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RESEARCH ARTICLE

Evaluation of microbial contents of table eggs at retail outlets in Sokoto metropolis, Nigeria

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Abstract

This study was carried out to evaluate the microbial contents of chicken eggs, sold at retail outlets in Sokoto metropolis. A total of 160 eggs were collected from 16 randomly selected retail outlets, in Sokoto metropolis, for microbial evaluation. Samples were cultured and isolated using nutrient and McConkey agar for bacteria while Sabouroud dextrose agar was employed for fungus and identified using Harvey and Green Wood method. All the 160 (100%) samples were positive for bacteria (nine different genera); while 104 (65%) egg shells were positive for fungi isolation from the genus *Aspergillus*; however, evaluation of the egg contents revealed 95(59.4%) positive for bacteria isolations from seven different genera and 86(53.8%) positive for fungi isolations from only one genus *Aspergillus*. The bacterial genera include *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Corynebacteria*, *Proteus* spp *Bacillus* spp *Staphylococcus* spp *Streptococcus* spp and *Klebsiella*. The only fungal genus was *Aspergillus*, which were identified to be *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*. The eggs from these areas should therefore be taken with caution and the public should be educated on the dangers associated with consumption of raw and under cooked eggs and egg products, retailers should be encourage to store their eggs in refrigerators and practice good hygiene in order to prevent microbial growth on the eggs.

Keywords: Bacterial isolation, Microbial contents, Retail outlets, Sokoto, Table eggs.

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Introduction

Food-borne diseases caused by micro-organisms are a large and growing public health problem (Casey *et al.*, 2012). Contamination of eggs and egg products with microorganisms can affect egg quality, which may lead to spoilage and pathogen transmission. This may induce food borne infection or intoxication to consumers.

Today, eggs remain a staple food within the human diet, consumed by people throughout the world. They are consumed worldwide in the form of pastries, stews and beverages and are considered very nutritious and a cheap source of protein

(Blumenthal, 1990; Papadopoulou *et al.*, 1997; MAFF, 2000). Though eggs are considered as complete food for growth and sustenance, studies indicate that micro-organisms often contaminate eggs (Abdullahi 2010). Freshly laid eggs are generally devoid of organisms. However, following exposure to environmental conditions for example, soil, faeces and dirty nesting materials, eggs become contaminated with different types of micro-organisms (Ellen *et al.*, 2000; Smith *et al.*, 2000). Furthermore, these microorganisms may contaminate the egg contents either by penetration

or withdrawal through pores of the shells (Harry, 1963; Schoeni *et al.*, 1995) and also through the transovarian route (Bruce & Dysdale, 1994). Predisposing factors such as environmental temperature and humidity influence the bacterial penetration thus enhancing infection and spoilage (Frazier & Westhoff, 1987; Theron *et al.*, 2003).

Several pathogenic micro-organisms have been isolated from the surface of chicken egg shells and contents. Among them, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter* spp (Leasor & Foegeding, 1989; Chiesa *et al.*, 1991; Schoeni & Doyle, 1994; Hope *et al.*, 2002; Adesiyun *et al.*, 2005). Other pathogens are fungal organisms *Aspergillus*, *Penicillium* etc (Neamatallah *et al.*, 2009). Aflatoxins produced by some species of fungi contaminate a vast array of food and agricultural commodities. Such mycotoxins pose profound challenges to food safety in many countries, especially in tropical and subtropical regions where temperature and humidity are optimum for growth of moulds and production of toxins. The possible transmission of such toxic residues to edible eggs results in potential hazards to human health (Martin *et al.*, 1998).

Agriculture contributes to the Nigerian economy providing employment for 70% of the population (Omobowale *et al.*, 2009). The sector is being transformed by commercialization at the small, medium and large-scale enterprise level (Omobowale *et al.*, 2009). In this sector, animal rearing especially commercial poultry production plays an important role in the creation of jobs, generating income and also provides food in the form of meat and eggs.

Due to the worldwide consumer demand for eggs, periodic assessment is required to offer safe and good quality eggs for consumption particularly in Nigeria, where the environmental condition combined with poor hygiene that characterizes poultry or egg production favors survival and proliferation of micro-organisms.

This study was conducted to assess the microbial quality of shell surface and of the internal contents of chicken eggs displayed for sale in various locations of Sokoto metropolis, Nigeria.

Materials and Methods

Sampling

One hundred and sixty (160) fresh table eggs were collected randomly from sixteen (16) retail outlets in

Sokoto metropolis. A total of ten (10) eggs were collected from each retailer by systematic random sampling which involved selecting five crates of eggs and selecting the fifth and tenth egg in each of the five crates selected. The sampled eggs were taken to the microbiology laboratory of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto in a batch of twenty eggs for bacterial and fungal analysis of both egg shells and contents.

Preparation of media

Nutrient agar and McConkey agar media were utilized for isolation of bacterial pathogens. Sabouroud dextrose agar media was used for isolation of fungal organisms after incubation for 3-5 days at room temperature. All the media were prepared following the manufacturer's instruction and sterilized by autoclaving at 121°C for 20 minutes (Osei-Somuah *et al.*, 2003).

Cultures from egg shell surfaces

For the culture of bacteria from the surface of the egg shells, cotton swab stick, on each occasion was moistened in 0.1% peptone water and used to swab the surface of the egg shells after which the swab was transferred to the two media. After smearing out on each of the plates, they were then incubated at 37°C for 24 hours.

Cultures from egg contents

In culturing of the content of eggs, surface of each of the egg was disinfected with 70% ethanol. For each of the selected egg, a sterile spatula was used to create an opening into the egg and the content was thoroughly mixed. A sterile needle and syringe was then introduced into the content and 0.1 ml of the mixture of the egg yolk and the white was drop on each of the media plate and was spread using sterile swab stick.

Identification of organisms

After incubation, the method of Harvey & Green Wood (1985) was used for the identification of the organisms. Morphological characteristics (such as size, shape edge elevation, consistency, color changes on various media); Standard microbiological techniques, such as cellular morphology and staining, among others, were used to identify the organisms isolated. Bacteria culture were stained using Gram staining techniques which differentiate isolates into Gram positives or Gram negatives bacilli, cocci or coccobacilli, spore formers or non

spore formers. Stained organisms were examined for Gram reaction using light microscope at x100 magnification with oil immersion.

Fungi were identified by colonial and cellular morphology using lacto-phenol cotton blue staining technique. Biochemically, tests such as citrate utilization, methyl red, urease test were carried out (Holt, 1994). Sugar test such as glucose, sucrose, mannitol, lactose and sorbitol were used (Clarke, 1974). Catalase test was used to differentiate between Staphylococci and Streptococci. Coagulase test was used to differentiate *Staphylococcus aureus* from other *Staphylococci* isolates.

Determination total viable count (TVC)

For total viable count, the pour plate method was used. One (1) ml each of the mixture of the egg white and the yolk was diluted serially, using ten (10) fold dilution factors. Ten test tubes were used, each containing 9ml of sterile distilled water different pipettes were used for various dilution. One (1) ml of dilution was aseptically transferred into sterilized Petri dishes after 10 ml of molten nutrient agar was poured into sterilized Petri dishes. It was mixed by

gentle rotation and was allowed to cool to solidify after which it was incubated at the temperature of 37°C for 24 hours. Plates showing colonies between 30 -300 were selected and counted using electronic colony counter.

Data analysis

Data generated were collated and analyzed using descriptive statistics (George & William, 1989).

Results

The results from this study revealed that all of the 160 (100 %) sampled from the 16 randomly selected retail outlets had their shells contaminated with microbe of different genera; however, only 95 (59.4%) growths were observed from the egg contents (table 1). The following bacteria genera were isolated from the samples from the sixteen retail outlets. The bacteria genera were identified as *Escherichia coli*, *Staphylococcus spp*, *Streptococcus*, *Bacillus*, *Salmonella*, *Klebsiella*, *Corynebacteria*, *Proteus* and *Shigella* and only one fungal genus *Aspergillus* was isolated.

Table 1: Number of positive samples for bacteria and fungi isolate from (160) Shells and contents of table eggs

Microbial agents	Egg shell	Egg content
Bacteria	160(100%)	95(59.4%)
Fungi	104(65%)	86(53.8%)

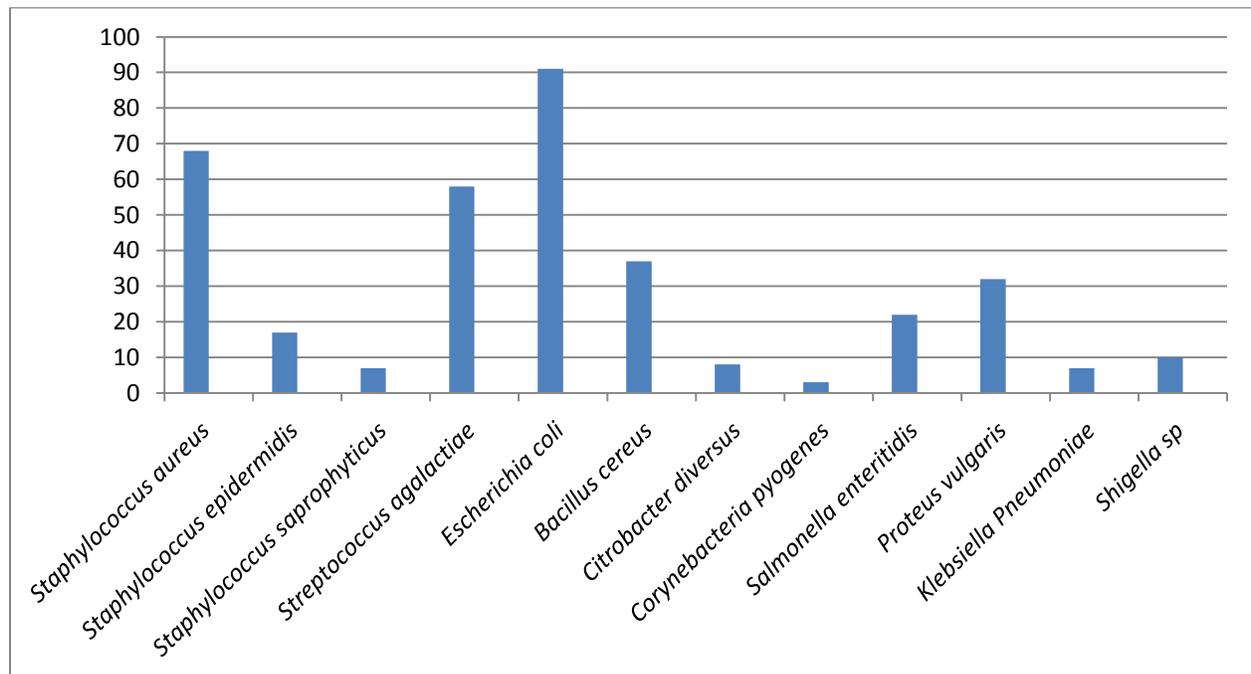


Figure 1: Bacterial organisms identified from shell surfaces of sampled eggs

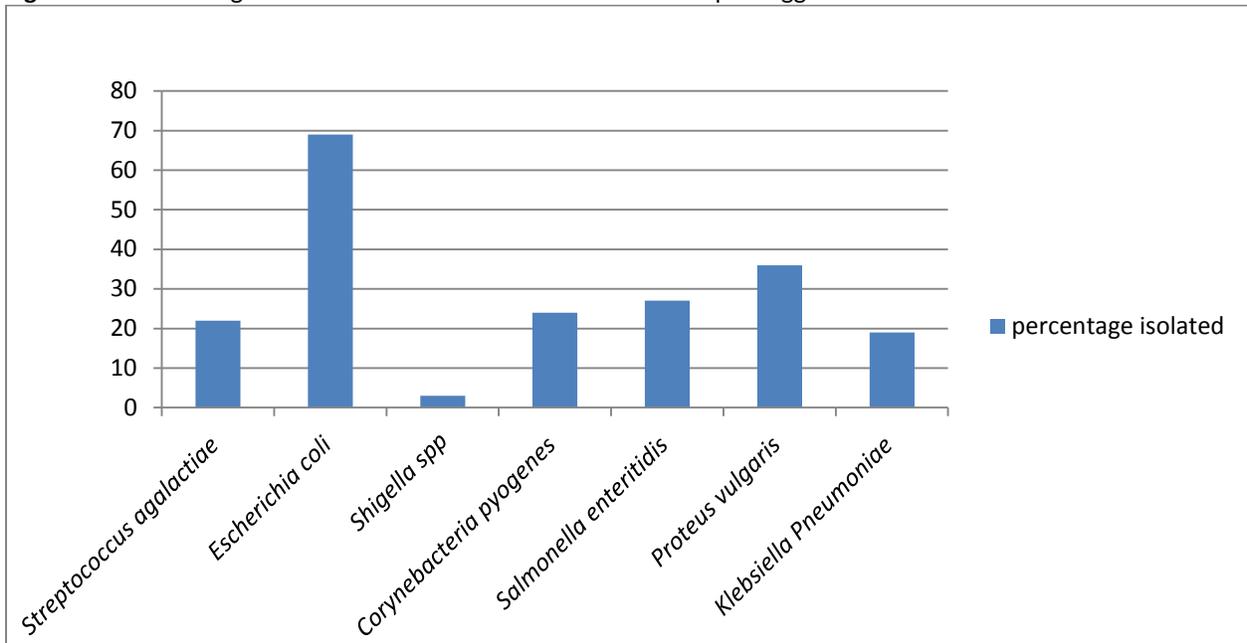


Figure 2: Bacterial organisms isolated from contents of sampled eggs

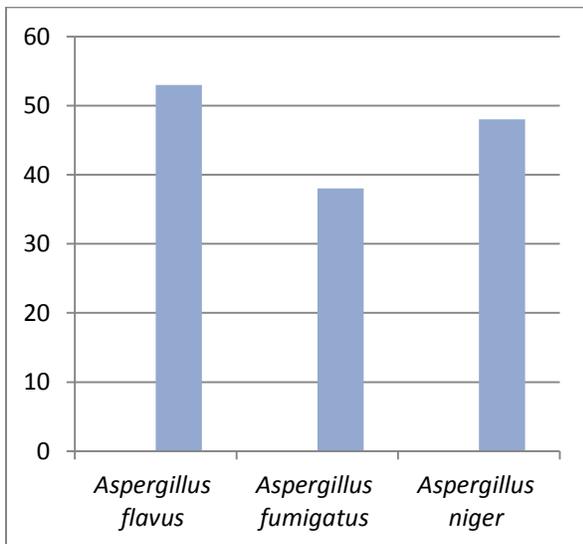


Figure 3: The Fungal organisms isolated from shell surfaces of sampled eggs

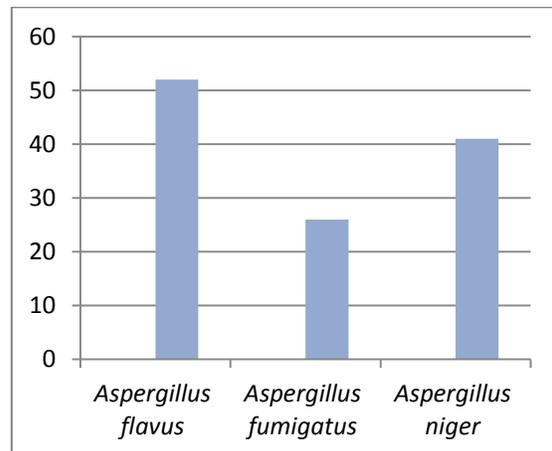


Figure 4: Fungal organisms isolated from the contents of sampled eggs

Discussion

The study was undertaken to evaluate microbial quality of table eggs sold for human consumption in Sokoto. The major contaminants are Gram negative bacteria belonging to the family enterobacteriaceae, although *Staphylococcus*, *Streptococcus* and *Bacillus spp* were also isolated in large percentage in addition to *Aspergillus spp*. This is in agreement with Hang' Ombe *et al.* (1999), who reported microbial

contamination of chicken eggs with predominantly members of the family enterobacteriaceae. These microbes were isolated from the shell surfaces and the contents of the egg they sampled. However more organisms were isolated from shell surfaces. This agrees with USDA (2011), that micro-organisms can be found on the outside and inside of the egg shell. This may be due to the fact that the egg

emerges from the hen's body through the same passageway the faeces is excreted, micro-organisms inside an un-cracked egg or intact egg may be due to the presence of pathogen within the hen's ovary or through oviduct, before the shell forms around the yolk and albumin. Faecal contaminants could also occur through the pores on the shell after they are laid. Ansah *et al.* (2009), reported that, as eggs stay longer, their resistance reduce enabling these organisms to penetrate into the egg content. Warm and moist litters, poor condition in the farm houses were reported to be sources of fungi growth and sporulation (Abdullahi, 2010).

Several factors have been implicated in egg contamination. Among these are faeces of the birds, litter material, improper handling of the eggs by retailers, unhygienic conditions of the markets where these eggs are being sold, contaminated egg crates, packing and poor storage method (Bruce & Drysdale, 1994).

Others are cloths and hands of poultry workers, the environment, dust transporting marketing and weather condition. Eggs in many stores were exposed to high temperature and low humidity which favors the growth of microbes, especially fungi and hence result into rapid decrease in the quality of eggs.

Among the microbes isolated from the egg samples were *Staphylococcus species*, *Streptococcus species*, *Salmonella spp*, *Shigella spp*, *Proteus*, *Klebsiella*, *Citrobacter*, *Corynebacterium*, *Bacillus spp*, *Escherichia coli*, and a fungi genus, *Aspergillus* of which three species were identified; *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*.

Escherichia coli is the major micro-organism isolated both from the surface and in the content of the egg in the study, this may be attributed to the fact that *Escherichia coli* are normal inhabitants of intestinal tracts of birds (Singleton & Sainsburg, 1981). They have also been known to contaminate the surface of egg while the mechanical process can spread the bacteria through the eggs. Contaminations with the pathogen while in the field occur through improperly decomposed manure and poor hygienic practice of farm workers. *Escherichia coli* can bring about urinary tracts infections, pneumonia meningitis and peritonitis in humans (Schoeni & Doyle, 1994).

Staphylococcus and *Streptococcus* which were isolated are frequently implicated with faecal contamination. This has a serious zoonotic implication since species of these organisms cause

diarrhea and fever in man and animals (Riley *et al.*, 1979).

Isolation rate of *Salmonella spp*, in this study is 22(6.11%) and 27(13.50%) from the shell surfaces and the contents of the egg samples respectively. The high number of isolates from the contents may be due to the organism's presence in the ovary or oviducts before the shell forms around it. This agrees with (Kinde *et al.*, 2000; WHO/FAO, 2002; Van *et al.*, 2005). These authors reported that the bacterium infects the eggs by either vertical transmission during development of these eggs within the ovary or horizontal transmission through trans-shell contamination. *Salmonella* are potentially dangerous because of their ability to producing potent enterotoxin (Singh *et al.*, 2010). *Shigella*, *Citrobacter*, *Corynebacterium*, *Klebsiella* and *Proteus spp* have the potential of causing human disease condition (WHO/FAO, 2002) and this (*Shigella*) isolation from egg surface is a confirmation of contamination with human faeces.

The total isolate of *Bacillus* was 37(10.28%), and was isolated only from the shell surfaces of egg sampled this may be attributed to the fact that this organism is found everywhere in the soil. Also some poultry farmers practice of deep litter system of management where by these eggs are laid on the floor.

Members of genus *Aspergillus* were isolated and identified to be *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*. These were isolated from the shell surfaces and the contents of egg samples. This may be attributed to the fact that most retailers lack good storage facilities, most of them store their eggs in open room with high humidity which favors fungal growths. Presence of *Aspergillus* in the egg contents is of a serious health concern because the organism has the ability to cause disease condition called aspergillosis, which affects respiratory system.

Aspergillus have been isolated in otomycoses, onychomycoses, in Seborrhoeic and verrucous papillomatous skin changes. It can also cause aspergilloma. Involvement of central nervous system (CNS) is also possible. *Aspergillus flavus* can produce toxins. It also produces highly toxic carcinogenic aflatoxin B₁. (Hartmann & Rohde, 1980).

These findings underscore the need for optimum hygienic practices in farms in order to decrease bacterial load in commercial chicken eggs in Sokoto.

Marketers should also ensure good hygienic standard at various retail outlets as indicated with the isolation of *Shigella* specie which is a strictly human pathogen as this contamination is likely from human faeces. Hence

consumption of raw and under cooked eggs or egg

products should be discouraged.

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