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Risk assessment due to dietary exposure of quinolones and Penicillins antimicrobials residues in milk

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Abstract
This study was conducted to determine exposure risk to quinolones and penicillins residues in milk consumed in the Hisar and aimed to estimate dietary exposure. The risk assessment due to dietary exposure was done on the basis of per capita availability of milk in Haryana and India by calculating hazard quotient for each analyte. On the basis of calculated dietary intake and acceptable daily intake available, the hazard quotient was calculated and it was found 0.032, 2.58 and 0.0004 for enrofloxacin, amoxicillin and cloxacillin respectively for population of India whereas for Haryana population, hazard quotient was 0.0145, 1.28 and 0.0002 for enrofloxacin, amoxicillin and cloxacillin respectively. The estimated dietary exposure based on the data on average consumption of milk suggested that people in India as well as Haryana are not at risk for dietary exposure of ciprofloxacin, enrofloxacin and cloxacillin residues. However, with respect to amoxicillin, the risk of dietary exposure is slightly more as compared to ADI value.

Keywords: Dietary exposure, risk assessment, milk, residues, hazard quotient

Introduction
Milk is a white, non-transparent liquid produced by the mammals. It is a healthy and nutritious daily drink that is consumed by people of all ages. Since the early 1960s, there is two-fold increase in per capita milk consumption of developing countries. This increased demand of milk made it essential to adopt extensive animal husbandry practices. Use of veterinary drugs for curing of variety of ailments in farm animals is an integral component of such extensive animal husbandry practices. In veterinary practice, antimicrobials are utilized at therapeutic levels primarily to treat diseases and to prevent infection and at subtherapeutic levels to increase feed efficiency, promote growth and prevent diseases (Ronquillo and Hernandez, 2016). Failure of following withdrawal period due to lack of awareness and extra lable use of drugs is responsible for the antimicrobial residues in foods of animal origin.

Presence of antimicrobial residues in foods can cause health hazards in consumers such as allergic reactions in sensitive persons (Thomson and Sporns, 1995) and increase in the number of antibiotic resistant pathogenic bacteria (Honkanen and Reybroeck, 1997) [4]. Another important fact regarding antibiotic residues in milk, which is a matter of worry, is that it disproportionately affects the younger segment of the population, as they are large consumers of this product. India is the first ranker in milk production which makes a good per capita availability (355 g/capita/day) of milk to Indian population and Haryana also stands for second highest per capita availability of milk (930 g/capita/day) (NDDB, 2018) [7]. So, more the availability of milk leads to high probability of milk consumption in larger amount and hence, more probability of dietary exposure to antimicrobial residues through milk consumption.

The international organizations involved in regulating the use of drugs in animal production activities have proposed MRLs for livestock products based on regular, monitoring, controlling and surveillance programmes. 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.

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 (Vragovic et al., 2009). Therefore, the present work was carried out to detect the residues of quinolones and penicillins in milk and estimate the dietary risk to the consumers.

Method

Collection of samples: The present work was carried out in the Department of Veterinary Public Health and Epidemiology, LUVAS, Hisar. For this, 100 milk samples were randomly collected from local market of Hisar, among which, 80 samples of raw milk and 20 samples of pasteurized milk of various brands were included. Samples were collected in sterile plastic bottles and stored at -20°C till analysis.

Chemicals and Reagents: The analytical standards of antimicrobials *viz*. norfloxacin, ciprofloxacin, enrofloxacin, amoxicillin and cloxacillin 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. HPLC grade solvents namely methanol and acetonitrile 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 quinolones *viz*. enrofloxacin, norfloxacin, ciprofloxacin and penicillins *viz*. amoxicillin and cloxacillin from milk as per method described by Stolker *et al.,* (2008) [10] with slight modification. Mobile phase used for the instrumental analysis of quinolones and penicillins 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 both quinolones and penicillins was performed at UV detector at 280nm wavelength. On the basis of linearity, accuracy and precision, quinolones and penicillins were standardized and validated. Residues were determined in all milk samples and on the basis of mean concentrations of enrofloxacin, norfloxacin, ciprofloxacin, amoxicillin and cloxacillin residues.

Risk assessment due to dietary exposure

Dietary intake of antibiotics under study by Indian population was calculated as per the guidelines provided by WHO. Estimates of dietary exposure to quinolone and penicillin antibiotics were calculated on the basis of the amount of analyte in the milk and the quantity of milk available on a daily basis. The data from site of National Dairy Development Board, India (NDDB, 2016) [6] and Department of Animal Husbandary and Dairying, Haryana (DAHD, 2016) was used for per capita availability of the milk of India and Haryana, respectively. The ADI is determined as a conservative estimate of the safety ingestion levels by the human population. The acceptable daily intake values for the antimicrobials under study are given in Table 2. Following formula was used for the estimation of dietary intake:

$$\text{Dietary intake (µg/kg BW/day)} = \frac{\text{Mean concentration of antimicrobial} \times \text{Daily per capita availability of milk}}{\text{Adult body weight (60kg)}}$$

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 and Discussion

In the current study, the analytical determination of three antimicrobials of quinolone group (enrofloxacin, norfloxacin and ciprofloxacin) and two antimicrobials of penicillin group (amoxicillin and cloxacillin) 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 quinolones and penicillins via milk consumption was evaluated for the population of Haryana and India. Table 1 and 2 show the dietary intake of antimicrobial residues expressed as µg per kilogram of body weight per day (µg/kg BW/day) for the population of Haryana and India, respectively in comparison with available acceptable daily intake (ADI) values prescribed by JECFA i.e. 0-6.2 µg/kg BW/day and 0-0.07 µg/kg BW/day for enrofloxacin and amoxicillin respectively (JECFA, 2002) whereas ADI value for cloxacillin was 200 µg/kg BW/day (OCS, 2013). The hazard quotient based on ADI values and dietary intake values was also calculated and presented in Table 1 and 2.

Table 1: Dietary intake of quinolone and penicillin antimicrobials through milk by consumers in Haryana

Analyte	Dietary Intake (µg/kg BW/ day)	ADI (µg/kg BW/day)	Hazard Quotient
Norfloxacin	1.00	NA	-
Ciprofloxacin	-	NA	-
Enrofloxacin	0.20	0-6.2	0.032
Amoxicillin	1.81	0-0.7	2.58
Cloxacillin	0.08	200	0.0004

ADI= Acceptable daily intake

Table 2: Dietary intake of quinolone and penicillin antimicrobial residues through milk by the consumers in India

Analyte	Dietary Intake (µg/kg BW/ day)	ADI (µg/kg BW/day)	Hazard Quotient
Norfloxacin	0.29	NA	-
Ciprofloxacin	-	NA	-
Enrofloxacin	0.09	0-6.2	0.0145
Amoxicillin	0.90	0-0.7	1.28
Cloxacillin	0.04	200	0.0002

ADI= Acceptable daily intake

On the basis of calculated dietary intake and acceptable daily intake available, the hazard quotient was calculated and it was found 0.032, 2.58 and 0.0004 for enrofloxacin, amoxicillin and cloxacillin respectively for population of India whereas for Haryana population, hazard quotient was 0.0145, 1.28 and 0.0002 for enrofloxacin, amoxicillin and cloxacillin

respectively. These suggest that people in India as well as Haryana are not at risk for dietary exposure of ciprofloxacin, enrofloxacin and cloxacillin residues. However, with respect to amoxicillin, the risk of dietary exposure is slightly more as compared to ADI value. Due to more per capita milk availability in Haryana as compared to India, possibility of milk consumption is also increased and hence, more risk to the population of Haryana as compared to Indian population.

Similar studies have been conducted on assessment of health risk due to dietary exposure of various antimicrobials through milk consumption. Elizabeta *et al.* (2011) [3] 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 results except for amoxicillin were also found in our studies, with lower toxicological risk associated with consumption of milk as compared to standards. 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 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) [1]. In a recent study conducted by Vragovic *et al.* (2012) [12], it was reported that the estimated dietary exposure based on the data on average consumption of milk and the estimated concentration of amoxicillin, ampicillin, benzyl penicillin, cloxacillin, cephapirin, cefazolin, cefoperazone and ceftiofur were not exceeding the relevant toxicological reference value (acceptable daily intake). Wang *et al.* (2017) [14] conducted a study in China for the detection of quinolones and β-lactam antibiotic in milk and found estimated daily exposure <1 µg/kg bw/day.

The issue concerning antibiotics inactivation during milk processing is 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. Even through pasteurized milk is also found laden with residues of various antibiotics. 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 antimicrobial residues concentrations instead of total elimination.

Conclusion

The results obtained in the present study suggest that people in India as well as Haryana are not at risk for dietary exposure of ciprofloxacin, enrofloxacin and cloxacillin residues. However, with respect to amoxicillin, the risk of dietary exposure is slightly more as compared to ADI value. These findings indicated that milk quality may have improved over the last few years, possibly owing to increased producer awareness of dairy cow management and waiting periods. Due to more per capita milk availability in Haryana as compared to India, possibility of milk consumption is also increased and hence, more risk to the population of Haryana as compared to Indian population.

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