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Research Article

Evaluation of Antibiotic Residues in Marketed Broiler Meat of Kathmandu Valley of Nepal

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Abstract

A descriptive cross-sectional study was conducted to evaluate the antibiotic residues in marketed broiler meat of Kathmandu valley of Nepal. Altogether 90 veterinary drug retailer shops were randomly selected and were interviewed with pre-designed questionnaire. Meat samples were collected from purposively selected 44 retail meat shops of each three districts. The samples were tested according to protocol of Rapid Residue test kits for tetracycline, macrolide/aminoglycoside/sulfonamide and penicillin groups. Education level of the drug retailers was varied, most of them were JT/JTA (54.44%) followed by 35-days veterinary training (22.22%), out of veterinary sector (15.55%) and veterinary doctors (7.8%). Several antibiotics were used in treatment of poultry among them macrolide/aminoglycoside/sulfonamide (44%) followed by tetracycline (39%) and penicillin (17%). Majority of the drug retailers (56%) were known about antibiotic residues in meat and only 38% retailers were known about drug withdrawal period. Only 22% have known about antibiotic residues in meat and their effects in animal and public health. Overall presence of antibiotic residues in marketed chicken meat of Kathmandu valley was 30.81%. The residues of tetracycline, macrolide/aminoglycoside/sulfonamide and penicillin were found 33.33%, 41.67% and 17.42% respectively. The location wise prevalence of tetracycline residue was found to be higher in Kathmandu (36.36%) followed by Lalitpur (34.09%) and Bhaktapur (29.54%). Macrolide/aminoglycoside/sulfonamide residue was found higher in Kathmandu (47.72%) followed by Lalitpur (40.90%) and Bhaktapur (36.36%). The location wise prevalence of penicillin residue was found similar in Kathmandu and Lalitpur districts (18.18%) and the lowest in Bhaktapur district (15.90%). According to statistical results, there was non-significant difference in overall prevalence and location wise prevalence of tetracycline, macrolide/aminoglycoside/sulfonamide and penicillin residue ($P > 0.05$) which shows no association between occurrence of antibiotic residues and markets. Thus, the findings of this study indicated that percentage of antibiotic residue in chicken meat in Kathmandu valley is one of the major public health issues and ascends interrogation to our regulatory mechanisms and public health concern.

Keywords: *Broiler; Antibiotic; Residue; Meat; Public Health*

Abbreviation

JT: Junior Technician; JTA: Junior Technical Assistant

Introduction

Poultry farming, one of the important farming occupations is emerging as a viable economic enterprise especially around the urban centers [13]. It has gone an unprecedented growth during last three decades and now transformed itself into the status of an industry [6].

The production of broiler type poultry has greatly expanded over the past several decades. Demand for the chicken is increasing because of its low price and healthy values. Nepal belongs to 112th position in chicken meat production ranking in the world having more than 1,000 broiler farms and 150,000 kg/day poultry meat demand [7] that shares 8% in total meat production.

Antibiotic is a chemical substance produced naturally by a microorganism or synthetically in the laboratory which has potential to kill or inhibit the growth of microorganisms. Substance produced synthetically (e.g. sulfonamides and quinolones) should not be termed antibiotics but the definition of antibiotics has also been used to include chemically derived synthetic antibacterial drugs [11]. Antimicrobials used for the treatment of disease in animals closely followed their uses in humans [8]. Antimicrobial drugs are being haphazardly used to prevent, to control and to treat infections.

Along with the growth in poultry business, antibiotic residues in edible poultry meat have been the serious issue. Residues as defined by the European Union (EU) and the Center for Veterinary Medicine, an agency under food and Drug Administration (FDA/CVM) in the USA are pharmacologically active substances (whether active principles, recipients or degradation products) and their metabolites which remain in foodstuffs obtained from animals to which the VMPs in question has been administered. Maximum residue limit (MRL) is defined as the maximum concentration of a residue resulting from the registered use of an agricultural or veterinary chemical which is recommended to be legally permitted or recognized as acceptable in or on a food, agricultural commodity (milligrams per liter the case of a liquid commodity) [4]. The MRL level of concerned antibiotics in the study is 200 µg/kg for tetracyclines, 100 µg/kg for sulfonamides and 50 µg/kg for penicillins. These are the standards set by CAI in 1998 and 2000. In context of Nepal, as there is no strong veterinary drug regulation, the country has adopted European Union standards for drug withdrawal period. According to the standard, the withdrawal period of veterinary medicines should not be less than 7 days for eggs, 7 days for milk and 28 days for meat from poultry and mammals including fat and offals.

In order to guarantee safety of global health, the international recognized bodies such as Codex Alimentarius Commission (CAS) which is a joint FAO/WHO body food standardization and the European Union have legalized a series of Maximum Residue Limits (MRLs) for veterinary drugs in edible tissues of animal origin [17]. The concerned regulating authorities like Veterinary Standard and Drug Administration Laboratory (VSDAL) conduct screening of different residues present in consumable meat and milk items to appraise public health ensuring from hazards of antibiotic residues. Consuming residues of drugs or their metabolites in meat and other foods of animal origin may cause adverse effects which include carcinogenicity, mutagenicity, bone marrow toxicity (Chloramphenicol) and allergies (Penicillin) [14] and also may create the selection pressure to the gastrointestinal flora of the human that results in advancement of antimicrobial resistant bacteria [3]. The incidence of these residues may be due to any one of the followings - a failure to discern the withdrawal periods of each drug, additional label dosages for animals, contamination of animal feed with the excreta of treated animals or the use of antibiotics by unauthorized person, use of drugs without laboratory diagnosis, veterinary prescription and supervision. The unawareness in drug withdrawal period which is the interval established to safeguard human from exposure of antibiotic added food leads to the supply of antimicrobial treated animal to market for slaughter before the residue of toxicological concern reach to safe concentration or provide animal products with drug residues above the MRLs.

Materials and Methods

The research work was divided into two parts, viz, Questionnaire Survey and Laboratory Examination. Under the laboratory diagnosis, RR test kit following G9 Manual of Test Kit was used to detect antibiotic residues in marketed poultry meat at Veterinary Public Health, Tripureshwor, Kathmandu.

Site profile

This study was conducted in different sites of Kathmandu Valley at Kathmandu, Bhaktapur and Lalitpur district. These districts lie in Bagmati zone, central development region province number 3. Kathmandu, Bhaktapur and Lalitpur cover an area of 395 km^2, 119 km^2 and 385 km^2 respectively.

Methodology

Questionnaire survey

The cross sectional survey was done to veterinary drug retailer shops based on pre-designed questionnaire to find out pattern of antibiotics being used in poultry diseases in that area. Altogether 90 veterinary drug retailer shops were selected, 30 shops from each study site and the retailers were interviewed with pre-designed questionnaire.

Laboratory Examination

Sample Size

Among 132 total meat samples, 44 samples were collected from each district Kathmandu, Bhaktapur and Lalitpur for identification of antibiotic residues in broiler meat.

Method of Sampling

Sample Collection

Purposive sampling technique was used for collection of samples. A total of 132 meat samples (breast muscle) weighing about 20 grams were collected from each retail shop. The samples were collected in transparent polythene bags. They were sealed, coded and recorded. Then collected samples were kept in cool box with ice packs, transported to Veterinary Public Health, Tripureshwor, Kathmandu and stored in deep freeze at -40°C until laboratory procedures began.

Laboratory Processing

Test Method

The samples were tested according to the G9 Manual of Test Kit for determination of drug residues in Meat (G9 Co., Ltd. Thailand). This test kit has 93.0% accuracy, 78.9% sensitivity and 96.7% specificity. It can be tested for at least 12 kinds of drug residues, viz, amoxicillin, bacitracin, chlortetracycline, erythromycin, gentamicin, neomycin, oxytetracycline, penicillin, streptomycin, sulfadimethoxine, tetracycline and tylosin.

Protocol of G9 Test Kit

Following procedures was followed in G9 Manual of Test Kit for determination of drug residues in meat:

- 5 grams of minced meat was sampled into 50 ml centrifuge tube.
- 5ml extraction solution A, B and C was added into another each centrifuge tube. The criteria for choosing the types of extraction solution depend on the group of drug residues to be determined as follows:
- Solution A for tetracycline group
- Solution B for macrolide, aminoglycoside and sulfonamide group
- Solution C for penicillin group
- The tubes were vigorously shaken by electronic shaker for 10 minutes. Then, tubes were placed in water bath and slowly heated it until reaching 60°C. After that, tubes were maintained there for5 minutes and later cooled down.
- Then, tubes were centrifuged at 3000 - 4000 rpm for 15 minutes.
- The supernatant from the sample tube was separated.
- The pH of the supernatant samples (chicken) was adjusted for further test

For determination of Tetracycline group

The pH of the supernatant samples was adjusted to 6.5 as follows:

- Chicken: 3.75 ml of the supernatant samples (initial pH - 6.1) were adjusted with 1 drop (33 µl) of 1 N NaOH.

For determination of Penicillin group:

The pH of the supernatant samples was adjusted to 6.5 as follows:

- Chicken: 3.75 ml of supernatant samples (initial pH - 6.1) were added with 1 drop (33 µl) of 1 NaOH.

For determination of Macrolide, Aminoglycoside and Sulfonamide group:

The pH of the supernatant samples was adjusted to 7.0 as follows:

- Chicken: 5.0 ml of supernatant samples (initial pH - 6.5) were added with 1 drop (33 µl) of 1N NaOH.

Each of inhibitor- free supernatant samples were performed as steps which describe above for testing as negative control sample.

Testing procedure

- 4 drops of supernatant obtained was added from using different extraction solution into each prepared tube.
- 4 drops of negative control was added into another prepared tube.
- All tubes were incubated for ≥ 2 hrs 45 minutes (chicken) in water bath at temperature of 64±2ºC. Keeping medium in the tube under water level (if using water bath for incubation) until the color of medium in negative control tube changes completely from purple to yellow.
- The color change of medium in sample tubes observed was purple tubes changed into yellow color in case of negative result and purple tubes still had purple coloration if the result was positive. Positive results were matched with standard chart to determine the level of the drug residues.

Reading and interpretation

- If the color or medium changes completely from purple to yellow, it indicates negative result for drug residues.
- If it shows purple color in medium, it indicates positive result for drug residues of which their quantities related to various level of purple color.

Statistical analysis

Data were collected, coded, computed and analyzed using Program Microsoft Excel Version 10. The association between different antibiotic residues present in different places were compared statistically by Chi-square (χ^2) analysis using SPSS version 20 with significance level defined at $p < 0.05$.

Results

Survey findings

Education level of drug retailers

The education level of the drug retailers was varied. Most of them were JT/ JTA (54.44%) followed by 35-days veterinary training (22.22%), out of veterinary sector (15.55%) and veterinary doctors (7.8%).

Information relating to drug

S.No.	Educational level	Districts			Total
		Kathmandu	Bhaktapur	Lalitpur	
1	B. V. Sc.	3 (10%)	2 (6.7%)	2 (6.7%)	7 (7.8%)
2	JT/JTA	16 (53.33%)	18 (60%)	15 (50%)	49 (54.44%)
3	Veterinary trainings	8 (26.7%)	7 (23.33%)	5 (16.7%)	20 (22.22%)
4	Others	3 (10%)	3 (10%)	8 (26.7%)	14 (15.55%)

Table 1: *Education Level of Drug Retailers during Questionnaire Survey.*

Several antibiotics were used in treatment of poultry among them macrolide/aminoglycoside /sulfonamide (44%) followed by tetracycline (39%) and penicillin (17%). Regarding the sale of veterinary drugs, highest percentage (60%) was from doctor's prescription followed by self-prescription (23.33%) and farmer's request (16.67%) in Kathmandu district. Similarly, the highest percentage (43.33%) was from doctor's prescription followed by self-prescription (36.33%) and farmer's request (20%) in Bhaktapur district. Likewise, the highest percentage (46.67%) was from self-prescription followed by doctor's prescription (40%) and farmer's request (13.33%) in Lalitpur district.

Antibiotic residues

Majority of the drug retailers 56 (62.22%) were known about antibiotic residue in meat while the rest 34 (37.78%) were unknown about it. Similarly, 52 (57.78%) of drug retailers were unknown about drug withdrawal period while the rest 38 (42.22%) retailers were known about it. Likewise, majority of the drug retailers 68 (75.56%) were unknown about antibiotic residues in meat and their effects in animal and public health while the rest 22 (24.44%) have known about it.

Laboratory examination

Overall presence of antibiotic residues

In this study, among 132 muscle samples taken for analysis, each sample was tested for three different antibiotic groups. Result revealed that out of 396 test samples, 122 samples were positive. Hence, the overall presence of antibiotic residues in marketed broiler meat of Kathmandu valley is 30.81%. The study revealed that the highest percentage (41.67%) of antibiotic residues found in collected sample is macrolide/aminoglycoside/sulfonamide followed by tetracycline (33.33%) and penicillin (17.42%).

District-wise comparison of antibiotic residues

Out of 44 meat samples subjected to 132 tests from every district, prevalence of positive case for antibiotic residues was found the highest in Kathmandu (34.09%) followed by Lalitpur (31.06%) and Bhaktapur (27.27%) as shown in table 2.

Districts	Total	Positive	χ^2	p value	Remarks
Kathmandu	132	45 (34.09%)	1.433	0.488	> 0.05
Bhaktapur	132	36 (27.27%)			NS
Lalitpur	132	41 (31.06 %)			
Total	396	122 (30.81%)			

Table 2: *Overall district-wise prevalence of antibiotic residues.*

Individual antibiotic residues in different districts

Each of 44 samples from three districts was tested separately for the presence of individual antibiotic group (tetracycline, macrolide/aminoglycoside/sulfonamide and penicillin). The prevalence of antibiotic residues in meat available in marketed broiler of different districts was studied.

Residue of tetracycline

Out of 132 samples, 44 samples were positive for the residue of tetracycline. Therefore, the overall residue of tetracycline was found to be 33.33%. 44 meat samples from each district were tested for the presence of tetracycline of which 16(36.36%), 13 (29.55%) and 15 (34.09%) were found positive from Kathmandu, Bhaktapur and Lalitpur respectively.

The residue of tetracycline in meat available in markets of different districts of Kathmandu valley was found non-significance difference ($p > 0.05$) (Table 3).

Districts	Total	Positive	χ^2	p value	Remarks
Kathmandu	44	21 (47.72%)	1.184	0.553	> 0.05
Bhaktapur	44	16 (36.36%)			NS
Lalitpur	44	18 (40.90%)			
Total	132	55 (41.67%)			

Table 4: *Residue of macrolide/aminoglycoside/sulfonamide in marketed meat of different districts.*

Residue of macrolide/aminoglycoside/sulfonamide

Out of 132 samples, 55 samples were positive for the residue of macrolide/aminoglycoside/sulfonamide. Therefore, the overall residue of macrolide/aminoglycoside/sulfonamide was found to be 41.67%. 44 meat samples from each district were tested for the presence of macrolide/aminoglycoside/sulfonamide. From Kathmandu, 21 samples (47.72%) were found to be positive, while 16 samples (36.36%) from Bhaktapur and 18 samples (40.90%) from Lalitpur were positive for macrolide/aminoglycoside/sulfonamide test.

The residue of Macrolide/Aminoglycoside/Sulfonamide in meat available in markets of different districts of Kathmandu valley was found non-significance difference ($p > 0.05$) (Table 4).

Districts	Total	Positive	χ^2	p value	Remarks
Kathmandu	44	21 (47.72%)	1.184	0.553	> 0.05
Bhaktapur	44	16 (36.36%)			NS
Lalitpur	44	18 (40.90%)			
Total	132	55 (41.67%)			

Table 4: *Residue of macrolide/aminoglycoside/sulfonamide in marketed meat of different districts.*

Residue of penicillin

Out of 132 samples, 23 samples were positive for the residue of penicillin. Therefore, the overall residue of penicillin was found to be 17.42%. 44 meat samples from each district were tested for the presence of penicillin. From Kathmandu, 8 samples (18.18%) were found to be positive, while 7 samples (15.90%) from Bhaktapur and 8 samples (18.18%) from Lalitpur were positive for penicillin test.

The residue of penicillin in broiler meat available in markets of different districts of Kathmandu valley was non significance difference ($p > 0.05$) (Table 5).

Districts	Total	Positive	χ^2	p value	Remarks
Kathmandu	44	8 (18.18%)	0.105	0.949	> 0.05
Bhaktapur	44	7 (15.90%)			NS
Lalitpur	44	8 (18.18 %)			
Total	132	23 (17.42%)			

Table 5: *Residue of penicillin in marketed meat of different districts.*

Note: Prevalence of different antibiotic residues were non-significantly associated with its associated factors (since p > 0.05). For the prevalence of antibiotic residues to be significantly associated with its factors the probability value should be less than 0.05 i.e. p< 0.05. Where in NS represents significance level (i.e. non significance).

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 research was designed to evaluate mainly for the antibiotic residues in marketed broiler meat of Kathmandu, Bhaktapur and Lalitpur districts of Nepal.

From questionnaire survey, following answers were obtained. Education level of the drug retailers was varied. Most of them were JT/JTA (54.44%) followed by 35-days veterinary training (22.22%), out of veterinary sector (15.55%) and veterinary doctors (7.8%). Several antibiotics were used in treatment of poultry among them macrolide/aminoglycoside/sulfonamide (44%) followed by tetracycline (39%) and penicillin (17%). Similarly, during the sale of veterinary drugs, the highest percentage (60%) was found by doctor's prescription followed by self-prescription (23.33%) and farmer's request (16.66%) in Kathmandu district. Similarly, the highest percentage (43.33%) was from doctor's prescription followed by self-prescription (36.33%) and farmer's request (20%) in Bhaktapur district. Likewise, the highest percentage (46.66%) was from self-prescription followed by doctor's prescription (40%) and farmer's request (13.33%) in Lalitpur district. Majority of the drug retailers (56%) were known about antibiotic residues in meat while the rest (34%) were unknown about it. Similarly, 52% of drug retailers were unknown about drug withdrawal period while the rest (38%) retailers were known about it. Likewise, majority of the drug retailers (68%) were unknown about antibiotic residues in meat and its effect in animal and public health while the rest (22%) were known about it.

The laboratory examination of 132 breast muscle samples represents 44 samples from each district which were tested for the presence of three different antibiotic groups, viz, tetracycline, macrolide/aminoglycoside/sulfonamide and penicillin. Results revealed that out of 396 test samples, 122 samples were positive. Hence, the overall presence of antibiotic residues in marketed chicken of Kathmandu valley was 30.81% which was lower than the study of Tajick and Shohreh [18] in which antimicrobial residues were found 50% of the samples. Similarly, according to Gwachha [9] presence of antibiotic residues in marketed meat of broiler of Kathmandu valley was 33.03% which was almost similar with the findings of our research and result was higher than Pandey., *et al*. [15] that was 18.91%. The result is similar to the study of Abdelmoaty [1] conducted in fresh samples of broiler meat in Egypt which was 34%. This variation may be due to difference in sample size, sample collection sites, use of feed additives and recently treated, use of antibiotics before marketing of poultry without emphasizing in their withdrawal period, sensitivity and specificity of the kits used, methodology and other limitations as well as violation in use of antibiotics.

The residues of tetracycline, macrolide/aminoglycoside/sulfonamide and penicillin were found 33.33%, 41.67% and 17.42% respectively. The study showed the highest residue for macrolide/aminoglycoside/sulfonamide followed by tetracycline and penicillin. The high macrolide/aminoglycoside/sulfonamide group may be due to the frequent use of this group for treatment of diseases and as feed additives. The positive result of sulfonamide (41.67%) is higher than [1] which was 23%, [9] 21.9%, [5] 22.67% and [10] 36.29%.

Residue of tetracycline antibiotics (33.33%) was lower than finding of Gwachha [9] 50.48%, [5] 58.67%, [10] 75.81% and [2] 69% and higher than [1] 32% and [20] 23.22%. The reason behind the occurrence of antibiotic residues in meat may be due to the haphazard prescription of drugs as a growth promotor or due to enormous use of antibiotics in a poultry farming and the use of various practices. The presence of penicillin was found to be lower than the finding of Rawal., *et al*. [16], a study conducted in marketed broiler meat of Gorkha, Parsa, Chitwan and Kathmandu district which was 60% [9], 18.10% [5], 33.33% [19] 96%. This may be due to various factors affected by limitation of study time and place, etc. along with the techniques used during work period or ignorance in the withdrawal period of drugs before marketing poultry for meat.

Out of 44 meat samples subjected to 132 tests, from every district, prevalence of positive case for antibiotic was found higher in Kathmandu (34.09%) followed by Lalitpur (31.06%) and Bhaktapur (27.27%). The location wise prevalence of tetracycline residue was found to be higher in Kathmandu (36.36%) followed by Lalitpur (34.09%) and Bhaktapur (29.54%). Macrolide/aminoglycoside/sulfonamide residue was found higher in Kathmandu (47.72%) followed by Lalitpur (40.90%) and Bhaktapur (36.36%). The location wise prevalence of penicillin residue was found similar in Kathmandu and Lalitpur districts i.e. 18.18% and lower in Bhaktapur district (15.90%) which do not match the finding of Gwachha [9]. The location wise prevalence of tetracycline residue was found to be higher in Lalitpur followed by Kathmandu and Bhaktapur. Similarly, according to his findings, sulfonamide residue was found higher in Kathmandu and Bhaktapur and lower in Lalitpur district. Likewise, penicillin residue was found higher in Kathmandu followed by Bhaktapur and Lalitpur districts. As the probability value is greater than 0.05, the result is non-significant ($p > 0.05$). This may be due to interchange and supply of meat between the valley districts. For example, farmers from Bhaktapur carry their chicken ready to slaughter to Kathmandu and Lalitpur to different vendors. According to [21], the seasonal factor also affects the prevalence of antibiotic residues.

According to statistical results, there was no significant difference in overall prevalence and location wise prevalence of tetracycline, macrolide/aminoglycoside/sulfonamide and penicillin residue ($P > 0.05$) which shows no association between occurrence of antibiotic residues and markets. This result may be obtained due to small sample size.

The incidence of antibiotic residues in marketed meat and subsequent occurrence of antibiotic resistant-bacteria [12] has become the global public health concern in recent times. So, Food Act and Feed Act should be implemented and followed strictly along with regular investigation of major retail shops by regulatory bodies including evaluation and use of antibiotic alternatives to reduce antibiotic resistance and uncontrolled use of antibiotics.

Conclusion

The threat of antibiotic contamination is one of the biggest challenges to public health that is faced not only by underdeveloped country like Nepal but also by the human population worldwide. The use of antibiotics has been the most essential for the better production and treatment purposes. In 2003, WHO recommended to stop using antibiotics for growth promotion. The FDA center for Veterinary Medicine must approve every drug used in animals to prevent the drugs that may harm animals and humans who consume the meat [1]. But the studies of antibiotic residues clearly indicate there exists inappropriate use of antibiotics and no proper inspection of withdrawal periods.

The overall presence of antibiotic residues in marketed broiler meat of Kathmandu valley is 30.81%. It can be concluded that the presence of antibiotic in such percentage is highly threat to the public health as it creates the antimicrobial resistance in the consumers. The antimicrobial resistance is the threat to the public health globally. It is highly recommended that further quantitative research should be conducted to assess the amount of antibiotic residues present in the meat samples.

It is deemed that the regular withdrawal periods should be strictly followed before chickens are slaughtered and delivered to retailers. Because of neglecting the withdrawal periods, meat and its products contain high level of antibiotics. Unless the veterinary medicine and treatment is regulated by veterinarians and prescribed by registered veterinarians, the current scenario can't be improved.

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