Journal of Etiology and Animal Health
Received: Feb 08, 2016, Accepted: Apr 18, 2016, Published: Apr 22, 2016
J Etiol Anim Health, Volume 2, Issue 1
http://crescopublications.org/jeah/JEAH-2-005.pdf
Article Number: JEAH-2-005

Research Article Open Access

Isolation and Characterization of *Candida albicans* Associated with Canine Conjunctivitis

Mailafia S, Olabode HOK*, Adah BMJ and Waziri HA

Department of Veterinary Microbiology, University of Abuja, Nigeria

***Corresponding Author:** Olabode HOK, Department of Veterinary Microbiology, University of Abuja, Nigeria; Tel: +2340823806741; E-mail: olabodeok@yahoo.com

Citation: Mailafia S, Olabode HOK, Adah BMJ and Waziri HA(2016) Isolation and Characterization of *Candida albicans* Associated with Canine Conjunctivitis. J Etiol Anim Health 2: 005.

Abstract

Isolation and characterization studies was conducted on fifty (50) ocular swabs from dogs presented to some selected Veterinary Clinics, in Abuja and its environs. This was to establish isolates of Candida associated with canine conjunctivitis. The samples collected were homogenized and cultured on Sabouraud Dextrose Agar (SDA). Biochemical characterization was conducted using Simmons citrate agar test, triple sugar iron agar test, and germ tube test in accordance with standard methods. Thirteen (13) isolates obtained showed typical characteristics of *Candida albicans* with overall occurrence rate of 20%. Although, this was not Significant (P>0.05) as other potential pathogens could be responsible for the observed signs of canine conjunctivitis. Therefore, this study provides preliminary information on possible occurrence and predominance of *Candida albicans* over other Candida species associated in canine conjunctivitis cases examined. Routine mycological investigation and further characterization of obtained Candida isolates, alongside bacteriological test should be conducted on reported suspicious cases of canine conjunctivitis.

Keywords: *Candida albicans*; Isolation; Characterization; Canine Conjunctivitis.

Introduction

Candidiasis commonly known as yeast infection is caused by fungal organism referred to as *Candida albicans* and usually found in the mouth, eye, ear, digestive (gastro intestinal tract) and the vagina of animals and human [1] with characteristic whitish (cheesy) discharges from affected organs [2]. *Candida* was discovered over 100 years ago which is becoming a major problem today [3]. Candida species include *C. tropicalis, C. stellatoides, C. albicans,C. parapsilosis, C. guillermondi, and C. krusei*. The first two are the most virulent of the group; however, *C. albicans*is the only organism capable of evoking fatal disease regularly in humans and animals [4]. *C. albicans* is an opportunistic, dimorphic normal flora of mucous membrane and alimentary canal [5] resulting into subclinical mucocuteaneous infection [6] and fatal systemic and invasive infection [7] under extreme conditions. It has been reported to co-exist with many kinds of harmless bacteria like *Escherichia coli*, without causing any infection [1], but in conditions of weakened immune system, prolonged antibiotics, corticosteroids and cancer drugs use or diabetics the fungus can multiply and cause disease [8].

The most frequent infections caused by *Candida* are superficial lesions of the oral cavity, vaginal, skin, nails, but also affects deeper organ tissues such as kidneys, heart, digestive tract, lungs, eyes and brain [9]. Predisposing factors of denture wearing, prolonged infant bottle nursing, occupational hazard associated with continuous hand or feet immersion in water, diabetes, pregnancy, and or prolonged use of oral contraceptives [4] enhances haematogenous spread of yeast infections. Immunologically compromised animals with lymphatic disease and or open-heart surgery transplant are also most susceptible to invasive Candida infection [7]. *Candida* conjunctivitis damages the eye structures causing keratitis, corneal ulcers, conjunctivitis, endopthalmitis, dacryocyst, and follicular conjunctivitis characterized by itching and irritations caused by Candida heat stable toxin affecting man and animals especially dogs of all breeds and ages [10]. This infection can be localized or systemic causing extreme discomfort in dogs [11]. The increasing reports of inflamed ocular signs in dogs presented to veterinary clinics in and around Abuja environs necessitated this study. This is to avail clinicians and other researchers a diagnostic and management guide as well as providing baseline information of Candida canine conjunctivitis occurrence in the study area.

Materials and Methods

Study Area

Federal Capital Territory (FCT), located in central Nigeria was created in 1976 from parts of former Nasarawa, Niger, and Kogi States. It situated between latitude 8.25° and 9.20° north of the equator and longitude 6.45° and 7.39° east of Greenwich Meridian and located North of the confluence of rivers Niger and Benue. FCT is bordered by Niger state to the North, Kaduna state to Northwest, Nasarawa to the South-East and Kogi to the West with a land mass of approximately 7,315km^2. FCT is currently made up of six Local Government Areas Councils namely: Gwagwalada, Kuje, Bwari, Kwali, Abaji and Abuja city situated within the savannah region with moderate climate conditions [12]. The study was carried out on a weekly basis amongst dogs presented to some selected veterinary clinics in Gwagwalada and Abuja Municipal City centers between November and December 2012.

Sample Collection and Sample Technique

Convenient sampling was conducted based on the availability of reported cases to the designated veterinary clinics. A total of 50 swab samples were collected from dogs with reported swelling and inflamed eyes characterized by discharges and itching, diagnosed tentatively as conjunctivitis. The samples were collected using sterile swab sticks and stored in clearly labelled specimen bottles according to the location of collection. The samples were stored in ice pack and transported to Microbiology Laboratory for analysis.

Sample Processing

All the samples were processed in accordance with standard methods [13]. All the materials used were sterilized and autoclaved at 121°C for 15 minutes.

Media Preparation

Sabouraud Dextrose Agar (SDA)

The media was prepared according to the manufacturer description (Hi media) U.S.A. Thirty one (31) gram of Sabourauds Dextrose Agar (SDA) was calculated following the manufacturers specification, weighed and dissolved in 500ml of distilled water. The mixture was autoclaved at 121°C for 15 minutes and allowed to cool and 50ml each was carefully dispensed aseptically into sterile petri dishes and allowed to gel at room temperature.

Fungal Culture and Identification

The inoculation and culture of swabs specimens was carried out as described by [14]. The inoculating wire loop was sterilized using the flame from a spirit lamp and streaks were made on the Sabouraud Dextrose Agar and incubated at 25°C room temperature for 24-48hours. Twenty four hours post inoculation growths were observed on the media plates. Distinct colonies with circular white to cream colored, smooth growth, as well as those with waxy to glabrous surface and yeast like appearance and odour were considered as Candida species. Those plates considered to have more growth of *Candida* were used for subcultures. Sub cultures were on new plates and incubated at room temperature for 7days until pure culture was obtained for further biochemical test. Visual examination of the cultures on media and microscopic identification of typical colonies was carried out on each plate and interpreted as described by Uruburu [15]. Also gram staining was executed as described by Isu and Onyeagba [13].

Biochemical Characterization

The biochemical activities were used to characterize fungi isolate according to their reaction to various test such as Triple Sugar Iron test (TSI), citrate utilization test and germ tube test as described [13]. In triple sugar iron test, the isolates was inoculated into the tube gently and removed by striken and observed for development of coloration change post incubation for 18 – 24hr which is indicated as: An alkaline slant – acid butt (red/yellow) indicated fermentation of dextrose only. An acid/slant – acid butt (yellow/yellow) indicated fermentation of dextrose lactose and sucrose. An alkaline slant - alkaline butt (red/red) indicated dextrose or lactose were not fermented (non fermenters). Cracks, splits or bubbles in medium indicated gas production.

Citrate Utilization Test

Simmon citrate (31.5 gram) was dissolved in 100mls of distilled water and 5mls was dispensed into test tubes and autoclaved at 121°C for 15 minutes and allowed to solidify in slant positions. The sloped surface was inoculated by streaking with a loopful of *Candida* isolates. It was incubated at 37°C for 48 hours. A change in color from green (bromothymol blue) to blue indicated an alkaline reaction from citrate utilization.

Germ tube test: Sheep serum (0.5ml) was dispensed into two sterilized test tubes and pure culture of the isolates were inoculated into each test tube and incubated at 37°C. The mixture was observed hourly for 2 – 3 hours. Germ tube growth which normally occurs within 2 – 3 hours indicated typical growth of *Candida albicans* and yeast like cell (blastoconidia) under the microscope.

Results

Ten samples swabs (20%) showed evidence of Candida species on cultural and morphological identification. Further biochemical characterization indicated the isolates as *C. albicans*. The occurrence of *Candid aalbicans* with canine conjunctivitis was not significant (P>0.05).

Table 1: Occurrence of *Candida albicans* isolates associated with canine conjunctivitis.

Weeks	Location	Number of Samples	+vepositive	-vesample
1	VTH	10	2(4%)	8
2	Kings Vet.	10	4(8%)	6
3	Vet. World	10	3(6%)	7
4	A/C Vet.	10	1(2%)	9
5	Aso Vet.	10	0(0%)	10
Total		**50**	**10(20%)**	**40**

$X^2 = 6.25$ df = 4 P = 0.181

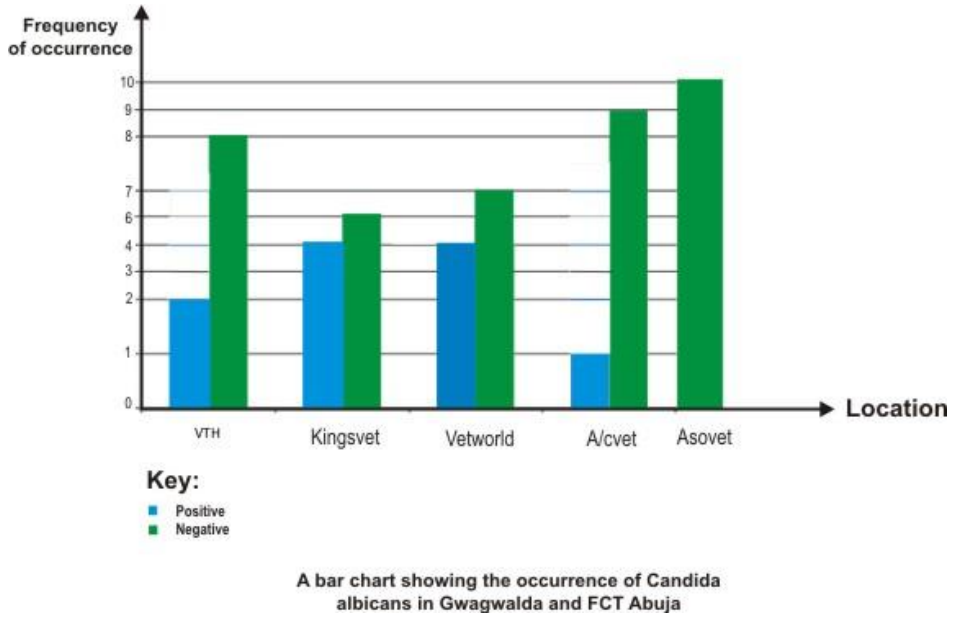

Figure 1: Distribution of *Candida albicans* isolates in Abuja and environs

Table 2: Morphological and Biochemical characteristics of *C. albicans*obtained from Canine Ocular Swabs

Test (procedures)	Characteristics/appearance
Colonial morphology on Sabouraud Dextrose Agar (SDA)	Circular to round creamy smooth glabrous to waxy surface and yeasty like appearance
Sabouraud dextrose agar (SDA)	Creamy color
Gram reaction(GSM × 100)	Spherical to oval budding yeast like cells (blastoconidia) and radially orientated pseudohyphae
Citrate test	+ve, which indicated a change in color from green (bromothylmol blue) to blue, indicates alkaline reaction arising from citrate utilization
Triple sugar iron test (TSI)	(Y/Y) indicated fermentation of dextrose, lactose and sucrose. (R/Y)+H_2s this indicated fermentation of dextrose only and production of gas.
Germs tube test	Positive, with typical growth of *Candida albicans* and yeast like cell (blastoconidia) under microscope (germ tube).

Key

R/Y = Alkaline slant/acid butt	+ve = Positive	R = Red
Y/R= Acid slant/alkaline butt	-ve = Negative	Y = Yellow

Discussion

The overall occurrence of *Candida* associated canine conjunctivitis in this study is 20% which shows the existence of *Candida albicans* in this study area. The presence of *Candida species*is of public health concern [16] as dogs were usually kept as pets in many household in Abuja and its environs. The distribution of the positive isolates as indicated in Table 1 and Figure 1 with Veterinary clinic in Gwagwalada having the highest Candida isolates. This could be associated with the low income and awareness on pet care by owners when compared with other pet (dogs) in highbrow areas in the city of Abuja presented to Veterinary clinics located within the neighbourhood of the city, although observations during case presentations indicates history of antibiotic misuse and abuse especially in Gwagwalada areas.

This highest frequency could also be incline with to poor hygiene, management, prolong antibiotics and corticosteroids use as previously reported by Ako-Naiet al. [17] which highlights a link between Candida infection and poor management. The higher occurrence of Candida in Gwagwalada in comparison with other parts of the FCT is an indication that *Candida* prevails more in high temperature prone areas as previous occurrence was reported by Patel et al. [18] in the hot tropical regions.

The visual morphology and cultural characteristics of isolates (Table 2) confirms typical *Candida albicans* with circular or round, smooth, glabrous to waxy surface with creamy and yeasty like appearance which also occurs as gram positive cocci and in chain as previously documented by Buttler et al. [18]. This was further confirmed by the positive citrate utilization with a characteristic color change from green (bromothylmol blue) to blue which indicates alkaline reaction arising from citrate utilization Pfalleret al. [20]. In triple sugar iron test (TSI) indicates fermentation of dextrose, lactose and sucrose with production of gas which confirms previous reports of Van Kesselet al. [21] that states yeast (*candida*) are microorganisms that can ferment sugars into alcohol, gas and other substance. Germ tube test confirms typical growth of *Candida albicans*as earlier documented by Pfalleret al. [20] as this method is simple, cheap and reliable means of rapid growth of *Candida albican* in serum.

Previous reports especially in Women and Poultry indicates high occurrence of *Candida* infection [18,22]. However, these low findings of *Candida albicans* amongst screened canine ocular swabs maybe associated to the small sample size and or aging dogs with compromised or failing immune system as previous documented. These affected dogs can spread the organism to other susceptible canine population as well as human especially among dog handlers following poor social sanitary habits [23]. Another reason for the low occurrence could be adjudge mis-diagnosis of signs of *Candida* conjunctivitis as allergy [6]. Also according to Linek J [24] stated that mycotic endopthalmitis in dog cause by *Candid aalbicans* is mostly seen in dog under prolong treatment with antibiotic and poor management.

This research shows that the positive samples of ocular swab screened for *Candida albicans* were isolates from sick dogs presented with cases of ocular swelling and discharges characterized by marked localized ocular inflammation. These signs of conjunctivitis could be associated with the activities of Aspartyl proteinases secreted by *Candida albicans*as reported previously by Naglik et al. [25] in the pathogenesis and virulence of ocular *Candida* infections [26]. Although, the diagnostic significance Candida as a definitive etiology of canine conjunctivitis may seem contentious as previous reports indicates Candida as mucosal normal flora [5]. Further experimental Candida specific case-control study would be required. There also, exists the possibility of potential activities of other bacterial agents in aliments observed. This was not in the outline of this study and hence, was not conducted.

In conclusion, this study provides preliminary findings on the occurrence and predominance of *Candida albicans* over other *Candida* species associated canine conjunctivitis infection in and around Abuja metropolis. Therefore, proper laboratory diagnostic tests should be conducted to establish the causative pathogens associated with canine conjunctivitis. In addition, public awareness on the possible mode of transmission and occurrence of *Candida albicans* infection within and between man and animals should be emphasized.

Acknowledgement

The authors appreciate the efforts of Mr. Hakeem Onigbanjo, Dr.Ifeanyi Casmir and Madam Grace Popoola of Veterinary Microbiology Department, University of Abuja, for their assistance towards the completion of this research work.

Conflict of Interest

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

References

1. Pappas PG, CA Kauffman, D Andes, DK Benjamin, Jr., TFCalandra, JE Edwards, Jr., SG Filler, JF Fisher, BJ Kullberg, L Ostrosky-Zeichner, AC Reboli, JH Rex, TJ Walsh and JD Sobel (2009) Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis.48:503-535.
2. Hatakka K, Ahola AJ, Yli-Knuuttila H, et al. (2007) Probiotics reduce the prevalence of oral candida in the elderly--a randomized controlled trial. J Dent Res 86(2):125-130.
3. Blonz ER. (1986) Is there an epidemic of chronic candidiasis in our midst? Journal American Medical Association256: 3138-9.
4. Greene CE, and Chandler FW (1998) Candidiasis, torulopsosis, and rhodotorulosis. In: Greene CE (ed.): Infectious Diseases of the Dog and Cat. 2nd ed. WB Saunders Co., Philadelphia. 414–417.

5. Robin CP, Herkhout P and McClary (2006) Factors affecting the morphology of *candida albicans*.Journal of biology Missouri botanical Garden 39(3):137-164.
6. Ryan KJ (2004) Candida In: Sherris Medical Microbiology, 4th ed., Ray CG (editors) McGraw Hill.
7. Kourkoumpetis TK, Velmahos GC, Ziakas PD, Tampakakis E, Manolakaki D, Coleman J.J and Mylonakis E (2011) "The effect of cumulative length of hospital stay on the antifungal resistance of Candida strains isolated from critically ill surgical patients." Mycopathologia 171 (2): 85-91.
8. *JamesWD, Berger*TG and *Elston* DM (*2006*).*Andrews' diseases of the skin: Clinical dermatology*(10th ed., international ed.). Philadelphia, PA: W.B. *Elsevier*-Saunders. 466-469.
9. Odds FC (1988) Candida and Candidosis In: A review and bibliography. Second ed. London: Bailliere Tindall; England. Pp 89.
10. Sobel JD (2006) The emergence of non-albicans Candida species as causes of invasive candidiasis and candidemia. Curr. Infect. Dis. Rep.8:427-433.
11. Calderone RA and Fonzi WA (2001) Virulence factors of *Candida albicans*. Trends in Microbiology9:327-335.
12. Anon (2012) Geographic Information on Federal Capital Territory (www.fcda.gov.ng).
13. Isu NR and Onyeaba RA (1998) Basic Practical in Microbiology. FASMEN Educational and researchpublications. Pp134-155.
14. Bello RH,Agbo EB and Olabode HOK (2011) Antibiogram of Bacterial and Fungal Isolates associated with otitis media amongst children in Bauchi state, Nigeria International Journal of Pharma and Bio Sciences 3(2): 200- 207.
15. Uruburu F (2003) "History and services of culture collections". International Microbiology6 (2): 101–103.
16. Mårdh PA, Rodrigues AG, Genç M, Novikova N, Martinez-De-Oliveira J and Guaschino S (2002) Facts and myths on recurrent vulvovaginalcandidosis - A review on epidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. *International Journal of STD and AIDS*13(8):522–539.
17. AkoNai AK, Adejuyigbe EA, Ajayi FM, Onipede AO (1999) The Bacteriology of Neonatal Septicemia in Ile-Ife, Nig. J. Trop. Paediatrics. 45: 146- 151.
18. Patel DA, B Gillespie, JD Sobel, D Leaman, P Nyirjesy, MV Weitz and B Foxman (2004) Risk factors for recurrent vulvovaginal candidiasis in women receiving maintenance antifungal therapy: results of a prospective cohort study. Am. J. Obstet. Gynecol.190:644-653.
19. Butler G, Rasmussen MD, Lin MF, Santos MA, Sakthikumar S, et al. (2009) "Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes". Nature459 (7247): 657–62.
20. Pfaller M.A (2000)Antifungal susceptibility testing: Progress and future developments. Brazilian Journal of Infectious Diseases 4 (2): 55-60.
21. Van Kessel JS, Karns JS, Gorski L, McCluskey BJ and Perdue ML (2004) Prevalence of salmonellae, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on US dairies. J Dairy Sci 87: 2822-2830.
22. Adah BMJ, Kwanashie CN and Ameh JA (2015) Isolation and characterization of yeast associated with Hatchery Dead-In-Shell Embryos in Zaria. Journal of Agriculture and Veterinary Science 9 (1): 12-17.
23. Hachem R, Hanna H, Kontoyiannis D, Jiang Y, Raad I (2008) The changing epidemiology of invasive candidiasis: *Candida glabrata* and *Candida krusei* as the leading causes of candidemia in hematologic malignancy. Cancer112:2493–2499.
24. Linek J (2004) Mycoticedophthalmitis in a dog caused by *Candida albicans*. Vet Ophthalmol7: 159-162.
25. Naglik JR, Challacombe SJ and Hube B (2003) *Candida albicans*secreted Aspartyl proteinases in virulence and pathogenesis. Microbiol. Mol. Biol. Rev., 67(3): 400-426.
26. Ligoxygakis PA and Ithazakis TC (2011) Virulence factor of yeast infection. Journal of Laboratory Microbiology 6 (12):236-311.