Full Length Research Paper

Isolation and antibiotic sensitivity patterns of *Salmonella* species from raw beef and quail eggs from farms and retail outlets in Jos, Plateau State, Nigeria

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This study was conducted to determine the prevalence and antibiotic susceptibility pattern of *Salmonella* species from raw beef obtained from markets and Japanese quail eggs from farms and retail outlets in Jos and environs, Nigeria, using colony morphology and biochemical tests. Of the 100 raw beef samples examined, 11 were contaminated with *Salmonella* given a prevalence of 11.0%. *Salmonella* was isolated from 3 out of the 225 quail eggs analyzed. The result revealed that only 0.8% of the quail egg contents were positive for *Salmonella* while the prevalence on egg shell was 1.7%. The prevalence of *Salmonella* on raw beef was found to be relatively higher in Tudun Wada and Rukuba Road 4(20.0%), while Terminus, Abattoir and Gada Biyu had a lower prevalence of 1 (5.0%). The relative prevalence of *Salmonella* on the egg shell and content base on location indicates considerable high (4.0%) levels of contamination in samples from Jos South Local Government Area while eggs collected from Jos North and Jos East revealed a prevalence of 0.0%. The antibiotic sensitivity pattern of the isolates showed varying degree of sensitivity. The isolates were moderately sensitive to Ciprofloxacin, Sulphademethoxazole, Chloramphenicol and Gentamycin recording the highest sensitivity. The isolates were completely resistant to Tetracyline, Neomycin, Oxacillin and Erythromycin. Multi-drug resistance was reported in isolates from quail eggs from Farm A. All the isolates from the three farms were resistant to Oxacillin and Erythromycin while two (from Farms A and C) were resistant to Sulphadimethoxazole. Regulatory control of antibiotics usage in livestock production, meat/poultry hygiene and pharmaco-epidemiological surveillance in food animals to ensure consumer safety were therefore recommended.

Key words: *Salmonella*, resistance, susceptibility, sensitivity, surveillance.

INTRODUCTION

Salmonella have been isolated from nearly all vertebrates, and infection has been associated with both animal and human disease. Human *Salmonella* infections in the United States have been estimated in millions

annually. In some of these cases, foods of animal origin have been implicated as the source of the pathogen (APHIS, 2001). Although,*Salmonella* infections are increasing worldwide, there are only few reports on *Salmonella* surveillance in African and other developing countries. This has made it difficult to estimate the actual burden of salmonellosis, especially in Africa (Saba et al., 2013).

Contaminated raw meat is one of the main sources of food-borne illness (Bhandare et al., 2007; Podpecan et al., 2007). Cattle have been incriminated as reservoirs of *Salmonella* species. In meat animals (cattle, swine and sheep), *Salmonella* infection arises from intensive rearing practices and the use of contaminated feeds. Cross contamination of carcasses with *Salmonella* can also occur during slaughtering operations which may include harvest time such as transport, lairage, slaughter and dressing. Other sources of contamination may be at post harvest time such as boning, processing, retail distribution, and preparation (Ejeta et al., 2004).

Salmonella infections have also been reported to result from the ingestion of contaminated eggs and these are associated with consumption of raw eggs and food containing eggs (Hogue et al., 1997).

Egg and egg products have been implicated in most cases of human Salmonellosis (Lepoutre et al., 1994). Infected ovaries and oviducts of the hen have been shown to be the major source of contamination of eggs with *Salmonella Enteritidis* (CDC, 1996). More so, eggs can also become contaminated on the surface either from feces or the environment. Contaminated poultry eggs serve as important vehicles of *Salmonella* infections, especially when the bacterium is in the egg contents. Hence, increased consumption of raw eggs (as with quail eggs in Jos) may increase the potential for exposure to *Salmonella* spp. The administration of antimicrobial agents in animals creates selection pressure that favors the survival of antibiotic resistant pathogens. Drug resistance has been shown to be the most important hazard of drug residues in the country (Dipeolu and Alonge, 2002). Resistant bacteria could cause disease that is difficult to treat in humans and may also transfer the resistant gene to some other human pathogens (Barton, 2000; Doyle, 2006).

Multidrug-resistant phenotypes have been increasingly described among *Salmonella* spp. worldwide (Gyels, 2008). Consumption of raw quail egg has increased over the years in Jos Plateau State of Nigeria while beef are sold in open market where flies and other dust particles can serve as source of contamination. At present, there is no documented study of prevalence of *Salmonella* spp. in food products especially beef and quail eggs available in Jos. For effective long-term control of *Salmonella* infection, assessment of safety and quality of food is important. This study was therefore conducted to determine the prevalence of *Salmonella* spp. in raw beef and quail eggs in Jos Plateau State Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted in Jos metropolis. It is located at Latitude: 10° 0' 0 N, Longitude: 9° 30' 0 E on the Jos Plateau (SCRD, 2011). The city is divided into three separate local government areas: Jos-North, Jos-South, and Jos- East with a combined population density of 391 persons/km^2. The city has an altitude of 1,217 m above sea level and so enjoys a more temperate climate than most of the rest of Nigeria (SCRD, 2011).

Sampling technique

The study was cross-sectional and samples were collected using the formula of Thrusfield (1997). A total of 100 beef samples were bought from randomly selected 5 beef retail points in Jos and its environs. Twenty beef samples were collected at each retail points. Five retail outlets each in Jos North, Jos South and Jos East Local Government Areas were identified. Fifteen Japanese quail eggs were bought in each retail outlet giving a total of 225 (This comprises of 105 for egg shell and 120 for egg content).

Samples were collected aseptically into clean, dry leak-proof wide-mouth sterile bags and placed into icebox. Samples were then transported to the Bacteriology Laboratory Department of National Veterinary Research Institute, Vom.

Bacterial culture, isolation and identification

Samples were analyzed as described by Wallace and Thomas (2003). Briefly, 25 g of each sample of beef was thoroughly homogenized with 15 ml of buffer peptone water and incubated at 37°C for 24 h. Swab technique was used to sample the surface of the intact eggs. The swabs were directly inoculated into 10 ml buffered peptone water for pre-enrichment, in screw capped bottles and incubated at 37°C for 24 h.

In order to collect the egg content, the egg shell was aseptically broken and the content of each egg was transferred into 10 ml of buffered peptone water, homogenized and incubated at 37°C for 24 h. A portion was then inoculated into an enrichment media (Rappaport Vasiliadis broth) and incubated for 24 h.

Bacteria culture

A wire loop full was then cultured into an enriched selective media for *Salmonella* (XLD agar) and incubated at 37°C for 24 h. Typical colonies of *Salmonella* based on cultural and morphological characteristics (that is, transparent colonies with black centre on SSA and pink colonies surrounded by a red medium on BGA, and small red translucent and or dome-shaped colonies, which may have central black spot due to hydrogen sulphide (OIE, 2012) production were picked.

Purification of isolates

The isolates were sub cultured onto SSA and nutrient agar for isolation of pure culture and subsequent biochemical characterization.

Biochemical characterization of *Salmonella*

Isolation and identification of organisms was carried out as described by ISO (2002), Habtamu et al. (2011) and OIE (2012). A

Table 1. Prevalence of *Salmonella* species in raw beef based on location.

Location	No. of sample	No. positive	Prevalence (%, Location based)	Overall prevalence
Abattoir	20	1	5.0	1.0
Terminus	20	1	5.0	1.0
Gada Biyu	20	1	5.0	1.0
Rukuba Road	20	4	20.0	4.0
Tudun Wada	20	4	20.0	4.0
Total	100	11.0	-	11.0

Table 2. Antibiotic susceptibility profiles of *Salmonella* isolates from raw beef.

Antibiotic	Antibiotic susceptibility profile of *Salmonella* isolates		
	Sensitive	Intermediate	Resistant
TE (30 µg)	0.0	0.0	100.0
CIP (5µg)	0.0	36.4	63.6
E (15 µg)	0.0	0.0	100.0
N (30 µg)	0.0	0.0	100.0
RL (25 µg)	0.0	36.4	63.6
C (30 µg)	0.0	54.5	45.5
OX (5 µg)	0.0	0.0	100.0
CN (30 µg)	18.2	18.2	63.6

TE: Tetracycline; CIP: Ciprofloxacin; E: Erythromycin; N: Neomycin; RL; Sulphademethoxazole; C: Chloramphenicol; OX: Oxacillin; CN: Gentamycin.

24 h pure culture of each isolate was used to determine their gram stain reaction. The following biochemical tests were carried out on each isolate in individual test tubes: Indole test (Yellow color-Negative), triple sugar iron test (H_2S production-black color along stab line), citrate test (green color-negative), methyl-red test (Red color-positive), Voges-Proskauer test (yellow brown color-negative), lysine decarboxylase test (purple color-positive), ornithine decarboxylase test, urease test (No change in color-negative), sugar (trehalose, sucrose (negative), inositol, glucose (positive), lactose (negative) dulcitol, maltose, mannitol (positive), maltose (positive), melibiose, salicin, rhamnose and arabinose fermentation test and motility test.

Determination of antibiotic sensitivity patterns of the isolates

The *Salmonella* isolates were further subjected to the antibiotic sensitivity test using the agar disc-diffusion method according to the guidelines of the Clinical Laboratory Standard Institute (CLSI) (2008). Briefly, the antibiotic discs (Oxoid, UK) were evenly dispensed unto the surface of the inoculated agar plate using a disc dispenser and were gently pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37°C for 18 h. The following 8 antibiotic discs were used: sulphamethoxazole 25 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (C) 30 µg, gentamycin (CN) 30 µg, tetracycline (TE) 30 µg, oxacillin (OX) 5 µg, and Erythromycin 15 µg, Neomycin 30 µg were applied in the test. The diameters of the zone of inhibition were measured in millimeters with a ruler and compared with a zone interpretation chart (Muragkar et al., 2004). McFarlland scale was used and the result was reported as sensitive, intermediate or resistant based on the size of zone of inhibition table 5.

RESULTS

Of the 100 raw beef samples examined, 11 (11.0%) were positive for *Salmonella*. Prevalence of *Salmonella* in raw beef was higher in Tudun Wada and Rukuba Road 20.0% (4/100) than in Terminus, Abattoir and Gada Biyu 5% (1/100) as shown in Table 1.

The antibiotic susceptibility profile of the *Salmonella* isolates from beef characterized in this study displayed resistance to one or more antibiotics (Table 2). The antibiotic sensitivity pattern of the isolates showed varying degree of sensitivity with Ciprofloxacin, Chloramphenicol and Gentamycin recording the highest sensitivity. The isolates were moderately sensitive to Sulphademethoxazole, Tetracyline and completely resistant to Oxacillin (Table 2). The overall prevalence of Salmonella in eggs was 1.3% (3/235). Of this, 1.7 and 0.8% represented prevalence rates of egg shell and egg content, respectively as shown in Table 3. Based on location, Jos South recorded prevalence rates of 5.7 and 2.5% for egg shell and content, respectively. However, both Jos North and Jos East recorded prevalence rates of 0.0% (Table 4).

A total of three (3/235) *Salmonella* isolates from Japanese quail eggs and content were tested for their susceptibility to 8 different antibiotics. Overall resistance or susceptibility among *Salmonella* from quail eggs is given in Table 3. The isolate from farm A was only

Table 3. Prevalence of *Salmonella* species in egg shell and content.

Source	No. of samples	No. positive	Prevalence (%)
Egg shell	105	2	1.7
Egg content	120	1	0.8
Total	225	3	2.5

Table 4. Prevalence of *Salmonella* on egg shell and in the content base on the location.

Location	No. of samples (egg shell)	No. of samples (egg content)	Prevalence of *Salmonella* (%)		
			Egg shell	Egg content	Total
Jos North	35	40	0 (0.0)	0 (0.0)	0.0
Jos South	35	40	2 (5.7)	1 (2.5)	3.0
Jos East	35	40	0 (0.0)	0 (0.0)	0.0
Total	105	120	2 (5.7)	1 (2.5)	3.0

Table 5. Antibiotic susceptibility profile of *Salmonella* isolates from egg shell and content.

Farm	Susceptibility profiles of *Salmonella* isolates							
	CN	C	N	E	CIP	TE	RL	OX
Farm A	S	R	R	R	R	R	R	R
Farm B	S	S	I	R	S	I	I	R
Farm C	S	S	I	R	I	I	R	R

TE: Tetracycline; CIP: Ciprofloxacin; E: Erythromycin; N: Neomycin; RL; Sulphademethoxazole; C: Chloramphenicol; OX: Oxacillin; CN: Gentamycin; R: Resistant; S: Susceptible; I= Intermediate.

susceptible to Gentamycin and was resistant to Chloramphenicol, Neomycin, Erythromycin, Ciproflaxacin, Tetracycline, and Sulphademethoxazole. All the isolates from the three farms were susceptible to Gentamycin and resistant to Oxacillin while isolates from Farm B was susceptible to almost all the antibiotics except Oxacillin. Isolate from Farms A and C were resistant to Tetracycline, Sulphadimethoxazole and Oxacillin.

DISCUSSION

Food-borne diseases are considered to be global public health concern. In this study, the overall prevalence of *Salmonella* spp. in beef samples from Jos and environs, Nigeria was found to be 11.0%. Samples within close proximity to the abattoir showed lower prevalence (1.0%) than those far from the abattoir (Rukuba Road 4.0%; Tudun Wada 4.0%). This might be attributed to the unhygienic post slaughter operations such as transportation, handling of the beef by the distributors and even the retailers/meat sellers in the study area. It is most likely that the tables where beef are kept and vehicles used in transporting the meat are not properly cleaned. More so, knives and other materials used during the sales may be sources of contamination. The beef sampled were also sold in open markets where flies and other dust particles can serve as sources of contamination. There is therefore the need to have minimum hygienic operation standards for retail meat premises in the area. The standard should include a range of requirements related to the receiving, storage, processing, display, packaging and transportation of meat. McDonough et al. (1999) reported that cattle are among the known reservoirs of *Salmonella*. This might be a potential contamination source in slaughter houses and in retail beef carcasses. Contamination arising from the butchers and beef retailers through poor toilet manners and poor hand hygiene is another possible explanation since their health status was not ascertain. Cross contamination of edible carcass tissue due to the presence of *Salmonella* in cattle at slaughter or contamination due to poor handling process present a significant food safety hazard. Because the presence of any *Salmonella* is potentially a health threat, consumption of raw eggs and or undercooked beef in the study area may serve as vehicle for the transmission of *Salmonella* to humans.

Prevalence of *Salmonella* contamination on the shells of quail eggs from farms and retail outlets in Jos, Plateau

State, Nigeria was found to be 1.7%. In a similar study conducted in India, Harsha et al. (2011) reported a high (9.0%) prevalence of *Salmonella* contamination in shell of quail eggs and 0.0% in the egg content. Though the 0.8% prevalence of *Salmonella* revealed by the study may seem negligible, the public health implication of this in causing human salmonellosis can never be over emphasized. Since most of the positive samples in this study and those of other places were from egg shell, one may conclude that the contamination may be horizontal due to cross contamination from faeces or from the cage environment. Guard-petter, 2001 in a study reported that eggs can be contaminated with droppings from chickens excreting *Salmonella*. In such cases *Salmonella* in droppings can penetrate egg shell pores as egg cools and before the establishment of the proteinaceous cuticular barrier (St Louis, 1988). In view of this, *S. enteritidis* is able to persist on the surface of the egg shell and potentially cross contaminate the liquid portion of the egg when eggs are broken for preparation of food, which could pose a potential health risk to the society (Charles and Takayuki, 2010). This further suggests that prompt removal of the quails waste and disinfection can greatly reduce *Salmonella* contamination on the shell and the content. The study showed that *Salmonella* contamination in quail eggs (shell and content) was only found in samples obtained from Jos South Local Government Area.

No isolate was obtained in samples from Jos East and Jos North Local Government Areas. Agada et al. (2014) in a related study reported that prevalence of *Salmonella* isolates from human faeces/hand swabs, poultry droppings, swabs from shells of intact eggs and feeds was higher in Jos South Local Government Area. The distribution may not be unconnected with certain factors such as differences in the socio-demography and biosecurity practices in the areas. *Salmonella* entry into the farms can occur through environmental related factors such as litter, dust, mice, flies and even the poultry feed (Galis et al., 2013). Therefore, lack of proper biosecurity measures in poultry farms could lead to cross contamination from one poultry house to another.

The antibiotic sensitivity pattern of the *Salmonella* isolates from the study showed varying degrees of sensitivity with Ciprofloxacin, Chloramphenicol and Gentamycin recording the highest sensitivity. The isolates were moderately sensitive to Sulphademethoxazole and completely resistant to Tetracycline, Erythromycin, Neomycin and Oxacillin. This partially agrees with the findings of Agada et al. (2014) who in a similar study in Jos reported that all the *Salmonella* isolated were resistant to Oxacillin. This resistance may not be unconnected with indiscriminate use of this drug. The highest level of susceptibility was detected in Chloramphenicol, Ciprofloxacin and Gentamycin. This is in agreement with Gordana et al. (2012) who reported similar susceptibility in chloramphenicol and ciprofloxacin.

High sensitivity to Ciprofloxacin, Chloramphenicol and Gentamycin adequately explains it increasingly and successfully used for treatment of scepticemic salmonellosis in humans (Agada et al., 2014).

Antimicrobial resistant foodborne 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). Indiscriminate antibiotics usage in food animals especially poultry could predispose consumers to risks of antibiotic resistant bacterial infections. This situation is further complicated by the potential of resistant bacteria to transfer their resistance determinants to resident constituents of the human microflora and other pathogenic bacteria. The antimicrobial susceptibility test of the *Salmonella* isolates encountered in quail eggs in the present study revealed that the isolate from farm A had acquired resistance to more than 5 antibiotics. This study has revealed a multi-drug resistance of isolate of *Salmonella* in farm A and a lower resistance in isolates from farm B and C. More so, the isolate from farm A was resistant to Ciprofloxacin while isolates from farms B and C were not. However, the isolates from farms A and C were resistant to Tetracycline and the isolates from the three farms were resistant to Oxacillin and Sulphadimethoxazole. These may not be unconnected to the frequent and indiscriminate use of these antibiotics in quail birds at sub-therapeutic level or prophylactic doses. This promotes on-farm selection of antimicrobial resistant strains and also increases the human health risks associated with consumption of contaminated quail eggs.

Conclusion

Despite the seeming poor and unhygienic practices involved in the handing of beef in the study area, prevalence of *Salmonella* spp. was relatively low. Samples within close proximity to the abattoir showed lower prevalence (1.0%) than those far from the abattoir. The antibiotic sensitivity pattern of the *Salmonella* isolates from the study showed varying degree of sensitivity with Ciprofloxacin, Chloramphenicol and Gentamycin recording the highest sensitivity. The isolates were moderately sensitive to Sulphademethoxazole, Tetracyline and completely resistant to Oxacillin.

The study has also revealed a low prevalence *Salmonella* species in commercial quail eggs from different retail outlets of the three local government areas in Jos, Plateau State. More so, multi-drug resistance was reported in isolate from farm A. All the isolates from the three farms were resistant to Sulphadimethoxazole while two (from farms A and C) were resistant to Tetracycline and Oxacillin.

Improving the conditions in which quails are raised by periodic disposal of waste and disinfection may help in maintaining a low level of *Salmonella* contamination in quail eggs. Strict hygiene during post slaughter operations such as transportation, handling of the beef by the distributors and the retailers/meat sellers may reduce *Salmonella* contamination in beef. We also, recommend that further studies be carried out to determine the serovars of the *Salmonella* isolates from beef and quail eggs in the area.

Conflict of Interests

The authors have not declared any conflict of interests.

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