ISOLATION OF *CANDIDA* SPECIES IN DOMESTIC CHICKEN (*Gallus gallus*) DROPPINGS IN KABIGERIET VILLAGE, NAKURU COUNTY KENYA

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Abstract

Over the last three decades, reports on yeast infections in humans have increased especially with respect to immunocompromised individuals. This is associated with increased morbidity and mortality especially in HIV/AIDS immunocompromised individuals. The purpose of this study was to isolate and characterize yeasts from domestic Chicken droppings. The droppings were collected from Kabigeriet Villages, Olenguruone Division, Nakuru County. Eighty four samples were collected by scooping and swabbing Chicken droppings and transported to the Mycology laboratory, Kenya medical research institute using a cool box for processing. Samples were inoculated onto Typan blue agar and further sub cultured on CHROM agar and Corn meal agar for presumptive identification of various *Candida* species. Analytical profile index test was used for confirmation. 35 (41.67%) *Candida* species (9 *Candida lusitanie*, 7 *Candida glabrata*, 5 *Candida albicans*, 5 *Candida tropicalis*, 3 *Candida parapsilosis*, 2 *Candida lipolytica* and 2 *Candida krusei*) were isolated. The results of this work demonstrated that domestic chicken harbor potentially pathogenic yeasts in their dropping.

Key words: *Candida*, chicken, yeasts, AIDS

Introduction

In the genus *Candida*, most species exist as commensals in most healthy individuals (Ramage *et al*., 2001, Sullivan *et al*., 2004). Pathogenic *Candida* species is a growing problem in medical Science (Calderonne, 2002). *Candida albicans* is the most common species causing human infections (Vazquez *et al*., 2002), however emergence of non-*albicans* species such as *C. krusei, C. parapsilosis, C. tropicalis* and *C. glabrata* has

been reported in the last decade as human pathogens, mainly among immuno-suppressed individuals and hospitalized patients (Brawner *et al.*, 2002, Fleming *et al.,* 2002, Sanchez *et al.,* 2005a). In United States the proportion of fungal infection in comparisons to all nasocomial infections doubled over a period of ten years (Calderonne, 2002). The increased reporting of non-*albican Candida* species could be due to increased recognition in the laboratory (Sanchez *et al.,* 2005b). Candidiasis present a growing challenge for patients who are immunocompromised due to HIV/AIDS, organ transplant, cancer and other risk factors have increased the emergence of other *Candida* species as opportunistic pathogens (Calderonne, 2002, Gutierrez *et al.,* 2002, Vazquez *et al.,* 2002). *C. dubliniensis* has been associated mainly with oral candidiasis in HIV positive individuals worldwide. However in HIV negative individuals it has been reported to cause systemic and superficial diseases with prevalence rate of less than 5% (Gutierrez *et al*., 2002, Jewtuchowicz *et al*., 2008).

In immunocompromised individuals, large proportions of fungal infections are caused by *Candida* species: *Candida* species are found in 40-65% of feaces from healthy individuals as normal flora, and oropharyngeal colonization in approximately 30-55% of healthy adults (Colombo *et al*., 2006), causing different infections, such as candiduria (Da Silva *et al*., 2007) candidemia (Colombo *et al*., 2006) and Oropharyngeal candidiasis (Costa *et al*., 2006). Oropharyngeal candidiasis is developed by more than 90% of individual infected with HIV and is not on HAART (highly active antiretroviral therapy) (Repentighy *et al*., 2004).

Materials And Methods

Study area

The study was carried out in Kabigeriet Village, Olenguruone in Nakuru County. Olenguruone is Approximately 282 KM from Nairobi, Kenya. The area lies at about 35° 41'E and 0.1° 35'S. The climate is sub-humid consisting of one rainy season (April to December) and dry season (January – March). The average annual rainfall is 1200 mm and the average temperature is 28^{o}C with small variations (±5^{o}C) throughout the year (Meteorological Kenya, 2009). The study area was chosen since it is a typical rural setting where domestic Chicken are reared in close proximity with humans. Unlike in other Divisions of Olenguruone, in Kabigeriet village most farmers rear only domestic chicken in a free range system.

Subjects: The study was a cross sectional laboratory based study carried out for a period of five months, (April 2010 to August 2010). Sixty four Chicken droppings were sampled in thirty two homesteads after obtaining the farmers consent.

Sample collection: Environmental collection of domestic Chicken droppings was done by scooping fresh droppings from Chicken houses, grass, soil and trees using sterile plastic spoons. Each spoon was used once and discarded into sterile ziplock bags. Droppings which could not be collected using a spoon were swabbed by passing a sterile swab over each sample until it turned "dirty". Plate 1 and Plate 2 show some of the collection sites.

Plate 1: Chicken droppings inside poultry house.

Plate 2: Chicken droppings outside a human house.

Specimen preparation: One Gram of domestic Chicken dropping was weighed on sterile film paper using a scale balance and then transferred to a 10 ml sterile round bottomed tube containing 5 mls distilled water then incubated for 1h with agitation (mix every 15 min) (Misuzu *et al*., 2004). Two 1.7 ml appendoff tubes were filled with 900 µl distilled water and 100 µl of the original sample added to the first tube, (dilution factor 1:10) then 100 µl of the 1:10 dilution added to the last tube containing 900 µl distilled water, giving a dilution factor of 1:100 in the second dilution. Approximately 100 µl of the second dilution were inoculated onto Niger seed agar plate.

For swabs, 100 µl of distilled water was added to a round bottomed tube and the tip of each swab containing sample dipped into distilled water with agitation to make a suspension**.** Similar amounts for swabs suspension were inoculated onto Trypan blue agar plate for primary isolation. All inoculated plates were incubated at 30°C for 72 h checking daily for growth. Yeast-like colonies were purifying by sub culturing onto Sabouraud dextrose agar pH 5.6±0.2 (Difco, Detroit, MI, USA) (Isenberg and Henry, 2004). Purified yeasts colonies were further sub cultured onto CHROM agar (CHROM agar company, Paris, France) for preliminary identification of yeasts (Sivakumar *et al*., 2008) and to Corn meal agar for detections of *Candida* species producing pseudohyphae, true hyphae, arthrospores and chlamydospores. However not all yeasts were identifiable using CHROM

agar and cornmeal agar those that gave contradicting result were subjected to confirmation tests (API 20 AUX) (Bio Merieux SA).

Results

Profile:

i. Trypan blue agar

All samples collected were plated onto Trypan blue agar for primary isolation and purification purposes (plate 3).

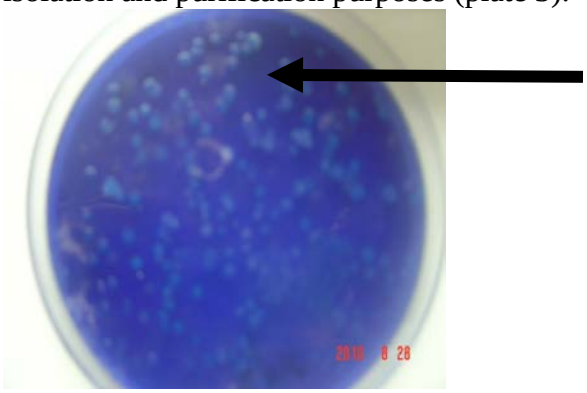

Plate 3: Trypan Blue agar, read after 3days incubation at 30°C. Only whitish colored colonies with smooth edged were seen. It was not possible to decide whether there was a pure or mixed culture of yeasts based on the appearance.

ii. CHROM agar

The CHROM agar supported the growth of all environmental yeasts isolates and its opaque to white background; this allows good discrimination among of colonies of different species with almost similar hues. A wide variety of colony colors were seen some which were species specific. Three *Candida* species, *C. tropicalis* (plate 4), *C. krusei* (plate 5) and *C. albicans* (plate 6), were presumptively identified.

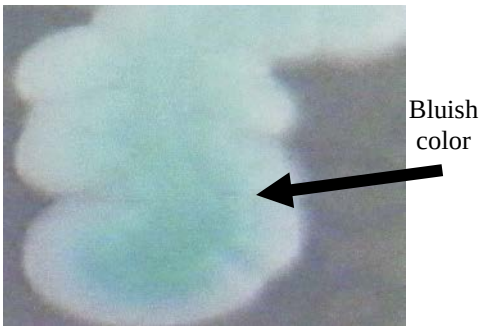

Plate 4: *C. tropicalis* showing bluish coloration of colonies on CHROM agar read after 48h at 30°C.

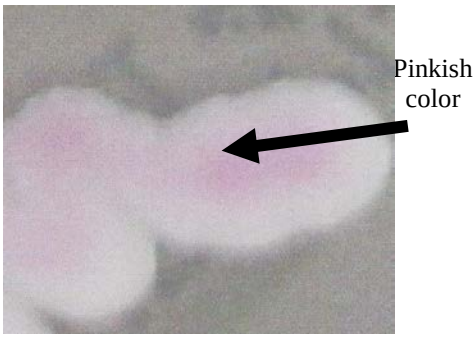

Plate 5: *C. krusei* showing pinkish coloration of colonies on CHROM agar read after 48h at 30°C.

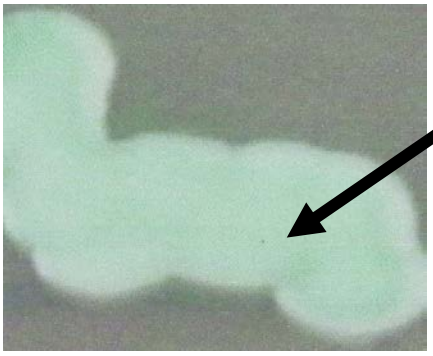

Plate 6: *Candida albican* note greenish coloration of colonies on CHROM agar read after 48h at 30°C.

iii. Cornmeal agar

All the *Candida* species which were not identified using CHROM agar were plated on cornmeal agar to study the formation of pseudohyphae, true hyphae, arthrospores and chlamydiospores. 47 *Candida* species were identified in cornmeal agar at 37°.

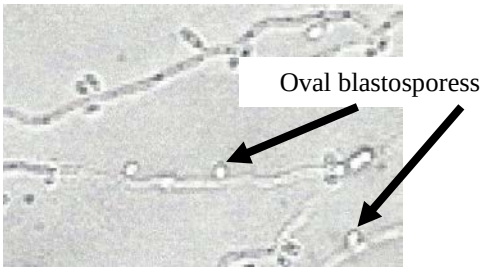

Plate 7: *C. tropicalis* in cornmeal agar showing oval blastospores read after 48h at 30°C.

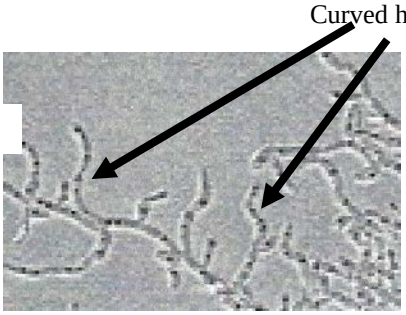

Plate 8: *C. parapsilosis* in cornmeal agar showing curved and large hyphae read after 48h at 30°C.

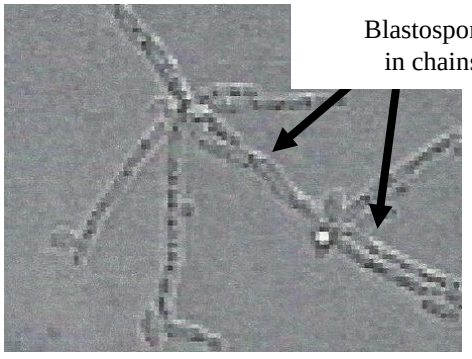

Plate 9: *C. krusei* in cornmeal agar showing pseudohyphae with moderate Branching read after 48h at 30°C.

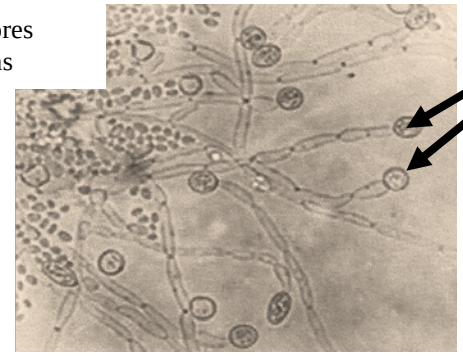

Plate 10: *C. albicans* in cornmeal agar showing chlamydiospores read after 48h at 30°C.

On cornmeal tween 80 agar *Candida tropicalis* (Plate 7) at 30°C after 72h, along the long pseudohyphae produces blastospores which are oval in shape and blastospores may occur in clusters or singly. These is differentiated from *Candida parapsilosis* which on cornmeal tween 80 agar at 30°C after 72h (Plate 8), produces blastospores usually located along pseudohyphae and pseudohyphae are curved and 'giant cells' (large hyphal) are observed, while *Candida krusei* (Plate 9) on corn meal tween agar produces branched pseudohyphae usually with chains blastospores. Along the hyphae and at the septa points *Candida albicans* produces round blastoconidia (Plate 10) and also a sexual spores (typical) know as chlamydiospores which are large, round and thick walled usually at terminal.

IV. Confirmation of *Candida* species using API 20C AUX

API 20C AUX was able to confirm 5 *Candida* species which were not possible to identify using cornmeal agar, which analyses each species based on carbohydrates assimilation profile. The identification of each species was possible by referring to the manufacturers analytical profile index provided by Bio Merieux SA.

Data analysis

The student T-test was used to compare the means of various types of yeasts isolated from soils and Chicken dropping at 95% confidence interval using SPSS packages. The finding showed that there was no significant difference in the yeasts isolated in the droppings to those isolated from the soils ($t = 2.073$, $df = 3$, $P= 0.130$). However, yeasts from droppings (mean 11.2500 ± 5.864) were higher than those from soil (3.750 ± 2.25).

Discussion

The aim of this study was to investigate the possible presence of *Candida* species in domestic Chicken droppings and soils enriched with droppings as a possible source of infections to humans particularly immunosupressed individuals e.g. HIV/AIDS, Cancer, and Diabetes. Since candidiasis is one of the most common opportunistic disease in HIV/AIDS individual. In the past three decade the discoveries and advances in the treatment and control of Viral and Bacterial diseases have been outstanding (----------). In contrast little work has been done in the discoveries of yeasts habitat and their control, despite its high impact on the Human beings and Birds. This is the first isolation of *Candida* species from Domestic Chicken droppings has been done in Kenya. Although in many homestead in Kenya peoples breed chickens in their yards, even some share the same room with chicken.

Candidiasis is the most common fungal infection cause by mainly *Candida albicans*, which it is also the most frequently isolated an etiological agent. Under appropriate environmental conditions it's has the ability to invade host tissues and initiate serious diseases (Morreti *et al*., 2001). *Candida* species caused different forms of Candidiasis namely; chronic mucocutaneous candidiasis syndromes, *Candida*-associated lesion, Atrophic and Hyperplastic candidiasis, Pseudomembranous and Erythematous candidiasis, Atrophic and Hyperplastic candidiasis e.t.c**.** High prevalent of *Candida* species, especially *Candida albicans* in the environment has been reported by many authors (Answar *et al*., 2012, Cafaechia *et al*., 2006) Thus, this study found out that domestic chicken excreta are possible reservoir and sources of infections to human. Human beings can acquire Candidiasis by inhaling spores from chicken droppings.

The weather in Kabigeriet village, Olenguruone is considered appropriate for the growth of *Candida* species; with a mean temperature of 28^{o}C with small variations ($\pm 5^{o}$C) throughout the year and mean annual rainfall of 1200 mm. It has been reported by Bell *et al*., (2001) that candidiasis is predominant during rainy season. However our results showed a different pattern, this may be due to the facts that in dry season patients inhaled a lot of spores from dry Chickens droppings and infections could have gone into latency period and later showed up during raining season (Blasi *et al*., 2001). In HIV infection *Candida albicans* is not only associated with the increased in the rate of colonization but also with development of the disease overt. The rate of *Candida* infections in HIV/ AIDS individual is inversely related to CD4 counts of the Individuals (Answar *et al*., 2012).

The kinds of Candida species isolated from chicken samples in this study agree with what has been isolated in the past by Cafarchia *et al*., (2006): *Candida albicans*, *C. tropicalis*, *C.glabrata*, *C. lusitanie* and *C. guilliermondii* have been in wild birds before. Also Refai *et al*., (1983) isolated *Candida albicans* from pigeon droppings in Egypt. There was higher isolation rates of yeasts in chicken dropping samples (72.13%) compared to soil (27.87 %). This could be attributed to soil exposure to harsh environmental condition like availability of nutrient in the soil, dilution of soil especially during raining seasons and sunlight that could not be conducive, for yeast survival for a long time.

The present results show that *C. lusitanie* is the most common *Candida* species isolated from the droppings in contrast with previously reported studies which indicated that *Candida albican* was the most common species (Brilhante *et al*., 2010). This difference could be due to regional variations associated with geographical location and environmental condition such as humidity, soil type and temperature and the species of birds.

Student t-test at 95% confidential intervals were used to compare the means of various types of *Candida* yeasts obtained from the soil with those obtained from the chicken droppings and it showed that there were no significant differences in occurrence of yeasts in soil and dropping (P=0.130). This could be due to the fact that droppings and soils enriched with droppings have same physiochemical factors.

Conclusions

The results of this work demonstrated that domestic chicken (*Gallus gallus*) harbor *Candida species* in their droppings and that humans cohabiting with Chicken are at a risk of contracting Candidiasis infections, especially immunocompromised individuals. This could partly explain the high incidence of candidiasis in HIV/AIDS patients in Kenya. The study signifies the need to discover more environmental niches for yeasts especially of *Candida albicans*.

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