



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2018; 7(8): 452-454

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www.thepharmajournal.com

Received: 10-06-2018

Accepted: 12-07-2018

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## Detection of antibiotic residues in raw cow milk in Thrissur, India

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### Abstract

Antibiotic residues have been recognized as an important threat to food safety ever since they created problems in dairy industry in the form of starter failure. The antibiotic residues in milk are of immense public health significance as they cause adverse health effects in humans. In the present study, the raw milk samples collected from farmer households in Thrissur district were analysed for the presence of antibiotic residues. The samples were subjected to screening by microbial inhibition assay (MIA). The positive samples in MIA were analysed by charm ROSA assay to detect the presence of tetracycline,  $\beta$ -lactams and enrofloxacin residues at violative levels. The results showed the occurrence of antibiotic residues in 8.48 per cent of samples. Tetracycline,  $\beta$ -lactams and enrofloxacin residues were detected at violative levels in three, four and three samples respectively. The presence of antibiotic residues clearly indicates misuse of antibiotics and insufficient withdrawal period followed.

**Keywords:** Antibiotic residues, milk, Thrissur, tetracycline,  $\beta$ -lactams, enrofloxacin

### 1. Introduction

Milk is an important and essential food for humans because of its richness in nutrients. It is highly valuable and consumed largely by people of all ages throughout the world. Milk is a perishable commodity susceptible for contamination with various chemical contaminants including Anthelmintic drugs, chlorinated pesticides, organophosphates, herbicides, fungicides, antibiotics, hormones and disinfectants. Antibiotic contamination of milk poses significant threat to safety of milk and is regarded as global public health hazard.

Antibiotic residues are the parent drug compounds and their metabolites excreted through edible tissues of animals to which antibiotics in question are administered. Antibiotic residues in milk are of major public health significance since it causes carcinogenicity, teratogenicity, drug allergy, bone marrow toxicity, mutagenicity and gastrointestinal disorders. It is also responsible for emergence of drug resistant microorganisms and starter failure in dairy industry. Indiscriminate and extra label use of antibiotics, insufficient withdrawal period and lack of records are responsible for occurrence of antibiotic residues in milk. Milk and milk products contaminated with antibiotic residue above maximum residue limit (MRL) are considered unfit for human consumption. Hence, it is necessary to routinely screen milk samples for antibiotic residues. So, the present study was undertaken to screen raw cow samples for antibiotic residues in Thrissur district

### Materials and methods

The present study was undertaken to investigate the presence of antibiotic residues in raw milk. The milk samples were screened for the antibiotic residues by Microbial Inhibition Assay (MIA). The positive samples in MIA were analysed with Charm Beta-lactam Tetracycline Combo Test (MRLBLTET) strips and Charm Enrofloxacin Test (ENRO) strips to determine the presence of tetracycline, enrofloxacin and lactams residues at violative levels.

### Collection and transportation of samples

A total of 165 raw cow milk samples were collected from individual households in Thrissur district under aseptic conditions. About 150 ml each of milk samples were collected in clean and sterile polypropylene tubes. The samples were transported to laboratory under refrigerated condition and stored in deep freezer at  $-20^{\circ}\text{C}$  until analysis.

**Microbial Inhibition assay (MIA)**

All samples were screened for the presence of antibiotic residues by microbial inhibition assay utilizing *Bacillus subtilis* as test organism (Dinki and Balcha, 2013) [4]. The plates containing 15 to 20 ml of Antimicrobial inhibitor agar at pH 7.2 were inoculated with *B. subtilis* by swabbing the entire agar surface using sterile cotton swabs dipped in suspension of the organism. Sterile discs were dipped in milk samples and excess milk was removed by pressing gently against sides of tubes. Six discs were placed in each plate at equal distance from each other. The plates were incubated at 37 °C for 18 to 24 h. The samples producing clear zone of inhibition around the discs were considered positive for antibiotic residues.

**Charm assay**

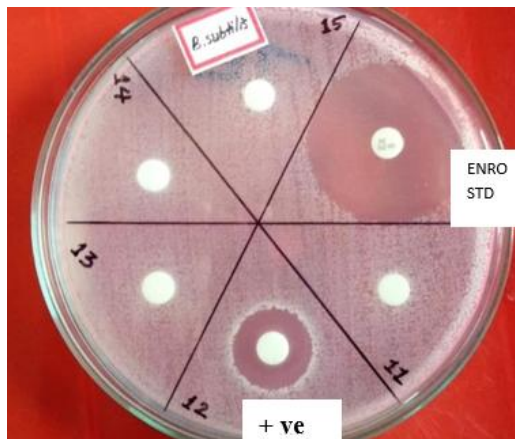
The milk samples showing positive results in MIA were subjected to Charm assay for determining the presence of tetracycline, β-lactams and enrofloxacin residues at violative levels. Each sample was analysed with Charm Beta-lactam tetracycline Combo Test (MRLBLTET) strips and Charm Enrofloxacin Test (ENRO) strips. About 300 µl of milk sample was added to milk chamber in the test strips. The strips were incubated at 56 °C for eight minutes. The strips with control line darker than the test line were considered positive for particular antibiotic residue.

**Results and Discussion**

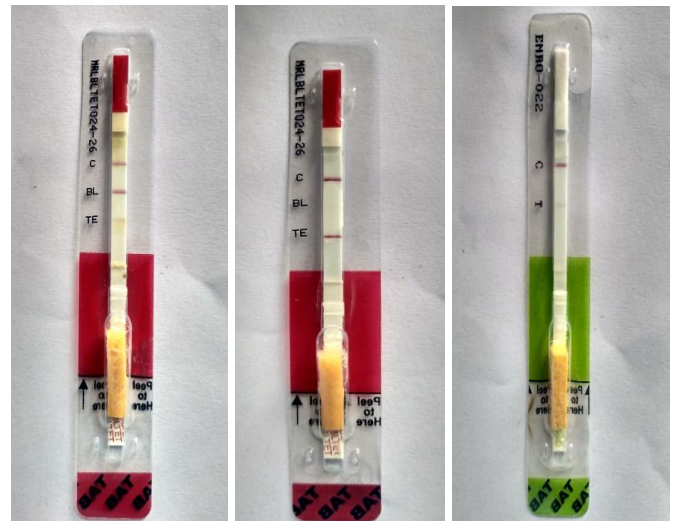
Out of 165 milk samples screened by MIA, it was found that 14 samples produced clear zones of inhibition (Fig. 1) and were considered positive for antibiotic residues. On analysing 14 positive samples with Charm MRLBLTET test strips, three and four samples were found to be contaminated with tetracycline and β-lactams residues at violative levels respectively (Fig. 2). Fourteen positive samples were also subjected to Charm ENRO test strips and results showed the presence of enrofloxacin residues at violative levels (Fig. 2) in three samples (Table 1).

**Table 1:** Occurrence of antibiotic residues in milk from individual households

S. No.	Antibiotics	Positive samples	
		Number	Per cent
1	Tetracyclines	3	1.82
2	β-lactams	4	2.42
3	Enrofloxacin	3	1.82
4	Others/ unknown antibiotics	4	2.42
5	Total antibiotics	14	8.48



**Fig 1:** Microbial inhibition assay for antibiotic residue detection



(a) Tetracyclines (b) β-lactams (c) Enrofloxacin

**Fig 2:** Charm assay positives for tetracycline, β-lactams and enrofloxacin residues

Milk and milk products contaminated with antibiotic residues can pose potential health effects in humans. The public health effects produced by antibiotic residues include skin allergy, bone marrow toxicity, carcinogenicity, mutagenicity, teratogenicity and gastrointestinal disorders. The milk containing antibiotic residues beyond recommended limits should be discarded from human use. In the current study, 165 raw milk samples were analysed by MIA and it was found that 8.48 per cent of samples were contaminated with antibiotic residues. The results are in accordance with Chung *et al.* (2009) [3] who observed occurrence of antibiotic residues in 7.81 per cent of samples using microbial assays in Korea. Similar results were obtained by Nero *et al.* (2007) [9] from Brazil, Nikolic *et al.* (2011) [10] from Montenegro and Tola *et al.* (2017) [13] from Bishoftu who found the occurrence of antibiotic residues in 11, 7.84 and 6.5 per cent of samples respectively. Higher occurrence of antibiotic residues in milk was reported by Dinki and Balcha (2013) [4] in Guwahati, India which was 23.3 percent.

The most commonly used in the food animal production belong to the group of β-lactams, tetracycline, aminoglycosides, quinolones, macrolides and sulfonamides. In the present study, occurrence of tetracycline residues in milk samples was found to be 1.82 per cent. Similar results were observed by Lejaniya *et al.* (2017) in Thrissur and Schlemper and Sachet, 2017 [12] in Brazil who observed the presence of tetracycline in two and six per cent of samples respectively. The study conducted by Abbassi *et al.* (2011) [1] in Ardabil revealed the higher occurrence of tetracycline residues above MRL in 25.4 per cent of milk samples. The β-lactams residues were detected in 2.42 per cent of samples which is similar to findings of Movassagh (2011) [7, 8] and Movassagh and Karami (2011) [7, 8] who observed β-lactams residues in 4.66 and 5.33 per cent of samples in Tabriz, Iran. But Al Zuheir, 2012 [2], found higher occurrence rate of 22.2 per cent β-lactams residues in milk. The occurrence rate noticed for enrofloxacin residues in the current study was 1.82 per cent which is in accordance with Nirala *et al.* (2017) [11] from Bihar who observed enrofloxacin residues above MRL in 1.2 per cent of samples. Moharana *et al.* (2015) [6] found the higher occurrence of enrofloxacin residues in milk in Chennai.

## Conclusion

The results from the study indicate the antibiotic contamination of raw milk that subsequently enters the milk supply chain and dairy plants. Antibiotic residues can be avoided in milk by judicious and proper use of antibiotics, enforcing governmental regulations and following withdrawal period. Screening tests used for detection of antibiotic residues helps in assuring the safety and quality of milk that finally reaches consumers. The implementation of nationwide food safety program and performance of routine surveillance studies on dairy products helps in preventing the marketing of contaminated products. Dairy farmers and para veterinary staff must be made aware of consequences of unnecessary use of antibiotics in dairy animals.

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