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FUNGAL PROFILE OF EGGSHELLS FROM COMMERCIAL AND FREE-RANGE HENS OF A SUPERMARKET CHAIN IN FORTALEZA, CE, BRAZIL

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Abstract

Eggs are perishable and fragile foods because of their easy contamination by different microorganisms, such as fungi. Hence, research on egg safety and quality has been recently developed worldwide to minimize illness transmitted by this food, although most present mainly pathogenic bacteria. Thus, this study identified potentially pathogenic fungal species in eggshells from free-range and commercial hens offered in supermarkets in Fortaleza, CE, Brazil. The research was performed at the Vettings laboratory at the State University of Ceará (UECE) with 40 eggs: 20 from commercial and 20 from free-range hens. The eggshells were assessed using the dextrose evaluation method for pathogenic cultivation, commensal, and yeast fungi. Subsequently, readings were taken to identify and count the fungi on a binocular light microscope in 100x increments. Both egg categories showed a significant number of colony-forming units (CFU). The study identified eight different fungal species in both egg production types. The most prevalent fungal colonies in the free-range hen lineage (brown shell) were yeast spp. and *Penicillium citrinum*, and the laying hen lineage (white shell) had mostly *Penicillium* spp. and yeast spp. Therefore, both egg production systems showed similar fungal contamination, which suggests that the manipulation and/or storage are adequate despite the lack of strict food safety regulations or supervision for eggs in Brazil.

Keywords: Contamination. Cultivation. Food safety. Fungi. Yeast.

1. Introduction

Eggs are one of the most complete human foods due to various essential nutrients, such as minerals, vitamins, fatty acids, and proteins (Ghasemi et al. 2022). Egg consumption has gained increasing interest worldwide over the last decades, mainly among families with low purchasing power because of easy access, commercial availability, cost-effectiveness, and easy/diverse preparation modes of this food (Henchion et al. 2021; Walker and Baum 2022). Despite these benefits, eggs for human consumption are most often available without adequate antimicrobial treatments (Kraus et al. 2022) and may present damaged shells. Unfortunately, that is a reality in several regions of Brazil where negligence persists due to a lack of knowledge or proper management of eggs intended for trade. Additionally, several commercial establishments report irregularities regarding damaged and/or expired products (Cardoso et al. 2021). Thus, new strategies must be developed to minimize pathogenic microorganism loads carried into this food, ensuring product safety for the human diet. Associated factors (e.g., the choice of laying hens, feed quality, raising mode, and farm environment) are also relevant for egg quality (Rakonjac et al. 2021).

Torres and collaborators (2022) claim that foods submitted to adequate antimicrobial treatments are safer for human consumption. However, egg industry workers (from collectors to marketing professionals) must pay attention to shell integrity, disinfection methods, and storage. These steps guarantee longer durability/shelf life and consumption safety. However, food biosafety recommendations inherent to the production and marketing of chicken eggs are often not followed correctly or disregarded, allowing the proliferation of countless microorganisms, many of which are pathogenic to humans. Among the fungi species identified in chicken eggs are *Allscheria* ssp., *Alternaria* spp., *Aspergillus* spp., *Chaetomium* spp., *Cladosporium* spp., *Curvularia* spp., *Fusarium* spp., *Helminthosporium* spp., *Mucor* spp., *Penicillium* spp., *Pullularia* spp., *Rhodotorula* spp., *Scopulariopsis* spp., *Stemphylium* spp., and yeast (Tomczyk et al. 2018; Behnamifar et al. 2020; Demjanová et al. 2020; Regecová et al. 2020).

Mycotoxins, secondary metabolites of certain fungal species, are produced under appropriate biotic and abiotic conditions, especially in cases of inadequate food storage, usually causing mycotoxicosis after ingesting these previously contaminated foods. Among various mycotoxins, specific ones are dangerous if consumed directly from food, including aflatoxins, trichothecenes, zearalenone, fumonisins, ochratoxins, and patulin (Chen et al. 2021; Ahmad et al. 2022). These toxins may occasionally lose their toxicity, but in some cases, they change their molecular structure and provide higher toxicity, forming more toxic metabolisms. Thus, sensitivity or immune responses depend on factors such as age, exposure to the toxin, absorbed amount, breed, and nutritional factors.

Professionals and regular consumers of chicken eggs must acknowledge that microorganisms in this product may behave as opportunistic pathogens. Food with signs of pigmentation, cracks, and color changes should be avoided. Therefore, this research identified the microbiota fungus in eggshells from free-range and commercial hens from supermarket chains in Fortaleza, CE, Brazil.

2. Material and Methods

Forty eggs were previously divided into two groups and evaluated for the prevalence of fungal contaminants: (I) 20 eggs from a laying hen lineage (white shell) and (II) 20 eggs from a free-range hen lineage (brown shell). All eggs were purchased from supermarket chains in Fortaleza, CE, Brazil. These chains were randomly selected, and one unit was allocated to each group. Eggshells cracked/broken, without certification of origin and matrices, or not stored in packed boxes were excluded. The study was conducted in the Veterinary Microbiology Laboratory at the State University of Ceará, Fortaleza, CE, Brazil.

All egg packages (both shell categories) were carefully manipulated in laminar flow and near the Bunsen burner. Immediately after opening the packages, swabs dampened in sodium chloride (NaCl) solution (0.5%) collected potentially pathogenic fungal species on shell surfaces. All samples were cultured in Petri dishes containing semi-solid Sabouraud dextrose agar (SDA) medium at 25°C for seven days. The pour-plate method evaluated mold and yeast profiles to obtain the total colony-forming units per gram (CFU/g). All CFUs were transferred to a new sterile SDA medium using a platinum loop for isolation and identification. The cultures remained at 25°C for five days. Then, the samples were removed and placed on a slide with a drop of lactophenol, and a coverslip was put over it. The readings were performed under a light microscope with a 100x objective. Fungi identification included analyzing their reproductive structures.

The total fungal colonies were grouped according to species. The study also analyzed these colonies using descriptive statistics based on differences in the fungal species identified in the eggshells of both studied groups. Finally, a comparative analysis was performed for the differences between the number of total colonies identified in the two groups.

3. Results

CFU counts evaluated the identification and counting of fungal colonies and are presented in Table 1. Both categories in this study showed a significant CFU number. Four different fungal species were identified: *Aspergillus niger*, *Candida sphaerica*, *Penicillium citrinum*, and *Mycelia sterilia*. Other taxonomic groups, including *Aspergillus* spp., *Candida* spp., *Penicillium* spp., and Yeast spp., were also seen in both eggshell groups. The most prevalent colonies in free-range hens' eggshells were yeast (CFU = 67) and *P. citrinum* (CFU = 51), and the laying hens' eggshells showed mostly yeast species (CFU = 42) and *Penicillium* species (CFU = 81).

4. Discussion

Decree #1 of February 21, 1990, from the Brazilian Ministry of Agriculture, Livestock, and Supply does not establish minimum tolerance standards for mold and yeast count in natural eggshells. However, based on the identified fungal species/groups and colony prevalences (Table 1), the hygienic conditions for handling (collection, cleaning, storage, and transportation of eggs) are precarious, as observed in all studied samples. Moreover, eggs may be colonized by the natural microbiota of hens (Damena et al. 2022). Dust, feces, and cracked shells, even when still in closed packages, seem common fungal dissemination forms (Kulshreshtha et al. 2022; Oliveira et al. 2022; Perić et al. 2022) depending on the microorganism nature and the egg storage environment.

Table 1. Fungal profile and the total colonies identified in eggshells from free-range and laying hen lineages.

Free-range hen lineage (brown shell)		Laying hen lineage (white shell)	
Fungal	Colonies found	Fungal	Colonies found
Aspergillus niger	1	*Aspergillus* ssp.	3
A. niger/Candida sphaerica	2	*C. sphaerica*	1
Aspergillus spp.	12	*Yeast/Penicillium* spp.	3
C. sphaerica	22	*A. niger/Candida* spp.	31
C. sphaerica/Penicillium spp.	10	Yeast spp.	42
Yeast spp.	67	*Mycelia sterilia*	8
Penicillium citrinum/C. sphaerica	10	*Penicillium* spp.	81
P. citrinum	51	*Penicillium* spp./Yeast	14

Eggshell cleaning methods, such as single wash or sanitization, have been extensively discussed in recent years regarding their effectiveness in eliminating pathogenic microbial loads, although this is a general practice. Overall, using these processes is more compelling commercially because they are dirt-free. Different microorganism species are found even after egg sanitization, but at lower concentrations than in eggs not submitted to this process (Liu et al. 2022). Microorganism pools in eggshells after washing or sanitizing treatments stand out: coliforms spp., mesophiles spp., bacteria (Segundo et al., 2020), and filamentous and yeast fungi (Chousalkar et al. 2021), according to the predisposition of specific species to imposed environmental conditions. Filamentous fungi in eggshells often induce changes in the internal chemical composition of eggs, such as coagulation, liquefaction, or taste changes. After fungi spp. dissemination, the eggs usually show an exacerbated pigmentation in external, internal, or both eggshell regions. Storage conditions and humidity of eggs may favor the proliferation of mucoid fungi on the eggshell surface, with rapid growth within and constituent decomposition.

All fungi species identified in this research may trigger pathogenic processes in humans. Aspergillosis, a set of illnesses caused by members of the *Aspergillus* genus, is characterized by opportunistic infections that mainly affect the lower respiratory tract after inhaling conidia. Conidias quickly develop into hyphae and migrate to blood vessels, where they may cause hemorrhagic necrosis and/or infarction (Okamura et al. 2022). The invasive character of aspergillosis may induce damage from the fungus or through an exaggerated inflammatory response of the host, depending on the underlying immune status of such host. Symptoms such as asthma, pneumonia, and sinusitis (Kahraman et al. 2022; Sabino 2022) seem recurrent pathological processes. Cerebral vasculitis in HIV-positive patients (Gao et al. 2022) and atypical oral and pulmonary infections in COVID-19 patients (Fiema et al. 2022), both caused by *A. niger*, were recently reported. Thus, the high mortality rate in immunocompromised patients with a positive diagnosis of a pulmonary illness may be maximized by *Aspergillus* infections. Clinical conditions, such as leukoencephalomalacia, liver diseases, and tumors associated with mycotoxin fumonisin synthesis by *A. niger* were also documented (Smith 1996; Sikandar et al. 2022).

P. citrinum was related to acute fibrinous and organizing pneumonia (Zhao et al., 2020), a rare clinicopathological condition characterized by an uncommon variant of acute lung injury with intra-alveolar fibrinous and organizing pneumonia. Another study found that *P. citrinum* and *A. niger* synthesize ochratoxins (named OT_A, OT_B, and OT_C) naturally (Sikandar et al. 2022). Among ochratoxins, the OT_A prevails and is the most lethal (Duarte et al. 2010) due to a strong nephrotoxicity and potential ability to induce phenomena such as hepatotoxicity, immunotoxicity, neurotoxicity, teratogenicity, and carcinogenicity (Beardall and Miller 1994).

M. sterilia is another fungus species identified and isolated in this study, representing an opportunistic fungus similar to *A. niger* and *P. citrinum*. *M. sterilia* has been associated with subcutaneous infections in transplant patients, and these cases are increasingly reported mainly due to the capacity of these infections to spread through blood and visceral pathways (Santos et al. 2013). *M. sterilia* infections have induced respiratory allergies (asthma and rhinitis) (Żukiewicz-Sobczak et al. 2013; Menezes et al. 2004) and keratomycosis.

However, non-albicans *Candida* spp., such as *C. sphaerica*, seem commonly capable of bloodstream infections in patients under hospital care and are more resistant to antifungal drugs (Gautam et al. 2022). *Candida* spp. is associated with a high prevalence of psoriasis (Elsner et al. 2022), a chronic and non-contagious skin disease characterized by pink or reddish patches covered with whitish scales. Other clinical conditions associated with *Candida* spp. are pneumonia (Meena and Kumar 2022), endocarditis (Sanku and Youssef 2022), denture stomatitis, thrush, urinary tract infections, meningitis (Giotaki et al. 2022), esophagitis (Chen et al. 2022), peritonitis (Chamroensakchai et al. 2023), and infectious arthritis (Han et al. 2022), but it may also cause more serious systemic diseases.

Therefore, consuming raw (e.g., eggnog) or semi-cooked eggs and ground eggshells as a calcium food supplement may trigger different illnesses of fungal etiology. Thus, this study indicates immediately implementing the norms/conducts by the Brazilian Health Surveillance Agency (ANVISA) to recommend/guide the safe manipulation of eggs in all instances (from production to marketing) to minimize microorganisms in this product, considering the consumption of this product *in natura* is usual in Brazil. Unfortunately, farm managers lacking basic food safety knowledge are commonplace as they promote unsuitable egg storage/maintenance, often under humidity, non-recommended temperatures, and environmental dirt. Another aggravating factor is the feed quality submitted to laying hens, as these usually carry mycotoxin-producing fungi. Thus, the animals in daily contact with the feed contaminated by mycotoxins may be vertically/horizontally transferring these toxins to eggs and via the food chain, damaging human health.

5. Conclusions

The fungal contamination between eggs from free-range and commercial farming systems presented similar values for the identified fungal colonies, possibly due to equivalent management techniques used from egg collection to storage for commercialization. The most prevalent contamination occurred with the genus *Penicillium* and yeast spp.

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