Dietary exposure and risk assessment of antibiotics residues in marketed bovine raw milk

NM Patel, R Kumar, CV Savalia, DN Desai and IH Kalyani

Abstract

Pathogenic microbes’ contaminations are another aspect of modern food production that leads to antibiotic residues with regard to pose human health risk. In animals the use of drugs in tandem to their treatment and prophylaxis is more difficult to prevent. In this analysis of potential risks of dietary consumption of different antibiotic residues was tested in marketed bovine raw milk. Risk assessment add the knowledge of the relation between food relative risk and consumer risk mitigation from adverse human health effects, making this specific pattern of creating to check a good food safety. The risk was measured the hazards quotient (HQ) of Gujarat region and PAN India on the basis of per-capita availability of milk under study. The results analysis of the risk for dietary exposure to residue in Gujarat and PAN India, indicate a risk quotient of 0.022, 0.13, 0.21 and 0.012, 0.079, 0.012 for tetracycline, fluoroquinolones and gentamicin respectively. The study concludes that risk assessed is incredibly small because the potential safe calculation of HQ values, so there was no toxicological risk with reference to antibiotic residue diet exposure in the studied samples. However, occurrence of residues above MRL in daily dietary milk intake could result in significant adverse effects on a long term basis.

Keywords: Antibiotic residue, hazard quotient, human health, risk assessment, food safety

Introduction

Across the nation of India, antibiotics are used in large volume in human and veterinary medicine. It is of great significant concern when these drugs are present in milk and meat. In recent years attempts have been made to examine foods of animal origin and public health implications of antimicrobial residual substances [18].

Antimicrobial in the food chain that leads to development of additional virulence characters of antimicrobial resistance and resistant bacterium pose a serious risk on human health. Animals are often treated with antibiotics and safe use of antibiotics in animal production line in most countries has received significant attention [20]. The crowning conditions of the animals on farms make infectious diseases to appear and, as a result, excessive use of antimicrobial agents is unfavorably regarded in farming practices. Therefore, synthetic allopathic medicines in relation to sickness should be limited to the minimum possible number and the withdrawal time should be observed critically [22].

Residues in milk are a significant public health issue as milk is commonly consumed by children, young, adults and invalid people worldwide. Possible acute and chronic adverse effects of antibiotic residues, such as transfer of antibiotic resistant bacteria in human, tetracycline may leads to mutagenicity, nephropathy, gentamicin and quinolones causes hepatotoxicity, reproductive disorders, toxicity to bone marrow and allergy were reported [21]. Related health hazards include effects triggered by drug excretion in milk, like beta-lactam group regardless of its low concentration, causes allergic reactions in susceptible persons following use, whereas chronic toxic effects on prolonged exposure to low antibiotics involve teratogenicity, reproductive disorders and development of resistance in bacteria in the treated animals and disruption of the human intestinal flora [23].

Antibiotics have become important to the rising dairy industries for animal production as therapeutics, prophylactics and as growth promoters. The antibiotics are reported to be given in sub therapy to almost 90% of the farm population. Approximately 70% is intended to prevent diseases and 30% to stimulate development [14]. Indiscriminately use of antibiotic at different scale lead to residues in raw milk that pose a significant risk to human health as well as hinder the treatment of milk and milk products [15].

Exposure to constant low levels of antimicrobial residue in food poses possible risk for public health, contributes to over growth of pathogenic bacteria or compromise human antimicrobial
therapy by exercising a selective pressure on the body’s intestinal microflora, thus promoting the growth of natural and acquired resistance to microorganisms in larger scale. This paper focuses on the risk assessment based on c-ELISA method, which can be applied in research for food safety quality assurance and evaluating the public health risks associated with simultaneous detection and determination of tetracycline, fluoroquinolones and gentamicin residues in raw milk.

Materials and Methods

Sample collection

A total of 120 milk samples comprised of 20 samples each of cattle (pooled), buffalo (pooled) and mix milk (pooled) of cattle and buffaloes, and 20 individual samples after treatment of fluoroquinolones, gentamicin and tetracycline were collected for monitoring from various non-organized dairy farms for 3 months (Nov 2018 to Jan 2019) period of locations of south Gujarat region. For the study 50 mL raw milk sample was collected in a labeled sterilized bottle and stored at -20°C till analysis. These samples were analyzed for tetracycline, fluoroquinolones and gentamicin residues by using BIOVISON competitive ELISA kit (California, USA.). Before subjecting to the test the milk sample was brought to room temperature (25-30°C) for quantitative estimation of the agents, and thereafter 100 μl of milk sample was mixed in 900 μl of extraction solution in centrifugal tube. From this 50 μl of extracted sample was used.

Antibiotic residues assay

The BIOVISON (California, USA.) kit used is a competitive enzyme immunoassay for the quantitative analysis as per manufacturer instruction for tetracycline, fluoroquinolones and gentamicin in milk. The test kits contained different standard concentrations (0, 1, 3, 9, 27 and 81 ppb in methanol solution for fluoroquinolones), (0, 0.3, 0.9, 2.7, 8.1 and 24.3 ppb in aqueous solutions for gentamicin) and (0, 0.5, 1.5, 4.5, 13.5 and 40.5 ppb in aqueous solutions for tetracycline). The standard curve was plotted between log concentration and absorbance of the standard solutions. The values were calculated for the standards were entered in a system of coordinates on a semi logarithmic graph paper against the antibiotic concentration in μg/kg. To obtain the actual concentration of antibiotic residues in the each milk sample, the concentration read from the standard curve was further multiplied by the respective dilution factor. The obtained calculated data was further subjected to calculate each residues mean values using the descriptive statistical package of the social sciences software version - 20 statistics.

Risk analysis in humans for intake of milk with residues

The ingestion of antimicrobials residual compound in food pose a danger to health of human by colonization, barrier disruption leading to pathogenic bacteria over growth or by exerting a selective pressure on the intestinal micro flora, thus favoring the growth of microbes with intrinsic or acquired resistance. The ADI is determined as a conservative estimate of the safety ingestion levels by the human population, based on the lowest “no effect level” (NOEL) among a battery of toxicological safety studies. The toxicological endpoint resulting in the lowest ADI ultimately drives the overall ADI.

Calculation of the estimated daily intake

The estimated daily intake (EDI) was calculated by the following equation of Juan et al., 2010:

\[ \text{EDI} = \frac{(\text{Mean of mg antibiotic per kg of food}) \times (\text{Daily Intake of food})}{\text{Adult body weight (60 kg)}} \]

Hazard Quotient (HQ) = Dietary intake of residues through milk/Acceptable daily intake

Hazard quotient is the ratio of the potential exposure concentration to a substance and the level at which no adverse effects are expected. Residues were determined in all milk samples and on the basis of mean concentration of tetracycline, fluoroquinolones and gentamicin residues, the risk was assessed on the basis concentration of acceptable daily intake recommended by JECFA. The HQ of tetracycline, fluoroquinolones and gentamicin was calculated by using acceptable daily intake. On the basis of dietary intake, risk was estimated in the form of HQ. The observed HQ ratio below 1 or equivalent of the potential level exposure to a substance for human health is not considered, at which no any adverse effects occurs, whereas the significant vulnerable risk on human health from exposure when HQ is more than 1 as considered risk to the consumers.

Results

The screening test and prevalence revealed that 98.75, 25 and 12.5% of the samples were positive with a mean residues value of 667.09, 80.44 and 47.92 μg/kg for tetracycline, fluoroquinolones and gentamicin residues at levels above the defined MRL, respectively. Tetracycline in particular had the highest share in this contamination and its mean concentrations in the positive and total milk samples were 8 and 13 times greater than the mentioned permitted limit, respectively. Their mean values were also measured at concentrations below the legislated level. Risk assessment is done by comparing the estimated daily intake of antimicrobial residues with their acceptable daily intake (ADI) values recommended by regulatory agencies. The present study analyzed residues of tetracycline, fluoroquinolones and gentamicin in marketed milk samples and evaluated the risk of dietary exposure to residual quantity of them through milk consumption based on current and representative evidence on average milk consumption of adults for the population of south Gujarat region 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 b.wt/day) in comparison with ADI values recommended by JECFA i.e. 0-30, 0.2 and 20 μg/kg b.wt/day for tetracycline, fluoroquinolones and gentamicin, respectively. The highest level of acceptable daily intake (ADI) of three antibiotics established by JECFA was taken into account for risk characterization.

On the basis of calculated dietary intake and acceptable daily intake, the HQ was found to be 0.012, 0.079 and 0.012 for tetracycline, fluoroquinolones and gentamicin, respectively for PAN India, while in Gujarat population, the HQ values were 0.022, 0.13 and 0.21, respectively. This suggests that the population is safe as far as dietary exposure of tetracycline, fluoroquinolones and gentamicin residues is concerned. In table 1 and 2, the result of HQ based risk characterization of antibiotics residue via milk consumption is presented. The calculation of antimicrobial residual exposure to the diet by milking was based on antimicrobial milk concentration multiplied by average daily intake of milk (taken as daily milk availability per capita). Per capita milk availability values in Gujarat and India are reported as 592 g and 375 g, respectively.
Table 1: Risk characterization of dietary exposures of antimicrobial residues through milk consumption in Gujarat

<table>
<thead>
<tr>
<th>Group of antimicrobials</th>
<th>EDI (µg/kg b.wt./day)</th>
<th>ADI (µg/kg b.wt./day)</th>
<th>Hazard Quotient</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>0.66</td>
<td>30</td>
<td>0.022</td>
<td>CAC, 2015 [4]</td>
</tr>
<tr>
<td>Fluroquinolones</td>
<td>0.80</td>
<td>6.2</td>
<td>0.13</td>
<td>JECFA, 2013 [11]</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4.2</td>
<td>20</td>
<td>0.21</td>
<td>JECFA, 2013 [11]</td>
</tr>
</tbody>
</table>

EDI: Estimated daily intake, ADI: Acceptable daily intake

Table 2: Risk characterization of dietary exposures of antimicrobial residues through milk consumption in PAN India

<table>
<thead>
<tr>
<th>Group of antimicrobials</th>
<th>EDI (µg/kg b.wt./day)</th>
<th>ADI (µg/kg b.wt./day)</th>
<th>Hazard Quotient</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>0.36</td>
<td>30</td>
<td>0.012</td>
<td>CAC, 2015 [4]</td>
</tr>
<tr>
<td>Fluroquinolones</td>
<td>0.49</td>
<td>6.2</td>
<td>0.079</td>
<td>JECFA, 2013 [11]</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.24</td>
<td>20</td>
<td>0.012</td>
<td>JECFA, 2013 [11]</td>
</tr>
</tbody>
</table>

EDI: Estimated daily intake, ADI: Acceptable daily intake

**Discussion**

The harmful effects arising from residues of indiscriminate use of veterinary antibiotics and antimicrobial resistance is now known to be a serious threat to human health. The presence of antibiotic residues in foods of animal origin has been shown to see, if their long-term consumption may lead to creation of human antibiotic resistance. The present study showed that antibiotic residues in the raw milk marketing in South Gujarat would likely exceed Codex Alimentarius Commission (CAC) safety standards limits. The average mean concentration of tetracycline, fluroquinolones and gentamicin residues in the milk samples for studied were found to be 667.09, 80.44 and 47.92µg/kg, respectively. All samples analyzed in this analysis were nevertheless in compliance with the CAC guidelines, since the total of tetracycline, fluroquinolones and gentamicin concentration were more than the MRL. This variation may be partly due to different patterns of dairy consumption in the US and Europe than in PAN India, which may suggest the need to set national levels standards based on dietary food pattern trends across the country.

Numerous worldwide comprehensive researches on the presence of tetracycline, fluroquinolones, and gentamicin residues in milk have recently been carried out. Contamination rates reported were however lower than those in the present study. The average daily intake of an adult in Macedonia, for antimicrobial products under investigation and obtains levels 2 to 100 percent lower than the World Health Organization’s appropriate daily intake rate, has been assessed by Elizabeta et al. [7] as an estimate. This suggested that the public health issue of the veterinary medicine could not be significant considered to be a toxicological risk of drinking examined milk. Similar findings were also reported in a Croatian study which showed that none of the samples analyzed contained veterinary drug residues above the maximum residue levels (MRLs) set by European Union and Croatian legislation. For an adult in croatia, the average estimated daily intake (EDI) of 300 ml was 20 to 1640 times less than the values defined by the European Medicines Agency and World Health Organization, for the average of dairy intake in the case of examined antibiotics. This shows that the toxicological risk of using examined milk cannot be seen as a public health problem with respect to these veterinary medicine [2]. The permissible daily intake for tetracycline (for a person 60 kg) was 1800 µg/person/day in the analysis by Vragovic et al. [23], and that is why the risk calculated is minimal i.e. belongs to less than 1% of the permissible daily consumption. The residual level observed for tetracycline in our sample was also 0.66 µg/ person/day and human health risk is negligible.

Tetracycline residues were investigated by Gaurav et al. [8] and Hazard Quotients were found in their pooled milk samples at 0.027 and 0.03 where in pasteurized milk samples 0.006 and 0.01, respectively. Moudgil et al. [16] also studied and identified hazard quotients for adult as 0.000049, 0.00011 and 0.000047 where 0.00026, 0.00034 and 0.00028 were observed in children in small, medium and large dairy farms, respectively. The concentration of most antibiotic residues determined does not exceed that defined by the World Health Organization in appropriate daily intakes via food. The concentrations of most antibiotic residues reported by Wang et al. [24] conducted an analysis of quinolones and β-lactam antibiotics in milk in China and estimated daily exposure <1 µg/kg b.wt/day were not higher than the appropriate daily intakes via food. Chauhan et al. [3] has carried out a risk assessment analysis to determine the risk of gentamicin findings indicating that the antimicrobial food consumption included in this research is not at risk to the patient. Moudgil et al. [16] studied tetracycline residue and it was found that there was no significant adverse impact on the health of consumers associated with consumption of the samples testing at the HQ of antibiotic residues at dairy and marketed samples below one.

Gradinaru et al. [9] has reported that estimated dietary exposure for antibiotic residues via milk was below the toxicological reference value. Prado et al. [19] considered low EDI to be at low risk or negligible, although, in pasteurized milk samples of different brands, De Albuquerque et al. [6] indicated high risk. The analysis was conducted in Aalipour et al. [1], which explores the risk of intake of tetracycline antibiotic residues via milk in Iran by various human age groups. Daily intake in antimicrobial drug residues was reported in a range of 58-62µg and 0.99.3µg per human, considering long-term and short-term milk consumption. Kabrite et al. [13], also found that the expressions of the dietary intakes of antibiotic residues of tetracycline as a percentage of ADI led to an ADI of 0.004% and the EDI of 1.28ng /kg b.wt/day for milk below its toxicological reference value is measured.

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 show the dietary intake of antimicrobial residues expressed as µg per kilogram of body weight per day (µg/kg b.wt/day) in comparison with ADI values recommended by JECFA i.e. 0-30, 0.2 and 20 µg/kg b.wt/day for tetracycline, fluroquinolones and gentamicin, respectively. Critical assessment of dietary exposure is necessary if fundamental food safety data, problems and trends in antimicrobial intakes are to be obtained and unusual residue...
sources established. The calculation of antimicrobial residual exposure to the diet from milk was based on antimicrobial milk concentration multiplied by average daily intake of milk (taken as daily milk availability per capita). Risk assessment is done by comparing the estimated daily intake of antimicrobial residues with their ADI values recommended by regulatory agencies. The ADI represents the amount of drug residues that can safely be consumed per day over a man’s lifetime without adverse effect. India ranks first in milk production and good per capita availability (375g/capita/day) of milk to Indian population and among all the states, Gujarat has 592 g/capita/day availability of milk. Thus, more milk available may lead to higher consumption and higher probability of dietary exposure to antimicrobial residues in long runs. As the HQ for antibiotic residues detected in milk samples was less than 1, assuming that there were no significant adverse effects on the health of consumers associated with the consumption of the analyzed milk samples.

Problems about inactivation of antibiotics during milk processing are more extreme than those with other food of animal origin because milk has to be heat-treated within a very short period of time. Residue thermal stability depends on the form of antibiotics. It has been shown that the concentration of tetracycline residues during the heat treatment of milk is only decreased partially and there will be no total removal. Since milk and milk products are essential foodstuffs for children and other age group of population. The presence of drug residues in milk must be taken into consideration. The detection of antimicrobial substances in milk samples should not only focus on the common antimicrobial substances used most frequently in the dairy farm animals therapeutically, but also as other group of serious food safety contamination in milk.

Conclusion
The present study analyzed the occurrence of antibiotic residues and assessment of human health risk due to the consumption of milk containing residues based on per capita availability of milk in humans revealed that the current levels of antibiotic residues in milk of tetracycline, fluoroquinolones and gentamicin pose no significant adverse effects on the health of consumers in PAN India and Southern Gujarat in present scenarios. This may be due to continuous improvement in last several years in milk hygiene practices in relation to use of antibiotics in dairy animals. Possibility it may have continued to be likely due to the increased knowledge of dairy farm animal care and waiting withdrawals periods mostly from dairy farmers and per capita availability of milk in South Gujarat according to “Presence Across Nation” India is may another reason of less risk. However, the occurrence of residues in milk above established MRL is certainly a matter of concern and daily dietary intake exposure of consumers to these residues through milk intake could result in adverse significant effects on their health as on a long term basis. The occurrence of antibiotic residues in milk collected from various non-organized dairy farms for 3 months (Nov 2018 to Jan 2019) period at the farm level indicates a need for implementing effective enforcement of appropriate regulatory legislations and regular residues monitoring to prevent the no prudent use of antibiotics in therapeutics veterinary practice and thus preventing unacceptable potential health risks to consumers consequently of South Gujarat and across Indian population upon milk consumption.

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References