International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 1862-1865

Received: 27-11-2018
Accepted: 30-12-2018

Sneh Lata Chauhan
Department of Veterinary Public Health & Epidemiology College of Veterinary Sciences, LUVAS. Hisar, Haryana, India

Priyanka
Department of Veterinary Public Health & Epidemiology College of Veterinary Sciences, LUVAS. Hisar, Haryana, India

SR Garg
Department of Veterinary Public Health & Epidemiology College of Veterinary Sciences, LUVAS. Hisar, Haryana, India

Vijay J Jadhav
Department of Veterinary Public Health & Epidemiology College of Veterinary Sciences, LUVAS. Hisar, Haryana, India

Correspondence
Priyanka
Department of Veterinary Public Health & Epidemiology College of Veterinary Sciences, LUVAS. Hisar, Haryana, India

Dietary exposure assessment of tetracycline residues in milk in Haryana

Sneh Lata Chauhan, Priyanka, SR Garg and Vijay J Jadhav

Abstract
In addition to biological hazards i.e. bacteria, viruses, parasites, the occurrence of antimicrobial residues in food is another characteristic of modern food production. The use of veterinary drugs is more in production of animals so it is difficult to avoid the harmful effects in the presence of their residues in food. This study was conducted to determine dietary exposure risk to tetracycline residues in milk consumed in the Hisar city. The risk was assessed on the basis of per capita availability of milk in Haryana and India by calculating hazard quotient for each analyte under study. The results of risk assessment due to dietary exposure to the residues through milk consumption in India showed hazard quotient of 0.009, 0.004, 0.002 for oxytetracycline, tetracycline, chlortetracycline. These results revealed no toxicological risk with regard to dietary exposure of tetracycline residues through milk.

Keywords: Milk, tetracycline, risk assessment, dietary exposure

Introduction
In India, although antibiotics have been used in large quantities in both human and veterinary medicine, the presence of residues of these drugs in milk and meat has received a little concern. It is only in the recent years that attempts have been made to investigate the role of antimicrobial substances in public health (Nirala *et al*., 2017) [10].
Animal infectious diseases are frequently treated with antimicrobial agents, which contribute to the development of resistant bacteria that could pose a human health hazard through the food chain (Tollefson and Karp, 2004) [14]. As a consequence, conventional farming is not favorably regarded due to the crowding conditions of the animals on farms, which facilitate the appearance of infectious diseases and, therefore, encourage the disproportionate use of antimicrobial agents. Thus, in regards to sickness encountered in organic farming, synthetic allopathic medicines should be limited to the minimum number possible, and the withdrawal period must be twice the established time for conventional production. Because of this regulation, organic products have become an attractive option for consumers who often perceive these products as being healthier and safer than the products obtained from conventional farming (Van boeckel *et al*., 2015) [15].
Tetracyclines should not be used by children up to the age of 6–8 years or by pregnant women because of the risk of developing secondary tooth discoloration. Other chronic effects include nephrotoxicity, hepatotoxicity, skin hyperpigmentation in the sun exposed areas, hypersensitivity reactions. Tetracyclines have also been reported to cause hypokalemia, proximal and distal renal tubular acidosis (Goldfrank *et al*., 2002) [7]. Antibiotic usage in livestock production as therapeutics, prophylactics and as growth promoters has become vital to the growing dairy industry. However, prolonged or inappropriate usage of antibiotics may lead to residues appearing in milk, which pose great risk to human health and also interfere with the processing of milk and milk products. Despite all this, there is a lack of understanding and knowledge about the antibiotic residues in milk and milk products due to absence of an organized residue monitoring programme in the country.
Some of the authors noticed that even low concentrations of these substances in foodstuffs of animal origin can present health risks. The exposure of intestinal microflora to low concentrations of antimicrobial residues contained in food cause the potential risk to public health, leading to pathogenic bacteria overgrowth, or of compromising antimicrobial therapy in humans by exerting a selection pressure on the intestinal microflora thus favouring the growth of microorganisms with natural or acquired resistance (Sudershan & Bhat 1995) [13].

Risk assessment can help in understanding the connection between reducing the risks that may be associated with food and reducing the risk to consumers from the harmful health effects, so this approach is of particular importance in the development of appropriate food safety control.

This paper focuses on risk assessment on the basis of the development and validation of a simple HPLC-UV method for the simultaneous determination of TC, OTC and CTC in milk which could be applied to quality control in the routine analysis and to assess the associated public health risk.

Methods

Sample collection

A total of 100 milk samples consisting 40 vendor milk samples, 40 mini diaries samples and 20 pasteurized milk samples were collected from Hisar city and nearby area. Sample size was determined on the basis of previously reported antimicrobial residues in milk. For each collected raw milk sample a quantity of about 100mL was collected in a labeled sterilized bottle and stored at -20°C till analysis.

Chemicals and reagents

The analytical standards of antimicrobials *viz*. tetracycline, oxytetracycline, chlortetracycline all having purity more than 98%, were procured from Sigma-Aldrich. Supelclean™ LC-18 SPE Tube having bed wt. 500 mg and volume 3 mL were also procured from Sigma-Aldrich. All HPLC grade solvents namely methanol, acetonitrile and iso-propyl alcohol (IPA) were procured from Fisher Scientific whereas anhydrous sodium sulphate was procured from Qualigens. HPLC grade water was prepared in the laboratory using Millipore (Bedford, MA, USA) Milli-Q system to give a resistivity of at least 18.2 M Ω cm.

HPLC Instrumentation and condition

A Shimadzu prominence UFLC system equipped with DGU-20A5R degasser, SIL-20A HT autosampler and LC-20AD pump connected to C_8 column (Enable 4.6 mm x 250 mm porosity 5 um) housed in CTO-10AS column oven with SPD-20A UV-VIS detector was used throughout the experiment. The system was controlled by Lab Solution Software.

HPLC analysis

HPLC-UV technique was standardized and validated for detection of TETs *viz*. tetracycline, oxytetracycline and chlortetracycline from milk as per method described by Stolker *et al*., (2008) [12] with slight modification. Mobile phase used for the instrumental analysis of tetracycline was composed of solvent A (water: formic acid as 1000:1 v/v) and solvent B (water: acetonitrile: formic acid as 100:900:1 v/v/v). The flow rate was 1ml/min. Detection of Tetracyclines was performed at UV detector at 280nm wavelength. On the basis of linearity, accuracy and precision, tetracyclines were standardized and validated. Screening of all milk samples and quantitation of residues of tetracyclines was done with the standardized and validated method.

Calculation of estimated dietary exposure

Daily intake are estimated using the formula in which chemical concentration refer to amount of chemical in food, consumption refer to the amount of food consumed and dietary exposure refer to the amount of chemical ingested via food.

Dietary exposure [μg/kg] = (Food chemical concentration [μg/kg] × Food consumption [kg/person])

Hazard Quotient

Residues were determined in all milk samples and on the basis of mean concentration of Oxytetracycline, tetracycline and chlortetracycline residues, the risk was assessed on the basis of acceptable daily intake recommended by JECFA. Hazard quotient of Oxytetracycline, tetracycline and chlortetracycline was calculated by using acceptable daily intake in Haryana and India.

On the basis of dietary intake, risk was estimated in the form of hazard quotient. Hazard quotient is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected.

$$\text{Hazard Quotient} = \frac{\text{Dietary intake of antimicrobial through milk}}{\text{Acceptable daily intake}}$$

Results

In the current study, the analytical determination of three types of tetracyclines (oxytetracycline, tetracycline and chlortetracycline) in milk samples was carried out, and then, based on the existing and representative data on the average milk consumption for adults, the risk of dietary exposure to residual quantity of TETs via milk consumption was evaluated for the population of Haryana and India. Risk assessment is done by comparing the estimated daily intake of antimicrobial residues with their acceptable daily intake (ADI) values recommended by regulatory agencies. Table 1 and 2 shows the dietary intake of antimicrobial residues expressed as μg per kilogram of body weight per day (μg/kg BW/day) in comparison with acceptable daily intake (ADI) values recommended by JECFA i.e. 0-30 μg/kg body weight/day for TETs. The hazard quotient based on ADI values and dietary intake values was also calculated and presented in Table 1 and 2.

Table 1: Dietary exposures of tetracycline residues through Milk consumption by the population of India

Group of antimicrobials	Analytes	Dietary Intake (μg/kg b.wt/day)	ADI (μg/kg b.wt/day) (JECFA, 2002)	Hazard Quotient
Tetracyclines	Oxytetracycline	0.73	0-30	0.009
	Tetracycline	0.30	0-30	0.004
	Chlortetracycline	0.16	0-30	0.002

ADI = Acceptable daily intake; NA= not available

Table 2: Dietary exposures of tetracycline residues through Milk consumption by the population of Haryana

Group of antimicrobials	Analytes	Dietary Intake (μg/kg b.wt/day)	ADI (μg/kg b.wt/day) (JECFA, 2002)	Hazard Quotient
Tetracyclines	Oxytetracycline	0.29	0-30	0.024
	Tetracycline	0.12	0-30	0.01
	Chlortetracycline	0.06	0-30	0.005

ADI = Acceptable daily intake; NA= not available

Discussion

Despite the extensive use of antimicrobials in developing countries, the information pertaining to their daily exposure is lacking. Critical assessment of dietary exposure is vital to obtain fundamental data concerning the safety of foods, problems and trends in the intake of antimicrobial and to identify the sources of unusual residues. Estimation of dietary exposure of antimicrobial residues through milk consumption was made on the basis of antimicrobial residue concentration in milk, multiplied by average daily intake of milk (which was taken as the daily per capita milk availability of milk). Risk assessment is done by comparing the estimated daily intake of antimicrobial residues with their acceptable daily intake (ADI) values recommended by regulatory agencies. ADI represents the amount of drug residues that can safely be consumed per day over a human's lifetime without adverse effect. India is the first ranker in milk production which makes a good per capita availability (337 g/capita/day) of milk to Indian population, Among all the states in the country, Haryana has the second highest availability of milk (835 g/capita/day). Thus, more milk availability may lead to higher milk consumption and higher probability of dietary exposure to antimicrobial residues through milk consumption.

The risk of dietary exposure to residual quantity of Tetracyclines via milk consumption was evaluated for the population of Haryana and India. Per capita milk availability values in Haryana and India are reported as 835 g and 337 g, respectively (DAHD, 2016 and NDDB, 2016) [3, 9]. Data of acceptable daily intake are available for OTC, TC, CTC only. On the basis of calculated dietary intake and acceptable daily intake, the hazard quotients were found to be 0.009, 0.004, 0.002 for OTC, TC, CTC respectively on national basis while in Haryana population, the hazard quotients were 0.024, 0.01, 0.005 for OTC, TC, CTC respectively. These suggest that the population is not at risk for dietary exposure of OTC, TC, CTC residues. Due to more per capita milk availability in Haryana as compared to national average, the risk is also greater.

Elizabeta *et al.* (2011) [5] calculated the estimated daily intakes (EDI) for the average daily consumption of 200 ml of milk for an adult in Macedonia, for the examined antimicrobials and obtained levels 2 to 100 times lower than the values of the acceptable daily intakes fixed by World Health Organization. This indicated that toxicological risk associated with the consumption of analyzed milk could not be considered as a public health issue with regards to these veterinary drugs. Similar findings were also mentioned in a study conducted in Croatia which showed that none of samples analyzed was found to contain veterinary drug residues above the maximum residues levels (MRLs) established by European Union and Croatian legislation. The calculated estimated daily intakes (EDIs) for the average daily milk consumption of 300 ml for an adult in Croatia for examined antibiotics showed levels 20 to 1640 times lower than the values of acceptable daily intakes (ADIs) fixed by the European Medicines Agency and World Health Organization. This suggested that toxicological risk associated with the consumption of analyzed milk could not be considered a public health issue with regards to these veterinary drugs (Bilandzic *et al.*, 2011) [2]. According to the study of Vragovic *et al.* (2011) [16] the acceptable daily intake for tetracycline (for a person weighing 60 kg) was 1800 µg/person/day, thus the assessed risk is negligible i.e. less than 1% of acceptable daily intake. Similarly in our study, the residue concentration found was 0.16-0.73 µg/person/day for tetracycline and risk is negligible for human health. Gaurav *et al.* (2014) [6] studied TETs residues and found Hazard Quotients for TC and OTC in pooled raw milk samples as 0.027 and 0.03 respectively. The Hazard Quotient for these in pasteurized milk samples were 0.006, 0.01 respectively. The determined concentrations of the majority of antibiotic residues do not exceed the acceptable daily intakes through food established by the World Health Organization.

In Yugoslavia, a similar study showed that the hazards associated with tetracycline residue intake via milk was negligible Gradinaru *et al.* (2011) [8]. Prado *et al.* (2014) found low EDI that is accounted for low risk or negligible where as Fernades *et al.* (2014) found high risk in pasteurized milk samples of different brand. Aalipour *et al.* (2015) [1] conducted a study to evaluate the risk of tetracycline residue intake via milk consumption amongst different age groups of human consumers in Iran. Daily exposure to tetracycline residues intake through milk considering long term and short term milk consumption was estimated to range from 58–62 µg and 0–99.3 µg per person.

The issues concerning antibiotics inactivation during milk processing are more serious than those with other animal origin foodstuffs which is given by the fact that milk is heat-treated over a very short time interval. Heat stability of the residues depends on the antibiotics type. It has been proved that during the heat treatment of milk, there is only a partial reduction occurs of tetracycline residues concentrations, total elimination not taking place.

Because milk and milk products are essential foodstuffs for small children, attention has to be paid to the presence of drug residues in milk. Antimicrobial substance detection in milk samples at the delivery in the dairy should not be focused only on the most frequently used antimicrobial substances (tetracyclines) but also on other agent groups.

References

1. Aalipour F, Mirlohi M, Jalali M, Azadbakht L. Dietary exposure to tetracycline residues through milk consumption in Iran. Journal of Environmental Health Science and Engineering. 2015; 13:80.
2. Bilandzic N, Solomun B, Varenina KI, Jurković Z. Concentrations of veterinary drug residues in milk from individual farms in Croatia. Mljekarstvo / Dairy. 2011; 61:260-267.
3. DAHD. Statistical information related to animal husbandry sector. Department of Animal Husbandry and Dairying, Haryana, 2016. Available at http, //www.pashudhanharyana.gov.in/html/statistics.htm
4. De Albuquerque Fernandes SA, Magnavita APA, Ferrao SPB, Gualberto SA, Faleiro AS, Figueiredo AJ *et al.* Daily ingestion of tetracycline residue present in pasteurized milk: a public health problem. Environmental Science and Pollution Research. 2014; 21:3427-3434.
5. Elizabeta DS, Zehra HM, Biljana SD, Pavle S, Risto U. Screening of veterinary drug residues in milk from individual farms in Macedonia. Mac. Vet. Rev. 2011; 34:13.
6. Gaurav A, Gill JPS, Aulakh RS, Bedi JS. ELISA based monitoring and analysis of tetracycline residues in cattle milk in various districts of Punjab. Veterinary World. 2014; 7:26-29.
7. Goldfrank LR, Flomenbaum NE, Lewin NA, Howland MA, Hoffman RS, Nelson LS. Goldfrank's Toxicologic Emergencies. The McGrawHill Companies, New York, 2002.

8. Gradinaru AC, Popescu O, Solcan G. Antibiotic residues in milk from Moldavia, Romania. HVM Bioflux. 2011; 3:133-141.
9. NDDB. Milk production in India. National Dairy Development Board, 2016. Available at http, //www.nddb.org/information/ stats/milkprodindia.
10. Nirala RK, Anjana K, Mandal KG, Jayachandran C. Persistence of Antibiotic Residue in Milk under Region of Bihar, India. International Journal of Current Microbiology and Applied Sciences. 2017; 6:2296-2299.
11. Prado CK, Ferreira FD, Bando E, Machinski Jr M. Oxytetracycline, tetracycline, chlortetracycline and doxycycline in pasteurised cow's milk commercialised in Brazil. Food Additives and Contaminants: Part B 2015; 8:81-84.
12. Stolker AA, Rutgers P, Oosterink E, Lasaroms JJ, Peters RJ, Van Rhijn JA *et al*. Comprehensive screening and quantification of veterinary drugs in milk using UPLC–ToF-MS. Analytical and Bioanalytical Chemistry. 2008; 391:2309-2322.
13. Sudershan RV, Bhat RV. A survey on veterinary drug use and residues in milk in Hyderabad. Food Additives & Contaminants. 1995; 12:645-650.
14. Tollefson L, Karp BE. Human health impact from antimicrobial use in food animals. Med. Maladies Infect. 2004; 34:514-521.
15. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP *et al*. Global trends in antimicrobial use in food animals. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112:5649-5654.
16. Vragovic N, Bazulic D, Njari B. Risk assessment of streptomycin and tetracycline residues in meat and milk on Croatian market. Food and chemical toxicology. 2011; 49:352-355.
17. Young I, Rajic A, Wilhelm BJ, Waddell L, Parker S, McEwen SA. Comparison of the prevalence of bacterial enter pathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: A systematic review and meta-analysis. Epidemiol. Infect. 2009; 137:1217-1232.