

Original Research

Study on Antibiotic Resistance in Escherichia Coli Isolates in Chicken Meat Marketed in Kathmandu Valley

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Abstract: *Escherichia coli* is a known most occurrence bacterial isolates food borne pathogens. It is found in contaminated chicken meat and importance indicator food borne infection with this bacterium. Antimicrobial resistance patterns of *E. coli* both clinical and food borne pathogens increased at a speedy rate (NARMS). The study was carried out in broiler chicken meat samples to identify the isolates of *Escherichia coli* and to evaluate the antibiotic resistance patterns in Kathmandu, Lalitpur and Bhaktapur districts from 2022 to 2023. Bacterial isolation and identification was done using conventional methods. Antibiotic susceptibility testing was conducted using the Kirby–Bauer method. Total 69 (82.14) *Escherichia coli* were isolated from 84 meat samples using epi version for definite population (for 10,000 population, 95% confidence level, 81 sample needed) using of broiler meat from different retail shops of Kathmandu valley. The number of *E. coli* isolates found was 31, 23 and 15 in Kathmandu, Lalitpur and Bhaktapur districts respectively. The proportion of *E. coli* isolates were found in the samples from Kathmandu, Lalitpur and Bhaktapur were 86% (31/36), 82% (23/28) and 75 % (15/20) respectively. The bacterial resistance did not differ by districts, whereas they were relatively susceptible to Gentamycin (65%) and Chloramphenicol (62.3%). The multidrug-resistant *E. coli* was found to be 52.19%. They were most resistant to Amoxycillin (73.91%), Enrofloxacin (55%), Doxycycline (52.17%), and trimethosulphamethazole (43.47). The comparison of different retail meat shop of each district of Kathmandu Valley for the *E. coli* isolates by using Chi-square test ($p > 0.05$) indicated no difference. From this study, it was observed that most of the shops either whole seller or retailer were contaminating meat due to poor personnel hygiene and biosecurity measures. To minimize this problem, a suitable awareness program may be conducted by related organizations for poultry farmers, meat whole seller and retailers to improve personal hygiene and control haphazard use of antibiotics in poultry.

Keywords: Broiler chicken meat, *Escherichia coli*, and antibiotic resistance

1. INTRODUCTION

Poultry industry is one of the leading industries among other livestock industries in Nepal and contributes about 4% to national GDP and 8% to AGDP with an investment of NRs. 80 billion per year (CBS, 2014). This sector has provided direct employment to 1.5 lakh people including people involved in exporting eggs to Bhutan, importing of parent stock, feed ingredients, supplements, medicines, vaccines (Singh et al., 2018). The highest concentration of commercial poultry is found in Chitwan (46%) and Kathmandu (41%) valley, both contributing around 87percent of total commercial poultry (FAO, 2014). One of the

important challenges in Nepalese poultry industry is facing the problem of diseases such as Ranikhet, Infectious Bursal Diseases, Infectious Bronchitis, Chronic Respiratory Diseases, Fowl Pox, Marek's, Avian Influenza (both highly and low pathogenic), Salmonellosis, Colibacillosis and parasitic problems etc. *E.coli* is most common species of facultative anaerobe found in the gastro- intestinal tract of both man and animals, most commonly encountered pathogen in the Enterobacteriaceae family (Lim et al., 2010). *E.coli* is one of the major food borne bacterial pathogen causing watery and bloody diarrhea e.g. *E.coli* 0157; H7, as life threatening disease such as hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura in human (Ameer *et al.*, 2021). Therefore, the presence of such organism in foods is the indication of fecal contamination (Rahman *et al.*, 2017). Due to increase consumption of the chicken meat and eggs, the risk of exposure to various animal origin pathogens such as pathogenic *E.coli* has also increased (Shivani *et al.*, 2014). Antibiotics are commonly used to treat bacterial infections as well as in viral diseases to prevent secondary bacterial infections. In context of Nepal, studies carried out on the total consumption of antimicrobial drugs in food animal found the total growth of 53.60% between 2008 and 2012, with an average annual growth of 11.40% and was concluded that the increased demand of veterinary pharmaceuticals including antimicrobials might be as a result of increasing commercialization of poultry and dairy sector (Khatiwada, 2013). If there are resistant bacteria circulating in the broiler meat market, there will be risk of transmission of these resistant bacteria to human population either through unhygienic handling of birds, consumption of contaminated undercooked poultry meats or indirectly through the environment. The present study was carried out to identify *E.coli* isolates and their antimicrobial sensitivity and resistance pattern in broiler chicken meat. Detailed quantitative analysis was not done due to time factor, availability of costly kits and reagents.

2. LITERATURE REVIEW

2.1 Use of antibiotics in Nepal

A study conducted by Acharya (2019) showed that 35.1% of drug sellers practiced self-prescription, whereas 40.4% of dispensed antibiotics were based on prescription by veterinarians. But in most of the cases veterinary drugs sold in Nepal were based on prescription by paraprofessionals and drug retailers. One study that examined prescription behavior by drug dispensers in Biratnagar, Kathmandu, Chitwan, Pokhara, and Surkhet (the main hotspots for drug sales in Nepal) found around 46% of veterinary drugs were sold under self-prescription and 12% were based on farmer demand (Rabin Raut 2017). The ignorance of drug withdrawal periods and its negative impact on animal and human health combined with long term use leads to animal products arriving on the market with residues above the permitted (MRL) which increases antimicrobial resistance (Acharya, 2019).

2.2 Colibacillosis in Poultry

Price (2018) studied on 2,452 meat samples to estimate "E-coli infection in humans linked to poultry", and of the total 2,452 samples they found *E.coli* in nearly 80% of meat samples and 72% positive in human urine and blood cultures from patients. *E. coli* ST131, which was also present on the meat samples, was the most common type infecting people. Bacteriology Unit of Central Veterinary Laboratory has shown an increasing trend of cases of colibacillosis (e.g. 620, 859 and 787 in 2015/16, 2016/17 and 2017/18); *E. coli* Isolates identified by CVL were 149, 190 and 125 in the same years. In Bangladesh, Hossain et al. (2008) reported that 25 (49.02%) *E. coli* isolates in chicken meat by using conventional methods.

2.3 E. coli in Broiler Meat

Chris Dall (2018) studied on "Analysis ties resistant E coli from poultry meat to human UTI" with 2,460 samples of poultry meat from a hospital and several retail grocery stores in Flagstaff, Ariz and recovered E coli in 72.4% human samples and 81.7% in meat samples. In Bacteriology Unit of Central Veterinary Laboratory the percent of E.coli isolates found in the poultry samples were 53.8 % 38.9% and 39.9% for 2014/15, 2015/16 and 2016/17 respectively.

2.4 Antibiotic sensitivity and resistance of E. coli

Tetracycline, enrofloxacin, neomycin-doxycycline, levofloxacin, colistin, and tylosin are the top seven antibiotics consumed in Nepal with ampicillin, amoxicillin, ceftriaxone, and gentamicin being the most inappropriately prescribed medicines (Ramdam et al. (2015). Some 13% of total veterinary expenditure was on antibiotics whose sales rose by 50% between 2008 and 2012 (Ramdam et al. (2015). Gregg and Davis (2018) studied on Antibiotic-resistant Escherichia coli taking meat samples from retail poultry meat market and each isolate was tested for susceptibility to gentamicin, and trimethoprim-sulfamethoxazole. E. coli isolates from chicken were found resistant to gentamicin and multidrug resistance in varying patterns. Zeus Seligbon Elumba (2018) studied on "Occurrence and Antibiotic Sensitivity of Escherichia coli and Salmonella spp. in retail chicken meat at Selected Markets in Valencia City". Out of 25 samples from wings and legs/ drumsticks, 17 drumsticks and 24 wings were positive for E.coli and out of the positive isolates 87.80 % E.coli were found to be resistance to Amoxycillin. Zachary and James (2016) researched on "One in four supermarket chicken samples contain antibiotic-resistant E. coli" and found 51% of E coli from poultry samples resistant to the antibiotic trimethoprim. A study was carried out by Gladys Taiwo Adeyanju and Olayinka Ishola in 2014 on antimicrobial sensitivity test in Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. Disc diffusion antibiotic sensitivity test was used to determining sensitivity of Escherichia coli. Gentamicin (10 µg), Trimethoprim-sulpha (25 µg) were used to determine sensitivity pattern and found the drugs 100 % resistance to E. coli. 43.4% samples were containing Escherichia coli in frozen retail poultry meat, whereas 3(5.7%) Escherichia coli isolates were obtained in the samples collected from the processing plants.

Gladys Taiwo Adeyanju (2014) had studied on Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Nigeria and reported that Antibiotic sensitivity for isolates of Escherichia coli showed 100% resistance to amoxicillin. Ali et al. (2014) studied on "Presence of Escherichia coli in poultry meat: A potential food safety threat" and found the presence of E. coli (pathogenic strain O157) in raw poultry meat and its antimicrobial sensitivity pattern to common antibiotics. Out of 152 samples 25% (38/152) were found contaminated with E. coli. Regarding the antimicrobial sensitivity pattern, 92.1% (35/38) the E. coli isolates were found resistant to ampicillin, while the same percentage of resistance was found against tetracycline. The highest resistances were found against ampicillin and tetracycline. Twelve out of thirty -eight (31.6%) E. coli isolates showed resistance to sulfamethoxazole/trimethoprim, whereas 47.4% (18/38) were found resistant to gentamicin, and 39.5% (15/38) were found resistant to chloramphenicol. Reza and Mehdi (2014) studied on antibiotic sensitivity of E.coli with a title "Multiple Antimicrobial Resistance of Escherichia coli Isolated from Chickens in Iran" and isolated. 318 pathogenic Escherichia coli (APEC) strains from commercial broiler flocks and coli-septicemia were examined for antimicrobials of both veterinary and human significance by disc diffusion method. Multiple resistances to antimicrobial agents were observed in all

the isolates. Resistance to the antibiotics was as follows: Sulfadimethoxine-Trimethoprim (39.62%), Enrofloxacin (37.74%), Florfenicol (35.85%), Doxycycline (16.98%), Chloramphenicol (20.75%), and Gentamicin (5.66%). This study showed resistance against the antimicrobial agents that are commonly applied in poultry, although resistance against the antibiotics that were only applied in humans or less frequently used.

2.5 Patterns of antibiotic resistance in bacterial isolates from the poultry samples in Nepal

A study was conducted by Manita Subedi and Himal Luitel in 2018 to find out the pattern of antibiotic resistance and virulence genes content in the APEC strains isolated from broiler chickens at National Avian Disease Investigation Laboratory and Veterinary Teaching Hospital, Rampur, Chitwan, Nepal in 2017 and the results showed that out of 50 isolates of *E. coli*, 47 (94%) showed resistant to three or more antimicrobials. The highest levels (22%) of multidrug-resistant *E. coli* were observed for five different types of antimicrobials. Antibigram profiles of 50 *E. coli* strains showed the maximum resistance to ampicillin (98%), followed by co-trimoxazole (90%), and doxycycline (62%). Anil shrestha (2017) reported the prevalence of multidrug resistant *E. coli* as 79.6%. The study showed that the *E. coli* had mutant and producing extended spectrum beta lactamase enzymes to survive followed by multidrug resistance. Resistant bacterial strains transmitted to humans from animals through consumption of undercooked meat, through contact with raw meat or meat surface. Central Veterinary Laboratory (... year?) performed drug sensitivity test of *E. coli* isolates and obtained 70 %, 61.8% and 52.1 % sensitivity towards Ceftriaxone, Chloramphenicol and amoxycillin respectively.

3. MATERIALS AND METHODS

Based on the concentration of poultry meat retail shops and poultry population the total area of district was divided into two to four parts as four different locations (Budhanilkantha, Gaushala, Kalimati, Baneswor area) in Kathmandu district, two locations ((Suryabinayak and Balkot area)) in Bhaktapur district and four locations (Chapagaun, Imadol, Jawalakhel and Lagankhel area) in Lalitpur district to collect meat samples. As the random sampling method was not possible due to large number of retail poultry meat shops in the Kathmandu valley, purposive sampling method was adapted. For the sample size used open epi version was used to calculate the frequency of prevalence present in a definite population (for 10,000 population, 95% confidence level, 81 sample needed) and a total of 84 broiler poultry breast muscles samples (36 from Kathmandu, 28 from Lalitpur and 20 from Bhaktapur districts) were collected from poultry meat retail shops for this study. Samples so collected were transported to Central Veterinary Laboratory, Tripureshwor in cool box and cultured for bacterial isolation, identified and tested for antibiotic resistance accordingly. Both heavy, light equipment, consumables were used to achieve the objectives of the study.

3.1 Isolation and identification of bacteria

For culture and subculture of bacteria buffer peptone, MacConkey agar and eosin methyl blue were used. Five major biochemical tests (Methyl Red, SIM, Urease, Citrate and TSI) were used for identification of bacterial isolates. Isolated *E. coli* isolates were preserved in Tryptone Soya broth with 50% glycerol at - 80° C for future use and analysis as described in Manual of quality control on Microbiological Analysis: ISSN 0254-4725, designed and approved by FAO (1992).

3.2 Sensitivity test

The confirmed *E.coli* isolates were tested for drug sensitivity using modified Kirby Bauer's disc diffusion method in Mueller Hilton agar. Standard suspensions of the isolates were adjusted to 0.5 McFarland Standard. The breakeven point was determined using CLSI (M 100S 26th edition and Vet01 first edition). Commercially available 6 antibiotic discs (gentamycin ug, Enrofloxacin ug, Chloramphenicol ug, Doxycycline ug, Amoxycillin ug and Trimethoprim - sulphamethoxazole ug) were used for drug sensitivity test (DST). Zone of inhibition was measured for each antimicrobial agent and it was interpreted as sensitive (S), intermediate (I) or resistant (R) as per Hudzicki (2009, 2016). The antibiotic susceptibility profile for selected antibiotics for *E. coli* is given in Table -1. The recorded data were entered and analyzed by the help of Microsoft excel to obtain results.

Table 01: Antibiotic susceptibility profile for selected antibiotics for *E.coli* isolate

Sr.#	Antibiotic name	Concentration	Sensitive	Intermediate	Resistance
1.	Doxycycline	30 µg	≥ 14	11-13	≤ 10
2.	Gentamicin	10 µg	≥ 15	13-14	≤ 12
3.	Chloramphenicol	30 µg	≥ 18	13-17	≤ 12
4.	Trimethoprim-sulfamethoxazole	25 µg	≥ 16	11-15	≤ 10
5.	Amoxycillin	(25 µg	≥ 18	14-17	≤ 13
6	Enrofloxacin	5 ug	≥21	18-20	≤ 17

Source: CLSI Standard (2019)

3.3 Statistical Analysis

Data were coded, computed and analyzed using Microsoft Office Excel programme. Descriptive analysis and other appropriate statistical procedures were used to analyze the antimicrobial resistance pattern of *E.coli*. The data were stored in Excel sheet and Open Epi version software to analyze the data. Chi-square (χ^2) test was used to test the significance of the difference in the Antimicrobial Resistance test of *E. coli* isolates in breast chicken meat samples from three districts following by using Drug sensitivity test in Gentamycin, Chloramphenicol, Amoxycillin, Enrofloxacin, Doxycycline and Trimethoprim-sulfamethoxazole drugs. Association between the different districts in isolation of bacteria were tested by Chi-square (χ^2) methods with significance level defined at the $P < 0.05$.

4. ANALYSIS AND RESULTS

During the data analysis overall 82.14 % (31 from Kathmandu, 23 from Lalitpur and 15 samples from Bhaktapur) isolates of *E. coli* were identified from the 84 samples collected from Kathmandu, Lalitpur and Bhaktapur respectively. Effort was made to evaluate the situation of *E. coli* isolates within the districts. In Kathmandu, 88.8 % (8/9,8/9,8/9) samples each from Budhanilkantha, Kalimati and Gaushala and 77.7%(7/9) from Baneshwor were found positive for *E.coli* isolates (Figure 1); 71.42%(5/7) from Imadole, 71.4%(5/7) from Lagankhel and 85.71%(6/7) from Jawalakhel and 100%(7/7) samples from Chapagaun of Lalitpur areas were containing *E.coli* isolates respectively (Figure 2); and 80%(8/10) samples from

Suryabinayak, and 70%(7/10) samples from Balkot of Bhaktapur were found containing *E.coli* isolates (Figure 3) in Appendices.

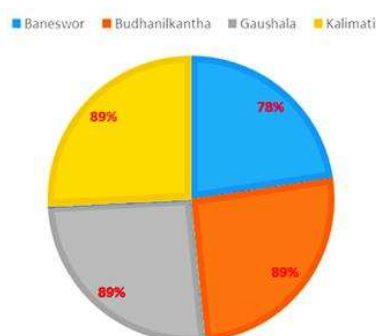


Figure 01: *E. coli* isolates in Kirtipur, Nepal

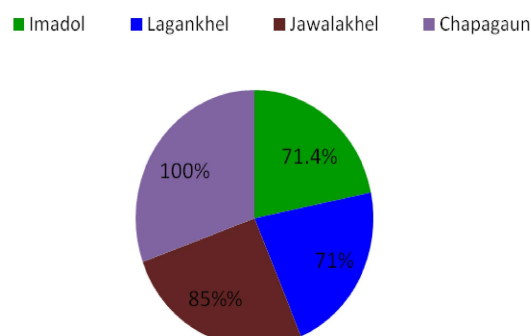


Figure 02: *E. coli* isolates in Lalitpur, Nepal

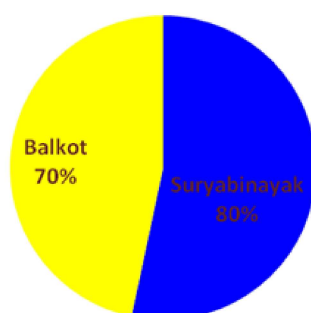


Figure 03: *E. coli* isolates in Bhaktapur district

The panel of antibiotics used to test AMR against *E.coli* was Doxycycline, Gentamycin, Chloramphenicol, Trimethoprim-sulfamethoxazole, Amoxycillin and Enrofloxacin. The concentration of these antibiotics is given in Table 1. Breakpoint values of antimicrobial agents were followed as given by CLSI standards, 2019. χ^2 Calculation of AST Results of *E.coli* in this study were represented in Table 2. From 69 positive *E. coli* isolates, Chloramphenicol was found 62% sensitive and Gentamycin and Trimethoprim-sulfamethoxazole were found 65 % and 45 % sensitive respectively. Amoxycillin was found only 10 % sensitive. Detail of this is presented in Figure 4. In Figure 5, antibiotic resistance of *E. coli* Isolates was evaluated during analysis and expressed in per centage. The percentage of *E.coli* isolates resistant to those antibiotics were found to be 73.91% for Amoxycillin, 55 % for enrofloxacin, 52 % for Doxycycline, 43 % for Trimethoprim-sulfamethoxazole, 26 % for Chloramphenicol and 21 % for Gentamycin (Figure 5). In this study multi- drug resistance *E.coli* were also observed from three different districts. Out of 31 positive samples from Kathmandu district 10 MDR *E.coli* were found. Similarly from Lalitpur districts 19 from 23 positive samples and 7 /15 positive samples from Bhaktapur districts were Isolated. This study showed that the sample number chosen could not affect on that for isolating *E. coli* prevalence and their resistance pattern in particular area. But it was found upon how often *E. coli* isolates is present in that particular area. This study also proved that antibiotic was commonly used in poultry in maximum numbers. Gregg *et al.* (2018) studied on Antibiotic-resistant *Escherichia coli* taking meat samples from retail poultry meat market and each isolate was tested for susceptibility to gentamicin, and amoxycillin,

florfenicol trimethoprim-sulfamethoxazole. *E. coli* isolates from chicken were found resistant to gentamicin and multidrug resistance in varying patterns.

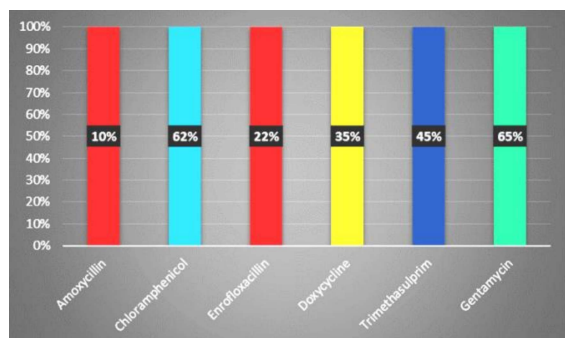


Figure 4 : *E.coli* isolates sensitive to antibiotics

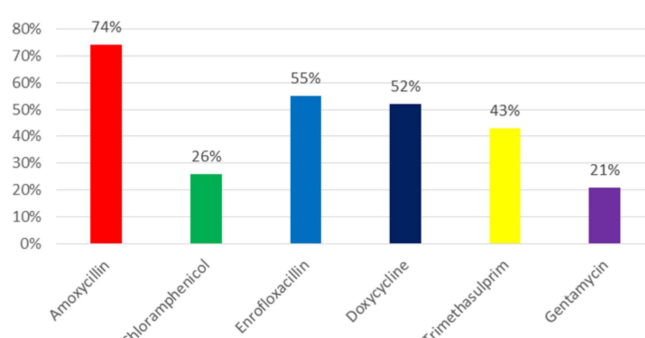


Figure 5: Percentage of *E.coli* isolates resistant to antibiotics

Table 01: Concentration of these antibiotics (Antibiotic Sensitive Test (AST) results of *E.coli* isolates)

Sr.#	Kathmandu Valley	E.coli positive	Sensitive	Intermediate	Resistance
1.	Kirtipur	31	82	28	70
2.	Bhaktapur	15	41	11	38
3.	Lalitpur	23	40	19	79
	Total	69			

Table 02: χ^2 Calculation of AST Results of *E.coli*

Districts	Total sample	E.coli positive	Sensitive	Intermediate	Resistant	P-value
Kathmandu	36	31	7(7.188)	9(6.739)	15(17.072)	0.734
Lalitpur	28	23	5(5.333)	4(5)	14(12.677)	
Bhaktapur	20	15	4(3.478)	2(3.261)	9(8.261)	

χ^2 value = 2.008 at DF = 4 at P-value = 0.734

Where,

$P_0 = 0.05$

H_0 = There is no significant difference between antibiotic resistant of *E.coli* present in meat available at markets of different districts in Kathmandu Valley.

H_1 = There is significant difference between antibiotic resistant of *E.coli* present in meat available at markets of different districts in Kathmandu Valley. $P > P_{0(0.734 > 0.05)}$, hence it was concluded that the antibiotic resistant of *E.coli* present in chicken meat available at markets of three districts of Kathmandu valley was found non-significant difference.

5. DISCUSSION

5.1 *E. coli* isolates

The study was focused mainly on prevalence of *E. coli* isolates with its sensitive resistance pattern with different but common antibiotics in marketed broiler meat of three districts of Kathmandu valley. Overall percentage of *E. coli* isolate in the present study was found to be 82.14 %. The prevalence of *E. coli* isolates found in the present study simulate with that reported by Gregg *et.al.* (2018) who had found *E.coli* in nearly 80% of meat samples. The results of this study also found similar to that found by Christensen *et al.* (2018), University of Copenhagen, Adeyanju and Ishola (2014) in Neigeria and Chris Dall (2018). The percent of *E.coli* isolates found at CVL in 2014/15, 2015/16 and 2016/17 was 53.8 % 38.9% and 39.9% respectively. The different in the results might be due to the fact that samples were taken from the poultry presented at CVL for diagnosis, not from the retail meat shop. The similar study conducted by Thapa and Chapagain (2020) in Chitwan found *E.coli* (64%) isolates, whereas Joshi *et al.* (2019) had found *E.coli* (33.3%) isolates in raw chicken. Such lower values might be attributed to use of clean water, season of sample collection and sanitation and hygiene maintained in the meat shops. Davis *et al.* (2018) had also found less prevalence of *E.coli* in chicken meat than the prevalence of *E.coli* in turkey meat using conventional method for *E.coli* isolation, indicating the variation in the prevalence rate in different species of poultry. By calculating χ^2 test it was found that $P > P_0$ ($0.734 > 0.05$) showed the prevalence of *E.coli* in different districts of Kathmandu were found different but not highly significance different. It indicated that it does not depend on the sample size because in small sample size it showed more than 50 percent prevalence.

5.2 Drug Sensitivity and drug resistance

From 69 positive *E. coli* isolates, Chloramphenicol was found 62% sensitive and Gentamycin and Trimetosulphamethoxazole were found 65.21 % and 45 % sensitive respectively. Amoxycillin was found only 10 % sensitive to the organism. This study found higher resistance to Amoxycillin (73%), Enrofloxacin (55%) and Doxycycline (52%) as previously reported by Khanal *et. al.* (2017). Doxycycline resistance pattern is found in increasing trend as compared to the research done by Thapa and Chapagain (2016), indicating the excessive use of this drug by poultry farmers. The results of this study was found similar to the finding of Gentamycin 65% sensitive with the previous study done by Van Den Vogard (2001), Talebiyan *et al.* (2014), Adeyanju (2014) in Ibadan. Gregg *et al.* (2018) found the average resistance to Gentamycin and Trimethasulprim in *E.coli* isolates. In this study about 52% Multidrug resistance *E. coli* was observed to Enrofloxacin, Doxycycline and Amoxycycline drugs.

5.3 Drug Resistance

In the present study the percentage of *E.coli* isolates resistant to those antibiotics were found to be 73.91% for Amoxycillin, 55 % for enrofloxacin, 52 % for Doxycycline, 43 % for Trimethoprim sulfamethoxazole, 26 % for Chloramphenicol and 21 % for Gentamycin. Subedi *et al.* (2018), had reported higher resistance pattern of *E.coli* in 5 common used antibiotics and maximum resistance followed by Doxycycline which was 62% in Chitwan. Thus, it indicated that the antibiotic resistance of *E. coli* to common commercial antibiotic is a potential threat to food safety and public health. Elumba *et al.*

(2018) studied on "Occurrence and Antibiotic Sensitivity of *Escherichia coli* and *Salmonella* spp. in retail chicken meat at Selected Markets in Valencia City". Out of 25 samples from wings and legs/ drumstics, 17 drumstics and 24 wings were positive for *E.coli* and out of the positive isolates 87.80 % *E.coli* were found to be resistance to Amoxycillin; this value seemed to be more serious than the present findings. Akbar *et al.* (2014) studied on "Presence of *Escherichia coli* in poultry meat: A potential food safety threat" and found 25% (38/152) samples contaminated with *E. coli* and 92.1% (35/38) the *E. coli* isolates were found resistant to ampicillin; 31.6% (12/38) resistance to sulfamethoxazole/trimethoprim; 47.4% (18/38) to Gentamicin; and 39.5% (15/38) resistant to chloramphenicol. Zachary and James (2016) found 51% of *E coli* from poultry meat samples resistant to the antibiotic trimethoprim.

5.4 Multi- drug resistance *E.coli*

In this study multi- drug resistance *E.coli* were also observed in the samples collected from three different districts. Out of 31 positive samples from Kathmandu district 10 MDR *E.coli* were found having such patterns. Similarly, 19/23 positive samples from Lalitpur district and 7 /15 positive samples from Bhaktapur district were Isolated. This study showed that the sample number chosen could not effect on that for isolating *E. coli* prevalence and their resistance pattern in particular area. But it depends upon how often *E. coli* isolates are present in that particular area. This study also proved that there is maximum use of antibiotics in poultry industry. Gregg *et al.* (2018) studied on Antibiotic-resistant *Escherichia coli* taking meat samples from retail poultry meat market and each isolate was tested for susceptibility to gentamicin, and amoxycillin, florfenicol trimethoprim-sulfamethoxazole. *E. coli* isolates from chicken were found resistant to gentamicin and multidrug resistance in varying patterns. Elumba *et al.* (2018) studied on "Occurrence and Antibiotic Sensitivity of *Escherichia coli* and *Salmonella* spp. in retail chicken meat at Selected Markets in Valencia City". Out of 25 samples from wings and legs/ drumstics, 17 drumstics and 24 wings were positive for *E.coli* and out of the positive isolates 87.80 % *E.coli* were found to be resistance to Amoxycillin. This study had also showed that the resistance of *E.coli* isolates in the chicken raw products was more or less similar to that in breast muscles (75%). This study had showed that the antibiogram of *E.coli* isolate differs from area to areas depending on the processing plant management, biosafety and biosecurity measures in the poultry farms and use of antibiotics in poultry. The similar results were reported in MDR *E.coli* (68%) by Hussain *et al.* (2017) in India in poultry meat. This study indicated that attention should be paid to those antimicrobials used in broiler feed, drinking water and as growth promoter in suboptimal doses like Enrofloxacin and sulphonamides. The high resistance levels and multidrug resistance levels observed for these antibiotic classes reflect the widespread use of them in poultry. Khanal *et al.* (2017) found 47 (94%) *E. coli* isolates in 50 broiler chicken meat samples and the organism showed resistant to three or more antimicrobials as ampicillin (98%) followed by co-trimoxazole (90%) and doxycycline (62%) at National Avian Disease Investigation Laboratory and Veterinary Teaching Hospital, Rampur, Chitwan, Nepal. Shrestha *et al.* (2017) reported the prevalence of multidrug resistant *E.coli* as 79.6%. Zachary and James (2016) researched on "One in four supermarket chicken samples contain antibiotic-resistant *E. coli*" and found 51% of *E. coli* from poultry samples resistant to the antibiotic trimethoprim which is less similar to this study where Trimethosulprim resistance showed 43%.

6. CONCLUSION

This study revealed that the presence of *E. coli* and its pathogenic strains is common in the poultry meat marketed in Kathmandu valley. Such contamination can easily lead to cause the infections related to this bacteria. Its presence in food materials can be more serious matter of concern for food safety and public health. It was concluded that the majority of commonly use antibiotics are not effective and appropriate use of antibiotics against *E. coli* isolates seemed necessary. To minimize antimicrobial resistance in poultry and poultry products prudent use of antibiotics or use of alternative of antibiotic may be indicated. Based on the present study following suggestions are made: i) this model can be used in further study with larger size of the samples and in wider areas to ensure the actual situation of *E.coli* isolates, antibiotic sensitivity and antibiotic resistance in the poultry population and their products ; ii) an appropriate awareness programs may be conducted by the national veterinary service for the prudent use of antibiotics in the poultry, withdrawal period of antibiotics in the poultry, sanitation and hygiene at slaughtering places and retail shops; iii) new strategies and proper food safety management may be needed to prevent the contamination of food materials and to reduce the drug resistance in poultry; iv) developing a new and natural antibiotic with a novel mode of action is necessary for the treatment of such multi-drug resistant bacteria; and HACCP (Hazard Analysis Critical Control point) approach should be applied in all food processing industries to eliminate or reduce significantly the load of *Salmonella*, *Escherichia coli* and other food-borne pathogens/contaminants in meat and meat products.

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