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Research Article

# Occurrence of antibiotic resistance Zoonotic Salmonella isolated from Table Egg in North West Ethiopia

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## Abstract

Salmonella is one of a major foodborne pathogen worldwide that table egg as one of the vehicles for its transmission. Across sectional study was conducted from November 2018 to April 2019 in Bahir Dar Town to identify Salmonella isolates from table egg and to determine their antimicrobial resistance profile. A total of 157 egg samples were collected by systematic random sampling technique and processed for isolation of Salmonella by conventional bacteriological methods. Antimicrobial sensitivity test was performed for 10 selected antibiotic discs by disc diffusion. The overall level of Salmonella prevalence in current study was 2.23 %. Prevalence of salmonella based on site and part of eggs were 3.85% from the open market and 1.28% from the poultry farm. About 3.80% of Salmonella was observed from internal contents of farm eggs. Isolation rate of Salmonella between sample sites and egg parts were not statistically significant (p = 0.69) and (p=0.702) respectively. All Salmonella isolates were susceptible to gentamycin; however, all of them were resistant to ampicillin, tetracycline and cephalothin. All isolates were developing multiple drug resistance. Confirmation of Salmonella from egg indicates hygiene protocols should be applied from farm to mouth to prevent the infection of human beings by Salmonella. Even though the isolation rate was low in current finding, isolates were developing multidrug resistance. Drug resistance is mobile; the genes for resistance traits can be transferred among bacteria of different taxonomic and ecological groups by means of transduction, conjugation, and transformation. Therefore, proper egg handling, strict biosecurity measures and rational antimicrobial usage should be practiced.

**Keywords:** Antimicrobial Sensitivity; Bahir Dar; Chicken Egg

## Introduction

Eggs are nutritious foods that are used throughout the world FAO [1]. The whole egg contains water (75%), proteins (12%), lipids (12%) and carbohydrates and minerals (1%). It is considered a good source of nutrients such as protein, lipids, vitamins and minerals Réhault-Godbert et al. [2]. They have pharmaceutical uses by acting as an anticancer agent, as an immune-modulator and the ability to suppress tumor cells, and have also antiviral agent Abeyrathne et al. [3]. However, eggs are nutritious and have a risk of food-borne illness if we are not maintaining food safety from farm to consumption. Salmonella is one of the causes of food-borne infection associated with egg origin foods worldwide Galiş et al. [4], U.S. Food and Drug Administration, [5]. Salmonella has been isolated from table eggs and egg foods. Nutritional constituents and water content available in table egg and egg foods have become useful growth ingredients for the proliferation of Salmonella. Salmonella, at room temperature highly proliferate and pathogenicity of the organism remained high and active in table egg Whiley and Ross & Long et al. [6,7]. The two most commonly identified serotypes that cause food-borne salmonellosis are: S. enterica serotype Typhimurium and S. enterica serotype Enteritidis. Both serotypes have the ability to colonize the reproductive organs of hens and transmit to humans through raw and under-cooked egg consumption Alghoribi et al. [8]. Salmonella Enteritidis is more commonly associated with contaminated eggs Jajere [9]. It may be

transferred to the human due to the poor hygienic conditions and raw eggs/under cooked eggs consumption Whiley and Ross [6].

The prevalence of Salmonella in table egg has been studied in different regions of the world and health risks were evaluated. In spite of steady observation and in depth efforts, food-poisoning flare-ups due to salmonellosis are expanding in western nations and egg items account for a critical parcel of reported flare-ups. In developing countries such as Ethiopia, there's no such ceaseless observing framework and the precise number of cases isn't known. Consequently there is need to study Salmonella occurrence and antimicrobial susceptibility profile in poultry table eggs in Ethiopia.

Antimicrobial resistance is a global problem due to the widespread and inappropriate use of antibiotic drugs in food animals Ayukekbong et al. [10]. Egg may contain drug residues by laying hens treated with pharmaceutical products Mund et al. [11]. Many drugs used in veterinary medicine have identical analogs that are used in human medicine Smith et al. [12]. Thus table egg can establish reservoirs of antimicrobial resistant bacteria those has veterinary and public health threat Afema et al. [13]. Studies conducted in Ethiopia suggest an increase in antimicrobial resistance of Salmonella to commonly used antimicrobials in both public health and veterinary sectors Beyene, Sibhat, Tesfaw & Dagnew [4,14-16].

Despite the high risk of egg-related salmonellosis, studies related to its epidemiology in Ethiopia are not adequately addressed and are not available in the study area. Thus, the aim of this study was to evaluate the presence of Salmonella in table eggs in North West Ethiopia. In addition, the antimicrobial susceptibility patterns of these isolates were evaluated to provide information for future intervention measures.

## Materials and Methods

### Description of Study Area

The study was carried out in the town of Bahir Dar, approximately 580 km north-west of Addis Ababa; Ethiopia. Geographically, Bahir Dar is located at latitude 11.59° North and 37.39° East. Its average elevation is estimated to be 1810 m above sea level. As the National Meteorological Agency reported in 2016, the town's mean annual temperature was 20.85 °C Abebe [17]. According to the Central Statistical Agency 2013, the total population of Bahir Dar Town is 202,157 [18]. According to Bahir Dar Town Agricultural Office's annual report, there were 13 intensive poultry farms and 36,870 layers in the town [19].

### Sample Size Determination

The sample size required for this study was determined based on the single population proportion formula Thrusfield, [20]. There was a previous study on Salmonella in chicken eggs of 11.5 % expected prevalence with 95 % desired confidence interval and 5 % absolute precisions were used. Based on the above formula, the total sample size was 157 eggs. Since the egg sampled internal content and surface, over all 314 samples were processed for microbiological isolation.

### Sampling Method

A systematic random sampling technique was used to sample. The 157 chicken table eggs collected from Bahir Dar Town poultry farms and markets were taken from November 2018 to April 2019. Eggs were collected aseptically, individually packed with a sterile plastic bag. The eggs were transported to Amhara Public Health Institute Microbiology Laboratory immediately after collection. Sterile plastic bags containing selected eggs were opened in the lab and samples were processed immediately with in the safety hood (Biobase II). The shell surface of intact eggs was sampled by swab technique. After sterile cotton swabs dipped in sterile buffered peptone water (BPW), the entire surface area of the egg shell was swabbed gently. The swab was inoculated directly into screw-capped bottles containing 10ml BPW [21].

The same eggs from which the shell sample was collected were also used for egg content sampling. To sample the internal contents, the surface of the eggs were sterilized by immersing in a solution prepared from 70 % alcohol and iodine in 3:1 ratio for 10 seconds, air dried in the safety cabinet for 8-10 min and then cracked with a sterile knife. An automatic vortex mixer was used to mix well with egg contents. After each egg content was homogenized, 25ml of mixed egg content was inoculated into 225 ml of BPW and homogenized for a two-minute period.

### Isolation of Salmonella

Isolation was conducted based on conventional methods for Salmonella detection, following standard ISO 6579 guidelines Mooijman et al. [22]. Egg surface swabs were inoculated directly into screw-capped bottles containing 10ml Buffered Peptone Water (BPW) and incubated at 37°C for 16-18 hours. Each egg's content was thoroughly mixed by using an auto vortex mixer and 25 ml of mixed egg content was inoculated into 225 ml of BPW and incubated at 37°C for 16-18 hours Suresh et al. [21]. The pre-enrichment broth after incubation for 16-18 hours was mixed and 0.1 ml of the broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium (RV broth). Another 1ml of pre-enrichment broth was transferred into a tube containing 10ml of Muller-Kauffmann tetrathionate broth (MKTT broth). The inoculated RV broth was incubated at 41.5°C ±1°C for 24 ± 3 hours. The inoculated MKTT broth at 37 ° C ± 1 °C for 24 ± 3 hours Suresh et al., [21].

A loop full of RV and MKTT broth inoculums was transferred and streaked separately onto the surface of Xylose Lysine Deoxycholate agar (XLD agar); Titan Biotech Ltd., Bhiwadi, India) and Salmonella-shigella agar (SSA agar; Titan Biotech Ltd., Bhiwadi,

India) separately. The plates were incubated at 37°C ± 1°C for 24±3 hours. After proper incubation, the plates were examined for the presence of suspected Salmonella colonies. XLD agar is pink with a darker pink center whereas lactose-positive Salmonellae are yellow with or without blackening. Confirmation was done by using a biochemical test according to ISO 6579 Mooijman et al. [22].

### Biochemical Confirmation of Salmonella Isolates

Each identified colony with typical Salmonella morphology was confirmed biochemically by inoculation into triple sugar-iron agar, methyl-red-Voges-Proskauer broth, indole test, Simmons' citrate agar, urea agar, lysine iron agar, kligler's iron agar and SIM medium and incubated at 370C for 18 to 48 hours. The result was interpreted according to International Organization for Standardization guidelines (ISO-6579-1, 2017).

### Anti-microbial Susceptibility Testing

Antimicrobial susceptibility test was performed for all Salmonella isolates based on the criteria of the Clinical and Laboratory Standards Institute [23] for Enterobacteriacea. For the susceptibility test, the anti-microbial was selected based on groupings of antimicrobial agents with United States food and drug administration which are commonly used for the treatment of Salmonella in animal and human in Ethiopia. The 10 antimicrobial anti-microbial susceptibility tests were carried out by Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA). The inhibition zone was measured with millimeter caliper meter (mm) and interpreted according to the CLSI guideline standard and manufacturer's recommendation as susceptible, intermediate or resistant Table 1 CLSI [23].

**Table 1:** Clinical Laboratory Standard Institute breakpoints for Enterobacteriacea (CLSI, 2018).

| Antimicrobial | Concentration (µg/disc) | Susceptible * | Intermediate* | Resistant* |
|---|---|---|---|---|
| Ampicillin (AMP) | 10 | ≥17 | 14-16 | ≤13 |
| Cephalothin (CEP) | 30 | ≥18 | 15-17 | ≤14 |
| Ciprofloxacin (CIP) | 5 | ≥21 | 16-20 | ≤15 |
| Chloramphenicol (C) | 30 | ≥18 | 13-17 | ≤12 |
| Gentamycin (GEN) | 10 | ≥15 | 13-14 | ≤12 |
| Kanamycin (K) | 30 | ≥18 | 14-17 | ≤13 |
| Nalidixic Acid (NA) | 30 | ≥18 | 14-18 | ≤13 |
| Rifampicin (R) | 5 | ≥14 | Not applicable | ≤14 |
| Tetracycline (TET) | 30 | ≥15 | 14-Dec | ≤11 |
| Streptomycin (S) | 10 | ≥15 | 14-Dec | ≤11 |

### Data Analysis

All data were entered into a Microsoft Excel spreadsheet and transferred to SPSS version 20 for analysis. Salmonella prevalence by source of samples and sample types was expressed as percentages. Descriptive analysis of the collected data was done for most variables in the study using statistical parameters such as percentage and mean. The association was identified by the chi-squire test and P-value less than 0.05 were taken as the cut point of significant.

## Results and Discussions

### Prevalence of Salmonella

Out of 314 samples processed (157 egg surfaces and internal contents each); Only 7 samples, 2.23 %, were Salmonella positive. Of the seven Salmonella positive samples, four isolates were from egg shall surface. The remaining three isolates were from egg internal content samples collected from small scale poultry farm (Figure 1). However, all samples collected from the egg contents of market eggs were negative for salmonella. The overall Salmonella prevalence levels of 2.23 % on eggs in the current study were in agreement with the finding of salmonella species recovered from table eggs in Denmark, with a prevalence of 2.6 %. Another study agreed with our finding was 2.7 % overall prevalence of Salmonella from chicken table egg in Harmaya district, Ethiopia Kemal et al. [24].

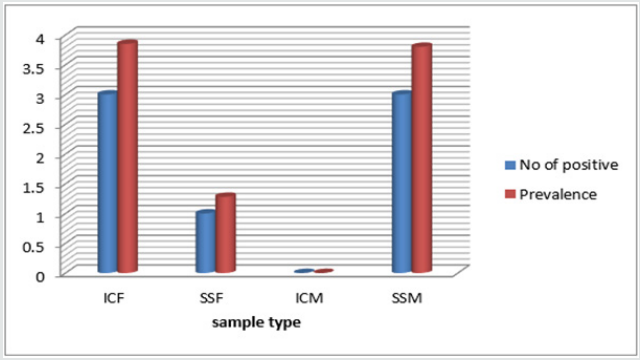


**Figure 1:** prevalence of Salmonella from farm, market and part of egg types in 2018/19.

ICF= Table egg internal contents from farm, SSF= Table egg surface swab from farm, ICM= Table egg internal contents from market, SSM= Table egg surface swab from market

The total isolation rate in the current study was 2.23 % higher than the study done in retail eggs samples in New Zealand 1.8 % and from retail egg samples reported in the United Kingdom 0.34 % (Authority, [25]), isolated Salmonella species. A recent survey of retail eggs from 5,000 egg samples in Northern Ireland and the Republic of Ireland found only 0.04 %, all of which were lower than the current study (Murchie et al., [26]). On the other hand, the current finding was lower than previous studies by Suresh et al. [21] Reported in South India: 7.7 % Salmonella prevalence in retail eggs and Tsegaye et al. [27] 13.3 % prevalence reported of exotic chicken eggs in Alage, Ziway and Shashemene, Ethiopia. Ejo et al. [28] also reported an 18 % prevalence of raw egg collected from restaurants, hotels, cafeterias pastry and retail shops in Gondar, Ethiopia. The probable reason for this variation in Salmonella prevalence could be due to difference in Salmonella disease epidemiology, difference in application of good egg handling practices, variation in hygienic farm situation, store retailers and lack of awareness of Salmonella status in chicken eggs. Poultry feeds and hatcheries could also be the reason for the variation in overall prevalence. Kassahun et al. [29] stated that the presence of chickens of different ages on the farm, the presence of arthropod pests, wet and soiled litter on the farm, the housing system and flock size could be important reasons for egg contamination with various micro-organisms.

Salmonella isolation rate on surface swabs of farm table eggs in this study was 1.28 % lower than reported by Nguse et al. [30] in Mekele, Ethiopia, which were 4 %. Kemal et al. [24] on the other hand did not isolate any Salmonella on the surface of the Harmaya District poultry farm egg. The prevalence difference may be due to Salmonella. The prevalence of eggs in this study may be explained by environmental contamination during storage and transportation and ineffective cleaning of eggs. Furthermore, the management system in practice could also be the probable reason for the variation in prevalence. Salmonella isolate levels were not varied among sampling sites and types. There was no significant difference in the level of Salmonella positivity in local markets and poultry farms (p=0.69) (Table 2).

**Table 2:** Distribution of Salmonella prevalence based on sampling sites and part of egg in Bahir Dar Town, 2018/19.

| Source of Sample | Total observation | Frequency of positivity | Prevalence | X2 value | p- value |
|---|---|---|---|---|---|
| Farm | 156 | 4 | 2.56 | 0.1594 | 0.69 |
| Market | 158 | 3 | 1.9 | | |
| Shell surface swab | 157 | 4 | 2.55 | 0.1461 | 0.702 |
| Internal contents | 157 | 3 | 1.91 | | |

The level of Salmonella prevalence in egg surface swab from the open market was higher than the level of Salmonella in egg internal contents. However there was no significant difference of Salmonella isolation rate between surface swab and internal contents (P value; 0.702) (Table 2). The prevalence of Salmonella samples taken from internal contents in current study were 1.91% which was lower than study done in Addis Ababa by Bayu et al. [31] reported 4.7%. In farm eggs, we recorded an overall isolation rate of 2.6 %.Various contamination levels were reported by numerous studies in Ethiopia 2.5% from chicken eggs in Kombolcha city Assefa et al. [32]; 2.9% in Haremaya on chicken of different breeds Kassahun et al. [29]2.9% and 3.3% in large farms from Grenada, West Indies (Sabarinath et al. [33]). Conversely, the present finding was higher than Kemal et al. [29] in Haremaya that was not isolated and lower than study done in Long et al. [34] were 33.3%.

Salmonella prevalence from eggs in this study might be explained by environmental contamination during storage and transportation and ineffective cleaning of eggs. Moreover, the management system in practice could also be the probable reason for the variation of the prevalence. Egg contamination can occur in the infected ovary of the hen (Hossain et al. [35]) and also through egg contact with fecal material or it might be due to contamination of eggs during their supply from chicken owners to wholesale and retail markets.

## Antimicrobial susceptibility testing

For the antimicrobials tested, all seven Salmonella isolates were resistant to ampicillin (AMP) and tetracycline (TET). In addition, 85.7 % of isolates were resistant to cephalothin (CEP), streptomycin (S) and rifampicin (R). On the other hand, all isolates were sensitive to gentamycin, 85.7 % of isolates were susceptible to ciprofloxacin and 57.1 % were susceptible to kanamycin (K), nalidixic acid (NA) and chloramphenicol (C) (Table 3). This finding was consistent with a study conducted in Harmaya, Ethiopia by Kemal et al. [24] who reported that all isolates were sensitive to ciprofloxacin and over 70 % isolates were sensitive to chloramphenicol(C). In the current study, all Salmonella isolates develop multiple drug resistance. Extensive and inappropriate use of antibiotic drugs in food animal agriculture can establish reservoirs of antimicrobial resistant bacteria, greatly affecting public health Afema et al. [13].

**Table 3:** Mono drug susceptibility profile of all isolates to the respective Antimicrobials.

| Type of drug | Susceptible (%) | Intermediate (%) | Resistance (%) |
|---|---|---|---|
| AMP | 0(0) | 0(0) | 7(100) |
| CEP | 0(0) | 1(14.3) | 6(85.7) |
| CIP | 6(85.7) | 1(14.3) | 0(0) |
| C | 4(57.1) | 1(14.3) | 2(28.6) |
| GEN | 7(100) | 0(0) | 0(0) |

| | | | |
|---|---|---|---|
| K | 4(57.1) | 1(14.3) | 2(28.6) |
| NA | 2(28.6) | 3(42.9) | 2(28.6) |
| R | 0(0) | 1(14.3) | 6(85.7) |
| TET | 0(0) | 0(0) | 7(100) |
| S | 0(0) | 1(14.3) | 6(85.7) |

AMP= Ampicillin, TET= Tetracycline, CEP= Cephalothin, S= Streptomycin, K= Kanamycin, NA= Nalidixic Acid, C= chloramphenicol, R= Rifampicin, GEN= Gentamycin, CIP= Ciprofloxacin.

In current study resistance to ampicillin and tetracycline becomes hundred percent which is higher than reported 72.7 % in Haremaya by Kassahun et al. [29] and 73 % in Germany (Miko et al. [36]). The finding of this result shows that resistance pattern of isolates to ampicillin and tetracycline were in consistent with 93.1% reported in Nigeria from Salmonella isolates in chicken eggs by (Ekundayo and Ezeoke, [37]). Isolates sensitivity to Streptomycin 14.3 % in current study were lower than 100 % and Kanamycin 57 % sensitivity also lower than 91% reported by (Kassahun et al., [29]). When bacterial isolate develop resistance to more than three antibiotics; it is said to be multidrug resistant (Sweeney et al. [38]). Accordingly all the 7 isolates were developed multi drug resistant. As shown in Table 4 of the isolates were multi drug resistant to at least for 5 different antimicrobials used. In addition, only one isolate was multi drug resistant for 6 and the other one isolate for 7 antimicrobials used in this study. Multiple drug resistance was observed in over 30 % of Salmonella isolates, which is lower than the current study (100%) of isolates were developed multi-drug resistant for tested antimicrobial disks. A high proportion of Salmonella isolates were developed to resist commonly prescribed antimicrobials. This may be a considerable risk in the treatment of clinical cases Addis et al. [39]. In contrast to the current study, Sibhat et al. [15] founds 18 (20.7%) Salmonella isolates were resistant more than three antimicrobials, which is lower than the current study [40-42].

**Table 4:** Multi-drug resistance pattern of Salmonella isolates from table eggs in Bahir Dar, 2018/19.

| Resistance pattern | Antimicrobials tested (number of isolate) | Total number of resistant isolate |
|---|---|---|
| 5 | CEP,AMP,TET,S,R(3) | 5 |
| | CEP,AMP,TET,S,K(1) | |
| | CEP,AMP,TET,R,C(1) | |
| 6 | AMP,NA,TET,S,R,C(1) | 1 |
| 7 | CEP,AMP,NA,TET,S,K,R(1) | 1 |

AMP= Ampicillin, TET= Tetracycline, CEP= Cephalothin, S= Streptomycin, K= Kanamycin, NA= Nalidixic Acid, C= chloramphenicol, R= Rifampicin, GEN= Gentamycin, CIP= Ciprofloxacin

## Limitation of This Pepper

Serotyping and molecular identification of resistance Salmonella strains, as well as analyses of its antimicrobial resistance genes were not done.

## Conclusion and Recommendations

The isolation rate was low in the current study. However, more egg contamination was observed by Salmonella isolates in eggs obtained from the farm than the market. External egg contamination was more frequently observed than the internal egg content. All Salmonella positive isolates were tested by ten selected antibiotic disks for susceptibility test. A hundred percent of the isolates were become resistant to more than five antibiotic disks. All isolates were developed to resist ampicillin and tetracycline, but all of them become sensitive to gentamycin. This indicates the importance of avoiding the use of antimicrobials in growth promoters and feed additives to reduce drug resistance. Strict biosecurity measures in poultry farms should be encouraged to reduce salmonella contamination of eggs. There is a need for further study on molecular characterization and serotyping of Salmonella strain found in table egg that is resistance.

Ethics approval and consent to participate.

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