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Candida Species As Potential Causes Of Dead–In–Shell Chick Embryos

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Abstract: *The study investigated the involvement of Candida spp as pathogens responsible for dead-in-shell chick embryos in a hatchery in Kaduna state, Nigeria. A total of two thousand dead-in-shell eggs from the hatchery facility of the Nigerian Agricultural Production and Research Institute (NAPRI), Samaru, Zaria were sampled over a period of 5 months to assess the prevalence of Candida species. Swabs from pooled samples were collected aseptically and processed according to standard bacteriological methods. The samples were cultured in Sabauraud`s Dextrose Agar (SDA) and Corn Meal Agar (CMA). A total of 15 isolates (7.5%) out of the 200 pooled samples were identified to be yeast organisms. Six (40%) of the yeast isolates or 3% of the pooled samples were confirmed to be Candida species based on morphological and physiological characteristics. Speciation using API-20C AUX KIT (bioMèriux) (Lyon, France) revealed that two distinct isolates (33.3%) from the Candida organisms belonged to Candida albicans or Candida tropicalis, while one (16.7%) out of the remaining two Candida isolates was either Candida guilliermondii or Candida pseudotropicalis respectively. A low level (3%) of Candida associated, dead-in-shell chick embryos was detected in this study. The fungal organisms could be a contributory factor to low hatchability and reduced production estimates in poultry farms. The findings underscore the need for proper awareness and adequate biosecurity measures against fungal contamination of eggs in poultry and hatchery establishments in Nigeria.*
Keywords: *Dead-in-shell, Embryos, Candida, Hatchery, Characterization.*

I. Introduction

The growth of poultry industry in Nigeria is constrained with numerous challenges. Fertile eggs are produced by the breeder stock and have ensured the continuity of the progeny through the hatching process. Basically, eggs are a major source of biologically active compounds which have found wide use in pharmaceutical, cosmetic and food industries due to their nutritive and health benefits [1,2]. However, many factors including nutritional requirements, managemental problems, inbreeding, chemical exposure, infectious diseases, and hatchery inadequacies have presented limitations to poultry production [3] especially in our country, Nigeria. *Campylobacter jejuni, Salmonella spp., Streptococcal spp. Escherichia coli* and many more virulent pathogens were incriminated in dead-in-shell embryos [4,5]. Yeast organisms specifically, *Histoplasma capsulatum, Blastomyces dermatitides, Filobasidiella neoformans, Coccidioides immitis* and *Candida albicans* were reported as major causes of food spoilage and diverse diseases in man and animals [6].

Yeasts are microbial eukaryotes; plant-like unicellular fungi which belong to ascomycetes and could be seen to thrive on every living organism [2]. Fungi require warmth, water, albumin or nitrogenous material and sugars to remain alive [7]. Candida species are responsible for the most common opportunistic fungal infection, candidaisis particularly in humans. Diseases caused by the organisms range from relatively benign infections of the skin to oral and vaginal thrush, deep-seated mycoses and life-threatening sepsis [8]. The genus Candida contains more than 300 species [9], and over 40 were associated with life-threatening infections in patients with compromised immune response [10,11]. However, yeast-like organisms are part of the lower genital tract flora in 20-50% of healthy asymptomatic women [12]. Candida species were isolated from diverse clinical specimens in Nigeria and different parts of the world [13,14]. Some commonly encountered Candida species include *C. tropicalis*, *C. stellatoides, C. parapsilosis, C. albicans, guillermondi, C. pseudotropicalis*, and *C. krusei*. *C. tropicalis* and *C. stellatoides* are regarded as the most virulent but *C. albicans* is the only organism capable of evoking fatal disease regularly [15]. *Candida species* were also documented to have induced reduced feed intake, weight lose and depression in birds due to ulcerative lesions in the oral mucosa, oesophagus and crop [16]. Fungal contamination and penetration of fertilized poultry eggs evoked deleterious effects and death of the embryo. The ability of *C. albicans* to damage the chorioallantoic membrane (CAM) of eggs coupled with immature effector functions of the embryonic immune system putatively contributed to mortality of chicken embryos experimentally challenged [17]. There is dearth of information on the contribution of *Candida species* to poor hatchability of chicken eggs generally in Nigeria. The study therefore sought to detect Candida

organisms associated with dead-in-shell chicken embryos in NAPRI, a major hatchery establishment in Zaria, Kaduna State, Nigeria.

II. Materials And Methods

2.1. Location of the hatchery

The study was carried out with dead-in-shell chicken eggs obtained from the hatchery facility of NAPRI, Samaru in Zaria metropolis, Kaduna State, Nigeria. NAPRI is a research and commercial poultry establishment with hatcheries (Funki, Hamerrun, DK 7400, Herning, Denmark). Zaria is located between latitude 11^0 4 N and longitude 7^0 42 E, covering an area of 300 km^2. The town has a population of about 408,198. The vegetation is Northern Guinea Savannah, with rainfall ranging from 0.0-816.0 mm/month and a temperature of 17-33^{0}C [Mortimore, 1970]. The hatchery complex had in-built egg turning devices with a total hatching capacity of four thousand eggs at a time.

2.2. Sample collection And Sampling Method

Dead-in-shell chicken eggs from the indeginous breed, 'Shika brown' were detected and selected among fertilized eggs during candling on day 18 of incubation. A total of two thousand dead-in-shell chicken eggs were collected from the hatchery over duration of 5 months (February-June, 2015). One hundred samples were collected on a weekly basis and promptly transported in ice packs to the Veterinary Mycology Laboratory, Ahmadu Bello University (ABU), Zaria. The eggs were processed using a pooled sample method with 10 eggs per beaker to obtain a total of 200 pooled samples. All the eggs were macroscopically examined for detection and subsequent removal of cracked or malformed ones before setting in the incubator. Fertile eggs were stored at 8 ^{0}C for a maximum of seven days prior to incubation at 37.6 ^{0}C and 50-60% relative humidity.

2.3. Culture media preparation and yeast identification Kit

Sabouraud's dextrose agar (SDA) obtained from Hi Media, USA, Corn meal agar (CMA) from Oxoid® Hampshire, England, and API-20C AUX KIT (bioMèriux, Lyon, France) were used in the study. A concentration (0.05 mg/ml) of chloramphenicol was incorporated into each culture medium as antibiotic before been poured into plates and bottles. The preparation of the culture media strictly followed the manufacturer's specifications and all tests were performed in accordance with standard procedures.

2.4. Processing of the samples and Innoculation of media

All the materials used were properly sterilized; laboratory benches were routinely disinfected using 70% alcohol, culture media and reagents were autoclaved at 121 ^{0}C for 15 minutes. Petri dishes were sealed with parafilm or placed in plastic bags to avoid dehydration or release of spores into the atmosphere. The external surface of dead-in-shell eggs was first disinfected with 70% alcohol or sodium hypochlorite to remove dirt and possible contaminants. The tip of spatula used in cracking the eggs was routinely flamed to keep it sterile before use. The site for cracking on each egg was repeatedly cleaned with alcohol just before cracking. The allantoic fluid from each batch of 10 sampled eggs was emptied into a sterile beaker and stirred uniformly with a sterile glass rod for proper mixing. The stock culture of the various fungal organisms was prepared with swab of the allantoic fluid of the sampled dead-in-shell eggs. A sterile swab stick was used for inoculation from a pooled sample onto a labeled culture medium. Inoculated samples were incubated aerobically at ambient temperature (26-28 ^{0}C) for 48-72 hours.

2.5. Maintenance of culture

The discrete isolated colonies (pure cultures) was picked out purified by re-streaking on SDA plate and maintained on slants of the same medium at 5 ^{0}C in the refrigerator.

2.6. Characterisation of yeast strains

2.6.1. Colonial Identification: The resulting colonies were stained using lactophenol cotton blue stain and Gram's stain following standard procedure, as described by Ochei and Kolhatkar [18] before it was viewed under microscope at low power objective magnification (x4 and 10) and oil immersion (x100) respectively.

2.6.2. Physiological tests: Biochemical analysis and other tests involving Germ tube test, Indian ink mount, Incubation at varying temperatures (30, 35, 37 and 42 ^{0}C), Assimilation and fermentation tests were carried out on the fungal isolates following standard procedures [19]. The carbon assimilation activities of the yeast species on carbohydrates were carried out using API-20C AUX KIT (Lyon, France) according to the method described by Espinel *et al.* [20] and Moghaddas *et al.* [21].

III. Results

3.1. Prevalence and morphology of Candida isolates from sampled dead-in-shell chick embryos

Two thousand dead-in-shell poultry eggs comprising 200 pooled samples were investigated for the presence of Candida species. A total of 150 yeast organisms from 15 pooled samples out of 200 (71-155) and representing 7.5% infection rate were detected among the dead-in-shell chick embryos. The morphological and vegetative characteristics of the isolated yeast strains showed, large, medium, tiny, creamy, swampy, smooth, spherical, raised and flat colonies with grey, yellow, bron, white, or shiny appearance. Microscopically, the vegetative cells were spherical, oval, elongated with single or multiple budding (Figures 1 and 2). Candida organisms had a prevalence of 6 (3%) among the 2000 eggs sampled. All the Candida species grew at various elevated temperatures of 30, 35, 37 and 42 ^{0}C (Table 2).

3.2. Morphological and physiological charactrerisation of Candida isolates from pooled sample 72 and 128

Two (13.3%) isolates from pooled sample numbers, 72 and 128 produced creamy, waxy, smooth colonies on SDA. The germ tube test was positive (Table 1). Spherical to ovoid clusters of blastoconidia and chlamydospores were seen microscopically budding from the pseudohyphae. Glucose and maltose were fermented but not sucrose or lactose and there was no hydrolysis of urea or nitrate utilisation. The isolates assimilated glucose, maltose, sucrose, galactose, xylose, soluble starch and trehalose but did not grow on lactose, melibiose, cellobiose, inositol, raffinose and dulcitol (Table 2). The isolates were therefore identified as *C. albicans.*

3.3. Identification of Candida isolates from pooled sample 79 and 144

Two (13.3%) other isolates from pool sample numbers, 79 and 144 produced white to cream-coloured smooth, yeast-like colonies on SDA. Germ tube test was negative (Table 1) and Indian ink preparation was also negative showing the absence of capsule. From the culture on cornmeal and agar, the isolates produced many long, wavy, branched pseudohyphae with numerous blastoconidia, budding off (Table 1). The two isolates fermented glucose and maltose with acid and gas production; fermentation of sucrose was variable while lactose was not fermented and there was no hydrolysis of urea or utilization of nitrate. However, there was assimilation of glucose, maltose, sucrose, galactose, cellobiose, xylose, and soluble starch but not lactose, melibiose, inositol, raffinose and ducitol (Table 2). The characteristics were typical of *C. tropicalis.*

3.4 Characterisation of Candida isolates from pooled sample 77 and 155

One (6.7%) out of the six isolated yeast organisms from the pooled sample number, 155 produced large, whitish, creamy, smooth colonies on SDA. Germ tube test was negative (Table 1) and there was no presence of capsule as indicated by a negative Indian ink preparation. Hydrolysis of urea and nitrate utilisation was negative. Microscopically, numerous long, wavy branched pseudohyphae with dense verticils of short-ovoid to long-ovoid, blastoconidia, which budded off singly, in pairs or chains, often in a verticillated position were observed. The isolated yeast organisms fermented glucose, galactose, sucrose and raffinose but did not ferment maltose, trehalose, melibiose, D-arabinose, D-glucosamine or D-gluconate. The fermentation of lactose, cellobiose, D-ribose, and D-glucitol was variable. The isolate also assimilated glucose, but was slow on galactose, ribitol and D-xylose while soluble starch and galactitol were not assimilated. The isolate was identified as *C. kefyr* based on the characteristics.

One (6.7%) of the six isolated *Candida* organisms from pooled sample number 77 formed white to cream-coloured smooth, glabrous yeast-like colonies on SDA. Germ tube test was negative (Table 1) and Indian ink preparation was also negative. Blastoconidia appeared as spherical to subspherical budding yeast-like cells on branched pseudohyphae microscopically. The isolate fermented glucose, sucrose, trehalose, raffinose, D-glucosamine, glycerol, D-ribose and 2-K-D-gluconate but did not ferment maltose, lactose, erythritol and D-glucuronate. The fermentation of melibiose, cellobiose, D-arabinose, D-glucitol, D-gluconate and DL-lactate was variable. Hydrolysis of urea was negative and nitrate utilisation was also negative. The isolate was identified as *C. guilliermondii.*

TABLE 1

The morphology of yeast isolates from dead–in–shell chicken embryos

Egg sample number	Pooled sample number	media	Colonial morphology	Conidia	Germ tube	Tentative organism
701-710	71	CMA	Moist, whitish, round colonies	+	-	Yeast cell
		SDA	Creamy, flat, waxy colonies	+	-	
711-720	72	CMA	Moist, whitish, flat smooth colonies	+	+	*C. albicans*
		SDA	Creamy, waxy, smooth colonies	+	+	
721-730	73	CMA	Creamy, large, round, raised colonies	+	-	Yeast cells
		SDA	No growth	+	-	
751-760	76	CMA	Moist, medium, brownish colonies	+	-	Yeast cells
		SDA	Moist, medium, brownish colonies	+	-	
761-770	77	CMA	Large, whitish, waxy colonies	+	-	Yeast cells
		SDA	Large, whitish, creamy colonies	+	-	
771-780	78	CMA	Large, whitish colonies	+	-	Yeast cells
		SDA	Small, creamy, raised, colony	+	-	
781-790	79	CMA	Whitish, raised, moist, swampy colonies	-	-	Yeast cells
		SDA	Whitish, raised, moist, swampy colonies	+	-	
791-800	80	CMA	Whitish, raised, moist, swampy colonies	+	-	Yeast cells
		SDA	Whitish, raised, moist, swampy colonies	+	-	
1241-1250	125	CMA	Yellow, round, flat, raised colonies	+	-	Yeast cells
		SDA	Yellow, round, flat, moist colonies	-	-	
1271-1280	128	CMA	Whitish, flat, smooth, moist colonies	+	+	*C. albicans*
		SDA	Creamy, waxy, smooth colonies	+	+	
1331-1340	134	CMA	Grey-yellow, large, moist, domed colonies	+	-	Yeast cells
		SDA	Grey-yellow, large, moist, domed colonies	+	-	
1341-1350	135	CMA	Whitish, tiny, colonies	+	-	Yeast cells
		SDA	Tiny, white, colonies	+	-	
1431-1440	144	CMA	Whitish, medium, flat, smooth colonies	+	-	Yeast cells
		SDA	Whitish, medium, flat, smooth colonies	+	-	
1461-1470	147	CMA	Whitish, tiny, powdery colonies	+	-	Yeast cells
		SDA	Whitish, tiny, powdery colonies	+	-	
1541- 1550	155	CMA	Large whitish, creamy, round, smooth colony	+	-	Yeast cells
		SDA	Large whitish, creamy, round, smooth colony	+	-	

KEY: CMA = Corn meal agar; SDA = Sabouraud`s Dextrose agar; + = present; - = absent

TABLE 2

Physiological characterization of Candida isolates from dead-in-shell chick embryos

Physiological tests					**Organism identified**
Fermentation with isolates from pooled samples (72 and 128)			**Other tests**		
Glucose +	Lactose -	Melibiose -	Germ tube +	Nitrate -	
Maltose +	Sucrose -	Trehalose v	Growth at 30 ^{0}C +	Urease -	
DL-Lactate +	D-Ribose -	D-Glucosamine v	Growth at 35 ^{0}C +		
N-A-D-glucosamine +	D-Gluconate -	D-Arabinose v	Growth at 42 ^{0}C +		
DL-Lactate +	Raffinose -	Glycerol v			
Sugar Assimilation					
Glucose +	Soluble starch +	D-Mannitol +	Melezitose v		
Galactose +	D-Xylose +	Galactitol -	Ribitol v		
Maltose +	Sucrose +	Lactose -	Ducitol-		***C. albicans***
Fermentation with isolates from pooled samples (79 and 144)			**Other tests**		
Glucose +	Lactose -	D-Glucosamine v	Germ tube -	Nitrate -	
Maltose +	Melibiose -	Sucrose v	Growth at 30 ^{0}C +	Urease -	
DL-Lactate +	D-Arabinose -	D-Gluconate v	Growth at 35 ^{0}C +		
Galactose +	Raffinose -	Glycerol v	Growth at 42 ^{0}C +		
Trehalose +,s	D-Ribose -,s				
D-Glucitol +	D-Glucuronate				
Sugar Assimilation					
Glucose +	D-Xylose +	Ribitol +,s			
Galactose +	D-Mannitol +	Galactitol -			
Soluble starch +	Maltose +	Melezitose v			***C. tropicalis***
Fermentation with isolates from pooled sample number 155			**Other tests**		
Glucose +	Maltose -	D-Glucosamine -	Germ tube -	Nitrate -	
Galactose +,s	Trehalose -	Lactose v	Growth at 30 ^{0}C +	Urease -	
Sucrose +	Melibiose -	Cellobiose v	Growth at 35 ^{0}C +		
Raffinose +	D-Arabinose -	D-Ribose v	Growth at 37 ^{0}C +		
		D-Glucitol v	Growth at 42 ^{0}C +		
Sugar Assimilation					

Glucose +	D-Xylose +	Melezitose v			
Galactose +	D-Mannitol +	Ribitol +,s			
Soluble starch +	Galactitol -				***C. kefyr***
Fermentation with isolates from pooled sample number 77			**Other tests**		
Glucose +	Maltose -	Melibiose v	Germ tube -	Nitrate -	
Sucrose +	Lactose-	Cellobiose v	Growth at 30 ^{0}C +	Urease -	
Trehalose +	Erythritol	D-Arabinose v	Growth at 35 ^{0}C +		
D-Glucosamine +	D-Glucuronate -	D- Glucitol v	Growth at 37 ^{0}C +		
Glycerol +		D-Gluconate v	Growth at 42 ^{0}C v		
D-Ribose +					
Sugar Assimilation					
Glucose +	Ribitol -	D-Mannitol v			
Galactose +	Soluble starch -				
D-Xylose +	Galactitol v				***C. guilliermondii***

Key: + = Positive; - = Negative; v = Variable, s = Slow, w= Weak

TABLE 3

The percentage prevalence of Candida isolates from dead-in-shell chick embryos

Organism	No. of isolates.	Frequency in 200 pooled samples.	Frequency among yeasts
Yeast	15	7.5%	-
Candida species	6	3%	40% of yeasts
C. albicans	2	1%	33.3% of yeasts
C. tropicalis	2	1%	33.3% of yeasts
C. kefyr	1	0.5%	16.7% of yeasts
C. guilliermondii	1	0.5%	16.7% of yeasts

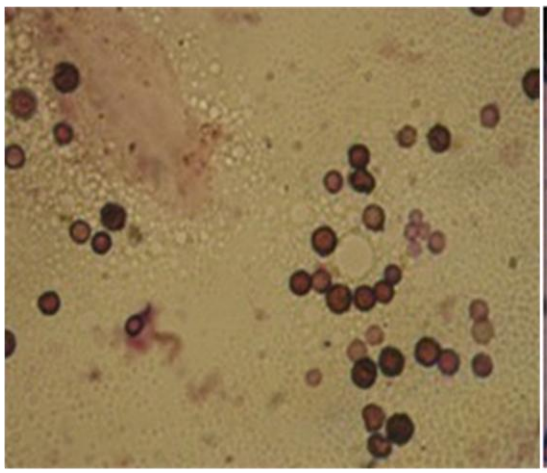

Figure 1:

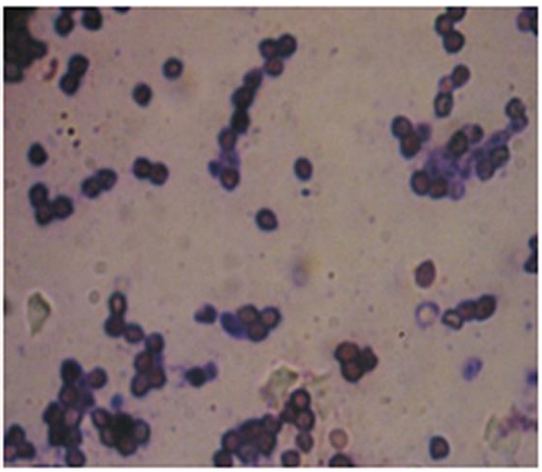

Figure 2:

Candida isolates showing spherical and oval vegetative cells with single and multiple budding

IV. Discussion

From the results, 15 yeast organisms were isolated; Candida species which formed 40% of the yeast isolates had a prevalence of 3% (Table 3) in 2000 dead-in–shell chick embryos within the hatchery. *Candida albicans* and *C. tropicalis* with 33.3% were the most frequently isolated yeast organisms in the samples (Tables 1 and 3). Both organisms fermented and also assimilated maltose (Table 2). The ability to ferment maltose shows that they possess uptake mechanism that involves two systems; an energy-dependent maltose permease (ATP ↔ ADP) which transports the maltose intact across the cellular membrane and a maltase (alpha-glycosidase) which hydrolyses maltose internally to yield two glucose units [22]. Thus, the mechanism is mediated genetically by three maltose utilization genes (MAL genes) which are involved in the operation of high-affinity maltose transporter [23]. A positive assimilation reaction is usually indicated by the presence of growth. The ability of yeast isolates to ferment sugars with production of ethanol and carbondioxide is a vital physiological characteristic for specie identification [24]. The organisms also metabolise xylose showing that the srains possess xylose reductase and xylitol hydrogenase genes responsible for xylose fermentation [25].

The yeast isolate from pooled sample number 77 was found to be *C. guilliermondii* (Table, 1 and 2). The organism utilized trehalose and this showed that the yeast cell has the gene responsible for synthesis of trehalose enzyme because the breakdown of trehalose to glucose is mediated by trehalose enzyme and both synthesis and degradation are regulated via cAMP [26]. *Candida albicans, C. tropicalis* and *C. kefyr* isolates did not assimilate trehalose indicating that they lack this gene. The yeast isolates employed different sugars as their main carbon and hence energy source.

The isolated yeast strains were able to grow at elevated temperatures slightly higher than room temperature (Table 2). The survival of the wild yeast strains obtained in this study grown at physiological temperatures (room temperature) and then subjected to mild heat shock (35-37) and lethal heat shock (42 ^{0}C) agrees with the report of Piper [27], that prior induction of heat shock responses enables cells to survive subsequent exposure to lethal high temperatures.

Candida organisms may penetrate the deep organ tissues including the kidneys, heart, digestive tract, lungs, eyes and brain through hematogenous and lymphatic dissemination [8]. Invasive Candida infection is more pronounced in immunologically compromised animals with lymphatic disease, chronic diabetics, and patients receiving prolonged cytotoxic drugs, steroids, broad-spectrum antibiotics, transplant recipients, cancer patients, Acquired immune deficiency syndrome (AIDs) patients, leukemia patients and others that have undergone open-heart surgery [28]. Candidiasis could affect warm blooded animals e.g. pigs and even the mammary gland of dairy animals. *Candida species* were also reported to have infected birds, producing lesions in the oral mucosa, oesophagus and crop [16]. Affected birds exhibited decreased feed intake, delayed crop emptying, reduced growth, depression and poor feathering. In some acute infections, mortality as high as 75% was recorded even when clinical manifestations were absent in young chickens and turkey poults [16].

Identification and characterization of yeast species have been based on morphological, physiological and biochemical properties [29]. Fungal infections present serious health challenges to treatment in man and animals generally due to non-availability of a novel antifungal drug. Amphotericin B, a highly toxic agent remains the 'gold standard' for treating disseminated life-threatening fungal infections [30]. Candida organisms have been recovered from the soil, hospital environments, inanimate objects, and food. The normal residence of *C. albicans* is the vagina, bowl, mouth and skin of man. *C. tropicalis* was obtained from seawater, sea sediments, marine fish and algae, shrimps, mangrove plants, various fruits, faeces and soil [31].

Optimum temperature and relative humidity are critical parameters necessary for fertilized poultry eggs to hatch. The internal temperature of the eggs is the most important physiological factor that affects the development of the chicken embryo. The developing embryo hatches earlier if it is incubated at high temperatures up to a maximum of 39 ^{0}C. However, a continuous 37.5 ^{0}C gives the best rate of embryo survival [32]. A relative humidity of 61% often gives the correct rate of loss of water, but other variable factors such as shell porosity, air movement and differences between strains can influence this. Fertile chicken eggs require an optimum temperature and relative humidity to hatch at 21 day of incubation. Embryonic death in poultry eggs is of great economic loss due to decreased productivity on hatch-day. Dead-in-shell chick embryos deprive farmers, consumers and scientists who depend on hatcheries for supply of day–old chicks for restocking and research purposes. Fertile eggs get infected with pathogens due to contamination. Contamination-promoting factors include lack of hygiene in the nests, presence of eggs on the floor, incubation of dirty eggs, or eggs with egg shell defects, and collection of dirty and clean eggs at the same time [5]. Poor fertile egg storage conditions, poor egg disinfection and high humidity levels during incubation may also promote low hatchability. Bacterial infection of chick embryos from egg contamination is also a major cause of reduced hatchability, early chick mortality and production losses [33].

Two isolates of *C. tropicalis* were detected in pooled samples (79 and 144) of dead-in shell chick embryos. The organism is associated with pyelonephritis, lower urinary tract infections, thrombophlebitis, arthritis, bursitis, meningitis,multiple organ infection, pericarditis, and candidal vulvovaginitis [31]. *Candida kefyr* (previously called *C. pseudotropicalis*) and *C. guilliermondii* formed a respective 16.7% of yeasts isolated from the dead-in-shell poultry eggs (Table 3). *Candida kefyr* was reportedly recovered from dairy products and clinical cases involving oesophagitis, nosocomial blood stream infections in neutropenic leukemia patients [34]. *C. guilliermondii* is a rare cause of invasive candidiasis, skin and soft tissue infections, onychomycosis, osteomyelitis, nosocomial catheter-related fungemia, and haematologic malignancies [35].

Candida albicans reportedly induced injurious damage to chorioallantoic membrane (CAM) of poultry eggs leading to death of chick embryos [17]. Some bioactive components of eggs are known to ensure the survival of the progeny. Ovalbumin, ovaglobulin G3 and ovaglobulin G2 are reported to be associated more or less intensively with high hatchability and low mortality [36]. Transferrin transports iron to storage cells and to protect embryo from bacterial infections [37] as well as complement the host innate immune defensive system [38]. Most reports on the effects of yeasts in poultry were related to spoilage of fresh and processed poultry products [39,2].However, fungal infection of poultry eggs occurs from contamination; eggs could be contaminated in the breeder farm, during transportation or in the hatchery [40].

Two isolates from dead-in-shell chick embryos were detected with germ tubes and were confirmed to be *C. albicans* (Table 2). A germ tube is a filamentous extension from the yeast cell, about half the width and three to four times the length of the cell. Only *C. albicans* produces germ tubes within three hours when cultured. Because germ tubes develop quickly, they are used as a rapid presumptive diagnostic identification of C. albicans, usually within 90 minutes [18]. Germ tube formation is believed to contribute to pathogenicity of *C. albicans* [41]. *C. albicans*, as an opportunistic, dimorphic fungus, is associated with a wide variety of disorders. These include fungal thrush, candidal enteritis, endocarditis, vulvovaginitis and urinary tract candidiasis [42], as well as mucocutaneous and invasive candidiasis [43]. The preponderance of *C. albicans* in causing a fatal disease places emphasis on early identification of the organism in clinical specimens. Certain essential growth nutrients required for germ tube formation such as platelet-derived factor [31], haemin, glutamine [44], and some amino acids [45] are likely to be present in sufficient amounts in human and animal host or poultry egg.

V. Conclusion

The study found a low level (3%) of Candida incriminated dead-in-shell chick embryos within NAPRI hatchery facility in Zaria, Kaduna State, Nigeria. The infection is a contributory factor to reduced egg hatchability and production losses in poultry farms.

VI. Recommendation

There is need for increased awareness, sound hygienic practices and efficient biosecurity measures against fungal contamination of eggs in poultry establishments in Nigeria.

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