

## Clinical Microbiology 2018 : Prevalence and Antibiotic Resistance Pattern of Salmonella Isolated from Caecal Contents of Exotic Chicken in Debre Zeit and Modjo, Ethiopia- Destaw Asfaw Ali- University of Gondar

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A cross-sectional study was conducted between December, 2013, and May, 2014, to determine the prevalence and antibiotic resistance feature of Salmonella isolated from broilers slaughtered in Debre Zeit and Modjo towns, Ethiopia. A total of 384 caecal content samples were collected for microbiological examination following the standard techniques and procedures outlined by the International Organization for Standardization to isolate Salmonella. The sensitivity of the isolates subjected to nine antimicrobials was tested by the Kirby–Bauer disk diffusion method. The overall prevalence of Salmonella was 14.6%, and its occurrence differ significantly by farm (). The occurrence of the bacteria was not statistically different in the midland (15.2%) and lowland (13.3%) () and between males (13.5%) and females (15.6) (). Of the 50 isolates, 48 were resistant to at least one drug. Multidrug resistance was recorded in 43 (86.0%) of the isolates. The study demonstrated considerable prevalence and high antimicrobial resistant Salmonella in exotic chicken and indicates the potential importance of chickens as source of foodborne salmonellosis and multiple antimicrobial resistance of Salmonella. Improving the hygienic practice of farms could help to reduce the occurrence of Salmonella in farms. Further studies are needed to describe the risk factors associated with the emergence of drug-resistant Salmonella in chicken.

### 1. Introduction

Despite global improvements in public health facilities, bacterial infections still remain an important public health problem worldwide. Salmonellosis is one of the main foodborne zoonotic and animal husbandry problem throughout the world. The bac-

teria cause foodborne poisoning in humans, mainly through animal products that include poultry, cattle, and pig products.

Salmonella is facultative anaerobic Gram-negative rod that grows optimally at 35°C to 37°C and catabolizes a variety of carbohydrates into acid and gas in addition to H<sub>2</sub>S gas production. The genus comprises two species: Salmonella enterica and Salmonella bongori.

Salmonellosis has been recognized in all countries but seems to be most prevalent in areas of intensive animal husbandry, especially poultry and swine production. Although primarily an intestinal bacteria, Salmonella is widespread in the environment and commonly found in farm effluents, human sewage, and in any material subject to fecal contamination. The natural habitat of Salmonella may be divided into three categories: (i) highly adapted to men and agents of typhoid fever; (ii) highly adapted to animals responsible for animal paratyphoid; and (iii) most of the serovars that affect men and animals.

In developing countries, domestic chickens live in close contact with humans in both urban and village communities, often being housed overnight in the family home. Infection of the chicken leads to faecal shedding into the environment. Contamination of meat and eggs is the main chain of human Salmonella infections in addition to the contact of workers in poultry farms and slaughtering houses . The risk development of antibiotic resistance in human and animal is one of the main reasons for control in animals.

Antimicrobial resistance is a global public health problem. Resistance to antimicrobials could be due to three basic mechanisms:(i) modification of the an-

tibiotic by decreasing absorption or increasing efflux of the antibiotic by using their enzymes; (ii) change in the target site of the antibiotic; and (iii) acquisition of the ability to break or modify the antibiotic. Several lines of evidence demonstrate that the use of antimicrobial agents in food animals contributes to the emergence and dissemination of antimicrobial resistance in food borne Salmonella. In recent years, antimicrobial resistance is a serious problem in the treatment of Salmonella infection.

## 2. Materials and Methods

### 2.1. Study Areas

The study was conducted in Debre Zeit and at Modjo, Ethiopia. Debre Zeit and Modjo are found in Oromia regional state 47 and 72 km South East of Addis Ababa, respectively. Debre Zeit is located at 9°N and 39°E, at an altitude of about 1900 m above sea level. Modjo is located at 8.3°N and 39°E, at an altitude of 1774 m above sea level. The areas experience a bimodal rainfall with the main rainy season extending from June to September. Debre Zeit and Modjo have annual average rain fall of 800 mm and 776 mm, average temperature of 18.7°C and 19.4°C, and mean relative humidity of 61.3% and 59.9%, respectively.

### 2.2. Study Design

The study was cross-sectional and conducted from December 2013 to May 2014. The animals were apparently healthy chicken slaughtered at commercial processing plants and private producers. The chickens were rose breed maintained under intensive management system and are of the same age (45–50 days) groups. The sample size was determined according to Thrusfield with an expected prevalence of 50%, 95% confidence interval, and 5% absolute precision. Accordingly, a total of 384 chickens were sampled: 133 from Alema Farms commercial processing plant, 120 from Modjo poultry slaughtering house, and 131 from three other private holders in Debre Zeit. Sampling was systematic and random.

### 2.3. Isolation and Identification

The isolation and identification of Salmonella was according to the techniques recommended by the International Organizations for Standardization (ISO-6579) [29] with Global Salmonella Survey (GSS) and WHO guidelines [30]. All the media used were prepared according to the instructions of the manufacturer.

#### 2.3.1. Culture Methods

(1) Non selective Preenrichment. The refrigerated samples were thawed at room temperature, and 5 grams of the caecal contents were preenriched with 45 ml of buffered peptone water at a ratio of 1 : 9 BPW (HiMedia M1494, Mubi, India). The suspension was harmonized by mixing using a vortex and incubated for 24 hr at 37°C.

(2) Selective Enrichment. A portion (1 ml) of the preenriched cultured was transferred to 10 ml of selenite-F (Titan TM389, Biotech, India) broth, and another 0.1 ml portion was transferred to 10 ml of RV broth (HiMedia M880, Mubi, India) and incubated at 37°C and 42°C for 24 hours, respectively.

#### 2.3.2. Biochemical Tests

Biochemical tests were performed by using the following tests: triple sugar iron agar (TSI) (Oxoid CM0277, Basingstoke, England), lysine iron agar (LIA) (Oxoid CM381, Basingstoke, England), urea broth (Oxoid CM53, Basingstoke, England), Voges Proskauer (VP) (HiMedia M070I, Mumbai, India), tryptone broth (HiMedia M463, Mumbai, India) for indole test.

#### 2.3.3. Antimicrobial Tests

The susceptibilities of the isolates to antimicrobials were tested by using the Kirby–Bauer disk diffusion method. The isolates were tested for the following antibiotics: ampicillin (AMP) (10 µg/ml), streptomycin (STR) (10 µg/ml), kanamycin (KAN) (30 µg/ml), ceftriaxone (CRO) (30 µg/ml), chloramphenicol (CHL) (30 µg/ml), oxytetracycline (OXT) (30 µg/ml), sulfamethoxazole-trimethoprim (SXT) (25 µg/ml), nalidixic acid (NAL) (30 µg/ml), and amoxicillin (AMC) (30 µg/ml), all from Oxoid, England. The isolates

were inoculated to brain heart infusion broth (Oxoid CM0225, Basingstoke, England) and incubated for 8 hours at 37°C till the turbidity of the suspension equals to that of a 0.5 McFarland standard.

#### 2.4. Statistical Analysis

The data were entered into a Microsoft Excel 2007 and imported to SPSS version 20 for analysis. Descriptive statistics was used to describe the data. Pearson's chi-square ( $\chi^2$ ) test was used to test associations. Alpha was set at 0.05.

### 3. Results

#### 3.1. Prevalence of Salmonella

Salmonella was recovered in 56 (14.6%) of the samples. The bacteria were isolated in 28 (21.1%), 16 (12.2%), and 12 (10.0%) of the samples that originated from Alema, Aleka Amba, and Modjo farms, respectively (Table 1). The occurrence of the bacteria differed significantly by farm (). Its occurrence was higher at Alema farms but not significantly different between Aleka Amba and Modjo farms ( $\chi^2 = 0.31$ ; ). The occurrence of Salmonella did not differ by altitude () and sex () (Table 2).

df = degree of freedom.

masl = meter above sea level; df = degree of freedom.

#### 3.2. Antibiotic Resistance

##### 3.2.1. Mono Drug Resistance

The antimicrobial susceptibility features of the isolates are given in Table 3. The highest resistance was recorded for oxy tetracycline (82.0%) followed by ampicillin (70.0%). Resistance to kanamycin and ceftriaxone were observed in 26.0% and 6.0% of the isolates, respectively. Intermediate resistance to each of the tested drugs was recorded in four or more of the tested isolates. Only two isolates were sensitive to all drugs, and five were resistant to a single drug.

##### 3.2.2. Multidrug Resistance

The multidrug resistance features of the isolates are

shown in Table 4. Of the tested isolates, 43 (86.0%) were resistant to two or more (up to seven) antimicrobials and 26 multidrug resistance profiles were observed. The number of isolates resistant to five drugs was higher followed by two drug-resistant isolates. The OXT, AMP, CHL, NAL, SXT, and STR (4/43) phenotype occurred more frequently followed by the OXT, AMP, CHL, NAL, SXT, and KAN (3/43) phenotype.

### 4. Discussion

The overall prevalence of Salmonella was 14.6% and differed by location. The prevalence difference in these sites could be due to differences in environmental contamination or management systems. However, the prevalence difference between Aleka Amba and Modjo farms did not differ statistically (). The prevalence difference of isolates between midland and lowland was 15.2% and 13.3%, respectively, while that of sex was 15.6% in female and 13.5% in male who did not also differ statistically (), and hence, there was no association between these risk factors and the prevalence of the pathogen.

The overall prevalence of Salmonella was 14.6%. The result is in agreement with the 16.1% prevalence estimate from faecal and cloacal swaps of intensive poultry farms in Hawassa [32]. Comparable results were also reported in Egypt [33] and in Nepal [34]. By contrast, a higher prevalence in Jima (41.9%) was recorded [35], and a lower prevalence (9.3%) in East Showa, Ethiopia [26], was recorded. The differences in the prevalence estimates could be due to differences in environmental contamination, management systems, breed, sample size, and method of isolation.

Except two isolates, all were resistant to at least a single antimicrobial. A considerably higher proportion of the isolates 43 (86.0%) was resistant to two or more of the antimicrobials, and 26 multidrug resistance profiles were observed. Multidrug resistant is considered when it is simultaneously resistant to two or more drugs [36]. The number of isolates resistant to five drugs was higher followed by two drug-resistant isolates.

### 5. Conclusion and Recommendations

The overall prevalence of Salmonella in chicken is considerable, and the antibiotic resistance feature of the subjected isolates is recorded high. Chicken are potential sources of foodborne salmonellosis and antimicrobial resistant Salmonella that pose a high risk to both animals and humans. Awareness should be created to avoid indiscriminate use of antimicrobials.

There should be intense and effective communication between veterinary organizations and human health care providers to control spread of salmonellosis and to target antibiotic resistance. Further studies are needed to describe the occurrence of Salmonella in chicken and the risk factors that favour the emergence of drug-resistant isolates.