OCCURRENCE AND ANTIBIOGRAM OF *Salmonella* IN QUAIL EGGS SOLD WITHIN ZARIA AND KADUNA METROPOLIS, KADUNA STATE NIGERIA

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Abstract: Quail eggs are considered a delicacy which is consumed raw by most consumers with the aim of deriving all the nutritional and medicinal benefits. Salmonellosis is a major public health problem around the world, affecting both animals and humans. This study was carried out to elucidate the prevalence of *Salmonella* spp. and antibiogram of the isolates in quail eggs sold in major markets within Zaria and Kaduna metropolis, Kaduna state, Nigeria using standard isolation techniques for *Salmonella* species and the standard disk diffusion method for the antibiotic sensitivity tests. A total of two hundred and eighty (280) quail eggs were collected from 7 different markets, 3 isolates of *Salmonella* was obtained, and this gave a prevalence of 10.71%. The *Salmonella* isolates showed a hundred percent (100%) sensitivity to Sulphademethoxazole/ trimethroprim, Chloramphenicol, Streptomycin, Kanamycin, Tetracycline, Trimethoprim, Gentamycin and Ciprofloxacin. While all isolates showed a hundred percent (100%) resistance to Amoxicillin/ clavulanic acid, Nitrofurantoin and Penicillin G. The consumption of raw or improperly cooked quail eggs was discouraged, also regulatory control of antibiotics usage in quail egg production to ensure consumer safety was therefore recommended.

Keyword: Antibiogram, quail eggs, resistance, *Salmonella*, sensitivity

Introduction

Eggs and egg products are nutritious foods and they form an important part of the human diet. Consumption of raw eggs however, has been associated with negative health impacts. Eggs and egg products that are improperly handled can be a source of food borne disease, such as salmonellosis (Anon, 2004). The genus *Salmonella* consists of rod shaped, aerobic or facultative anaerobic, gram negative bacteria belonging to the Family *Enterobacteriaceae* (Holt *et al*., 1994). *Salmonella* Typhimurium and *Salmonella* Enteritidis are the commonest causes of non-typhoidal salmonellosis in humans (Acheson & Homann, 2001). It is estimated that non-typhoidal *Salmonella* causes 1.2 million illnesses a year in the United States, resulting in 450 deaths (CDC, 2013). *Salmonella enterica* serovar Enteritidis is transmitted to the human food supply through eggs of hens that appear healthy (Porwollik *et al*., 2005). *Salmonella enterica* serova Enteritidis has also been isolated from quail eggs (Ozlem & Nuh, 2001).

Salmonellosis has become an important public health problem in Nigeria and other parts of the world. It causes heavy economic losses through substantial morbidity and mortality ((Muhammed *et al*., 2010). Various *Salmonella* species have been isolated from quail eggs from different studies carried out and they include: *Salmonella* Enteritidis, *Salmonella* Worthington, *Salmonella* Typhimurium and *Salmonella Bareilly* (Ozlem & Nuh, 2001; Harsha *et al*., 2011). Quail eggs are often consumed raw with the hope of deriving all the nutritional benefits and this could pose a major challenge if they habour food pathogens. With paucity of information on the occurrence of *Salmonella* in this egg type within the study area, it was therefore necessary to undertake this research to elucidate the prevalence of *Salmonella* specie and antibiogram of the isolates from quail eggs from major markets in Zaria and Kaduna metropolis.

The aim of the study is to isolate *Salmonella* from quail eggs sold in markets within Zaria and Kaduna metropolis and to determine the antibiogram of the *Salmonella* isolates.

Materials and Methods

Study area/ sampling sites

The study area is Zaria and Kaduna metropolis of Kaduna State. Kaduna State is located within the semi-arid and sub-humid regions of the north western zone of Nigeria. It lies between longitude $06.5^{o} – 08.6^{o}$ East of Greenwich meridian and latitude $09.2^{o} – 11.3^{o}$ North of equator (Quick Bird Imagery, 2013). In Zaria, samples were collected from retail outlets in the following major markets, that is Tudun Wada, Sabon Gari and Samaru markets. In Kaduna metropolis the major markets sampled were Kawo, Angwan sarki, Barnawa and Sabon Tasha markets

Sample collection

Sampling was done on a weekly basis in order to obtain the required and varied number of samples. Two hundred and eighty (280) fresh quail eggs were collected randomly from twenty eight (28) retail outlets in Zaria and Kaduna metropolis. A total of ten (10) eggs were collected from each retailer by systematic random sampling which involved selecting five crates of eggs randomly and selecting the fifth and tenth egg in each of the five crates selected. The sampled eggs were taken in batches of ten eggs each in clean crates to the Bacterial Zoonoses laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for bacterial analysis of the egg contents.

Procedure for examination of samples for Salmonella

Each egg collected was swabbed with 70% alcohol for disinfection. Using a sterile forcep, a punch was made on the tapered end of each egg and the contents of a pool of ten eggs that is, egg yolk and albumin, from each retail outlet was emptied into a sterile polythene bag and homogenized using a stomacher. 10 ml of each pool of homogenized eggs was inoculated into 90 ml buffered peptone water (BPW) and incubated at 37°C for 18 h. After incubation, 1 ml of the BPW broth, was then inoculated into 9 ml of Rappaport Vasilliadis broth using a sterile syringe and incubated at 37°C for 18- 24 h.

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Plating: A loopful of the inoculate was streaked on SSA (*Salmonella* shigella agar) plates to ensure isolated colonies which were then incubated at 37°C for 24hrs.

Preliminary identification: One or more characteristic colonies appearing colourless, with or without black centers on SSA agar were picked and inoculated into Triple Sugar Iron (TSI) agar and Urea agar slants. Colonies which gave reactions suggestive of *Salmonella* by showing alkaline over acid with or without gas and hydrogen sulphide on TSI and were urease negative were kept at 4°C on Nutrient agar (NA) slants until characterized.

Biochemical characterization of Salmonella isolates
Biochemical characterization was carried out based on standard techniques (Barrow & Feltham, 1995). All isolates that gave reactions typical of *Salmonella* in all or most of the substrates were considered to belong to the genus *Salmonella*; typical *Salmonella* reactions are indole negative, methyl red positive, Voges Proskauer negative, citrate positive, motile in motility medium, produces H_2S, nitrate positive, lysine decarboxylase positive, oxidase negative, ferments glucose, mannitol, dulcitol, and maltose but fails to ferment lactose, sucrose, adonitol and raffinose.

Polyvalent O Antisera Test
Isolates suspected to be *Salmonella* were serologically tested using *Salmonella* polyvalent 'O' group A-Z antiserum latex kit.

Evaluation of the in vitro susceptibility of the isolates to antimicrobial agents
All *Salmonella* isolates were tested for their susceptibility to twelve (12) antimicrobial agents with the following disc contents: tetracycline TE (30 µg), streptomycin S (10 µg), amoxicillin/clavulanic acid AMC (30 µg), kanamycin K (30 µg), chloramphenicol C (30 µg), penicillin GP(10IU), trimethoprim W (5µg), sulphamethoxazole/trimethoprim SXT (25 µg), gentamicin CN (10 µg), ciprofloxacin CIP (5 µg), nitrofurantoin F (50 µg), neomycin N (10 µg), by the disc diffusion method based on recommendations of Clinical and Laboratory Standards Institute (2011).

Data analyses
The prevalence of *Salmonella* was calculated by dividing the total number of samples positive relative to the total samples that were collected, and expressed as a percentage. Results were presented using tables.

Results and Discussion
The results (Table 1) of the three (3) isolates of *Salmonella* obtained from 28 pooled quail eggs collected from markets within Zaria and Kaduna metropolis gave a prevalence of 10.7% for *Salmonella* in quail eggs.

Based on sampling location, one (1) *Salmonella* isolate was obtained from Samaru market and two (2) isolates from Sabon Tasha market. Raw eggs can be contaminated with *Salmonella*, either presenting as shell contamination due to contact with feaces after laying or as egg content contamination due to colonization of the hen's oviduct (EFSA, 2013). The isolation of *Salmonella* organism in this study is similar to that found in a study carried out in India in which *Salmonella* Worthington, *Salmonella* Typhimurium and *Salmonella* Bareilly were isolated from commercial quail eggs (Harsha *et al.*, 2011). The prevalence of 10.71% obtained in this study is in agreement with the 5.69% found in a study carried out in Turkey (Ozlem & Nuh, 2001). It is also similar to the report of Erdoúrul & Akiroúlu (2002), who also reported a prevalence of 5.69% for *Salmonella* in quail eggs. Although the prevalence in this study was slightly higher (10.71%), this could be due to environmental

contamination and poor storage conditions within the study areas. In another study carried out in Ado-ekiti, Ekiti state Nigeria, a prevalence of 11.67% was obtained from quail eggs sampled within the locality (Atere *et al.*, 2015). In a similar study carried out in Plateau state Nigeria, 0.8% prevalence of *Salmonella* was obtained from quail eggs (Bata *et al.*, 2016).

Table 1: Occurrence of *Salmonella* in Quail eggs sold in Zaria and Kaduna Metropolis based on sampling location

Markets	No. of pooled eggs sampled	No. +ve	Percentage
Samaru	4	1	25%
Sabon Gari	4	0	0%
Tudun Wada	4	0	0%
Kawo	4	0	0%
Angwan Sarki	4	0	0%
Sabon Tasha	4	2	50%
Barnawa	4	0	0%
Total	28	3	10.71%

Table 2: Percentage susceptibility of three *Salmonella* isolates to a panel of twelve antibiotics

Antimicrobial agents	Disc content	Percentage susceptibility
Streptomycin	S (10 µg)	100%
Chloramphenicol	C (30 µg)	100%
Kanamycin	K (30 µg)	100%
Sulphamethoxazole/trimethroprim	SXT (25 µg)	100%
Amoxicillin/clavulanic acid	AMC (30 µg)	0%
Tetracycline	TE (30 µg)	100%
Trimethoprim	W (5 µg)	100%
Ciprofloxacin	CIP(5 µg)	100%
Gentamicin	CN (10 µg)	100%
Nitrofurantoin	F (50 µg)	0%
Penicillin G	P (10IU)	0%
Neomycin	N (10 µg)	50%

Table 3: MAR index of eight *Salmonella* isolates from quail egg samples

Salmonella isolates	MAR index
STQ (14)	0.33
STQ (13)	0.25
SQ1	0.17

Latex Agglutination Tests revealed that all three (3) isolates identified as *Salmonella* by conventional biochemical test were positive. All *Salmonella* isolates from this study were resistant to Penicillin (100%), Amoxycillin/Clavulanic acid (100%) and Nitrofurantoin (100%). In this study, the resistance to three or more antimicrobial agents was regarded as multi-drug resistance (MDR) according to defined standards (Magiorakos *et al.*, 2012). All *Salmonella* isolates showed 100% susceptibility to streptomycin, chloramphenicol, kanamycin, sulphamethoxazole/trimethroprim, tetracycline, trimethoprim, ciprofloxacin and gentamicin (Table 2). This is in agreement with Gordana *et al.* (2012) who reported similar susceptibility in chloramphenicol and ciprofloxacin. This finding on high frequency of susceptibility of salmonellae to these antimicrobials also agreed with those of Soufi *et al.* (2012), who reported phenotypic susceptibility of *Salmonella* enterica isolated from poultry in Tunisia to multiple antibiotics. It is also similar to the findings of Omoigberale *et al.* (2014) in Ekpoma, Nigeria and Sangeeta *et al.* (2010) in North India. High sensitivity to ciprofloxacin, chloramphenicol and gentamycin adequately explains why it is increasingly and successfully used for treatment of septicemic

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salmonellosis in humans (Agada *et al.*, 2014). Antimicrobial resistant food borne pathogens are considered to be acquired primarily through consumption of contaminated food of animal origin or water (Mead *et al.*, 1999). Antimicrobial use and misuse has been considered to be the most vital selecting force to antimicrobial resistance of bacteria development and spread in both veterinary and human medicine (Okeke *et al.*, 2005).

MAR (Minimum antibiotic resistance) index analyses(Table 3) revealed that two out of the three *Salmonella* isolates had MAR index value (>0.2). Bacteria having MAR index value >0.2 originates from an environment where several antibiotics are used (Tambekar *et al.*, 2006).

Conclusions

Three (3) isolates of the genus *Salmonella* were isolated from quail eggs with a prevalence of 10.71%. All the isolates of *Salmonella* recovered from this study, exhibited multiple antimicrobial resistance. The 100% susceptibility of all three isolates to eight (8) antibiotics suggests that infections with the isolates will be satisfactorily treated with the antibiotics. This study has established the presence of *Salmonella* in quail eggs sold in retail outlets at markets within Zaria and Kaduna metropolis. *Salmonella* organism is of significant public health importance. Consumers of eggs from these retail outlets are at risk of exposure to these organisms.

Consumption of raw, improperly or under cooked eggs should be discouraged. Eggs should be cooked until both the albumin white and yolk are firm and eaten promptly after cooking. Eggs should be properly stored after collection until it gets to the final consumer. There should be enforcement of regulations on the use of antimicrobial agents in quail farms by relevant authorities so as to minimize the development of multiple drug resistance amongst organisms of public health importance. There should be continuous monitoring and surveillance of *Salmonella* and other pathogens and of antimicrobial resistance in quail eggs.

Acknowledgements

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