

## SCREENING OF POOLED MILK SAMPLES FOR BETA LACTAM AND TETRACYCLINE ANTIBIOTIC RESIDUE

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**Abstract:** Pooled morning milk samples were collected from milk cooperatives and organized farms in Thrissur district. Samples were tested for beta lactam and tetracycline antibiotic residue using Antibiotic Test Kit (BEIJING YF-MARISGLOBAL CO., LTD, CHINA). Somatic cell count and methylene blue dye reduction test were carried out with the same samples. Out of the 50 samples collected 6 samples were positive for beta lactam antibiotic and one sample was positive for tetracycline. The somatic cell count was lower in samples containing antibiotic residues. Methylene Blue Reduction Test time of more than 5 hour were noted in positive samples. The study shows a prevalence of beta lactam antibiotic and tetracycline exogenous residues at a level of 12 per cent and 2 per cent respectively in the collected samples.

**Keywords:** Pooled milk, beta lactam, tetracycline residue, somatic cell count, MBRT.

### INTRODUCTION

India ranks first in milk production with 15% of world total production. Milk, a highly perishable commodity is susceptible to contamination with Pesticides, heavy metals, mycotoxins, antibiotics, other veterinary drugs and microorganisms. Mastitis is the most widespread disease in dairy cattle results in serious financial losses to dairy farmers. Currently, intra mammary infusion of antibiotics for mastitis reported as reason for antibiotic residue in milk (Pol and Ruegg, 2007). Veterinary drugs are substance applied or administered to food producing animal, for therapeutic, prophylactic or diagnostic purpose or modification of physiological function (IDF, 1997). In that antibiotics are low to medium molecular weight compounds which show a variety of chemical and biological properties. Detecting violated levels of antimicrobial residues in milk through the use of residue screening tests can help prevent contaminated milk from entering the human food supply. This study evaluated the percentage of incidence of antibiotic residue in pooled milk samples

in and around Thrissur district of Kerala and the influence of antibiotic residue on somatic cell count and methylene blue dye reduction test.

## **MATERIALS AND METHODS**

Pooled morning milk samples were collected from milk cooperatives and organized farms in and around Thrissur district of Kerala. A total number of 50 samples were collected in sterile containers comprising of 11 samples from farm and 39 samples from milk cooperatives

### **Screening test using kit method**

Antibiotic test kits were procured from BEIJING YF-MARISGLOBAL Co., Ltd, China. Wells and strips with appropriate identification for individual samples were taken. Then 200µl of the test samples were poured into the wells, then repeatedly mixed for 5 times with the reagent in the wells thoroughly. The mixture should be pink, and then start the timer. Wells were kept for 5 minutes at room temperature (25°C) and the test strips were inserted into the wells with the "MAX" end of strip fully dipped in to the mixture. The mixture was incubated at room temperature for 5 minutes. Then strip was removed and the results were read.

There were 3 lines in the strip, Control line, Beta-lactams Line and Tetracycline Line, which were termed as "C", "B" and "T" respectively. The test results will be based on the colour of these lines. Negative: Control line, B Line and T Line are all red; Beta-lactams Positive: Control Line is red, B Line has no colour; Tetracyclines Positive: Control Line is red, T Line has no colour; Beta-lactams and Tetracyclines Positive: Control Line is red; B Line and T Line have no colour.

### **Somatic cell count (SCC)**

Somatic cell count of the samples was estimated by Direct Microscopic Somatic Cell Count (DMSCC) by the procedure described by Schalm *et al.* (1971). Newman-Lampert staining techniques were followed for microscopic examination of somatic cells in the milk samples. Milk samples were uniformly mixed and 10 µl of the given samples were centrally placed in the 1 sqcm area of a grease free slide. After drying slides were flooded with modified Newman-Lampert stain (Himedia) for 2 minutes. Then excess stain should be drained, washed with tap water and air dried the slide. 20 different fields in the stained slides were counted under oil immersion in a horizontal progressive manner.

Cell count per ml of sample = average number of cell per field X microscopic factor X 100

### **MBRT (Methylene Blue Reduction Test)**

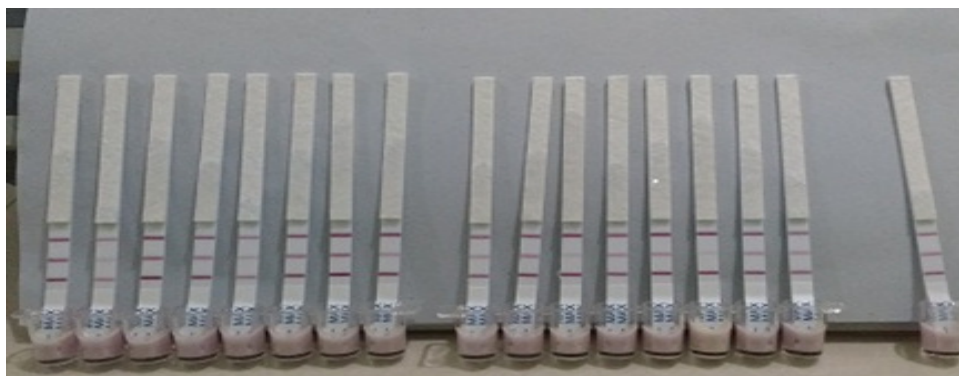
As per BIS 1479 (Part 3): 1977 criterion, 10 ml milk sample was taken in a sterile test tube and then 1 ml MBRT dye solution was added (dye concentration 0.005%). Test tubes were incubated in a water bath at  $37\pm 1^{\circ}\text{C}$ . Record the time period required for reduction of dye added.

### **RESULT AND DISCUSSION**

Testing of milk samples for antibiotic residues showed six positive samples for beta lactam antibiotics and one positive sample for tetracyclines. The rate of incidence for residue in milk was 12 per cent and 2 per cent for beta lactam and tetracycline antibiotics respectively. The principle of antibiotic kit was a specific reaction of antibody-antigen and immune chromatography. Beta-lactam antibiotics or tetracyclines in the sample compete with the antibody on the test strip membrane and the results are made visible after a colour reaction. Residue screening test kits will be more rapid, qualitative, and can detect a broad range of antibiotic residues in a better way (Navratilova, 2008). Dairy facilities and government agencies use residue screening tests to comply with the NCIMS requirement that all tankers of milk in the United States must be screened for  $\beta$ -lactam antibiotic residues (Kijak, 2004). The antimicrobial actions of normal constituents of milk can subsequent inhibition of growth of the bacteria in microbial inhibitory assay (Nair *et al.*, 2005). The residue screening tests were made to detect antibiotics below their permissible limit for safe guarding human health by food safety (FDAa, 2010). Veterinary drugs residues were detected at the level of 4.3 % of milk samples collected from Macedonia (Angelovski *et al.*, 2011). The occurrence of oxytetracycline, tetracycline, chlortetracycline and doxycycline in 439 samples were 32.7 %, 13.1 %, 3.4 % and 2.5 % respectively. The EDIs study proves that the contribution of dietary intake of milk with antibiotics residues was 2 to 100 times below ADIs proposed by EMEA and WHO. There by the raw milk in Macedonia contains low levels of veterinary drugs, so safe for consumption (Elizabeth *et al.*, 2011). Screening of milk samples in Czech Republic showed presence of tetracyclines at 50, 6 % level which is safe concentrations under MRL (Navratilova *et al.*, 2009). Study by Movassagh (2011) in Iran on milk samples 7 (4.66%) were positive for beta lactam antibiotic residues. In Iran, Movassagh and Karami (2010) also studied that 5% of cow raw milk were positive for betalactum antibiotics residues. Aydin *et al.* (2010) studied in 204 raw milk samples and detected that 44% of the samples were positive for beta lactam antibiotic residues in Turkey. Khaskheli *et al.*, (2008) studied that

36.5% of the milk samples collected were contaminated by beta lactam antibiotic residues at Pakistan.

Picture shows the beta lactam antibiotic positive in 2<sup>nd</sup>, 5<sup>th</sup>, 11<sup>th</sup> and 14<sup>th</sup> samples



The SCC ranges normally from 250,000 to 400,000 cells/ ml across breeds and more than 400,000 is deemed unfit for consumption by European Union. In dairy industry, the SCC acts as an indicator of the milk quality. White blood cells constitute the majority of somatic cells and number of cells increases in response to pathogenic microbes like *Staphylococcus aureus*, which mainly causing clinical mastitis. The SCC present in cells per milliliter. DeLaval cell counter (DeLaval Inc., Kansas City, MO) and direct microscopic count method were used for detecting SCC in positive samples. In the study by Sischo and Burns (1993) mentioned an increased SCC was in accordance with a raise in rate of false positive outcomes for the Delvotest-P®. This relation was also studied by Van Eenennaam *et al.* (1993). Milk with greater than 10<sup>6</sup> cells/mL of SCC was associated with a higher number of false-positive results in the Delovtest-P® and CITE® SNAP tests (Van Eenennaam, 1993). In this study the samples with beta lactam and tetracycline antibiotic shows a drastic reduction of somatic cell count below normal range. Even less than 10,000 cell per ml of milk.

Methylene Blue Reduction Test was used to assess the microbiological quality in milk. MBRT works based on the reduction of blue colour of methylene blue dye solution to colour less leuco-methylene blue by microbial activity. Faster the decolourization, quality of the milk should be inferior. Widely used at the dairy reception dock where it is followed as acceptance/rejection criteria for milk. As per BIS 1479 (Part 3): 1977 criterion grading of raw milk were done. The milk samples with incidence of beta lactam and tetracycline antibiotics were examined for MBRT. The samples with antibiotic residue show higher reduction time above the control sample. According to Mandy and Venkatesh (2009) it was clear that

presence of antibiotic residue in milk will not show dye reduction and thereby colour will not change. Aggarwal *et al.* (1970) reported that pesticide residue in milk can also adversely affect the MBRT result.

Table shows the SCC and MBRT values obtained for suspected samples

Antibiotic residue suspected samples	Somatic cell count obtained (cell per ml)	Methylene blue reduction test (time in hours)
1 (Beta lactam)	1000	Above 5 hrs
2 (Beta lactam)	4600	Above 5 hrs
3 (Beta lactam)	2902	Above 5 hrs
4 (Beta lactam)	6075	Above 5 hrs
5 (Beta lactam)	7049	Above 5 hrs
6 (Beta lactam and Tetracycline)	4005	Above 5 hrs

### Conclusion

There is no doubt that human misuse and overuse of antibiotics are large contributors to resistance, particularly in relation to bacteria associated with human infection. Interventions in medical, veterinary settings and the community are clearly needed to preserve the efficacy of antibiotics. Here in this study the beta lactam antibiotic and tetracycline residue were twelve per cent and two per cent respectively in the pooled milk samples examined by antibiotic test kit. Antibiotic contaminated milk should not be used for consumption. Milk from antibiotic treated animals should not be mixed with good quality milk before the labelled withdrawal period. Practice of using cow side screening kits for rapid detection of antibiotic residue thereby it help in providing good quality and safe milk for consumers. Challenging open global market demands international standards for food safety requirements. So, Indian dairy industry should be proactive. Dairy development agencies should conduct awareness programme targeted towards farmers and para-veterinary staff against unnecessary use of antibiotic in dairy animals.

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