International Journal of Veterinary Sciences Research
2016 Vol. 1, No. 4, pp. 8-14
ISSN(e): 2410-9444
ISSN(p): 2413-8444
DOI: 10.18488/journal.110/2016.2.2/110.2.8.14

OCCURRENCE AND ANTI-FUNGAL SENSITIVITY TESTING OF *CANDIDA SPEC* ISOLATED FROM CANINE CONJUNCTIVITIS

Olabode, H.O.K[1†] --- Ameh, J.A[2] --- Egwu, G.O[3] --- Bello, R.H[4] --- Nwniyi, F.C[5]
[1,2,3]*Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja*
[4]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Ilorin*
[5]*Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, University of Abuja*

ABSTRACT

Fungal infection in dogs and other domestic pets caused by Candida species have been previously reported. Several available antifungal drugs for treatment of yeast associated infections have been used with diverse effects. This study establishes antifungal susceptibility profile on identified Candida species isolates associated with canine conjunctivitis using disks diffusion method as described by NCCLS (M44-A). The antifungal disks used include Amphotericin-B (20mcg), Nystatin (100units), Itraconazole (10mcg), Ketaconazole (10mcg), Fluconazole (25mcg). The identified Candida species were C. albicans, C. krusei, C. tropicalis and C. parapsilosis. Percentage susceptibility was high in both Amphotericin B (77.27%) and Nystatin (58.18%), while Itraconazole showed (54.54%), Ketoconazole (55.45%) and Fluconazole was least (11.82%). The sensitivity ranking showed Amphothericin B > Nystatin > Itraconazole > Ketoconazole > Fluconazole and increased resistance amongst the Azoles especially Fluconazole (70.90%) in comparison with Polyenes. C. albicans showed the highest resistance (84.51%) to both Fluconazole and ketoconazole (67.61%). C. parapsilosis showed 63.64% and 61.54% percentage resistance to Nystatin and Itraconazole respectively. In conclusion, this finding showed that Amphotericin B and Nystatin were most effective against isolated Candida species. Proper diagnosis of the underlying ailment is necessary in conjunction with routine anti-fungal susceptibility testing to avoid drug abuse and resistance.

Keywords: Anti-fungal sensitivity testing, *Candida* species, Canine conjunctivitis.

Received: 3 June 2016/ **Revised:** 10 November 2016/ **Accepted:** 16 November 2016/ **Published:** 21 November 2016

Contribution/ Originality

This study contributes to the existing literature of canine Candida species epidemiology and control using conventional cultural, characterization and antifungal sensitivity test methodology. This first preliminary study conducted in Abuja-FCT, Nigeria revealed effectiveness of Amphotericin B and Nystatin on *Candida* species isolates associated with canine conjunctivitis.

1. INTRODUCTION

Candidiasis is an infection caused by yeast of the fungal genus called *Candida* [1] characterized by whitish, chessy discharges accompanied with flakes from infected sites such as vagina, eye, ear, and mouth of man and animals. Systemic candidiasis is however rare in animals with scanty literatures describing multi-systemic infection [2]. *Candida* species are ovoid budding yeast cells, 2 - 4 µm in diameter with thin walls occurring in chains. They

† *Corresponding author*

produce pseudohyphae with attached blastospores and true regular filamentous hyphae post budding [3]. The cultural morphology of *C. albicans* is circular, round, smooth, glabrous to waxy surface with creamy yeast like appearance and characteristic microscopic gram positive chained cocci [4]. Other virulent species in dogs include *C. tropicalis*, and *C. stellatoides*, while *C. parapsilosis*, *C. guillermondi*, and *C. krusei* are less virulent [5]. Candida *albicans* is an opportunistic dimorphic, sugar digesting yeast [6] and a natural inhabitant of the genital, alimentary and upper respiratory tract mucous membranes in animals [3]. Superficial infections have been limited to mucous membranes of intestinal tracts in pigs, foals and Chickens [7]. Identification and characterization of yeast species are based on morphological traits and physiological capabilities [8]. The major biochemical characteristic is the ability to ferment sugars for the production of ethanol and hydrolysis of citrates and urea [4]. The increasing incidence of human candidisis have been associated with greater use of cytoxic, immunosuppressive and antibiotic therapies [3]. Amphotericin B, Nystatin, 5-Fluoro-cytosine, and Fluconazoles have been used for candidiasis treatment [9]. However, Amphotericin B showed superior effect over 5-Fluoro-cytosine in combating candida infections [10]. Ketoconazole indicated promising antifungal effect, with little available clinical trial data on its use for systemic candidiasis [11]. In domestic pets *Candida albicans* has been reported as the etiological agent of multi-systemic infections in dogs [12]. The zoonotic nature of *C. albicans* especially in canine species [13] and treatment failures due to drug resistance [14] necessitated the assessment of some commercially available anti-fungal drugs on Candida isolates associated with canine conjunctivitis in Abuja and its environs.

2. MATERIAL AND METHODS

2.1. Study Design

This study was conducted between November and December 2012 to report only isolates of Candida species predominately associated with suspected canine conjunctivitis cases presented to some veterinary clinics in Abuja and its environs. The isolates was subjected to in-vitro antifungal susceptibility testing to establish the best drug of choice for treatment in order to minimize cost and avoid indiscriminate drug use. All dogs during the period of study with complains of inflamed eyes were also considered and sampled.

2.2. Sample Collection

Ocular swabs were collected using sterile swab sticks (pre-moistened with sterile saline) from tentatively diagnosed dogs with canine conjunctivitis presented to the clinics. A total of 150 swab samples were collected, packed and immediately taken to the laboratory for mycological investigation.

2.3. Isolation of Candida

Collected samples were inoculated on Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 48 hours as described [15]; [16]. Plates were inoculated under aseptic conditions and incubated aerobically. They plates were later observed macroscopically at intervals for yeasty growth. Plates that yielded creamy, mucoid and musty colonial appearance post 48 hr aerobic incubation were maintained on SDA slant at 4°C and used for further characterization.

2.4. Identification and Characterization of Isolates

Pure Isolates on Sabouraud Dextrose Agar (SDA) were identify and characterized by sub-culturing on CHROMagar Candida (CHROMagar Candida®, France) plates and incubated at 37°C for 48hours. All isolates were identified based on their colonial morphology (Pigment formation) on CHROMagar Candida, germ tube production

at 45°C, sugar fermentation reactions and gram staining to observe for the thick walled, round and budding clamydospores [15].

2.5. Preparation of Yeast Inocula

Yeast inocula were prepared using the Mcfarland turbidity standard as described [15]; [17]; [18]. Pure isolate of yeast were inoculated into Sabouraud Dextrose Broth (SDB) and incubated at 37°C for 5 hours until the turbidity was 0.5 McFarland turbidity scale. This turbidity scale was prepared by adding 0.6ml of 1%w/v aqueous solution of barium chloride in 9.4ml of 1% v/v sulphuric acid giving an approximate equivalent bacterial density of 1-5 x 10^6 CFU/ml.

2.6. Yeast Susceptibility Test

Susceptibility testing was conducted using disk diffusion technique on Mueller Hilton Agar (MHA) plates as described [19]; [20]. On MHA, Methylene blue-glucose solution (assisted yeast growth and enhanced visualization of inhibition zones) was added to the surface of the agar and allowed to air dry prior to the addition of yeast inocula. Inoculation was carried out by dipping a sterile swab into the inoculum suspension adjusted to turbidity of 0.5 McFarland standards (10^6cells/ml) and streaking was done across the agar surface in four directions. The plates were dried at ambient temperature for 15 minutes in a Laminar flow cabinet before applying the commercially impregnated anti-fungal disk drugs [Itraconazole (10mcg), Fluconazole (25mcg), Amphotericin B (20mcg), Ketoconazole (10mcg), Nystatin (100 U)]. The diameter zone of inhibitions was measured in millimeter 24hrs post-incubation.

The interpretation criteria for susceptibility and resistance of the anti-fungal used (Fluconazole, Nystatin, Amphotericine B, Ketoconazole, and Itraconazole disks) were as indicated in the Table below [2, 7].

2.7. Interpretation Criteria for Susceptibility and Resistance of Antifungal Disks Used

Anti-fungal Agent	Zone of Inhibition (mm)		
	Sensitive	Dose- Dependent	Resistant
Amphotericin B	>15	10 -14	< 9
Ketoconazole	≥ 30	23 – 29	≤ 22
Fluconazole	≥ 19	15 -18	≤ 16
Nystatin	≥ 25	17 – 24	< 16
Itraconazole	>16	10 – 15	< 9

Source: Pakshir, et al. [21] and Pfaller, et al. [22]

2.8. Statistical Analysis

All the data obtained in this study were expressed in simple descriptive statistics (frequency and percentages). The number of isolates was expressed as frequency, while the susceptibility and resistance patterns were presented in percentages.

3. RESULTS

Out of one hundred and fifty (150) swabs analyzed, 110 showed typical yeasty appearance while 40 samples showed no evidence of microbial culture and or yeast growth. Further identification and characterization of Candida isolates showed highest number (71) of *Candida albicans* isolates 64.55%, *C. krusei* 14.55% (16), *C. tropicalis* 9.09% (10) and *C. parapsilosis* 11.82% (13) as shown in Table I.

Antifungal susceptibility pattern indicated all isolates of Candida species 77.27% (85) were highly susceptible to Amphotericin B while Nystatin, Fluconazole, Ketoconazole, and Itraconazole showed varied percentage susceptibility with different Candida species. This sensitivity ranking was represented as Amphotericin B (AMP) > Nystatin, (NSY) > Itraconazole (ICZ) > Ketoconazole (KCZ) > Fluconazole, (FCZ) as shown in Table II

The percentage antifungal drugs resistance indicated the occurrence of varied levels of resistance amongst all the Candida species especially against the Trizoles in a sensitivity ranking represented as Fluconazole, (FCZ) > Ketoconazole (KCZ) >Itraconazole (ICZ) as shown in Table III.

Table-1. Distributions of *Candida* species isolates from Canine ocular swabs in Abuja –FCT, Nigeria

Species	Number of Isolates	% of Isolates
Candida albicans	71	64.55
C. krusei	16	14.55
C. tropicalis	10	9.09
C. parapsilosis	13	11.82

N = 110

Prevalence Rate: 73.33%

Table-2. Percentage susceptibility profile of Candida species isolated from ocular swabs in Abuja (n=110) %

Antifungal Agents	C.albicans(n=71)	C. krusei (n=16)	C.tropicalis (n=10)	C.paraplosis (n=13)	Total
Amphotericin B	54	13	7	11	85
	(76.06)	(81.25)	(70)	(84.62)	(77.27)
Nystatin	47	11	2	4	64
	(66.20)	(68.75)	(20)	(36.36)	(58.18)
Fluconazole	11	7	5	9	32
	(15.49)	(43.75)	(50)	(69.23)	(29.09)
Ketoconazole	23	9	8	10	50
	(32.39)	(56.25)	(80)	(76.92)	(45.45)
Itraconazole	38	7	10	5	60
	(53.52)	(43.75)	(100)	(38.46)	(54.54)

Table-3. Percentage resistance profile of Candida species isolated from ocular swabs in Abuja (n=110) %

Antifungal Agents	C.albicans(n=71)	C. krusei (n=16)	C.tropicalis (n=10)	C.paraplosis (n=13)	Total
Amphotericin B	17	3	3	2	25
	(23.94)	(18.75)	(30)	(15.38)	(22.73)
Nystatin	24	5	8	9	46
	(33.80)	(31.25)	(80)	(63.64)	(41.82)
Fluconazole	60	9	5	4	78
	(84.51)	(56.27)	(50)	(30.77)	(70.90)
Ketoconazole	48	7	2	3	60
	(67.61)	(43.75)	(20)	(23.08)	(54.55)
Itraconazole	33	9	0	8	50
	(46.48)	(56.25)	(0.00)	(61.54)	(45.45)

4. DISCUSSION

In this study, *Candida* species associated with canine conjunctivitis were *C. albicans*, *C. krusei*, *C. tropicalis* and *C. parapsilosis* with overall prevalence of 73.3%**.** *Candida albicans* was most predominant while *C. krusei* and *C. tropicalis* were least isolated. The occurrence of *Candida* associated with canine conjunctivitis in this finding is similar to the mycotic endopthalmitis reported in dogs [23] caused by same species. All the *Candida* species isolated in this study, especially *Candida albicans* and *Candida parapsilosis*, conforms with previous Candida isolated in the ears, nose, oral

cavity, and anus of clinically healthy dogs [3]. *Candida tropicalis, and Candida krusei* that were associated with immunocompromised dogs as well as *Candida parapsilosis*, reported in cats [24] were also identified in this study. This suggests that samples analyzed were either obtained from uncontrolled healthy dogs with immune disorders [25] or dogs exposed to chronic antibacterial therapy [26] and or dogs that co-exist with cats and humans under poor management and environmental conditions.

The Antifungal sensitivity testing of selected Triazoles (Fluconazole, Ketoconazole and Itraconazole) and Polyenes (Amphothericin B and Nystatin) test against *Candida* species indicates Amphothericin B as the most effective antifungal agent with percentage susceptibility 77.27%, Nystatin (58.18%) and fluconazole (29.09%) resistance. This finding is similar to the reports of Nweze [27]; Apurva, et al. [28]. This high efficacy of Amphotericin B and Nystatin on the isolates (*C. albicans, Candida krusei, C. tropicalis and C. parapsilosis*) following anti-fungal sensitivity testing is also in-line with other earlier reports [9] as previous candida treatment with fluconazole (FCZ) [10] terbinafine (TER) Favre, et al. [29] and flucytosine (FCY) [3] were ineffective and unsuccessful.

The highest resistance was observed in *C. albican* against fluconazole with a percentage resistance of 84.50% and 67.61% against Ketoconazole. *C. parapsilosis* also showed percentage resistance of 63.64% and 61.54% against Nystatin and Itraconazole respectively. This finding is in line with Manfredi, et al. [14] that reported increasing resistance of *C. albicans* to antifungal medicines especially azoles. This study indicates Amphotericin B and Nystatin high effectiveness in management of Candida *albicans* and other *Candida* species associated canine conjunctivitis. In addition, Itraconazole and Ketoconazole could also be helpful as shown in this study. Although, treatment of fungal infection in veterinary medicine is being limited to conventional polyenes and triazoles, these drugs have adverse effects and are relatively of high cost, especially when used in animals [30].

In conclusion, this study provides a preliminary report on the in-vitro antifungal susceptibility testing of Candida isolates associated with suspected cases of canine ocular conjunctivitis. Hence further specific Ocular Candida case-control study and drug clinical trial is thus recommended. Definitive etiology of canine conjunctivitis alongside routine anti-bacterial susceptibility testing post cultural investigation is also suggested, to reduce drug misuse, cost and development of resistance.

5. ETHICAL APPROVAL

The research procedure was approved by the staff and seminar committee of the Faculty of Veterinary Medicine, University of Abuja, FCT, Nigeria. The samples were collected, stored and analyzed using standard laboratory protocols as approved by the committee and recommended by Clinical Laboratory Standards US.

Funding: This study received no specific financial support.

Competing Interests: All authors indicated their consent prior to the commencement of this study. We hereby declare no conflict and competing interests.

Contributors/Acknowledgement: The authors appreciate the efforts of Mr. Hakeem Onigbanjo, Dr. Ifeanyi Casmir and Madam Grace Popoola for their assistance during the preliminary stages of this research as well as the staff of Microbiology Department, University of Ilorin for characterization and interpretation of this work.

REFERENCES

[1] S. Carvalhinho, A. M. Costa, A. C. Coelho, E. Martins, and A. Sampaio, "Susceptibilities of Candida albicans mouth isolates to antifungal agents, essentials oils and mouth rinses," *Mycopathologia*, vol. 174, pp. 69–76, 2012.

[2] M. R. Brown, C. A. Thompson, and F. M. Mohamed, "Sys¬temic Candidiasis in an apparently immunocompetent dog," *Journal of Veterinary Diagnostic Investigation*, vol. 17, pp. 272–276, 2005.

[3] C. E. Greene and F. W. Chandler, *Candidiasis, torulop¬sosis, and rhodotorulosis. In: Greene CE (Ed.): Infec¬tious diseases of the dog and cat*, 2nd ed. Philadelphia: WB Saunders Co, 1998.

[4] W. Chester, C. H. Emmons, and J. P. Binford, *Medical microbiology*, 2nd ed. Philadelphia USA: Lea and Febiger, 1970.

[5] C. Yurayart, "Comparative analysis of the frequency, distribution and population sizes of yeasts associated with canine seborrheic dermatitis and healthy skin," *Veterinary Microbiology*, vol. 148, pp. 356-62, 2011.

[6] R. A. Covadonga, J. K. Burns, L. M. Friedrich, R. M. Goodrich, and M. E. Parish, "Yeast species associated with orange juice: Evaluation of different identification methods," *Applied and Environmental Microbiology*, vol. 68, pp. 1955-1961, 2002.

[7] A. Moretti, F. D. Piergili, L. Boncio, P. Pasquali, and R. Del, "Isolation of Candida rugosa from turkeys," *Journal of Veterinary Medicine, Series B*, vol. 47, pp. 433-439, 2000.

[8] C. P. Robin, P. Herkhout, and F. McClary, "Factors affecting the morphology of Candida ablicans," *Journal of Biology Missouri Botanical Gardens*, vol. 39, pp. 137-164, 2006.

[9] F. Rochete, M. Engelen, and B. H. Vanden, "Antifungal agents of use in animal health pratical applications," *Journal of Veterinary Pharmacology and Therapeutics*, vol. 26, pp. 31-53, 2003.

[10] M. Moosa, Y. Harrison, and J. Scofield, "Fungal activity of fluconazole against Candida albicans in synthetic vagina simulative motium auto-microbial agent," *J. Chemotherapy*, vol. 48, pp. 161-167, 2004.

[11] B. Vande, "Susceptibility testing of fluconazole by the NCCL broth macrodilution method, E-test, and disk diffusion for application in routine laboratory," *Journal of Clinical Microbiology*, vol. 40, pp. 918-21, 2010.

[12] M. Skoric, P. Fictum, I. Slana, P. Kriz, and I. Pavlik, "A case of systemic mycosis in a Hovawart dog due to Candida albicans case report," *Veterinarni Medicina*, vol. 56, pp. 260–264, 2011.

[13] A. Ates, M. Likit, R. Ozdermir, and K. Ozdean, "Dermatophtes isolated from asymptomatic dogs in Adana, Turkey: A preliminary study," *Journal de Mycologie Médicale/Journal of Medical Mycology*, vol. 18, pp. 154-7, 2008.

[14] M. Manfredi, M. J. McCullough, L. Polonelli, S. Conti, Z. M. Al-Karaawi, and P. Vescovi, "In vitro antifungal susceptibility to six antifungal agents of 229 Candida isolates from patients with diabetes mellitus," *Oral Microbiology and Immunology*, vol. 21, pp. 177–82, 2006.

[15] F. J. Baker, R. E. Silverton, and C. J. Pallister, *Baker and silverton's introduction to medical laboratory technology (Hodder Arnold Publication)*: Hodder Arnold Publication, 1998.

[16] M. Cheesbrough, *District laboratory practice in tropical countries*. Cambridge: ELBS University Press, 2002.

[17] National Committee for Clinical Laboratory Standards (NCCLS), "Antifungal susceptibility testing committee report, No. 17," NCCLS, Villanova, Pa1986.

[18] Clinical and Laboratory Standards Institute [CLSI], *Reference method for broth dilution antifungal susceptibility testing of Yeast: Approved standard -Third Edition, M27A3E*. Wayne, Pa: Clinical and Laboratory Standards Institute, 2008.

[19] National Committee for Clinical Laboratory Standards [NCCLS], *Reference method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline. NCCLS document M44-A*. Wayne: National Committee for Clinical Laboratory Standards, 2004.

[20] Z. M. Ali, Z. Majid, and B. Maryam, "Antifungal susceptibility testing of candida species isolated from Candiduria," *Journal of Microbiology*, vol. 6, pp. 24- 28, 2013.

[21] K. Pakshir, L. Bahaedinie, Z. Rezaei, M. Sodaifi, and K. Zomorodian, "In-vitro activity of six antifungal drugs against clinically important derma¬tophytes," *Jundishapur Journal of Microbiology*, vol. 2, pp. 158-63, 2011.

[22] M. A. Pfaller, D. J. Diekema, A. L. Colombo, C. Kibbler, K. P. Ng, and D. L. Gibbs, "Candida rugosa, an emerging fungal pathogen with resistance to azoles: Geographic and temporal trends from the artemis disk antifungal surveillance program," *Journal of Clinical Microbiology*, vol. 44, pp. 3578-82, 2006.

[23] J. Lineck, "Mycotic endopthalmitis in dogs caused by Candida albicans," *Veterinary Ophthalmology*, vol. 7, pp. 159-162, 2004.

[24] B. M. Pressler, "Candida spp. urinary tract infections in 13 dogs and seven cats: Predisposing factors, treatment, and outcome," *Journal of the American Animal Hospital Association*, vol. 39, pp. 263-70, 2003.

[25] U. Joann and O. S. A. Henry, *Doggie health care*, 2nd ed. The Netherlands: Elsevier Science Publishers BV, Amsterdam, 2002.

[26] P. Eira, H. Outi, A. Veli-jukka, and R. Petri, "Candidemia in Finland, 1995-1999," *Emerging Infectious Diseases*, vol. 9, pp. 985-989, 2003.

[27] E. I. Nweze, "Oral candida isolated among HIV- infected subjects in Nigeria," *Middle East Journal of Family Medicine*, vol. 49, pp. 7-10, 2011.

[28] K. P. Apurva, R. J. Navin, and J. Kuchi, "Antibiogram of Candida species isolated from multispecies oral candidal carriage using paper disk diffusion method," *Saudi Journal for Health Sciences*, vol. 1, pp. 132- 138, 2012.

[29] B. Favre, B. Hofbauer, K. Hildering, and W. S. Ryder, "Comparison of in-vitro activities of- 17 antifungal Drugs against a panel of 20 Dermatophytes by using a midilution assay," *Journal of Clinical Microbiology*, vol. 41, pp. 4817-4819, 2003.

[30] D. Sanglard and F. C. Odds, "Resistance of Candida species to antifungal agents: Molecular mechanisms and clinical consequences," *Lancet Infectious Diseases*, vol. 2, pp. 3-85, 2002.