RESEARCH PAPER
J. Food Sci. Technol. Nepal, Vol. 8 (60-64) 2013
ISSN: 1816-0727

Sulfonamides and Penicillin Residue in Market Milk

MANISH THAPALIYA[1*], TIKA B. KARKI[2] and DAMODAR SEDAI[3]

1 Department of Microbiology, National College

2 Department of Biotechnology, Kathmandu University

3 Veterinary Standards and Drug Administration Office

*Corresponding author: **manish.thapaliya@gmail.com**

The present study was conducted at laboratory of Veterinary Standards and Drug Administration Office, Tripureshwor, Kathmandu in the month of October to December 2007 with aim of determining the prevalence and level of sulfonamide and penicillin residue semi-quantitatively in market milk samples from Kathmandu, Lalitpur, Bhaktapur and Kavrepalanchowk districts. All the samples were processed using standard procedure given in the protocol of the rapid residue test kit provided by the Division of Food, Department of Medical Sciences, Ministry of Public Health, Thailand. The prevalence of antibiotic residue was found to be 17.3% (n=26) of the total of 150 samples collected. Out of the total samples 12% (n=18) were found to contain penicillin residue and 5.3% (n=8) were found to contain sulfonamide residue. Sulfonamide residue was detected in the range of 0-1 ppb in 6 samples and 2-4 ppb in 2 samples. Similarly, penicillin residue was found in the range of 0-1 ppb in 14 of the samples and 2-4 pbb in 4 samples. The residues level detected were below their MRLs as set by the Codex Alimentarius Commission. The occurrence of the antibiotic residue in the tested milk samples was compared whether it differs from district to district. Statistically, there was no significant difference on the occurrence of the antibiotic residue in the tested milk from district to district.

Keywords: antibiotic residue, rapid residue test kit, milk

Introduction

Antibiotics are substances produced by living organisms that are able to kill or inhibit the growth of microorganisms. According to the literal sense of the word, 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). Nowadays, antimicrobial drugs are used to control, prevent and treat infection and to enhance animal growth and feed efficiency (Tollefson and Miller, 2000). Treatment of animals reared for food, especially pigs and poultry, is generally directed at groups or herds of animals (Johnston, 1998). Currently, approximately 80% of all food-producing animals receive medication for part or most their lives (Lee *et al*., 2001). The use of antimicrobials in food-producing animals may result in the presence of residues in foodstuffs of animal origin. The presence of certain antimicrobial agent residuals in milk constitutes a potential hazard for the consumer and may cause allergic reactions, interference in the intestinal flora, and resistant populations of bacteria in the general population, thereby rendering antibiotic treatment ineffective (Currie *et al*., 1998). Important losses are also provoked in the fermented products, by inhibiting the bacterial processes involved in the elaboration of cheese and cultured milk products (Brady and Katz, 1988).

World Health Organization (WHO, 1991) has reported that each antibiotic has label instruction that indicates the approval reasons for using the antibiotic, the dose or amount of the antibiotic, the dose or amount of the antibiotics, how often the antibiotic dose should be repeated, the route of administration and the type of cattle permitted to be treated with the antibiotic. WHO further reported that each antibiotic preparation also has a specific withdrawal time for both milk and meat.

Codex Alimentarius Commission (CAC), (2005) and WHO (2006) have set the Maximum Residue Limit (MRL) for different types of antibiotics in milk and meat samples and it is mandatory for the member countries of World Trade Organization (WTO) and Office International des Epizooties (OIE) for trade purpose. Recent information on antibiotics residue hazards has focused on the necessity to conduct tests for detecting

the level of veterinary drug residue for food safety.
In Nepal, most of the drugs are used without any restriction in such a huge amount and care of withdrawal period and assessment of antibiotic residue in meat and milk are not monitored properly by government and privet sectors (Sedai, 2007).

Many standard methods have been developed worldwide for antibiotic residue analysis. But use of very high-tech method to evaluate the residue in milk may be impractical in our context. Considering the above mentioned facts the present study investigates the prevalence rate of sulfonamides and penicillin groups of antibiotics in market milk and also quantifies the sulfonamides and penicillin groups of antibiotics drug residue semi-quantitatively.

Materials and Methods

Sample collection

The sample size (n) of the study was 150, which included 50 samples from Kathmandu, 50 samples from Lalitpur, 25 samples from Bhaktapur and 25 samples from Kavrepalanchowk. Sampling was done via random sampling method. Samples were collected at early morning. Milk collection was done in sterile Mecorney bottle. A milk sample of 450 mL was collected from each animal just after milking. The Mecorney bottle was then placed in icebox and brought to the laboratory of Veterinary Standards and Drug Administration Office (VSDAO) for testing.

Sample Processing

The processing of the sample was done according to the protocol of the rapid residue test kit provided by the developer. Before performing the analysis, the milk samples were heated in water bath at 82±2ºC for 2 minutes to destroy heat-labile natural inhibitors and microorganisms contaminated in raw milk.

Test for the presence or absence of drug residue

A sample containing 0.1mL of milk was added into the prepared tube then 0.1mL of UHT fresh milk and the provided negative control into another prepared tube for negative control. All the tubes were incubated for 2 hours 45 minutes in water bath at the temperature of 64±2ºC, keeping medium in the tube under water level, or were incubated until the colour of medium in negative control tube changed completely from purple to yellow. Then the colour changes of medium in sample tubes were observed.

Confirmation for the presence of penicillin groups

Penicillinase enzyme (0.05mL) of was added into 2-3ml of positive milk sample and were mixed well together. Then, 0.1mL of mixture was added into test kit and were then incubated for 2 hours 45 minutes in water bath at the temperature of 64±2ºC, keeping medium in the tube under water level, or were incubated until the colour of medium in negative control tube changed completely from purple to yellow. Then the colour changed of medium in sample tubes was observed.

Confirmation for the presence of sulfonamide groups

Para Amino Benzoic Acid (PABA) solution (7.5µl) of concentration1ppm was added into 1mL of positive milk sample and was mix well together. Then, 0.1mL of the mixture was transferred into test kit and and were then incubated for 2 hours 45 minutes in water bath at the temperature of 64±2ºC, keeping medium in the tube under water level, or were incubated until the colour of medium in negative control tube changed completely from purple to yellow. Then the colour changed of medium in sample tubes was observed.

Quantitative test for drug residue of Penicillin and Sulfonamide group

The quantity of Penicillin and Sulfonamide residues as indicated by the level of purple colour in medium were read in the range of 0-1, 1-2, 2-4, 4-8, 128-256 ppb by comparing it with standard chart.

Results and Discussion

Standardization of test kit

The test kits used in this study was standardized using the standards of both sulfonamide and penicillin as provided by the producer along with the test kits. The standards which were used in the standardization process were of 1 ppb, 2 ppb, 4 ppb, 8 ppb, 16 ppb, 32 ppb and 64 ppb. The level of the purple colour obtained was measured using a vernier caliper and then was compared with the standard chart provided by the kit developers (Figure 1 and 2).

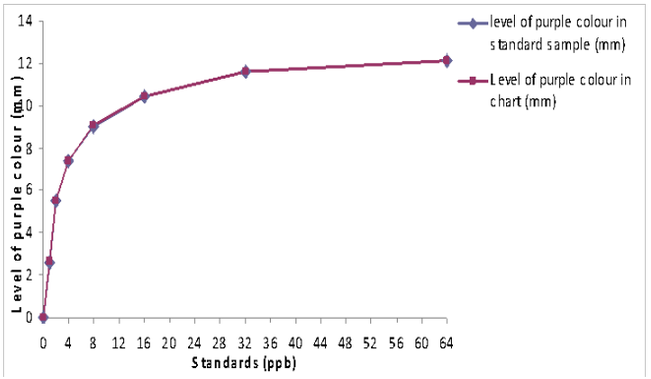

Figure1. Standardization of test kit for sulfonamide

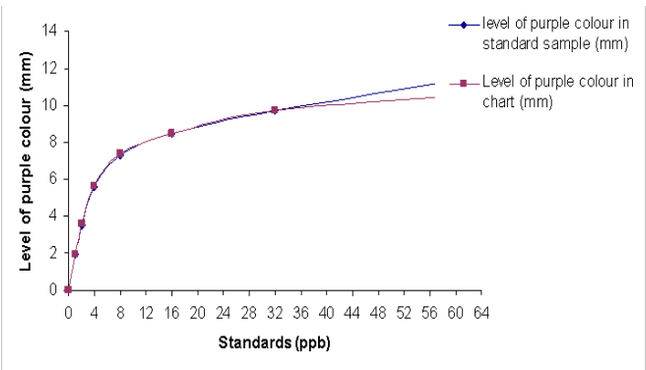

Figure 2. Standardization of test kit for penicillin

Result of the samples

Out of a total of 150 samples tested, 17.3% (n=26) samples were found to be positive for antimicrobial residue, whereas 83.7% (n=124) samples were found to be negative for antimicrobial residue (figure 3). Out of 26 positive samples, sulfonamide residue was detected in 8 (5.3%) of the samples and penicillin residue in 18 (12%) of the samples (Table 1). The prevalence of antibiotic residue in market milk during the study is similar to that of Seymour et al., (1988). But the data showed the higher prevalence of antibiotic residue than that in market milk of Kathmandu valley as reported by Sedhain, (2008) and bulk milk of Barbados and Jamaica Baynes *et al*. (1999).

Table 1. Amount of the antibiotic residue detected

S.No	Sample Code	Antibiotic detected	Amount of residue (ppb)	Above/ Below MRL
1.	KRM1	Sulfonamide	0-1	Below
2.	KRM2	Sulfonamide	0-1	Below
3.	KRM5	Sulfonamide	0-1	Below
4.	KRM28	Sulfonamide	2-4	Below
5.	LRM35	Sulfonamide	2-4	Below
6.	LRM43	Sulfonamide	0-1	Below
7.	LRM44	Sulfonamide	0-1	Below
8.	KpRM7	Sulfonamide	0-1	Below
9.	KRM16	Penicillin	0-1	Below
10.	KRM21	Penicillin	2-4	Below
11.	KRM24	Penicillin	0-1	Below
12.	KRM25	Penicillin	0-1	Below
13.	KRM26	Penicillin	2-4	Below
14.	KRM41	Penicillin	0-1	Below
15.	KRM42	Penicillin	0-1	Below
16.	KRM43	Penicillin	0-1	Below
17.	LRM10	Penicillin	2-4	Below
18.	LRM19	Penicillin	0-1	Below
19.	LRM20	Penicillin	0-1	Below
20.	LRM27	Penicillin	0-1	Below
21.	LRM31	Penicillin	2-4	Below
22.	KpRM14	Penicillin	0-1	Below
23.	KpRM15	Penicillin	0-1	Below
24.	KpRM16	Penicillin	0-1	Below
25.	BRM24	Penicillin	0-1	Below
26.	BRM25	Penicillin	0-1	Below

However the prevalence of antibiotic residue was lower than that found by Tovelentino *et al*. (2005) in four different brands of the Mexican pasteurized milk. Similarly, the prevalence was also lower as compared to the prevalence of the residue in the market milk of Kathmandu valley tested in peak rainy season when the

disease incidence might be high and so is the use of the antibiotic VSDAO (2007). Lesser amount of residue in positive sample may be due to lesser use of antibiotics during early winter season (time when study was conducted) when disease occurrence is comparatively lower. According to Yamaki *et al*. (2004), the seasonal factor also affects the prevalence of the antibiotic residue, because in his study the highest percentages of "positive plus doubtful" results were observed in late summer–early autumn.

The average level of the residue was in the range of 0-1 ppb for both penicillin and sulfonamides except in 6 samples were the level of the residue detected was in the range of 2-4 ppb i.e. 4 samples of penicillin and 2 samples of sulfonamide. This was also found to be some what similar to the result obtained by VSDAO (2007) which had reported the residue level ranging from 1-2 ppb for the penicillin. The level of antibiotic residue was compared with the MRLs set by CAC it was found below to their respective limits in all residue-detected samples and was found to be similar to the result of Raebroeck et al. (2002), who also detected all tested β-lactam compounds (except Cefquinone) and other antibiotics at a level below their respective MRLs.

District-wise analysis of the presence of antibiotic residue in the tested milk samples revealed 24% (n=12), 16% (n=8), 8% (n=2) and 16% (n=4) in Kathmandu, Lalitpur, Bhaktapur and Kavrepalanchowk, respectively (figure 3).

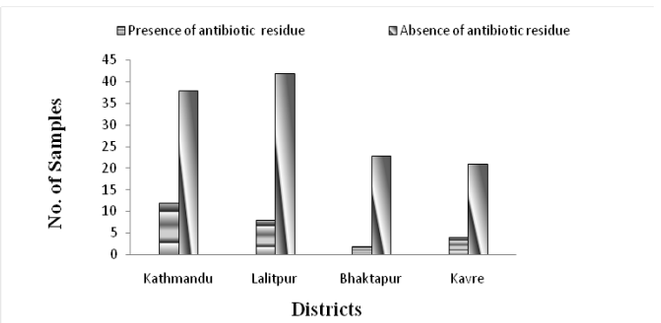

Figure 3. Number of milk samples containing antibiotic residue district-wise

When the occurrence of antibiotic residue obtained was compared with that of the different districts in cow milk, large number of cow milk samples from Kathmandu (n=7) was found to contain penicillin residue, whereas low number of cow milk from Bhaktapur (n=2) was found to contain penicillin residue. Similarly, sulfonamide residue were found to contain in large number of samples from Lalitpur (n=3) and none of the cow milk sample from Bhaktapur (n=0) was found to contain sulfonamide residue (figure 4).

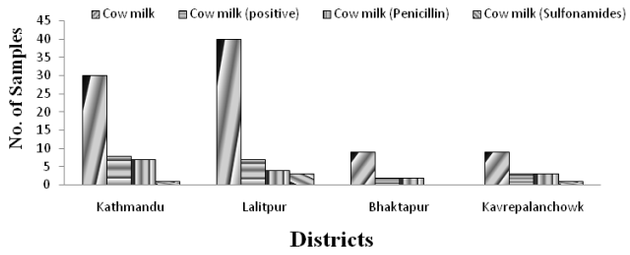

Figure 4. Penicillin and sulfonamide residue in the cow milk samples

When the occurrence of antibiotic residue obtained was compared with that of the different districts in buffalo milk, only one of buffalo milk samples from Kathmandu and Lalitpur (n=1) was found to contain penicillin residue whereas none of the buffalo milk sample from Bhaktapur and Kavrepalanchowk (n=0) found to contain penicillin residue. Similarly, sulfonamide residue were found to contain in large number of samples from Kathmandu (n=3) and none of the milk samples from Lalitpur and Bhaktapur found to contain sulfonamide residue (figure 5).

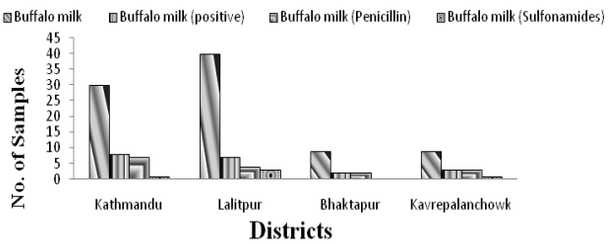

Figure 5. Penicillin and sulfonamide residue in the buffalo milk samples

This uniform prevalence at district levels suggests that the problem mainly originates at the farm level and on the concentration of the residues. This also correlates with the fact that, the sample were collected from the sub-urban areas of Kathmandu, Lalitpur, Bhaktapur and Kavrepalanchowk district where farmers are very conscious about animal health, mostly unqualified practitioners prescribe high doses of antibiotics not having the proper knowledge of withdrawal period of the antibiotics.

In conclusion, the samples used in this study were without preliminary medication information, so the samples might have contained residues of more than one

antimicrobial. Simultaneous identification of more than one antimicrobial in a sample would require reference patterns constructed with different antimicrobial combinations and concentrations, or alternatively one or more extraction steps. It is, however, possible with the present methods to gain some information on the presence of more than one antimicrobial in a sample.

Acknowledgement

The author would like to thank VSDAO (Veterinary Standards and Drug Administration Office) for providing the test kits and the laboratory space.

References

Baynes R.E., Lyman R., Anderson K.L. and. Browne C F (1999). A preliminary survey of antibiotic residues and viable bacteria in milk from three Carribean basin countries. Journal of Food Protection 62(2): 177-180.

Brady M.S. and Katz S.E. (1988). Antibiotic/antimicrobial residues in milk. Journal of Food Protection 51: 8–11.

CAC (2005). Veterinary drugs & Pesticide residue in food. FAO. Vet Drug Service Letter.

Currie D., Lynas L., Kennedy D.G. and McCaughey W.J. (1998). Evaluation of a modified EC Four Plate Method to detect antimicrobial drugs. Food Additives and Contaminants 15: 651–660.

Guardabassi L. and Dalsgaard D. (2004). Occurrence and faith of antibiotic resistant bacteria in sewage. Environmental Project No. 722. The Royal Veterinary and Agricultural University. Department of Veterinary Microbiology. Copenhagen, Denmark. e-publication (www.mst.dk) 3.4.2004

Gustafson R. (1993). Historical perspectives on regulatory issues of antimicrobial resistance. Veterinary Human Toxicology 35: 2-5.

Johnston A. (1998). Use of antimicrobial drugs in veterinary practice. British Medical Journal 317: 665-667.

Kennedy D.G., McCracken R.J., Cannavan A. and Hewitt S.A. (1998). Use of liquid chromatography - mass spectrometry in the analysis of residues of antibiotics in meat and milk. Journal of Chromatography 812: 77-98.

Lee H.J., Lee M.H. and Ruy P.D. (2001). Public health risks: chemical and antibiotic residues. Asian-Australian Journal of Animal Science 14: 402-413.

Raebroeck W., Ooghe S. and Grijspeerdt K. (2002). First results of the validation of the Copan milk test for the detection of residues of sulfonamides in milk. Applied microbiology: 806-808.

Sedai D. (2007). Risk of Veterinary drug residue in food from animal origin marketed in Nepal. In: The Blue Cross Annual Bulletin. Vol. 9. NVSA.

Sedhain P. (2008). Screening of sulfonamide and tetracycline antibiotics residue in market milk at Kathmandu valley. An internship report submitted to IAAS, Rampur, Chitwan.

Seymour E.H., Jones M.G., and MeGilliard M.L. (1988). Comparisons of On-Farm Screening Tests for Detection of Antibiotic Residues. Journal of Dairy Science 71: 539-544.

Spencer G.R. (1950). Recent progress in mastitis control. Journal of Milk and Food Technology 13: 44-49.

Tollefson L. and Miller M.A. (2000). Antibiotic use in food animals: controlling the human health impact. Journal of AOAC International 83: 245-256.

Tvolentino R.G., Perez H.N., Gonzalez G.D., Yleon S.V., Lopez M.G. and Flores G.P. (2005). Determination of the presence of 10 antimicrobial residues in Mexican pasteurized milk. Interciencia 3(5): 291-294.

VSDAO (2007). Annual Technical Report 2006/07.

WHO (1991). Joint FAO/WHO Expert Committee on Food Additives (JECFA). Toxicological evaluation of certain veterinary drug residues in food: Monograph prepared by the thirty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No.815. WHO, Geneva.

WHO (2006). Joint FAO/WHO Expert Committee on Food Additives (JECFA). Toxicological evaluation of certain veterinary drug residues in food: Monograph prepared by the forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series. No.38. WHO, Geneva.

Yamaki M., Berruga M.I., Althus R.L., Molina M.P. and Molina A. (2004). Occurance of antibiotic residue in milk from Manchega ewe dairy farms. Journal of Dairy Science. 87: 3132-3137.