



# Public Health Risk Assessment of Fluoroquinolones, Gentamicin and Tetracycline Residues in Bovine Milk of Peri-urban Area of Surat and Navsari of South Gujarat, India

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## ABSTRACT

**Background:** The occurrence of antibiotic residues in milk constitutes a potential risk to the health of consumers. In the veterinary sector, various forms of antibiotics are now being used worldwide for promoting livestock growth and treatment. Fluoroquinolones, Gentamicin and Tetracycline are commonly used in disease prevention and disease control. The current issues of food safety and used antimicrobial agent's residues in food products of animal origin are a serious public health issue and health risk globally.

**Methods:** In this field-laboratory investigation during Nov 2018 to Jan 2019, different localities peri-urban area of Surat and Navsari of Gujarat were surveyed. A total of 120 milk samples comprised of (n=20) cattle (pooled), (n=20) buffalo (pooled) and (n=20) mix milk (pooled) of cattle and buffaloes and samples from unhealthy individual milking animal provided treatment with each antibiotics was collected after completion of recommended withdrawal period (n=20) Fluoroquinolones, (n=20) Gentamicin and (n=20) Tetracycline from various non-organized dairy farms. In the laboratory, the collected samples were evaluated for residues in bovine raw milk by c-ELISA.

**Result:** The investigations in the Surat and Navsari evaluated 3 antibiotics residues in bovine raw milk. Among the 3 antibiotics are deemed dangerous level risks for human health. The detectable level was higher than of recommended antibiotic residue levels (MRLs) were observed as per European Union and the Codex Alimentarius Commission (CAC). The present work will be a complementary contribution to the comprehensive study of the stringent control measurements to guarantee that the milk is safe for public to drink.

**Key words:** Antibiotic residues, Assessment, ELISA, MRLs, Public health, Raw milk, Risk.

## INTRODUCTION

The fact that the livestock sector plays a major role in India in rising socio-economic status as well as guaranteeing employment, food protection for residents. The Food and Agriculture Organization (FAO, 2011) has projected that by 2050 consumption of meat and dairy products will rise by upwards of 70 per cent (Robinson *et al.*, 2011). An attempt to reach the need of food products increasing export potential, the livestock industry is increasingly deepening also with widening administration of antimicrobial drugs in food-producing animals to treat and prevent diseases.

Annually 64 tons usage of antibiotics reported in animals (Van Boeckel *et al.*, 2015). The global use of animal antimicrobials has been doubled as against other species (Aarestrup, 2012). Several studies have shown that large sections (30%-70%) of antibiotics are released into the environment without alteration (*i.e.*, with possible antimicrobial activities). Milk is a widely consumed foodstuff in the world (Ventola, 2015). Antibiotic residues are mostly present in milk because of their usage in the treatment of infectious diseases in animals (Zhang *et al.*, 2014).

The risk of residues in milk is higher in developing countries due to poor detection facilities and lack of adequate residue control systems. Regulatory agencies have generated and enforced MRLs to ensure the limited presence of antibiotic residues in foods of animal origin and restricting the usage of prohibited veterinary drugs

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(European Commission, 2010; Codex Alimentarius Commission, 2017). The presence of antibiotic residues in foods of animal origin is an issue of intense public health concern. Therefore, the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA), at its 40<sup>th</sup> Session of the CAC, updated the MRLs for veterinary drug residues in foods (Codex Alimentarius Commission, 2017).

Fluoroquinolones, Gentamicin and Tetracyclines, are synthetic antibacterial agents widely used in veterinary

practice for the treatment and prevention of infections in food-producing animals (Jank *et al.*, 2017). They are widely used for therapeutic purpose in veterinary practice due to their low cost, easy over the counter availability and broad spectrum of activity (Martins *et al.*, 2016). These antibiotics can lead to deposition of residues in foodstuffs intended to be used in human consumption, such as meat, milk, and eggs. The consumption of milk containing antibiotic residues may lead to allergic reactions, disorders of intestinal flora, immune pathological effects, and emergence of resistant strains of bacteria (Moudgil *et al.*, 2019; Kumar *et al.*, 2020).

The World Health Organization has also viewed antibiotic resistance as one of the most important health issues of the 21<sup>st</sup> century (CDC, 2010). Antibiotic irrationality in animal farming contributes to the production of resistant pathogens in livestock and to the incidence of antibiotic residues in foodstuffs of animal origin (Rossi *et al.*, 2018). For fast screening of antibiotics in milk and other food commodities; microbiological tests, immunoassays and biosensors are widely used (Ahmed *et al.*, 2017; Ning *et al.*, 2017).

Thus, in the view of Public Health risk assessment was aimed to detect and quantify Tetracycline, Fluoroquinolones, and Gentamicin antibiotics residues from bovine raw milk samples using competitive ELISA (c-ELISA).

## MATERIALS AND METHODS

### Sample collection

In total, 120 milk samples were collected from various non-organized dairy farms for 3 months (Nov 2018 to Jan 2019) period from peri-urban area of Surat and Navsari of Gujarat region, consisting of (n=20) cattle (pooled), (n=20) buffalo (pooled) and (n=20) mix milk (pooled) of cattle and buffaloes and samples from unhealthy individual milking animal provided treatment with each antibiotics was collected after completion of recommended withdrawal period (n=20) Fluoroquinolones, (n=20) Gentamicin and (n=20) Tetracycline. The approximately 50 ml raw milk of samples was collected in a sterilized labeled bottle and transported to the laboratory at 4°C and were preserved at -20°C for further analysis. These samples were tested and analyzed for Fluoroquinolones, Gentamicin and Tetracycline, antibiotics residues by using BIOVISION competitive ELISA kits (Biovision, California, USA).

### Methodology

The BIOVISION competitive enzyme immunoassay kit

(Biovision, California, USA) including LOD with 0.5 ppb (µg/kg or L) was used according to the manufacturer's instructions for antibiotic determination.

The Fluoroquinolones, Gentamicin and Tetracycline antibiotics residues was performed as per the protocol outlined in the user manual supplied in the kit. The standard curve was plotted for different analytics between log concentration and absorbance of the standard solutions. The values calculated for the standards were entered in a system of coordinates on a semi logarithmic graph paper against the antibiotic concentration in µg/kg or L. To obtain the actual concentration of antibiotic in the milk sample, the concentration obtained from the standard curve was further multiplied by the dilution factor.

The results were calculated by obtaining the O.D. values at 450 nm and calculating the % absorbance.

$$\text{Percentage of absorbance value (\%)} = A / A_0 \times 100$$

Where,

A is the average OD value of sample or the standard solution and  $A_0$  is the average OD value of the 0-ppb standard solution.

## RESULTS AND DISCUSSION

Residues of antimicrobial agents have a potential hazard for the consumer and may cause allergic reactions, interference in the intestinal flora and to transfer of multiple antibiotic resistance to human and animal bacterial pathogens, thereby rendering antibiotic treatment ineffective. The control of antibiotic residues is necessary to ensure food safety and prevent exposure of the consumers to drug residues.

### Public health risk assessment of fluoroquinolones residues

Detailed sample wise data provided in Table 1 and the highest antibiotic residue in bovine raw milk above the MRL values was noted in 20(25 %) samples (n=80) than recommended limits for individual samples collected after treatment with specific antibiotics and cattle pooled, bulk (cattle and buffalo milk) and buffalo milk was noted as residue below maximum residue levels. The MRL recommended by EU and CAC for Fluoroquinolones Antibiotic residues is 100 µg/kg or L.

The results of Fluoroquinolones residues analyzed in bovine raw milk in mean, median and interquartile range is

**Table 1:** Public health risk assessment of fluoroquinolones residues.

Sample wise	No. of samples analyzed	No. of samples detected positive	Minimum/Maximum of positive sample (µg/kg or l)	Median (µg/kg or l)	Interquartile range	Mean±SD (µg/kg or l)	No. of samples found above MRL (100 µg/kg or l)
Cattle pooled	20	20	4.30/8.92	4.08	0.76	4.30±1.85	NIL (0%)
Buffalo pooled	20	20	4.22/5.08	4.26	0.62	4.24±0.44	NIL (0%)
Mix pooled (C+B)	20	20	3.94/4.65	4.00	0.62	3.94±0.45	NIL (0%)
Individual	20	20	309.14/329.14	320.40	20.16	309.32±23.17	20 (100%)
Total	80	80	4.22-329.14	-	-	-	20 (25%)

reported in Table 1. The detection rate of Fluoroquinolones in milk (n=80) samples was 25 % with a calculated mean residues value of 80.44 µg/kg or L. The maximum and minimum content of residues in milk was 4.22 and 329.14 µg/kg or L, respectively. Zhang and Wang (2009) tested 19 milk samples in China and found 10 samples positive for quinolone residues. Other researchers have reported that quinolone residues in milk were extremely stable (Fisher *et al.* 2010; Roca *et al.* 2010). That might be the reason for high detection rates of quinolone residues in milk.

In the present study, the highest Fluoroquinolones level (667.09 µg/kg or L) was detected, which exceeded the prescribed MRL value. While in another study (Elizabetha *et al.* 2011), the MRL value was 149.1 µg/kg or L. These findings where mean residue values were higher than the values obtained in present study might be due to pooling effects of sample of the milk obtained. The higher rates for quinolones were reported in Mexico and China, were a maximum detected value for samples from Mexico was 5047.3 µg/kg or L, which was more than 8 times lower than the present study and 32 times higher than china (20.49 µg/kg or L).

In another study conducted by Junza *et al.* (2010) in Spain for detection of quinolones and β-lactams in milk using Liquid chromatography (LC), 3% samples were found to be positive for quinolones out of 49 samples. A very high prevalence of 87.3% of Fluoroquinolones was reported by Navratilova *et al.* (2011) in bulk samples of raw cow's milk from Czech Republic. Similarly, Zhang *et al.* (2014) analyzed 120 samples in China and found 95.7% samples with detectable levels of quinolone residues. Another study conducted by Bilandzic *et al.* (2011) in Croatia, total of 1259 raw milk samples were examined over a three-year period for various antibiotics. The contamination levels were lower than the maximum levels proposed by the European Legislation.

#### Public health risk assessment of Gentamicin residues

Detailed sample wise data of (n=80) bovine raw milk samples is summarized in Table 2 and the highest antibiotic residue in bovine raw milk above the MRL values was noted in 10 (12.5%) samples after treatment with specific antibiotics (n=20) and cattle pooled, mixing bulk cattle and buffalo and buffalo milk (n=0) was noted as residue below maximum residue levels. The MRL recommended by EU and CAC for

Gentamicin antibiotic residues is 200 µg/kg or L. 70 milk samples did not exceed the MRLs of 200 µg/kg or L.

The analyzed samples resulted positive for Gentamicin in bovine raw milk with mean, median and interquartile range is reported in Table 2. The detection rate of Gentamicin in milk (n=10) samples was 12.5% with a calculated mean residues value of 47.92 µg/kg or L, respectively.

Gentamicin is potentially ototoxic and nephrotoxic and is known to cause immune deficiencies leading to drug resistant bacteria in animals and humans. Therefore, its residues in animal originated foods are of public concern (Tan *et al.* 2009). Several studies were conducted to detect Gentamicin were Fathalrhman *et al.* (2016) reported Gentamicin residues in 25% raw milk samples. In another study Bilandzic *et al.* (2011) analyzed 119 samples collected from five counties for the detection of Gentamicin residues in raw milk and estimated mean value of Gentamicin residues to be 1.10 µg/kg or L, whereas in the present study, calculated mean value of residue was 47.92 µg/kg or L indicating higher contamination of Gentamicin.

In another study conducted by Zeina *et al.* (2013) mean residual level of Gentamicin was 90 and 80 µg/kg or L, which was below the allowable maximum residue limit of 200 µg/kg or L as set by the FAO/WHO. Whereas in the present study, estimated mean value of Gentamicin residue below then the above study.

Gradinaru *et al.* (2011) studied antibiotic residues in milk samples collected from farms in the NE Romania (Moldavia). Out of 2785 total milk samples Gentamicin identified in 25% of samples, with average concentration of 198.68 µg/kg or L. In recent study, SL Chauhan *et al.* (2019) analyzed 100 samples in Hisar for the detection of aminoglycosides residues in milk and found 10% samples contained average concentration of 65.06 µg/kg or L of Gentamicin which slightly higher than the above study.

A study reported by Martins *et al.* (2016) in which total 180 milk samples of mammary quarter evaluated before and after mastitis treatment with Gentamicin and found 1440 milk samples (24.95%) contaminated to Gentamicin residues after the withdrawal period which higher than the values obtained in present study.

#### Public health risk assessment of Tetracycline residues

The (n=80) bovine raw milk samples from different dairy farms were collected and were quantified by c-ELISA.

**Table 2:** Public health risk assessment of gentamicin in residues.

Sample wise	No. of samples analyzed	No. of samples detected positive	Minimum/Maximum of positive sample (µg/kg or l)	Median (µg/kg or l)	Interquartile range	Mean±SD (µg/kg or l)	No. of samples found above MRL (100 µg/kg or l)
Cattle pooled	20	20	4.98/11.91	7.24	1.65	7.59±1.85	NIL (0%)
Buffalo pooled	20	20	4.62/8.06	6.54	1.15	6.62±0.88	NIL (0%)
Mix pooled (C+B)	20	20	5.80/7.38	6.76	0.48	6.74±0.40	NIL (0%)
Individual	20	20	21.23/391.85	214.75	233.72	169.34±129.84	10 (50%)
Total	80	80	4.62-391.85	-	-	-	10 (12.5%)

**Table 3:** Public Health risk assessment of Tetracyclines residues.

Sample wise	No. of samples analyzed	No. of samples detected positive	Minimum/Maximum of positive sample (µg/kg or l)	Median (µg/kg or l)	Interquartile Range	Mean±SD (µg/kg or l)	No. of samples found above MRL (100 µg/kg or l)
Cattle pooled	20	20	149.03-900.55	723.93	238.37	655.24±183.06	20 (100%)
Buffalo pooled	20	20	80.00-959.36	577.63	369.36	630.11±256.72	19 (95%)
Mix pooled (C+B)	20	20	535.85-861.55	697.59	123.8	705.22±98.85	20 (100%)
Individual	20	20	116.50-968.19	735.86	253.72	666.58±239.72	20 (100%)
Total	80	80	80.00-968.19	-	-	-	79 (98.75%)

Detailed sample wise data presented in Table 3 indicated that overall exceeded Tetracycline antibiotic residues were observed in 79 (98.75%) samples than prescribed MRLs of 100 µg/kg or L, according to the regulations set by EU and CAC. The highest prescribed maximum residue levels were noted in cattle pooled (n=20), mix bulk of cattle and buffalo (n=20) and individual animal samples after treatment with tetracycline (n=20) following decreasing in buffalo pooled milk (n=19). Only one milk sample did not exceed the MRLs of 100 µg/kg or L.

The analyzed results of samples positive for Tetracycline with, mean, median and interquartile range is reported in Table 3. The detection rate of Tetracycline in milk (n=79) samples was 98.75% with a calculated mean residues value of 667.09 µg/kg or L.

Several studies were conducted to detect Tetracycline antibiotics in milk samples round the world. Moghaddam *et al.* (2014), Noori *et al.* (2013) and Aalipour *et al.* (2015), reported contamination rate of milk samples with Tetracyclines 33.39%, 17.5% and 34.75%, respectively. Orwa *et al.* (2017) and Gaurav *et al.* (2014) found highest contamination rate of milk samples with Tetracyclines residues to be 31.4% and 13.5%, respectively. Another study was conducted in Czech Republic indicated Tetracyclines in 50.6% of the samples with concentrations under the MRL value (Navratilova *et al.* 2011). In the present study, Tetracycline level detected has exceeded the MRL value and it was 667.09 µg/kg or L. However Elizabeta *et al.* 2011, reported the MRL value as 149.1 µg/kg or L. Which was lower than the values obtained in present study.

Monitoring of locally produced and imported milk and dairy products collected in different seasonal periods from farms and retail outlets in Kuwait indicated that 29.1% of the fresh milk samples were above the MRL (Alomirah *et al.* 2007). The study conducted in Croatia (Bilandzic *et al.* 2011), the highest tetracycline level detected was 49.5 µg/kg or L. However, the mean Tetracycline concentration (2.83 µg/kg or L) was more than 35 times lower than the MRL level.

A study conducted by Zhang *et al.* 2014, with (n=94) samples of ultra-high temperature (UHT) milk and (n=26) samples of pasteurized in China, using the ELISA method were assessed for contamination with Tetracyclines. It was found that the percentage of UHT milk samples and pasteurized milk samples containing detectable levels of Tetracyclines were 0 and 7.7% respectively.

Due to the presence of reducing factors such as beta-lactams and milk microflora, antibiotics were decomposed during milk storage and in a high microbial population. Decomposition in processed milk is higher at room temperature and ready for spoilage samples (Ahlberg *et al.* 2016). Therefore, due to high storage time and possibly high microbial populations, particularly in local raw milk samples, one or more antibiotic groups may have been decomposed and might not have detected in the milk samples in this study.

## CONCLUSION

Fluoroquinolones, Gentamicin and Tetracyclines were detected in the raw bovine milk and their levels were above MRL remarkable and challenging health risks for consumer and public health issue globally. The present work showed a need for more rigorous work to monitor the use of antibiotics in livestock enterprises. Dairy farm practices needs efficient knowledge for preventive steps of infectious diseases is a key recommendation in the reduction of antibiotic residues in dairy products and comprehensive public health risk surveillance system for distribution antimicrobial prescription therapeutics antibiotics. Thoughtful consideration of withdrawal time in suspect animals before milk collection and supply; risk surveillance, monitoring and control of antibiotic residues in raw milk; Train and educate veterinarians in order to correctly use antibiotics and make food safety concerns a problem faced by producers and food authorities when antibiotics are found by milk.

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