Orinigal Article

Study on Enrofloxacillin Antibiotic Residue in Chicken Meat Marketed Of Kathmandu Valley

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Abstract: Poultry industry is one of the leading industry among other livestock industries in Nepal and contributes about 4% to national GDP and 8% to AGDP with an investment of NRs. 80 billion. Antibiotic residue in food stuffs can cause hazards to human as well as animal health as an indicator for safety and quality of meat products with Antimicrobial Resistance in different Antimicrobial. This study was to estimate the antibiotic residue in broiler chicken meats marketed at Kathmandu valley to safe guard the poultry industry and public health. The study was carried out to 84 broiler chicken meat samples to identify the antibiotic residue in Kathmandu, Lalitpur and Bhaktapur districts from 1st December 2019 to 21 march 2020. A number of 84 meat samples were tested for Antibiotic Residue for Enrofloxacilin antibiotics from ELISA Kit Test. Samples from Kathmandu 22(61.1%), Lalitpur 15 (53.5%) and Bhaktapur 9 (45%) were found positive, that indicated the presence of Enrofloxacillin drug in the meat samples were more than 50%. The comparison of different retail meat shop of each district of Kathmandu Valley for the Enrofloxacillin residue test 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 by poor personnel hygiene and biosecurity measures with no follow antibiotic use withdrawal period. To minimize this problem, a suitable awareness program may be conducted by related organizations for poultry farmers, meat and drug whole seller and retailers to improve personal hygiene and control haphazard use of antibiotics in poultry.

Keywords: broiler chicken meat, antibiotic residue, antibiotic resistance

1. INTRODUCTION

Poultry industry is one of the leading industry among other livestock industries in Nepal and contributes about 4% to national GDP and 8% to AGDP with an investment of NRs. 80 billion. This sector has provided direct employment to 1.5 lakh people including people involved in exporting eggs to Bhutan, import of Parent stock, feed ingredients, supplements, medicines, vaccines (Singh et al., 2018). There are about 16000 registered poultry farms, nine chicken meat processing industries, 6 veterinary/livestock pharma industries, 2800 Medicine dealers and sub-dealers, 16 egg- tray and cartoon industries in Nepal. New sector of poultry industry are ostrich farm, turkey farms, duck farms and quail farms. In Nepal, annual per capita consumption of chicken meat and eggs is 4.1kg and 44 pieces respectively which is well below the global average (CBS, 2014). One of the important challenges in Nepalese poultry industry is facing the problem of diseases. Some of the common diseases prevalent in Nepal are ranikhet, infectious bursal diseases, infectious bronchitis, chronic respiratory diseases, fowl pox, narek's, avian influenza (both highly and low pathogenic), salmonellosis, colibacillosis and parasitic problems. Antibiotics are commonly used to treat bacterial infections as well as in viral diseases to prevent secondary bacterial infections. In addition, antibiotics are also used as growth promoters in feed (Darwish et al., 2013). The annual use of antimicrobials globally for cattle, chicken and pig production is 45,148 and 172 mg/kg of animal respectively (Van Boeckel et al., 2015). They also predicted a 67% increase in global consumption of antimicrobial in livestock production from 2010 to 2030. 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 they 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). Currently, approximately 80% food-producing animals receive medication for part or most of their lives (Darwish et al., 2013), and hence has the potential to generate residues in animal derived products (meat, eggs and honey) and poses a health hazard to the consumer (Beyene, 2016). Antimicrobial resistance (AMR) is recognized as one of the issue that involves links along the potential human-animal-environment infection chain (Aidara-Kane et al., 2018; Collignon and McEwen, 2019). Multidrug resistance is increasingly reported in poultry worldwide, and due to the impact of these organisms on public health, there is increasing interest in the origin and spread of these organisms in the poultry production chain (Dandachiet al., 2018; Amador et al., 2019). In food producing animals, the most commonly used antibiotics are Beta-lactams, Tetracyclines, Aminoglycosides, Quinolones, Macrolides and Sulfonamides (Ramdam, 2015). As a result of this, residues persist in foodstuffs (milk, meat etc.) of animal origin which poses a serious hazard to the consumer causing allergic reactions, mutagenic, carcinogenic and toxigenic effects. Harmful residual effects are arising as a consequence of using antibiotics for the purpose of growth promotion and increasing feed efficiency (Ramdam,2015). Likewise, in previous year antimicrobials were illogically used in synchronizing the reproductive cycle and breeding performance. The foodstuffs of animal origin like meat, milk, honey and eggs may contain antibiotic residues from their direct application to these animals during their medication (Merve, 2019) Antibiotic residues in foods produced by animals may be the cause of numerous health hazards in humans (National Academies Press, 1999). In Nepal, anecdotal evidence suggests that antibiotics are haphazardly used in livestock industry, especially in poultry industry. As prescription is not taken seriously for the use of antibiotics, non-veterinarians including Veterinary technicians, agro-vet operator and even farmers themselves buy and use antibiotics as they wish. This rampant use of antibiotics in poultry production for therapeutic and non-therapeutic use might have contributed in the development of resistance by bacteria to commonly used antibiotics. Most of these available data are based on the examination of dead birds or clinically sick birds. Besides, Maximum Residue Limit (MRLs) have been recommended by FAO/ WHO/ JECFA for various antibiotics in foods of animal origin (FAO/WHO, 2018). Therefore, animal foodstuff/ products should be tested for antibiotic residues for the welfare of the consumers. Implementation of quality assurance programmers to protect public health against adverse effects of antibiotics is a major challenge for developing countries where there is misuse of veterinary drugs and sale of animal source food are primarily informal. If there are resistant bacteria circulating in the marketed broilers meat, there will be risk of transmission of these resistant bacteria to human population either through unhygienic handling of the birds, consumption of contaminated under-cooked poultry meats or indirectly through the environmental contamination. The present scenario is that there is no sufficient data to estimate the prevalence of different bacteria in broilers marketed in the whole Kathmandu valley meat shops. This study was carried out with objective to provide evidence of antibiotic residue in meats from broiler chickens marketed in the different districts of Kathmandu valley of Nepal. As this study was carried out using a purposive sampling technique and within a limited time period of the internship program and with small sample size and limited financial resources, quantitative analysis was not done. Limited costly kits and reagents were other factors to limit the study.

2. LITERATURE REVIEW

A study conducted by Acharya (2019) showed that 35.1% of drug sellers practised 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. Another study showed that over 70% of veterinary drug sales were obtained from para-professionals or retail outlets (which do not have proper storage facilities and whose staff usually have no veterinary training) and not prescribed by veterinary professionals. 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 and farmers didn't aware about antibiotic residue and withdrawal periods in foodstuffs (Rabin, 2017). This 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 et al., 2019). A study was conducted by ManitaSubedi and HimalLuitel 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. Antibiogram 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.colihad 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. E.coli is highly prevalence bacteria in the environment so from the source of central veterinary laboratory, performed drug sensitivity test of E. coli isolates and obtained 70 %, 61.8% and 52.1 % sensitivity towards cetriaxone, chloramphenicol and amoxicillin. Enrofloxacin (ENR) is commonly used in intensive poultry farming to treat chronic respiratory disease, colibacillosis, and fowl cholera. Their long-term use may result in residues in animal tissue causing resistance to antibiotics. Fluoroquinolones (FQs) also affect humans who consume food-producing animals and the environment. Imperfect elimination of Campylobacter jejuni from poultry’s intestinal tracts by ENR can lead to survival of those bacteria and result in resistance to FQs. Prajapati (2018), conducted a study to assess the residue of Enrofloxacin in broiler tissues before and after heat treatments. A total number of 60 were given Enrofloxacin (10%) at a dose of 1ml /2 letter of drinking water at 38-40 days old. Birds were euthanized at 41, 43 and 45 days and assessment of Enrofloxacin residue was estimated in the muscles and livers before and after heat treatments. Results illustrated a significant difference of Enrofloxacin residues in pectoral muscles at 41, 43 and 45 days before heat treatment but found non-significant after heat treatment in the same days. Sarmina et al., (2014) studied on antibiotic residues in broiler and layer muscle (breast and thigh), kidney, and liverin Chittagong district of Bangladesh using thin layer chromatography (TLC) and ultrahigh performance liquid chromatography (UHPLC) method and found Enrofloxacin residues as 40% in liver, 34% in kidneys, 22% in thigh muscles, and 18% in breast muscles. Yousuf Ali Sarker (2018), studied on 160 meat samples (breast, thigh muscle and liver) collected from markets and farms from different region of Bangladeshto screen out antibiotic residues in chicken meats by thin layer chromatography method. In breast muscle, Ciprofloxacin was found highest (39%) followed by Doxycycline (26%), Amoxicillin (24%), and Oxytetracycline (23%) and lowest was Enrofloxacilin (21%). Obaid (2019), had studied on “Estimation of Enrofloxacin residues in broilers tissues before and after heat treatments”. 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The mean values of enrofloxacin residue found in chicken breast by ELISA and HPLC were 8.63 and 12.25 µg kg–1, respectively and all positive samples for enrofloxacin residues detected by LC-MS/MS were also positive by ELISA. "High occurrence rates of enrofloxacin and ciprofloxacin residues in retail poultry meat revealed by an ultra-sensitive mass-spectrometric method”. The achieved limits of quantification were 1 ug kg−1 for enrofloxacin and 10 ng kg−1 for ciprofloxacin. The analysis of 40 retail poultry samples originating from Estonia, Latvia, Lithuania, Poland and France revealed that 93% of samples contained residues of enrofloxacin in the range from 3.3 to 1126 ug kg−1. In Nepal, previous years the antibiotic residue was performed by Rapid kits in central veterinary public health laboratory, Tripureswor. In VSDRL meat samples of different species poultry, goats, pork and buffalo were collected from from Kavre, Kailali, Bhaktapur, Lalitpur, Kathmandu, Illam and Morang and tested the antibiotic residue test for the presence of different antibiotics (Tetracyclin, Penicillin, Aminoglycoside, and sulfonamide) by using RR kit. In total 130 meat samples 46 were positive for drug residue and 130 samples were negative for antibiotic residue. From 148 meat samples from poultry 34 were positive and samples were 114 negative for antibiotic residue test by RR test. Lee and Cho (2018), studied on “Prevalence of antibiotic residues and antibiotic resistance in E. coli isolates in chicken meat" with a total of 58 chicken meats; and 51 E. coli strains were isolated and identified. All isolates were analyzed to determine resistance patterns to appropriate antimicrobial agents. The antibiotic residue level of enrofloxacin was found to be 0.35–0.73 µg/kg in 12.1%7 samples. The aim of study was to investigate the correlation between the level of 17 antibiotic residues and 6 antibiotic resistances of Escherichia coli isolates in chicken meats. A total of 58 chicken meats were collected from retail grocery stores in five provinces in Korea. The total detection rate of antibiotic residues was 45% (26 out of 58). Ten out of 17 antibiotics were detected in chicken meats. None of the antibiotics exceeded the maximum residue level (MRLs) in chicken established by the Ministry of Food and Drug Safety (MFDS). The most detected antibiotics were amoxicillin (15.5%), followed by enrofloxacin (12.1%) and sulfamethoxazole (10.3%). In a total of 58 chicken meats, 51 E. coli strains were isolated. E. coli isolates showed the highest resistance to ampicillin (75%), followed by tetracycline (69%), ciprofloxacin (65%), trimethoprim/ sulfamethoxazole (41%), ceftiofur (22%), and amoxicillin/clavulanic acid (12%). The results of study showed basic information on relationship between antibiotic residue and resistance for 6 compounds in 13 chicken samples. Further investigation on the antibiotic resistance patterns of various bacteria species is needed to improve food safetyThe aim of study was to investigate the correlation between the level of 17 antibiotic residues and 6 antibiotic resistances of Escherichia coli isolates in chicken meats. A total of 58 chicken meats were collected from retail grocery stores in five provinces in Korea. The total detection rate of antibiotic residues was 45% (26 out of 58). Ten out of 17 antibiotics were detected in chicken meats. None of the antibiotics exceeded the maximum residue level (MRLs) in chicken established by the Ministry of Food and Drug Safety (MFDS). The most detected antibiotics were amoxicillin (15.5%), followed by enrofloxacin (12.1%) and sulfamethoxazole (10.3%). In a total of 58 chicken meats, 51 E. coli strains were isolated. 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3. MATERIALS & METHODS

Kathmandu Valley comprises of three districts, Kathmandu, Lalitpur, and Bhaktapur, together which cover an area of 899 square kilometers, whereas the area of the Valley as a whole is 665 square kilometers. The Valley encloses the entire area of Bhaktapur district, 85% of Kathmandu district and 50% of Lalitpur district. The climate of Kathmandu Valley is sub-tropical cool temperate with maximum of 35.6°C in April and minimum of –3°C in January and 75% annual average humidity. The temperature in general is 19°C to 27°C in summer and 2°C to 20°C in winter. Commercial Poultry population in Kathmandu Valley are, Kathmandu/ Kirtipur 720 611 lakhs, Bhaktapur 8, 60 000 lakhs & Lalitpur 12, 65,550 lakhs (CBS, 2014).

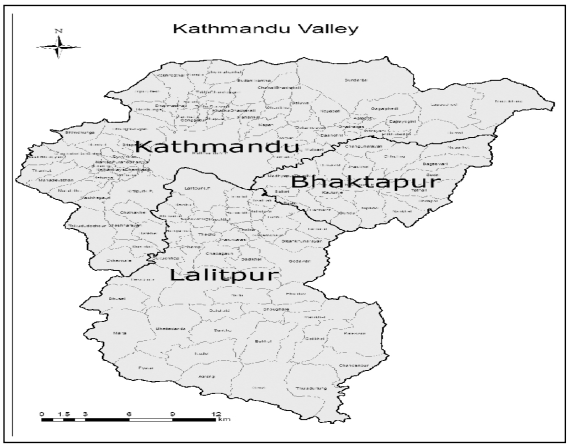

Figure 01: Map of Study Area

Cross Sectional Study method was used, based on the concentration of poultry retail shops, total area of district four different locations were choosen to collect samples from each district to represent the respective district. Such as at Kathmandu, Budhanilkantha (North), Gaushala (East), Baneswor (South), Kalimati (west) etc. Likewise, for Lalitpur district four areas as Lagankhel, Chapagaun, Imadole, Jawalakhel areas were selected for poultry meat sample collection. Similarly for Bhaktapur district areas poultry belt and market place for Newar community were selected. Purposive sampling technique was applied for the collection of sample. Sample size was estimated by using epi software according to the samples of broiler present in that area and according to the prevalence of selected isolates in Kathmandu valley is shown in Table no. 1 and 2 from CVL data. The samples so collected were kept in polyethylene bags and transported to central veterinary laboratory in a cool box. And Samples were preserved at 40C Celsius till tests were done (i.e. within 1-2 days). If process was delayed by some reasons, then the sample were preserved for -200c for a certain periods of time. Meat samples from Suryabinayak area and fromBalkot of Bhaktapur district were collected for antimicrobial resistance of E. coli Isolates and from this samples antibiotic residue test for Enrofloxacillin were performed. The samples for the tests were collected from the retail shops of three different districts of Kathmandu metropolitan city viz, Bhaktapur, Lalitpur, and Kirtipur/Kathmandu district. Suryabinayak and Balkot area were selected in Bhaktapur district. Similarlry, Budhanilkantha, Gaushala, Kalimati, Baneswor in Kathmandu district and Chapagaun, Imadol, Jawalakhel and Lagankhel areas were selected in Lalitpur district based on the concentration of poultry population and meat shops. 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 openepi version to calculate the frequency of prevalence present in a definite population (for 10,000 population, 95% confidence level, 81 sample needed). Total 84 poultry breast muscles samples were collected from poultry meat retail shops.The samples were cultured for bacterial isolation, identified and tested for antibiotic resistance and antibiotic residue accordingly. 36 meat samples, 9 from each 4 location from Kathmandu, were collected. Likewise, 28 meat samples, 7 from each 4 location of Lalitpur districts, were brought and 20 meat samples, 10 from each area, were taken from Bhaktapur district. Data were coded, computed and analyzed using Microsoft Office Excel program. Descriptive analysis and other appropriate statistical procedures were used to analyze the antibiotic residue. The data were stored in Excel sheet and Open Epi version software to analyze the data. Association between different antibiotic residues found at different locations and antibiotic residues present in different breast muscles of three different districts were compared statistically by Chi-square ($\chi 2$) analysis using epidemiological software Open Epi version 2.3 with significance level defined at the $P<0.05$. The Correlation of antibiotic residues and antibiotic resistances in chicken meats were also assessed. Chi-square (χ 2) test was performed to test the significance of the difference in the detection of antibiotic residue were tested by Chi-square ($\chi 2$) methods with significance level defined at the $P<0.05$. Dichlormethane, N-hexane, Enrofloxacin ELISA Kit catalog Number. CSB-E12035f. Total 84 samples were tested for antibiotic residue. From this meat samples, antibiotic residue of enrofloxacillin was identified and analysed. For this method, MaxSignalREnrofloxacin ELISA Test Kit was used to detect the presence of antibiotic residue in the tested meat. After processing the laboratory work the data were entered and analyzed by the help Microsoft excel to extract the results. For Enrofloxacin antibiotic residue test, the meat samples were tested by using Enrofloxacin ELISA Kit (Catalogue Number: CSB-E12035f) and according to the protocol of the test kits. The immunoassay kit was used for in -vitro quantitative determination of Enroflxacin concentrations in chicken tissue. This assay employs the competitive enzyme immunoassay technique. The coupling antigen is pre-coated on the micro-well stripes. The Enrofloxacin in the testing sample competes with the coupling antigens pre-coated on the micro-well stripes for the antibodies against Enrofloxacin. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the content of

Enrofloxacin in it. This value is compared to the standard curve and the content of the corresponding enrofloxacin is subsequently obtained. When samples were collected, it was preserved in 40 to -200c depends on the time of processing. Before processing, the frozen samples and all reagents were thawed at room temperature (20 – 250C refrigerator) before use for antibiotic residue test procedure.

4. RESULTS & DISCUSSION

Out of 84 breast muscles samples, 43(51.19%) samples were positive for Enrofloxacin residue .The value were observed by using SkanIt Software 4.1 for Microplate Readers RE, ver. 4.1.0.43, from calculating concentration (ppb), for First 42 samples, 52 % samples were positive for Enrofloxacin antibiotic residue and the concentration ranged from 0.878016807 ppb to 3.89693009(3.89ug/kg) ppb byinterpretating based on (median =1.92). Out of second 42 samples, 50 % samples were found positive for Enrofloxacin residue with the concentration range from 0.368998651ppb (0.36 ug/kg) to12.84757481ppb (12.8 ug/kg) by interpreting based on (median = 6.24).

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

S.No.	Antibiotic name	Concentration	Sensitive	Intermediate	Resistance	Remarks
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.	Trimethoprimsulfame thoxazole	25 µg	≥ 16	11-15	≤ 10	
5.	Amoxicillin	(25 µg	≥ 18	14-17	≤ 13	
6	Enrofloxacillin	5 ug	≥21	18-20	≤ 17	

Source: CLSI standard, 2019

It had found 93% of samples contained residues of enrofloxacin in the range from 3.3 to 1126 ug kg−1. A total of 72% of the analysed samples contained enrofloxacin residues detected by the ELISA and 22.2% were detected by LC-MS/MS.

Table 02: Enrofloxacin Antibiotic residue in broiler meat

No.	Area	Pos	ENX neg	Total
t1.	Kathmandu	22	14	36
2.	Lalitpur	15	13	28
3.	Bhaktapur	9	11	20
Total		46	38	84

X2 test

Districts	Enrofloxacin residue positive	Enrofloxacin residue negative	Total	X2	P value	Remarks
Kathmandu	22(19.71)	14(16.29)	36	1.371	0.504	>0.05

Lalitpur	15(15.33)	13(12.67)	28			NS
Bhaktapur	9(10.95)	11(9.05)	20			
Total	46	38	84			

X2 value =1.371 at DF = 2 at P-value= 0.504.

Where, P0= 0.05, H0= There is no significant difference between antibiotic residue in meat available in markets of different districts of Kathmandu Valley. H1= There is significant difference between antibiotic residue in meat available in different districts of Kathmandu Valley. P>Po, hence it is concluded that the residue of enrofloxacin in meat available in markets of different districts of Kathmandu valley was found non-significant difference. One obstacle to gathering more comprehensive data on the use of antibiotics in poultry is the majority of the poultry industry utilizes vertical integration. As a consequence, farmers are often unaware of what components go into the health and its impacts for future generation, Also in antibiotic usage in general, there are criteria to define bacterial resistance to specific antibiotics, however, there are no standards to divide the bacteria into resistant and susceptible categories based on antibiotics utilized. The study was carried out to identify the antibiotic residue in Kathmandu, Lalitpur and Bhaktapur districts. Purposive samples were collected from 4 locations of each districts, except in Bhaktapur district, and uniformly to represents the whole districts accordingly. A randomly selected 84 meat samples were tested Enrofloxacillin antibiotic residue were studied quantitatively by performing on MaxSignalREnrofloxacin ELISA Test Kit. The present study found that most of the area were contaminated from food from antibiotic residue. Poor husbandry practices with inappropriate infection prevention and control (IPC), lack of awareness on good management practices (GMP), prudent use of antibiotics with self judgement without prescribed medicines and lack of awareness to hold during withdrawal periods causes the accumulation of antibiotic rsidue in breast muscles of poultry, have contributed to development of AMR and presence of antibiotic residue in animal health. This emphasizes the presence of antibiotic residue in the poultry mostly.

4.1 Discussion

The results presented in the form of tables and figures in the previous section are now discussed for the possible interpretations, justifications and analyses aided by possible causes and literature supports. The study was focused mainly in Enrofloxacillin antibiotic and Enrofloxacillin antibiotic residues in marketed broiler meat of different districts of Kathmandu valley. The detected antibiotics, residue concentrations. Antibiotic residues were detected in 46 out of 84 samples (Supplementary Table – 10). The results of this study were found similar to the study of Tajick and Shohreh at Majandaran in 2020 in which residue were found in 50% samples. Another similar study was carried by Mohammad Younus (2017) and found 53% the enrofloxacillin positive, whereas Prajapati (2018) had found only 8.7% positive from Kathmandu, pokhara and Chitwan area. This variation may be due to difference in sample size, sample collection sites, use of feed additives and samples from recently treated cases, use of antibiotics before marketing of poultry without emphasizing in their withdrawal period. Overall present results showed that enrofloxacillin was most detectable compound because of its widely used in livestock farm in Nepal. These findings suggested that antibiotic residues of chicken meats and antibiotic resistance of isolates from chicken meats were closely related with antibiotic uses. Public health concerns regarding antimicrobial residues and antimicrobial resistance pathogens in food and the environment reinforce the need for more research on safer alternatives to antibiotics as feed additives. Country has to set a monitoring action plan, rules and regulation, develop guidelines, record keeping of antimicrobial usage, prescription from veterinarian to reduce antimicrobial use in animals.

5. CONCLUSION

This study revealed that the presence of antibiotic residue and is common in the poultry meat. Such contamination can easily lead to cause the infections related to different harmful pathogens. Its presence in food materials is a problem, while the developments of drug resistance by these common pathogens are more serious matter of concern for food safety and public health. In the context of antibiotic residue, this study also showed that antibiotics residue have been abundantly found in poultry meat which can have serious effect in human health. This study also found that the correlation between antibiotic residue and is closely significant. So, to minimize antimicrobial resistance in poultry minimize the use of antibiotics or use alternative of antibiotic seems necessary. This study also provided some clear information to minimize antimicrobial resistance in poultry and poultry products minimum use of antibiotics or use alternative of antibiotic may be used.

6. RECOMMENDATION

Based on the present study following suggestions are made:

- This model can be used in further study with larger size of the samples and in wider areas to ensure the actual situation of antibiotic residue in the poultry population and their products
- 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.
- To mitigate the present problem a routine antibiotics residue monitoring and surveillance programmers in poultry and their products should be conducted by the related authority to ensure the consumer safety.
- 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.
- Developing a new and natural antibiotic with a novel mode of action is necessary for the treatment of such multi-drug resistant bacteria.
- To mitigate the present problem a routine antibiotics residue monitoring and surveillance programmers in poultry and their products should be conducted by the related authority to ensure the consumer safety.
- HACCP (Hazard Analysis Critical Control point) approach should be applied in all food processing industries to eliminate or reduce significantly the prevalence of Salmonella, Escherichia coli and other food-borne pathogens/contaminants in meat and meat products.

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