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Original Article

Prevalence, assessment, and antimicrobial resistance patterns of *Salmonella* from raw chicken eggs in Haramaya, Ethiopia

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Abstract

Introduction: The presence of antimicrobial-resistant *Salmonella* in poultry and poultry products, including eggs, is a global public health concern. This study aimed to estimate the levels and patterns of antimicrobial resistance of *Salmonella* from chicken eggs and assess consumers' raw egg consumption and farmers' handling practices.

Methodology: A total of 300 egg samples were collected from Haramaya open market (n = 150) and Haramaya University poultry farm (n = 150) in Ethiopia. Questionnaires were administered to egg sellers and buyers. A sterile cotton swab was used to sample the surface of eggs. The shells were sterilized and the egg content sampled. Isolation was done using the conventional methods for the detection of *Salmonella*, following the standard guidelines from ISO 6579. Sensitivity to 12 selected antibiotics was tested following the procedure of the Clinical and Laboratory Standards Institute.

Results: A level of 5.3% was observed among eggs shells from the open market and 0% among egg shells from the poultry farm, for an overall level of 2.7%. There was a significant difference (p = 0.004) between the prevalence of *Salmonella* spp. in sample site and sample type. Of the antimicrobials tested, *Salmonella* isolates were all resistant to erythromycin and clindamycin. Isolates were sensitive to ciprofloxacin (100%) and chloramphenicol (87.5%). All isolates were resistant to multiple antibiotics. One-third of the consumers were found to have eaten raw eggs for perceived medicinal values.

Conclusion: To minimize the potential contamination of eggs by pathogens, the eggs should be properly handled, transported, and stored.

Key words: antimicrobial sensitivity; chicken egg; Ethiopia; *Salmonella.*

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Introduction

Poultry meat, eggs, and egg products are among the most nutritious foods on earth and are an important part of the human diet. However, they are perishable, just like other meat, fish and other food items, and consumption of improperly handled poultry and egg products is closely associated with foodborne illnesses, such as salmonellosis [1].

The Food Standards Agency (FSA) [2] of the United Kingdom has drawn attention to the risk associated with eating raw and lightly cooked eggs and issued public health advice on the safe handling and use of eggs. It is estimated that, in the United States, *Salmonella* transmission through contaminated shelled eggs or egg products results in 700,000 cases of salmonellosis and costs $1.1 billion annually [3]. One study in Ethiopia found that from a total of 400 chicken eggs examined for *Salmonella*, 46 (11.5%) were positive; 25 (6.3%) and 27 (6.8%) of isolates were found on egg shells and in egg contents, respectively [4].

Overuse of antimicrobials in food animals can generate genomic selective pressures to enable microbes to adapt and acquire resistance [5]. Ultimately, increases in bacterial antimicrobial resistance pose a considerable threat to public health, especially for vulnerable populations including young children, the elderly, and immunocompromised individuals [6]. Concentrated animal feeding operations (CAFOs) in agricultural practices have evolved to accommodate food consumption rates with increased agricultural output at the risk of introducing antimicrobial-resistant pathogens into the environment. Several studies have suggested that characteristics of agricultural environmental settings, including animal crowding, CAFO hygiene, temperature, ventilation control, and stress can influence antimicrobial resistance and pathogen risk [7]. There are reports of high levels of resistance in *Salmonella* isolates from countries such as Taiwan [8], India [9], the Netherlands [10], France [11], Canada [12], and Ethiopia [13-16].

The presence of resistant organisms in poultry and poultry products for consumption is a safety concern to the population [17]. In Ethiopia, the extent of *Salmonella* contamination of eggs sold at local markets and farms, and the antimicrobial profile of the *Salmonella* isolates has not been adequately studied; there is very limited information available, and none from eastern Hararghe. This study aimed to estimate the levels and determine antimicrobial resistance patterns of *Salmonella* isolated from chicken eggs collected from Haramaya University (HU) poultry farm and the nearby local market and to assess the consumption and handing (storage) practices of farmers and consumers of raw chicken eggs.

Methodology

Study design, sample collection and transportation

Sampling of chicken eggs from HU poultry farm and local chicken eggs directly from the farmers in Haramaya district was undertaken from November 2012 to April 2013. The sample size required for this study was determined based on a study on the prevalence of *Salmonella* among chicken table eggs in Kombolcha, northern Ethiopia, by Minte *et al*. [4]. The estimate was desired to be with 5% absolute precision and 95% confidence [18]. Accordingly, a total of 300 eggs (150 eggs from HU poultry farm and 150 eggs from Haramaya district open market) were collected directly from the farmers. Ten eggs from the market (one egg from one egg seller) and 10 eggs from HU caged white leghorn birds were collected once per week using a simple random sampling technique. Eggs were collected individually in sterile plastic bags and transported on ice in an ice box for analysis in the microbiology laboratory of the College of Veterinary Medicine at HU within 2–4 hours of collection.

Sample processing

The sterile plastic bags containing sampled eggs were opened with scissors and the samples were processed immediately. A swab technique was used to sample the shell surface of the intact eggs. Sterile cotton swabs dipped into sterile buffered peptone water (BPW) were used to swab the entire surface area of the egg shell. The swabs were then incubated separately in a test tube that contained 10 mL BPW (Oxoid Ltd, Hampshire, UK; Lab M Ltd., Quest Park, UK). The same eggs from which the shell samples were collected were used for interior (egg content) sampling. The egg's surface was sterilized by immersing the egg in 70% alcohol for 2 minutes; the egg was then air dried in a sterile chamber for 10 minutes, and then cracked with a sterile scalpel blade. The egg contents were homogenized in stomacher bags containing 225 mL sterile BPW for 1 minute in a stomacher. The isolation was conducted using the conventional methods for the detection of *Salmonella*, following the standard guidelines from ISO 6579. Confirmation was done by using biochemical tests such as triple sugar iron (TSI) agar, urea agar, citrate utilization test, L-lysine decarboxylation medium, and indole test according to ISO 6579 [19] (Table 1-S).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done by the agar disk diffusion method as described by the Clinical and Laboratory Standards Institute [20]. The pure *Salmonella* isolates confirmed by the biochemical testing procedure as described in ISO 6579:2002 were tested for antimicrobial susceptibility. A total of 8 isolates were tested against 12 commonly used antimicrobials (Oxoid Ltd., Cambridge, UK): amoxicillin (AMX), 25 μg; ampicillin (AMP), 10 μg; chloramphenicol (CHL), 30 μg; ciprofloxacin, (CIP), 5 μg; clindamycin (CLN), 2 μg (CLN); erythromycin (ERY), 15 μg; gentamycin (GEN), 10 μg; kanamycin (KAN), 30 μg; nitrofurantoin (NIT), 300 μg; spectinomycin (SPC), 100 μg; tetracycline (TET), 10 μg; and trimethoprim (TMP), 5 μg. The antimicrobials used were selected from the currently available and commonly used chemotherapeutic agents for the treatment of *Salmonella* infection in humans and animals (Table 2-S).

Questionnaire survey

A questionnaire was used to collect information from 75 farmers and consumers, who were chosen randomly, to identify conditions of handling (storage) practice, transportation, and preparation and utilization patterns of chicken eggs in the study area.

Data management and analysis

Data management was carried out using Microsoft Office Excel 2007, which was used to generate descriptive statistics such as percentage and proportion. The Chi-square (χ^2) test was used to determine the difference in levels between study groups. A p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software package, version 15.0 (IBM, Armonk, USA).

Results

Prevalence of Salmonella spp. in raw chicken egg shell and egg contents

Out of the 300 eggs examined for bacteriological status, only 8 samples yielded *Salmonella*. All of the isolates were from egg shell samples collected from Haramaya local market. The other 150 eggs collected from the HU poultry farm were negative for *Salmonella* spp. (Table 1).

The levels of *Salmonella* varied among the sampling sites and types. The level of *Salmonella* in the local market was significantly higher than the level of *Salmonella* in the poultry farm ($p = 0.007$). The level of *Salmonella* in egg shells from the open market was also significantly higher than the level of *Salmonella* in egg content from both the open market and poultry farm ($p = 0.007$). Similarly, the level of *Salmonella* in egg shells from the open market was significantly higher than the level of *Salmonella* in egg shells from the poultry farm ($p = 0.007$). The overall level of *Salmonella* in egg shells was significantly higher than the overall level of *Salmonella* in egg contents (Table 1).

Farmers' and consumers' chicken egg use and handling (storage) practices

The questionnaire administered to farmers and egg consumers indicated that 28% of the respondents had a preference for raw egg consumption, while 72% disliked eating raw eggs. The habit of washing eggs before consumption was also investigated, and the results showed that 90.7% did not have the habit of washing eggs, and the other 9.3% had this habit only when the eggs become extensively dirty. Egg-keeping practices of respondents showed that 57.3% used open containers such as baskets, cartons, and trays, while 42.7% of the respondents kept eggs together with different cereal crops and coffee (Table 2).

Antimicrobial resistance testing

For the antimicrobials tested, all 8 *Salmonella* isolates were resistant to clindamycin (8/8) followed by erythromycin (5/8), ampicillin (3/8), amoxicillin (3/8), and tetracycline (2/8). The overall frequencies of sensitivity to nitrofurantoin, gentamicin and trimethoprim were 50%, 37.5%, and 50%, respectively. All and 87.5% of the isolates were found to be sensitive to ciprofloxacin and chloramphenicol (Table 3), respectively. In this study, all of the *Salmonella* isolates were resistant to two or more of the tested antimicrobials (Table 4, Table 3-S).

Discussion

In Ethiopia, Minte *et al*. [4] reported that the prevalence of *Salmonella* from egg shells and egg contents was 6.3% and 6.8%, respectively, with a total of 11.5%. The number of *Salmonella* isolates observed from market egg shells was 11 (5.5%), and from farm egg shells was 14 (7.0%). The overall level of *Salmonella* spp. and the finding in egg shells of the previous study was higher than that in the present study, and the result in the egg content was different from that in the current study in that no *Salmonella* was isolated

Table 1. Prevalence of *Salmonella* from raw chicken eggs of local market and poultry farm.

Sample source	No. examined	Egg shell No. positive (%)	Egg content No. positive (%)	Total	p value
Open market	150	8 (5.3)	0 (0.0)	8 (5.3)	0.007
Poultry farm	150	0 (0.0)	0 (0.0)	0 (0.0)	
Total	**300**	**8 (2.7)**	**0 (0.0)**	**8 (2.7)**	

Table 2. Handling and consumption practice of eggs among farmers and consumers from Haramaya.

Items	Respondents response			
	Yes	%	No	%
Raw egg consumption	21	28	54	72
Egg washing before preparation for consumption	7	9.3	68	90.7
Egg handling (keeping) at home	75	100	0	0
Egg handling (keeping) days at home: 1–7 days	48	64	27	36
7–15 days	22	29.3	53	70.7
15–30 days	5	6.7	70	93.3
Egg-keeping strategy: Open containers [a]	43	57.3	32	42.7
Together with crops [b]	32	42.7	43	57.3
Fate of cracked eggs: Consumed	71	94.7	4	5.3
Discarded	4	5.3	71	94.7

[a] Carton, box, basket, tray; [b] Barley, sorghum, maize, wheat and together with coffee.

Table 3. Antimicrobial resistance profiles of *Salmonella* isolates (n = 8).

Antimicrobials tested	Resistant isolates		Intermediate isolates		Sensitive isolates	
	No.	%	No.	%	No.	%
AMP	3	37.5	5	62.5	-	-
AMX	3	37.5	-	-	5	62.5
CHL	-	-	1	12.5	7	87.5
CIP	-	-	-	-	8	100
CLN	8	100	-	-	-	-
ERY	6	75	2	25	-	-
GEN	1	12.5	4	50	3	37.5
KAN	3	37.5	3	37.5	2	25
NIT	2	25	2	25	4	50
SPC	2	25	2	25	2	50
TET	3	37.5	5	62.5	-	-
TMP	1	12.5	2	25	5	50

AMP: ampicillin; AMX: amoxicillin; CHL: chloramphenicol; CIP: ciprofloxacin; CLN: clindamycin; ERY: erythromycin; GEN: gentamycin; KAN: kanamycin; NIT: nitrofurantoin; SPC: spectinomycin; TET: tetracycline; TMP: trimethoprim.

Table 4. Resistance patterns exhibited by *Salmonella* isolates against 12 antimicrobial agents.

Salmonella isolates	Isolates with same pattern	Antimicrobial resistance pattern	No. of antimicrobials developed resistance
H37S, H40S, H81S	3	CLN, ERY	2
H138S	1	CLN, ERY, KAN	3
H62S	1	CLN, ERY, KAN, TEY	4
H42S, H102S	2	CLN, AMP, AMX, ERY, KAN, TMP	6
H33S	1	AMP, AMX, CLN, GEN, NIT, SPC, TET	7

CLN: clindamycin; ERY: erythromycin; KAN: kanamycin; TET: tetracycline; AMP: ampicillin; AMX: amoxicillin; TMP: trimethoprim; GEN: gentamycin; NIT: nitrofurantoin; SPC: spectinomycin; TET: tetracycline.

from egg contents. However, the report [4] on market egg shells (5.5%) was in line with the prevalence found in the current study (5.3%). A study conducted by Wiriya *et al.* [21] reported a prevalence of 5.2% from egg shells, whereas none of the egg content samples were *Salmonella* positive from different poultry housing systems, which is in agreement with a study carried out in Bangladesh by Kohinur *et al.* [5], who found 4 isolates (0.4%) out of 1,000 poultry eggs and 3 isolates (0.27%) out of 1,100 domestic eggs. A survey of retail eggs in the United Kingdom found an overall prevalence of *Salmonella* contamination of 0.34% [22], and a survey of 5,000 samples in Northern Ireland and the Republic of Ireland found only 2 positive samples (0.04%) [23].

Overall *Salmonella* levels on eggs in this study (2.7%) (Table 1) were in agreement with the finding of *Salmonella* spp. recovered from table eggs in Denmark, with a prevalence of 2.6% [24]. *Salmonella* on egg shells from the local market in this study (5.3%) was also in agreement with the work of Adesiyun *et al.* [25], who reported 5.4% *Salmonella* prevalence on egg shells in Trinidad. Suresh *et al.* [26] from India also reported that the prevalence of *Salmonella* on egg shells was 6.1%. However, in the United Kingdom, the prevalence of *Salmonella* on egg shells was 0.38% [27]. Furthermore, 0.29% *Salmonella* egg shell prevalence was reported from the United Kingdom [2]. This *Salmonella* egg shell prevalence was lower than that of the present finding and could be due to egg shell surface contamination with feces, dust, and hands of egg collectors.

In this study, the negative results of the egg contents for the presence of *Salmonella* were, in general, in agreement with the literature, but there was a high difference in the number of egg samples used. In the study reported by UK Food Standards Agency in 2003, none of the 4,753 pooled egg contents of retail samples were *Salmonella* positive. Similarly, in a study conducted by a research group in Belgium, none of the 490 table eggs from 49 different producers were *Salmonella* positive [28]. In another study conducted in England and Wales, a total of 12,615 eggs were collected from catering premises from September 2002 to November 2004. *Salmonella* was detected in 5.5% of eggs imported from Spain, in 6.3% of eggs of unknown origin, and in 1.1% of eggs imported from the UK. *Salmonella* was not detected in eggs from other countries (France, Germany, Portugal, USA: 0%) [29,30].

Based on the results of the questionnaire, one-third of the consumers practiced eating raw eggs for medicinal values; this could have some negative effects on health. Around half of the respondents used open containers and kept eggs together with other cereals or coffee husks. This situation might have led to a warmer micro-environment conducive to multiplication of a small number of *Salmonella* isolates acquired from the environment or from handlers during egg collection. A study conducted in Ethiopia by Minte *et al*. [4] indicated that 6.2% of egg consumers preferred to consume raw eggs, while 93.8% did not.

In a study carried out by Ayalu *et al.* in eastern Ethiopia [14], the sensitivity of the *Salmonella* isolates was 0.0% to ampicillin and amoxicillin, 14.2% to tetracycline, 28.6% to chloramphenicol, and 92.8% to gentamicin. In a report by Sibhat *et al*. [16], the highest level of resistance (66.7%) was seen in *S.* Newport to streptomycin. Zewdu and Cornelius [31] assessed antimicrobial resistance of 98 isolates of *Salmonella* serovars recovered from food in Addis Ababa, Ethiopia, and revealed that 32 *Salmonella* isolates were resistant to one or more of the 24 antimicrobials tested. The most common resistance was to streptomycin (75%), ampicillin (59.4%), tetracycline (46.9%), and spectinomycin (40.6%), which is in agreement with the present study. Miko *et al.* [32] determined antimicrobial resistance of *Salmonella* serovars isolated from German foodstuff; they showed that the most prevalent resistance among the multidrug-resistant *Salmonella* serovars isolated from foods were to streptomycin (94%), sulfamethoxazole (92%), tetracycline (81%), ampicillin (73%), spectinomycin (72%), chloramphenicol (48%), and trimethoprim (27%). The result regarding nitrofurantoin with 50% resistance is in agreement with that of Tassios *et al.* [33] in Brazil, who reported 57.82% resistance among *Salmonella* isolates. These drugs are among the most commonly used drugs in Ethiopia both in humans and animals.

A study conducted by Kohinur *et al.* [5] reported that 100% strains isolated from poultry eggs were found to be sensitive to ciprofloxacin and chloramphenicol. On the other hand, different strains have shown resistance to ampicillin, tetracycline, and kanamycin. Chen *et al.* [34] analyzed 133 isolates of *Salmonella* serovars and found that 73 (82%) of these *Salmonella* serovars were resistant to at least one antimicrobial agent. Resistance was common to tetracycline (68%) and ampicillin (29%). The problem is probably associated with the widespread use of these antimicrobials in humans and animals for treatment of enteric infections.

Conclusions

The study showed that *Salmonella* contamination of eggs in Haramaya area is low, with an overall prevalence of 2.7%. However, people consume raw and cracked eggs in the area, indicating a lack of awareness of zoonosis. The presence of *Salmonella* contamination in local chicken eggs is of public health concern, as these are the most widely available and used egg types. Therefore, the public should be made aware of risks associated with consumption of raw chicken eggs and raw eggs cracked during storage and transportation.

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Authors' contributions

BS and JK designed the study, conducted the work, and prepared and wrote the paper. SM and DB advised and reviewed the paper. All authors read, evaluated, and approved the manuscript.

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Annex – Supplementary Items

Table 1-S. Biochemical characteristics of *Salmonella* spp. isolated from chicken egg shells, Haramaya, 2013.

Isolates	Citrate utilizat ion test	Urease test	Indole reaction	Lysine decarbo xylation	TSI agar test			
					Slant Alkaline (red)	Butt Acidic (yellow)	Gas	H_2S
H33S	+	-	-	+	+	+	+	+
H37S	+	-	-	+	+	+	+	+
H40S	+	-	-	+	+	+	+	+
H42S	+	-	-	+	+	+	+	+
H62S	+	-	-	+	+	+	+	+
H81S	+	-	-	+	+	+	+	+
H102S	+	-	-	+	+	+	+	+
H138S	+	-	-	+	+	+	+	+

TSI: triple sugar iron; H: Haramaya; S: shell; +: positive reaction; -: negative reaction.

Table 2-S. Inhibition zone diameter size interpretive standards for *Enterobacteriaceae* (for selected antimicrobial disks appropriate for *Salmonella* spp.).

Antimicrobial agents	Disk potency	Diameter zone of inhibition to nearest mm		
		Resistant	Intermediate	Susceptible
AMX	25 µg	≤ 13	14-17	≥ 18
AMP	10 µg	≤ 13	14-16	≥ 17
CHL	30 µg	≤ 12	13-17	≥ 18
CIP	5 µg	≤ 15	16-20	≥ 21
GEN	10 µg	≤ 12	13-14	≥ 15
CLN	2 µg	≤ 14	15-20	≥ 21
ERY	15 µg	≤ 13	14-22	≥ 23
NIT	300 µg	≤ 14	15-16	≥ 17
KAN	30 µg	≤ 13	14-17	≥ 18
SPC	100 µg	≤ 11	12-14	≥ 15
TET	10 µg	≤ 14	15-18	≥ 19
TMP	5 µg	≤ 10	13-14	≥ 16

Source: CLSI, 2005; AMX: amoxicillin; AMP: ampicillin; CHL: chloramphenicol; CIP: ciprofloxacin; CLN: clindamycin; ERY: erythromycin; GEN: gentamycin; KAN: kanamycin; NIT: nitrofurantoin; SPC: spectinomycin; TET: tetracycline; TMP: trimethoprim.

Table 3-S. Summary of disk diffusion zone diameter of isolated *Salmonella* spp. for selected antimicrobials in millimeters.

Salmonella isolates	Antimicrobials and diameter of disk diffusion zone of inhibition (mm)											
	AMX	AMP	CHL	GEN	CIP	CLN	ERY	NIT	KAN	SPC	TET	TMP
H33S	10	10	25	14	26	0	15	10	15	0	20	15
H37S	20	16	24	14	27	0	0	17	17	16	16	22
H40S	19	15	26	14	27	0	10	19	18	27	15	22
H42S	0	0	17	20	30	0	0	18	15	25	15	0
H62S	20	15	23	18	28	0	10	16	14	25	14	15
H81S	21	15	23	14	28	0	10	17	18	26	17	21
H102S	0	0	22	16	24	0	16	12	18	0	10	17
H138S	21	15	23	19	27	0	10	19	14	15	16	20

AMX: amoxicillin; AMP: ampicillin; CHL: chloramphenicol; GEN: gentamycin; CIP: ciprofloxacin; CLN: clindamycin; ERY: erythromycin; NIT: nitrofurantoin; KAN: kanamycin; SPC: spectinomycin; TET: tetracycline; TMP: trimethoprim.