MICROBIAL EVALUATION OF BOILED EGGS SOLD IN RETAIL OUTLETS IN ZARIA, NIGERIA

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ABSTRACT
One hundred and fifty samples of boiled eggs were collected from three designated sampling areas in Zaria, Nigeria, for the isolation of Escherichia coli and Staphylococcus aureus, widely known to be contaminants and in some cases pathogens in ready-to-eat foods. Total aerobic plate count was carried out using nutrient agar. The total aerobic plate count ranged from 1.0 x 10^9 to 7.1 x 10^{10} cfu/g. The average aerobic plate count was 1.8 x 10^{10} cfu/g. The samples were inoculated on Eosin Methylene Blue (EMB) and staphylococcus 110 agars and the susceptible isolates were subjected to standard biochemical tests and pathogenicity tests for proper biochemical characterization and identification of the pathogens respectively. The results showed that out of 7 suspected isolates of Escherichia coli and 9 of the suspected Staphylococcus aureus isolates, there were no Escherichia coli confirmed in all 7 (100%) isolates and 9(100%) was confirmed as Staphylococcus aureus. Therefore, the work shows that boiled eggs can be contaminated and that unsanitary, and unhygienic practices, during preparation and presentation especially cracked shell eggs for consumption can pose serious health hazards.

KEYWORDS: Escherichia coli, Staphylococcus aureus, boiled eggs, food safety.

INTRODUCTION

There is a growing tendency to eat in places other than the homes and food consumption in restaurants and public eateries (Kinton and Ceserani, 1986; Soriano *et al*., 2000), as well as road-side ready-to-eat foods which are convenient sources of nourishment available at reasonable costs and sold along road sides, market places, schools and at door steps in Zaria and many other towns in Nigeria (Raji, 2001).

Most of these establishments and places are implicated or considered (by the Health Authority or by consumers) as being implicated in foodborne disease outbreaks (Bryan, 1988; Hedberg *et al.,* 1993; Wieneke *et al.,* 1993; Igoe *et al.,* 2003).

Foodborne illness is a major public health problem in all developed and developing countries (Akbar and Anal 2014a; Akbar and Anal 2014b). In Nigeria, like most other public health foodborne disease outbreaks, have been attributed to high ambient temperatures, unhygienic surrounding and insanitary manner in which food is hawked and sold in Nigeria predisposes the food to contamination with foodborne micro-organisms (Williams, 1984).

Several foodborne micro-organisms are common contaminants in poultry and livestock products e.g. *Campylobacter* sp., *Salmonella* sp., *Staphylococcus* sp., *Escherichia coli* (Ghasemian, 2011; Akbar and Anal, 2013a). In case of *Salmonella* infection, egg products were identified as the major food vehicle for outbreaks (CDC, 2000) as well as for sporadic infections (Hedberg *et al.,* 1993; Morse *et al.,* 1994; Trepka *et al.,* 1999), though other harmful pathogens are capable of causing foodborne disease outbreaks.

Total Enterobacteriaceae count and total coliforms count are more frequently used to assess enteric contamination and commonly used in slaughterhouses as indicators of faecal as well as environmental contamination (Gonzalez and Domingues, 2006). However, total staphylococci count and Staphylococcus aureus counts, which are present on hand, mucous membrane and skin of man, birds and animals, are good indicators of poor personal hygiene, poor handling and temperature control (Rindhe *et al*., 2008).

Contamination of poultry products with foodborne pathogens remains an important public health issue, where many food poisoning bacteria contaminate chicken meat (Mbata, 2005), eggs and egg products (Kolo, 2009). Therefore, the present study aimed to evaluate the bacteriological quality of boiled eggs sold in retail outlets in Zaria, Nigeria.

METHODOLOGY

Study area: The study was carried out at the bacterial laboratory of the Department of Public health and preventive medicine, Faculty of Veterinary medicine, Ahmadu Bello University, Zaria. The institution is located in Samaru, a suburb

of Zaria Nigeria; situated on latitude 11^0 12" N and longitude 07^0 37" E, and located in the Northern Guinea Savannah zone of Nigeria.

Source of experimental samples: A total of one hundred and fifty samples of boiled eggs were purchased from three designated sampling areas in Zaria, namely: Samaru, Sabon-Gari and Zaria city. The samples were purchased from the sellers after usual packaging without any prior instruction on mode of packaging.

Laboratory procedure / analyses of sample: Ten grams of each of the boiled egg samples was aseptically weighed out using the weighing balance after sterilizing the egg shell with 70% alcohol using a clean cotton wool and then de-shelling with a sterilized forceps (Loongyai, *et al*. 2011). A sterilized pair of scissors was used to cut the products into small pieces and then homogenized with 90ml of 0.1% peptone water in sterile transparent polythene bags using a Stomacher machine (Stomacher L-B 400).

Total Aerobic Plate Count: Nutrient agar was used for the total aerobic plate count. A hundred fold serial dilutions were carried out using sterile pipettes to aspirate 0.1mL of the homogenate and 9.9mls of sterile physiological saline solution. Serial dilutions up to 10^{-9} were carried out. Aliquots of 0.1mL of the 10^{-9} dilution were usually inoculated on to nutrient agar plates and spread uniformly using sterile glass spreaders (Hockey sticks). The plates were incubated at 37^0C for 24 hours, after which the colonies were counted.

Isolation of *Escherichia coli*: About 0.1mL of the initial homogenate at 10^{-3} dilution was inoculated onto separate Eosin-Methylene Blue agar plates and spread using sterile glass spreaders. The plates were appropriately labelled and incubated at 37^0C for 24 hours. The plates were observed for colonies with characteristic greenish metallic sheen on Eosin-Methylene Blue agar plates. A loopful of the suspected *E. coil* was picked and sub cultured on nutrient agar slants for further confirmatory tests.

Biochemical Characterization: The suspected *E. coli* isolates were subjected to biochemical characterization using Triple Sugar Iron agar (TSI), Simmon's citrate agar, Sulphide Indole Motility agar (SIM), Urease agar and Methyl Red Vogues Proskaur broth for their various reactions.

Isolation of *Staphylococcus aureus*: A volume of 0.1mL of the initial homogenate at 10^{-2} dilution was inoculated onto Staph 100 agar plates using sterile glass spreaders. The plates were appropriately labelled and incubated aerobically for 24 hours at 37^0C. Growths which appeared as round, smooth creamy white to yellowish colonies were suspected as *Staphylococcus aureus* colonies. The colonies obtained were identified using Gram staining technique. The isolates were streaked on nutrient agar slants and stored in the refrigerator for further analyses.

Pathogenicity Test for *Staphylococcus aureus*: To confirm the presence of pathogenic *Staphylococcus aureus*, catalase and coagulate tests were conducted.

For the catalase test, a loopful of the growth was picked from the nutrient agar slant and emulsified with a drop or two of hydrogen peroxide on a clean glass slide. Appearance of bubbles of gas signified positive reaction to catalase test.

For the coagulase test, a drop of physiological saline was dropped on a clean glass slide with the aid of a sterile inoculating loop. The loop was then used to pick the growth of the suspected *Staphylococcus aureus* isolate from the nutrient agar slant. The isolates were emulsified in drops of normal physiological saline on clean glass slide. Two drops of human plasma were added and mixed with the emulsified isolate. The mixture was observed for clumping or agglutination.

Statistical analysis: Microbial counts (CFU/g) were represented as $\log_{10}$ CFU/g and Means were calculated. Microbial counts were compared by ANOVA using SPSS Software 13.0.

RESULTS AND DISCUSSION

Isolation of *Escherichia coli* from boiled eggs: Growths of suspected *Escherichia coli* were observed on 7(4.6%) out of the 150 samples cultured on Eosin-Methylene Blue. The growths had flat surface with blackish centre and a greenish metallic sheen.

Out of the 7 suspected *Escherichia coli* isolates, there were no positive results to confirm *E. coli* following the biochemical tests (Table 1).

Isolation of *Staphylococcus aureus* from boiled eggs: Growths of the suspected *Staphylococcus aureus* isolates were observed on 9(6%) out of the 150 samples (Table 2) cultured on Staph110 agar. The growths appeared round, smooth creamy-white to golden yellow with convex appearance. Gram staining of the suspected isolates revealed gram-positive cocci in clusters, like grapes, with purple appearance.

Catalase and Coagulase tests: All the 9(100%) suspected isolates were catalase positive, and

coagulase positive hence confirming the presence of *Staphylococcus aureus* (Table 2).

Total Aerobic Plate Count: The mean $\log_{10}$ total aerobic plate count ranged from 9.0 to 10.9 $\log_{10}$ cfu/g with an overall mean of 10.3 $\log_{10}$ cfu/g. The colonies were small to medium-sized, rounded and smooth, with a creamy to yellowish colouration on nutrient agar (Table 3).

Exogenic contamination of eggs (also termed horizontal contamination), by far more frequent than vertical contamination in terms of overall levels and diversity of bacteria, corresponds to the contamination of the egg (Baron and Jan, 2011) after the egg defense system such as cuticle, calcium egg shell and shell membrane (Jerzy and Dagmara, 2009; Chaemsanit *et al*., 2015) have been bridged.

Egg can be contaminated at both egg shell and egg contents by a variety of microbes including *Escherichia coli* and *Staphylococci aureus* (Board and Tranter, 1995; Ricke *et al*., 2001). *Staphylococci* are most common bacteria contaminating egg shells. Contamination is more likely linked with cracked egg, dirty shells and storage in contaminated surroundings. This agrees with our findings for the presence of *Staphylococci aureus* across all the sampled areas in Zaria.

From the study, the total aerobic plate count ranged from 1.0 x 10^9 to 7.1 x 10^{10} cfu/g and the average aerobic plate count was 1.8 x 10^{10} cfu/g. This agrees with other reported level of eggshell contamination by mesophilic aerobic microbiota ranges from $10^{3.8}$ to $10^{6.3}$ cfu/egg, with an average of around $10^{4.5}$ cfu/egg (Moats, 1980; Lucore *et al*., 1997; Favier *et al*., 2000; Jones *et al*., 2004; De Reu *et al*., 2005, 2006a; Musgrove *et al*., 2005). De Reu *et al* (2006a) also observed a predominance of *E coli* (5.5 x 10^4 cfu/egg), and *Staphylococcus* (4.3 x 10^4 cfu/egg), thus indicative of contamination of boiled eggs with aerobic microbes through various sources of contamination.

CONCLUSION AND RECOMMENDATIONS

This study shows that boiled eggs sold in Zaria, Nigeria, are capable of a relative contamination with foodborne micro-organisms which can pose a threat to consumer's health due to unsanitary and unhygienic locations as well as egg and egg product handlers are culpable to varying levels of contamination. It is recommended that:

1. Boiled eggs especially with a cracked shell should not be purchased and consumption of raw or undercooked eggs should be avoided so that they do not serve as medium for transmission of foodborne pathogens.
2. An enlightenment campaign should be carried out to educate food handlers and consumers on the need for proper personal hygiene practices to minimize contamination.

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Table 1: Isolation of *Escherichia coli* in boiled eggs sold in retail outlets in Zaria, Nigeria

	Sampling area	Total number of samples examined	Number of samples with suspected growth (cfu/g) and percentage	No. of samples positives for *E. coli*
1	Samaru	50	7(14%)	0
2	Sabon-Gari	50	0(0%)	0
3	Zaria city	50	0(0%)	0

Table 2: Isolation of *Staphylococcus aureus* in boiled eggs sold in retail outlets in Zaria, Nigeria

	Sampling area	Total number of samples examined	Number of samples positive for *S. aureus* and percentage
1	Samaru	50	4(8%)
2	Sabon-Gari	50	3(6%)
3	Zaria city	50	2(4%)

Table 3: Total Aerobic Plate count (TAPC) of boiled eggs sold retail outlets in Zaria, Nigeria

	Sampling area	Total number of samples examined.	Number of samples with growth observed (cfu/g), the percentage and Standard Deviation.	Mean $\log_{10}$ of TAPC (cfu/g).	Range of $\log_{10}$ TAPC (cfu/g).
1	Samaru	50	47(94%)± 16.3	10.3	9.3-10.9
2	Sabon-Gari	50	45(90%)± 17.3	10.3	9.0-10.8
3	Zaria city	50	49(98%)± 9.7	10.0	9.0-10.6

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