

Drug- and Multidrug-Resistance Pattern of Enterobacteriaceae Isolated from Droppings of Healthy Chickens on a Poultry Farm in Southwest Ethiopia

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Background: In Ethiopia, the precise attribution of animals and their food products as the sources of resistant strains and the consequences of it on human health have not yet been seriously evaluated. Therefore, the aim of this study was to assess the drug- and multidrug-resistance pattern of Enterobacteriaceae isolated from chicken droppings at Jimma University poultry farm, College of Agriculture and Veterinary Medicine, southwest of Ethiopia.

Methods: A cross-sectional descriptive study was conducted from April, 2018 to June, 2018. A total of 140 fresh chicken dropping samples were collected and transported to Jimma University Medical Microbiology Laboratory for analysis. All samples were inoculated on MacConkey agar and xylose lysine deoxycholate agar. Gram stain and relevant biochemical tests were done for identification of isolates. Antimicrobial susceptibilities were tested by the Kirby–Bauer disk diffusion method.

Results: Out of 140 chicken dropping samples, 61 (43.6%) showed bacterial growth. Of these, *E. coli* accounts for 39.0% followed by *K. pneumoniae* (22.0%), *P. mirabilis* (19.3%), and *Salmonella* species (17.7%). With regard to antibiotic resistance pattern, *E. coli* demonstrated a high rate of resistance against ampicillin (91.7%), tetracycline (75.0%), and trimethoprim-sulfamethoxazole (70.8%). *K. pneumoniae* showed a high resistance rate against ampicillin, trimethoprim-sulfamethoxazole, and tetracycline, with a resistance rate ranging from 76.9% to 85.6%. *P. mirabilis* and *Salmonella* spp. also showed high resistance against ampicillin, tetracycline, and trimethoprim-sulfamethoxazole with a resistance rate ranging from 72.7% to 83.3%. All isolates relatively showed lower resistance rates with a range of 20.8% to 41.7% against third-generation cephalosporins (ceftazidime and ceftriaxone), ciprofloxacin, and gentamicin. Totally, 32 (52.5%) of the isolates showed MDR against three or more antibiotics.

Conclusion: Antibiotic resistant isolates against commonly prescribed single and multiple drugs were common. This highlights that chickens in the farm may serve as the reservoirs of antibacterial resistant bacteria that might infect humans through the food chain. Therefore, emphasis on the usage of antibiotics in chicken farms has to be considered.

Keywords: drug- and multidrug-resistance, Enterobacteriaceae, chicken droppings, Ethiopia

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Background

Time-to-time emergence of antimicrobial resistant (AMR) strains of bacteria challenges the dependable treatment for many infectious diseases. The extensive and misuse of antibiotics in animals for growth promotion and disease prevention

triggers high selection pressure among microbial agents which might contribute to the emergence of drug- and multidrug-resistance bacteria and put human health at risk of becoming infected with these transferred zoonotic resistant bacteria. Especially in low and middle-income countries (LMICs), due to the highest incidence of infection and inappropriate use of antibiotics in human and animals, there are significant gaps in combating the infectious diseases.¹⁻³

Despite its benefits, inappropriate use of antibiotics leads to the emergence of antibiotic resistant bacteria and this situation has threatened the current and future advanced modern treatment efficacy.⁴ In veterinary practice, in addition to using antibiotics for therapy and disease prevention, antibiotics are regularly added to animal feed in sub-therapeutic doses for growth promotion. For instance, approximately 8,164,662 kg of antibiotics are used annually out of which 70% is used for non-therapeutic purposes.⁵ Chickens are consumed worldwide, hence poultry and poultry products for increment of human demand drive the agro-based industries of poultry to grow fast and large. The increment of demand poses a concern to the producers on the issue of care and keeping chicken health. In doing this, poultry producers employ antibiotics at therapeutic doses to prevent disease, and increase efficiency of feed utilization and growth performance.⁶

It was reported that Enterobacteriaceae, a large family of Gram negative bacteria, is a highly prevalent infectious agent that has increasingly shown a high rate of antimicrobial resistance.⁷⁻⁹ These organisms are regularly found in the intestine of animals including poultry mostly as harmless, while the pathogenic form has threatened the effective prevention and treatment interventions.⁹ The truth is that animal production is an important vehicle for the spread of antimicrobial resistance (AMR) to consumers.⁹ For instance, the emergence of AMR from poultry and poultry farms is recognized as a potential community health concern as it can be transmitted through food chains and direct contact with poultry and poultry products.^{3,9} The situation influenced international organizations to give emphasis over decades to recognizing its current threat on public health and veterinary medicine.¹⁰ The World Health Organization has recognized the need for an improved and coordinated global effort to contain the problem of emerging antimicrobial resistance based on global surveillance.¹⁰ The One Health paradigm has also recognized that the combined assessment of health risks

across the three domains – humans, animals, and the environment – involves design and implementation of intervention strategies that preserve the usefulness of existing antibiotics for as long as possible.¹¹

In Ethiopia, chickens are an important income source in addition to offering eggs and meat for poor smallholder households.¹² The application of antimicrobial use to poultry in the country has been also practiced. Taking into account the issue, it is important to monitor the resistance of microbial agents, not only human pathogens but also pathogens of animal origin for the human. Therefore, this study aimed to assess the bacterial profile and antimicrobial resistance patterns of Enterobacteriaceae isolated from chicken droppings at Jimma University poultry farm, College of Agriculture and Veterinary Medicine, southwest of Ethiopia.

Methods

Study Area and Period

This study was conducted at Jimma University poultry farm which is found in Jimma town (administrative town of Jimma zone) from April, 2018 to June, 2018. The town is located in Jimma zone, Oromia region at a distance of 335 km away from Addis Ababa to the southwest of Ethiopia. Jimma University poultry farm was established for two basic goals, for academic and research purposes for animal science studies and for community based service through supply of chickens for the society and Jimma area farmers at affordable cost. This poultry farm was managed by two veterinary professionals and three assistants (caretakers) during the study period and holds a total of 631 chickens of exotic and endogenous types.

Study Design and Study Units

A cross-sectional study was conducted and apparently healthy chickens and those aged above one week were considered eligible. A total of 140 fresh chicken dropping samples were collected from randomly selected healthy chickens of age more than one week. Based on the document of the poultry farm a total of 631 chickens were found during the study period. All chickens with age under one week and those suspected of having a symptom of illness were excluded from this study.

Sample Collection and Transport

The farm was visited once every week for consecutive eight weeks and approximately 10 g of freshly passed poultry dropping samples were aseptically collected

using sterile spatulas from randomly selected apparently healthy chickens by attendants and placed into sterile universal sampling bottles. The samples were kept in an ice box containing an ice bag and immediately transported to Jimma University microbiology laboratory to be processed on the same day.

Assessment of Chicken Care Practices at the Poultry Farm

The poultry farm was managed by two veterinary professionals and three assistants (caretakers) during the study period. Chicken care practices of the workers were assessed by the use of an observational checklist and closed-ended questionnaires. During the farm visit, variables such as age of chickens, regular cleaning of chicken droppings, feeding condition (ie, whether modified feeding or antibiotic was given or added as growth promotion, whether vaccine was given for prevention of disease), living conditions/spaces, and whether diseased chickens were isolated and empirically treated were assessed.

Isolation and Identification Process

Culturing Method

About 1 g of chicken droppings were suspended in sterile universal sampling bottles containing 9 mL of 0.1% buffered peptone water (BPW) and Selenite F broth and homogenized by shaking for 5 minutes in a sterile stomacher and incubated at 37 °C for 24 hrs for enrichment purposes. One loop-full (10 µL) of the overnight broth cultures were streaked on MacConkey agar and xylose lysine deoxycholate agar (XLD) and incubated at 37 °C for 24 hrs. At the end of incubation the plates were examined for growth. First, the isolated colonies were screened by their colony morphology, lactose fermentation (pink color colonies), and Gram staining and further identifications were made with relevant biochemical tests, such as the Triple sugar iron (TSI) agar test, indole test, urease, methyl red test, gas and acid production tests, motility test, and citrate utilization test as described Cheesbrough.^{13,14} Colonies with red color with a black center on XLD and producing an alkaline slant with acid butt and hydrogen sulfide production on TSI, positive for lysine, negative for urea hydrolysis, negative for Indole test, and positive for citrate utilization were considered to be *Salmonella* spp.

Antimicrobial Sensitivity Testing

The isolated and identified bacteria were subsequently subjected to antimicrobial susceptibility testing according to the Kirby–Bauer disk diffusion technique using guidelines established by the Clinical and Laboratory Standards Institute (CLSI).¹⁵ The following 11 antibiotics were used (Oxoid Ltd, Hampshire, UK): amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ampicillin-sulbactam (10/10 µg), cefoxitin (30 µg), gentamicin (20 µg), ciprofloxacin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), and chloramphenicol (30 µg). After 24 hrs of incubation, the zone of inhibition was measured and interpreted as sensitive (S), Intermediate (I), and resistant (R) according to CLSI guidelines, 2018.¹⁵ *E. coli* ATCC*25922 was used as a quality control strain to monitor the quality of susceptibility testing. Multidrug resistance (MDR) is defined as resistance to three or more classes of antibiotics.¹⁶

Statistical Analysis

Data were edited, cleaned, and checked for completeness and entered into a Microsoft Excel spreadsheet and descriptive statistics, including count and percentage, were calculated using Statistical Packages for Social Sciences (SPSS) version 20. Study findings were explained in words and tables.

Results

Isolation of Bacteria from Chicken Droppings

Out of 140 chicken dropping samples, 61 (43.6%) showed bacterial growth. Only those growths with individual colonies were included, and three to five of the separate colonies were taken for identification. The predominant bacterial isolate was *E. coli* (n=24, 39.0%), followed by *K. pneumoniae* (n=14, 22.6%), *P. mirabilis* (n=12, 19.3%) and *Salmonella* spp. (n=11, 17.7%) (Figure 1).

Feeding Practices of Chicken Attendants

Data from the interview of chicken attendants showed that none of them had any form of formal educational and short course training certificate on how to feed and on safe handling practices of chickens. There was no regular cleaning of chicken excreta which made it to be mixed with chicken feed. In addition, chickens above three months of age were supplied with antibiotics in their feed as usual practice of

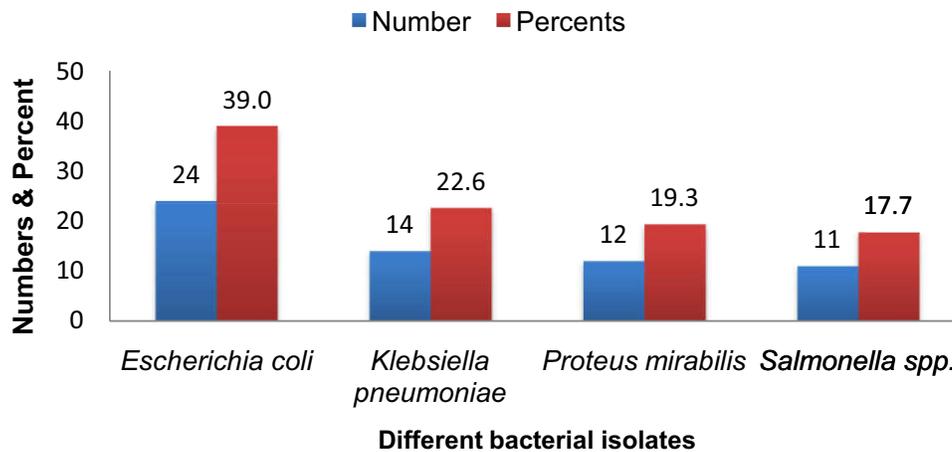


Figure 1 The proportions of bacterial isolates from chicken droplets.

modified feeding. As any other feeding components, antibiotic treatment of drinking water supply for chickens was practiced. Preventive vaccine for Newcastle viral disease and other infection for all adult chickens was given on a programmed and scheduled booster dose approach. They also responded that diseased chickens might not be treated and isolated timely from the groups (Table 1).

Antimicrobial Susceptibility Testing

In the current study a varying degree of susceptibility against the tested antimicrobial agents was observed. Accordingly, *E. coli* demonstrated a high rate of resistance against ampicillin (n=22, 91.7%), amoxicillin-clavulanic acid (n=16, 66.7%), ampicillin-sulbactam (n=16, 66.7%), tetracycline (n=18, 75.0%), trimethoprim-sulfamethoxazole (n=17, 70.8%), and chloramphenicol (n=13, 54.2%). Likewise, *K. pneumoniae* isolates showed a high resistance rate against ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, tetracycline, cefoxitin, and chloramphenicol with resistance rates ranging from 50.0% to 85.7%.

P. mirabilis and *Salmonella* spp. isolates also showed high resistance rate to ampicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, cefoxitin, tetracycline, trimethoprim-sulfamethoxazole, and chloramphenicol with resistance rates ranging from 45.5% to 83.3%. All isolates showed lower resistance rate with a range of 20.8% to 41.7% against third-generation cephalosporins (ceftazidime and ceftriaxone), ciprofloxacin, and gentamicin (Table 2).

Multidrug-Resistance (MDR) Pattern

In this study, 13 (54.2%) *E. coli* isolates showed resistance against three or more antibiotics, whereas 8 (57.1%) of *K. pneumoniae* showed resistance against three or more antibiotics. In addition, 6 (50.0%) *P. mirabilis* isolates and 5 (44.4%) *Salmonella* spp. isolates showed resistance against three or more antibiotics. Totally, 32 (52.5%) of the isolates showed MDR phenotype (resistance to three or more antibiotics belonging to different families (Table 3)).

Table 1 Summary of Activities Done by Chicken Attendants at Jimma University Poultry Farm

Activities Checked	Responses
Having formal education or short course training certificate on how to keep chickens	None of them
Regular cleaning of chicken droppings	In every 2 to 3 days
Giving antibiotics as growth promotion and disease prevention	Regularly given
Timely isolation and separation of diseased chickens	Sometimes
Living condition/space	Crowded
Contact of feeds with chicken droppings	Frequently contacted
Whether chickens are exotics and/or endogenous	Both

Table 2 Antibiotic Resistance Pattern of the Bacterial Isolates

Bacterial Isolates	Number of Strains Resistant to the Tested Antibiotics N (%)										
	AMP	AMC	AMS	CRO	CAZ	FOX	CN	CIP	TET	SXT	CHL
<i>Escherichia coli</i> (n=24)	22 (91.7)	16 (66.7)	16 (66.7)	10 (41.7)	9 (37.5)	14 (58.3)	5 (20.8)	5 (20.8)	18 (75.0)	17 (70.8)	13 (54.2)
<i>Klebsiella pneumoniae</i> (n=14)	12 (85.7)	9 (64.3)	8 (57.1)	5 (35.7)	4 (28.6)	8 (57.1)	4 (28.6)	3 (21.4)	11 (78.6)	11 (78.6)	7 (50.0)
<i>Proteus mirabilis</i> (n=12)	10 (83.3)	7 (58.3)	7 (58.3)	4 (33.3)	4 (33.3)	6 (50.0)	4 (33.3)	3 (25.0)	10 (83.3)	9 (75.0)	7 (58.3)
<i>Salmonella</i> spp. (n=11)	9 (81.8)	6 (54.5)	6 (54.5)	4 (36.4)	4 (36.4)	7 (63.6)	4 (36.4)	3 (27.3)	8 (72.7)	8 (72.7)	5 (45.5)
Total R [N (%)]	52 (83.9)	38 (61.3)	37 (59.7)	23 (37.1)	21 (33.9)	35 (56.4)	17 (27.4)	14 (22.6)	46 (74.2)	44 (71.0)	32 (51.6)

Abbreviations: AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CAZ, ceftazidime; CRO, ceftriaxone; AMS, ampicillin-sulbactam; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline; CIP, ciprofloxacin; CN, gentamicin; FOX, cefoxitin; CHL, chloramphenicol; MEM, meropenem

Discussion

In this study, a total of 140 chicken droppings were collected from Jimma University poultry farm, southwest of Ethiopia for bacteriological analysis. Out of these, 61 (43.6%) samples showed bacterial growth. The proportion of culture positive samples in this study was lower than study findings in Nigeria^{17,18} and Kenya.¹⁹ However, this finding is higher than study findings in Ethiopia²⁰ and Bangladesh.²¹ Differences in prevalence rates in various studies may be due to method used to isolate the bacteria, types and numbers of samples, animal management practices, and hygienic conditions. For instance, in this study, none of the chicken attendants had any form of formal educational and short course training certificate on how to feed and on safe handling practices of chickens. That is why there was no regular cleaning of chicken excreta which made it to be mixed with chicken feed that is prone for cross-contamination of chickens.

In this study, the most predominant isolate was *E. coli* followed by *K. pneumoniae* and *P. mirabilis* and *Salmonella* spp. The predominance of *E. coli* isolates as well as *P. mirabilis* and *Salmonella* spp. were also reported from Bangladesh²¹ and Kenya.^{19,22} The fact is that most Enterobacteriaceae, especially, *E. coli*, are predominantly found as normal flora gastrointestinal tracts of animals and humans.²³ This study covered only one poultry farm from a focal area and thereby has generated the additional prevalence data for the bacteria in the poultry industry of this country. The outcomes of this study would be helpful in designing further extensive investigations covering more poultry farms from expanded areas of the country to find nationwide prevalence of zoonotic Enterobacteriaceae pathogen.

Emergence of MDR bacteria, especially Enterobacteriaceae, has increased in recent years. In this study, more than half (52.5%) of isolates showed MDR. The higher proportions of MDR were also reported from Bangladesh,²¹ Vietnam,²⁴ and China.²⁵ The occurrence of MDR may be linked with indiscriminate use of antimicrobial agents such as wrong indication, wrong duration, and improper route of administration, use of leftover antibiotics from a family member, and improper discontinuation of antibiotics.²⁶⁻²⁹

This study also revealed that isolates from chicken droppings showed high resistance properties toward ampicillin, augmentin, tetracycline, and trimethoprim-sulfamethoxazole followed by cefoxitin and chloramphenicol.

Table 3 Multidrug-Resistance (MDR) Pattern of the Bacterial Isolates

Profiles of Antibiotics Resistance	MDR Bacterial spp. N (%)				
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. mirabilis</i>	<i>Salmonella</i> spp.	Total MDR N (%)
AMP, AMS, AMC, TET AMP, AMS, AMC, SXT	9 (37.5%)	5 (38.5%)	4 (33.3%)	3 (33.3%)	21 (34.4)
AMP, AMC, CRO, CAZ, SXT, TET AMP, AMS, CAZ, FOX, SXT, TET AMP, AMC, CRO, FOX, SXT, TET	7 (29.2%)	4 (28.6%)	3 (25.0%)	3 (33.3%)	17 (27.9)
AMP, AMS, AMC, FOX, CN, SXT, TET AMP, AMC, CAZ, FOX, CIP, SXT, TET	4 (16.7%)	2 (15.4%)	2 (16.7%)	1 (11.1%)	9 (14.8)
AMP, AMC, CAZ, FOX, CIP, CHL, SXT, TET AMP, AMS, AMC, FOX, CN, CIP, SXT, TET	2 (8.3%)	2 (15.4%)	1 (8.3%)	1 (11.1%)	6 (9.8)
Total MDR N (%) ≥3 antibiotic classes	13 (54.2%)	8 (57.1%)	6 (50.0%)	5 (44.4%)	32 (52.5)

Abbreviations: AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CAZ, ceftazidime; CRO, ceftriaxone; AMS, ampicillin-sulbactam; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline; CIP, ciprofloxacin; CN, gentamicin; FOX, cefoxitin; CHL, chloramphenicol.

The significant proportion of resistance against the above antibiotics was also reported from Bangladesh,²¹ Nigeria,^{18,30} Ethiopia,²⁰ Vietnam,³¹ and China.²⁵ Moreover, the current study recorded higher resistance to ampicillin-sulbactam. This demonstrates that apart from therapeutic use of antibiotics in poultry, other factors influence development of antibiotic resistance. For instance, in this study, chicken attendants responded as they were supplied antibiotics in their feed as usual practice and as they used as growth promotion and as disease prevention. In addition, chicken droppings were not cleaned regularly and it has a contact with chicken feedings. Studies from Kenya and Bangladesh showed that significant numbers of poultry feeds were contaminated with *E. coli* and *Salmonella* spp. isolates.^{22,32}

In contrast, in this study, isolated bacterial species showed relatively lower grade of resistance against third-generation cephalosporins (ceftriaxone and ceftazidime), ciprofloxacin, and gentamicin. This finding is supported by other studies showing lower resistance rate against the above antibiotics.^{22,26,33} However, higher resistance rates were reported from Bangladesh²¹ and Nigeria.³⁰ This might be a challenge for effectiveness of modern antimicrobial agents commonly used to treat human infections.

Limitations of This Study

The study was done from a single source of sample (one chicken farm) for the determination of prevalent bacterial isolates and antibiotic resistance patterns. Molecular

techniques for more informative finding to indicate the specific antibiotic resistant genes were not conducted due to resource problems.

Conclusions

In this study, significant numbers of chicken droplets contain bacterial isolates that are resistant to single and multiple antibiotics that are commonly prescribed to humans. These findings suggested that avian farms might serve as reservoirs of antibacterial-resistant bacteria that can infect humans through the food chain. Emphasis on the usage of antibiotics in poultry farms has to be considered in order to make longer effectiveness of existing antibiotics.

Data Sharing Statement

All data were incorporated in the manuscript.

Ethical Approval and Consent to Participate

This study was done as dissertations for MSc thesis in Jimma University. That is why ethical approval of the study was obtained from the Institutional Review Board of Jimma University. Communication with the Agricultural College of Jimma University was made through formal letter before running data collection. Verbal informed consent that was approved by the Institutional Review Board of Jimma University was obtained from chicken farm attendants after fully explained the objective of the study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declared that they have no conflicts of interest for this work.

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