



Assessment of Antibiotic Residues in the Marketed Meat of Kailali and Kavre of Nepal

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Abstract

This is a cross-sectional study to detect presence of antibiotic residues in meat sample of Kailali and Kavre districts. Each sample out of 55 meat samples (muscles and liver) of poultry-41, goat-12, buffalo-9 and pig-4 from Kailali and Kavre was tested for different groups of antibiotic residues separately. The antibiotics tested were Penicillin, Tetracycline, Aminoglycosides, Macrolides and Sulfonamides. Samples were preserved at -84°C in the deep freezer after collecting in sterile plastic pouch. Rapid test kit (78.9% sensitivity and 96.7% specificity) of G9 Co. Ltd., Thailand was used to detect residues in the samples. The protocol of kit was followed for the test. The overall prevalence of antibiotic residues in meat was around 22%. Meat samples exceeding the Maximum Residue Limit (MRL) were 6. Liver was found with higher number of residues than muscles. The organ and location wise prevalence of residue was found statistically significant at $p < 0.05$. The samples contained alarmingly high level of antibiotic residues which is because of neglecting the withdrawal period. The prescription of antibiotic is not prudent.

Keywords: Antibiotic residue; Withdrawal period; MRL; Meat

Introduction

Antibiotic is a chemical substance produced naturally by a microorganism or produced synthetically in the laboratory which has potential to kill or inhibit the growth of microorganisms. Substance produced synthetically (e.g. sulphonamides and quinolones) should not be termed antibiotics (Guardabassi and Dalsgaard, 2004), but the definition of antibiotics has also been used to include

chemically derived, synthetic antibacterial drugs (Kennedy *et al.*, 1998).

The use of antimicrobials for the treatment or prevention of disease in animals closely followed their uses in humans (Gustafson, 1993), and they were first employed in veterinary medicine for the treatment of mastitis in dairy cows (Spencer, 1950). Antimicrobial drugs are being haphazardly used to prevent, control and treat infections.

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Treatment of animals reared for food, especially pigs and poultry, is generally directed at groups or herds of animals (Johnston, 1998). 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. Likewise antimicrobials are illogically used in synchronizing the reproductive cycle and breeding performance. Antibiotic Resistance; due to consumption of animal products and tissues containing these residues, has imposed a serious threat in public health and the existence of human beings. The chance of drug failure is increasing upon the production of new and new strains. In order to ensure safety of global health, the international recognized bodies such as Codex Alimentarius Commission (CAC) which is a Joint FAO/WHO body for food standardization and the European Union have established a series of Maximum Residue Limit (MRL) for veterinary drugs in edible tissues of animal origin (Reig and Toldrá, 2008; Turnipseed and Andersen, 2008; Peters *et al.*, 2009). The concerned regulating authorities like Veterinary Public Health Office (VPHO) and Veterinary Standard Drug Administration Office (VSDAO) conduct screening of different residues present in consumable meat and milk items time to time to assess public health resulting from hazard of antibiotic residue.

The superbugs that resist the effects of antibiotics transfer their resistant plasmid to other thereby creating other resistant bacteria. The necessity of addressing the large number of antibiotics used in livestock and poultry sectors has been felt to overcome the burden of antibiotic resistance. The administration of sub-therapeutic dosage of antibiotics by farmers to accelerate growth or to prevent animals from getting sick due to unsanitary practices is also one of the key factors for developing antibacterial resistance. While mastitis is the most common disease of adult dairy cows and accounts for most cases of antibiotics (Pol and Ruegg, 2007) lactating cows are also treated for other infectious diseases, including respiratory and uterine infections and infectious foot disease.

Medicine had brought about revolution around 1940s through the introduction of antibiotics to treat infectious disease. But the progress couldn't be maintained for long time because of the rapid spread of antibiotic resistance that ultimately resulted treatment failure. Allergic reactions to antibiotics are well recognized and hypersensitivity to β -lactam compounds is especially prevalent. The literature regarding allergic responses of humans after exposure to drug residues found in milk is sparse and focused primarily on risks associated with exposure to β -lactams (Dewdney and Edwards, 1984; Ormerod, *et al.*, 1987; Wicher *et al.*, 1969). Nepal being one of the member countries of World

Trade Organization (WTO) and OIE has obligation to follow standards and rules regarding to Maximum Residue Limit (MRL). The MRL set by VSDAO, Budhanilkantha is followed in our country though CAC (2005) and WHO (2006) have also set MRL for different antibiotics.

Methodology

Cross Sectional Study method was used for research. Samples were collected from different retail shops of Kavre and Kailali of Nepal. Total 55 samples were tested (41 muscle samples and 14 liver samples) for different antibiotics separately. Hence, 165 tests were done in total. The antibiotics tested were Penicillin, Tetracycline and Aminoglycoside, Macrolides & Sulfonamides. Similarly 98 milk samples were collected from different dairy cooperatives of Kavre. Purposive sampling technique was applied for the collection of sample. Samples preserved in zipper bag by maintaining cold chain was transferred to VPHO and further preserved at -84°C in deep freezer till tests were done. The protocol of the test kits was followed for different antibiotics as per the instructions given in manual provided by the G9 Co. Ltd.

Procedure

5 grams of meat was taken into one or more about 50 mL centrifuge tubes. 5 mL of extraction solution A or B or C was added into each centrifuge tube respectively for Tetracycline group, Macrolide, Aminoglycosides and Sulfonamide group and Penicillin group. The tubes were shaken vigorously by electrical shaker for 10 minutes. The tubes were then placed in water bath and heated slowly until reaching 60°C and later cooled down slowly. The supernatant was taken after centrifuging at 3,000 – 4,000 rpm for 15 minutes. The pH of the supernatant samples was adjusted for further test by adding 4 drops of the supernatant solution in each of the prepared tubes and adding 4 drops of the negative control into another tube. The tubes were incubated into the hot water bath at 64°C for 2 hour 45 minutes. The color change of tubes was observed where pink tubes changed into the yellow color in case of negative result and pink tubes still had pink coloration if the result was positive. Positive results were matched with standard chart to determine the level of the drug residues.

Statistical Analysis

Data were collected, coded, computed and analyzed using program Microsoft Office Excel version 7. The association between different antibiotics was compared statistically by a Chi-square (χ^2) analysis using commercial software SPSS version 16 with significance level defined at the $p < 0.05$. OpenEpi version 2.3 software with significance level defined at the $p < 0.05$ was also used to know the association of different antibiotic residues present at different locations and in different organs.

Result and Discussion

Prevalence of Antibiotic Residues in Meat

Antibiotic residue was found 22% positive for at least one of the antibiotic tested (Table 1). This result is nearly equal to the study conducted by Hussein & Khalil (2013) which is 21% in broiler meat. Tajik (2006) reported 50% prevalence for at least one of the antibiotics by using Thin Layer Chromatography (TLC).

Table 1: Presence of Antibiotic Residues in Meat

Presence of antibiotic residues in mean	Total
Total number of samples tested for antibiotics	165
Total positive	22% (36/165)
Total negative	78% (129/165)

Prevalence of overall residues in meat

Tetracycline is intensively used in poultry sector and that's why its residues may be higher than that of penicillin (Table 2).

Table 2: Prevalence of overall residues in meat

Antibiotics	Positive	Negative	Total
1. Tetracycline	16	39	55
2. Macrolides, Sulfonamides & Aminoglycosides	13	42	55
3. Penicillin	7	48	55
Total	36	129	165

Prevalence Based on Location

Table 3: Location wise prevalence of antibiotic residues in meat

Total positive sample	36
Positive sample from Kailali	27.77% (10/36)
Positive sample from Kavre	72.22% (26/36)

There is significant difference in location wise prevalence of antibiotic residue ($p < 0.05$). The reason behind high prevalence of antibiotic residue in Kavre may be due to the haphazard prescription of drugs as a growth promoter (Table 3).

Prevalence of Antibiotic Residues in Muscle and Liver

Among the 41 muscle and 14 liver samples tested, 16 (39%) muscle and 10 (71%) liver samples were found positive for antibiotic residue. There is significant difference in prevalence of residue in muscle and liver ($p < 0.05$). The higher percentage of residues in liver than muscles in this study may be due to the fact that liver is the chief site for drug metabolism. The study clearly indicates that residues persist in marketed meat. The findings of this study differ from those mentioned and is might be due to the variation in methodology used and test kits used. Different authors have taken sample sizes ranging from small to large and technique used are HPLC, TLC, ELISA, Plate Bioassay whereas Rapid Test Kit is used here to detect the antibiotic residues (Fig. 1).

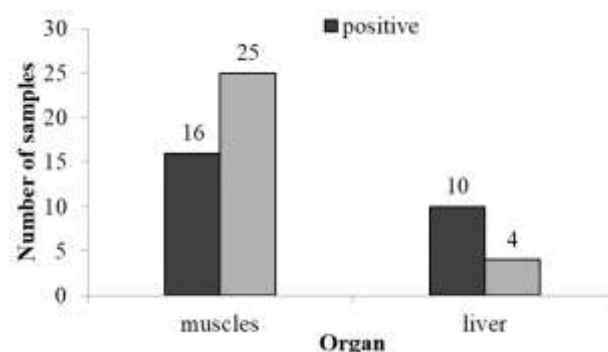


Fig 1: Organ wise distribution of antibiotic residue in meat

Conclusion

Resistant and multi-resistant bacteria have been developed due to indiscriminate and prolonged use of antibiotics. Consumable broiler chicken liver showed very high percentage of antibiotic residue and withdrawal period for antibiotic use is highly violated. Meat contained higher amount of residues than that of milk. The consumption of these veterinary drugs as a residue poses a serious threat to public health such as allergy, bacterial resistance, teratogenic and carcinogenic effect. A few studies regarding antibiotic residues in meat and milk products of Nepal have limited the data for comparison. The action taken by government to prevent antibiotic residue bearing meat and their products from reaching to consumers isn't satisfactory. Strict following of withdrawal periods only make the meat safer for consumption. Special training and awareness help the farmers to prevent infections so that the use of antibiotics can be minimized.

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