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

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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 gonorrhoea 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 fungicidal, 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 37°C 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:

\[
Pz = \frac{\text{Colony diameter}}{\text{Colony diameter} + \text{Zone of Precipitation}}
\]

**Statistical analysis**

Chi-square (χ²) 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

\[\text{Bio-Research Vol.17 No.1 pp.1044A-1055A (2019)}\]
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 **<em>Candida</em>** species**

Table 4 illustrates the distribution of human and animal <em>Candida</em> species showing different virulence attributes while Figure 1A shows the percentage of different clinical <em>Candida</em> isolates producing extracellular enzymes. All the <em>Candida</em> species (100%) recovered from human blood were hemolysin and lipase producers. The blood <em>Candida</em> 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 <em>Candida</em> isolates from different animal samples with extracellular enzyme production. <em>Candida</em> 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 **<em>Candida</em>** species**

A high resistance rate was noted in this study. Table 5 shows the in vitro antifungal susceptibility profile of the recovered <em>Candida</em> 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 <em>Candida</em> 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 <em>Candida</em> isolates recovered from the blood of animals. Out of 14 <em>Candida</em> 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 <em>Candida</em> species. Overall, resistance was higher in human than in animal isolates. However, the difference was not considered statistically significant (P > 0.05).

**Discussion**

<em>Candida</em> 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 (<em>Candida</em> 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. <em>Candida albicans</em> was more predominant in human samples. Similar findings have been reported in Nigeria (Nweze and Ogbonnaya, 2011). <em>C. albicans</em> was more frequently recovered from the blood of animals (pig and sheep) (43.3%) and were also the most common <em>Candida</em> spp. in the whole animal samples. However, when comparing the <em>C. albicans</em> and non-albicans recovered from both human and animal samples, the non-albicans <em>Candida</em> species were significantly (P<0.05) more than <em>Candida albicans</em>. A predominance of non-albicans <em>Candida</em> species (68%) was observed in animal samples screened in the study and <em>C. parapsilosis</em> was the most frequently occurring. This is contrary to a report by Kemoi et al., (2013) who reported <em>C. lusitaniae</em> as the most common <em>Candida</em> spp.
Table 1: Distribution of *Candida* species in different human samples

| Candida species | Total |
|-----------------|-------|
| C. albicans     | 47 (36.4%) |
| C. glabrata     | 28 (21.7%) |
| C. tropicalis   | 14 (10.9%) |
| C. krusei       | 16 (12.4%) |
| C. parapsilosis | 10 (7.8%) |
| Others          | 14 (10.9%) |

*Human Urine* | 24 (63.2%) |
|---------------|-----------|
| Human HVS     | 25 (47.2%) |
| Human Blood   | 25 (47.2%) |

| Total          | 96 (43.6%) |

Key: HVS - high vaginal swab

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

| Candida species | Total |
|-----------------|-------|
| C. albicans     | 25 (32.9%) |
| C. glabrata     | 10 (13.2%) |
| C. tropicalis   | 11 (14.5%) |
| C. krusei       | 6 (7.9%) |
| C. parapsilosis | 15 (19.7%) |
| Others          | 9 (11.8%) |

*Animal Rectal swab* | 0 (0.0%) |
|---------------------|---------|
| Animal Ear swab     | 3 (23.1%) |
| Animal Blood        | 13 (43.3%) |
| Animal Faeces       | 3 (23.1%) |
| Animal Milk         | 0 (0.0%) |

| Total              | 41 (32.0%) |

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

| Candida species | Total |
|-----------------|-------|
| C. albicans     | 96 (43.6%) |
| C. glabrata     | 38 (17.3%) |
| C. tropicalis   | 17 (7.7%) |
| C. krusei       | 23 (10.5%) |
| C. parapsilosis | 19 (8.6%) |
| Others          | 27 (12.3%) |

*Human* | 137 (39.4%) |
|---------|------------|
| Animal  | 41 (32.0%) |

| Total  | 178 (39.4%) |

Bio-Research Vol.17 No.1 pp.1044A-1055A (2019)
Table 4: Distribution of human and animal *Candida* species showing different virulence

| Enzyme       | *Candida* spp       | Human                          | Animal                          | P value |
|--------------|---------------------|--------------------------------|---------------------------------|---------|
|              | C. *albicans*       | n  | Mean ± SD | Range | n  | Mean ± SD | Range |         |
|              |                     | 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 |         |
|              |                     |    |           |       |    |           |       |         |
|              | 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±0.18 | 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 | 0.512  |
|              |                     |    |           |       |    |           |       |         |
|              | C. *albicans*       | 71 | 0.57±0.22 | 0.33-1 | 34 | 0.57±0.19 | 0.40-1 |         |
|              | 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±0.27 | 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.

Bio-Research Vol.17 No.1 pp.1044A-1055A (2019)

1049A
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

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

*Bio-Research* Vol.17 No.1 pp.1044A-1055A (2019)
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 and 52% 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 Candida 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. subject 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

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