due to selective pressures induced by the exposure to azole products used in clinical practice or agriculture. It could also be due to the presence of these compounds in the feed, water, and fruit consumed by these animals. The high rate of azole resistance among *Candida* spp recovered from humans could be due to the widespread use of azoles in the therapeutic and prophylactic management of candidiasis and other fungal infections. Even intrinsic resistance shown by various yeasts to the antifungal agents cannot be overlooked. It is also possible that drug-resistant strains in animals may have originated from humans.

Conclusion

This study showed that there is a difference in *Candida* species distribution in humans and animals, as the non-albicans *Candida* spp. appear to be more frequently isolated from animals. Sequel to the high extracellular enzyme production and high level of azole resistance observed in this study, it is safe to conclude that there is a strong correlation between the extracellular production of enzymes and azole antifungal resistance. Furthermore, this study showed that *Candida* isolates from animals and humans exhibit similar virulence and antifungal resistant attributes. We, therefore, conclude that healthy animals and their droppings harbor potentially pathogenic *Candida* species, including multi-azole-resistant strains that are capable of secreting extracellular hydrolytic enzymes. Humans are at risk of contracting candidiasis when they eat or come in contact with the animals and their products. However, a throughput molecular genotyping investigation is needed to confidently justify this claim.

CONFLICT OF INTEREST

The authors declare that they have no competing interests

REFERENCES

Akinjogunla OJ, Ajayi AO, Nkanga IE (2018) Characterization, in-vitro detection of extracellular hydrolytic enzymes and antifungal susceptibility of faecal*Candida* isolates from diarrhoeal patients. Niger J Pharm Appl Sci Res **7**:8-14.

Alkharashi N, Aljohani S, Layqah L, Masuadi E, Baharoon W, Al-Jahdali H, Baharoon S (2019). *Candida* bloodstream infection: changing pattern of occurrence and antifungal susceptibility over 10 years in a tertiary care Saudi Hospital. Canadian J Infect Dis Med Microbiol 2015692.

Brilhante RSN, Alencar LP, Cordeiro AR, Collares DS, Branco MC, Cordeiro CE, Macedo RB, Lima DT, Paiva MAN, Monteiro AJ, Alves ND, Oliveira MF, Sidrim JJC, Rocha MFG (2013) 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 **62**:889–895.

Butola R, Agwan V, Thakuria B, Madan M (2015) A comparative study of virulence factors in clinical isolates of *Candida* species. Int J Curr Microbiol Appl Sci **4**:716-722.

Clinical and Laboratory Standards Institute (CLSI) (2009) Method for antifungal disk diffusion susceptibility testing of yeass: approved guidelines-second edition Vol.29 No.17, Aug-2009 CLSI document M44-A2.

Clinical and Laboratory Standards Institute (CLSI) (2008) Reference method for broth dilution antifungal susceptibility testing of yeasts, 3rd ed. Clinical and Laboratory Standards Institute, Wayne, PA (M27-A3). 2008.

Cordeiro RA, Bittencourt PV, Brilhante RS, Teixeira CE, Castelo-Branco Des S, Silva ST, De Alencar LP, Souza ER, Bandeira Tde J, Monteiro AJ, Sidrim JJ, Rocha MF (2013). Species of *Candida* as a component of the nasal microbiota of healthy horses. Med Mycol **51**:731-736.

Cordeiro RA, Oliveira JS, Castelo-Branco DSCM, Teixeira CEC, Marques FJF, Bittencourt PV, Carvalho VL, Bandeira TJPG, Brilhante RSN, Moreira JLB, Pereira-Neto WA, Sidrim JJC, Rochas MFG (2015). *Candida tropicalis* isolates obtained from veterinary sources show resistance to azoles and produce virulence factors. Med Mycol, **53**:145-152.

El-Diasty EM, Ibrahim MAE, El-Khalafawy GK (2017). Isolation and molecular characterization of medically important yeasts isolated from poultry slaughterhouses and workers. Pakistan J Zoo, **49**:609-614.

Ho¨rmansdorfer S, Bauer J (2000).Yeast infection in veterinary medicine. ContribMicrobiol5:54-78.

Jasim ST, Flayyih MT, Hassan A (2016). Isolation and identification of *Candida* spp. from different clinical specimens and study the virulence factors. World J Pharm Pharm Sci **3**:121-137.

Júnior A, Silva A, Rosa F, Monteiro S, Figueiredo P, Monteiro C (2011). In vitro differential activity of phospholipases and acid proteinases of clinical isolates of *Candida*. J Braz Soc Trop Med **44**:334-338.

Kemoi E, Okemo PBCC (2013). Isolation of *Candida* species in domestic chicken (Gallus gallus) droppings in Kabigeriet village, Nakuru county Kenya. Eur Sci J, **9**:1857-7881.

Lamoth F, Lockhart SR, Berkow E, Calandra T (2018). Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother **73**:4-13.

Luo G, Samaranayake LP, Yau JYY (2011). *Candida* species exhibit differential in vitro hemolytic activities. J Clin Microbiol **39**:2971-2974.

Maheronnaghsh M, Fatahinia M, Dehghan P, Mahmoudabadi AZ, Kheirkhah M (2019). Comparison of virulence factors of different *Candida* species isolated from the oral cavity of cancer patients and normal individuals. Jundishapur J Microbiol **12**:e91556.

Mba IE, Nweze EI (2020). Mechanism of *Candida* pathogenesis: revisiting the vital drivers. Eur J Clin Microbiol Infect Dis **39**(10):1797-1819.

Nadeem SG, Hakim ST, Kazmi SU (2010) Use of CHROMagar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings. Libyan J Med **5**(1):2144.

Nweze EI, Ogbonnaya UL (2011). Oral *Candida* isolates among HIV-infected subjects in Nigeria. J Microbiol Immunol Infect **44**:172-177.

Osman K, Abdeen EE, Mousa WS, Elmonir W, El-Diasty EM, Elbehiry A (2019). Genetic diversity among *Candida albicans* isolated from humans and cattle with respiratory distress in Egypt. Vector Borne Zoonotic Dis **19**:199-220.

Owotade FJ, Gulube Z, Ramla S, Patel M (2016). Antifungal susceptibility of *Candida albicans* isolated from the oral cavities of patients with HIV infection and cancer. S Afr Dent J **71**:8-11.

Pandey N, Gupta MK, Tilak R (2018). Extracellular hydrolytic enzyme activities of the different *Candida* spp. isolated from the blood of the intensive care unit-admitted patients. J Lab Physicians **10**:4.

Price MF, Wilkinson ID, Gentry LO (1982). Plate method for detection of phospholipase activity in *Candida albicans*. *Sabouraudia.* J Med Vet Mycol **20**:7-14.

Rozanski P, Pluta M, Rozanska D (2017) Mycological profile of the integumentary system in feline ponies. Ann Anim Sci **17**:1019-1028.