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Antibiotic residue screening of milk samples using microbial inhibition assay: A preliminary study

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Abstract

A preliminary study was conducted to detect the presence of antibiotic residue in milk samples collected in and around Chennai using the microbial inhibition assay with freeze dried vegetative form of *Geobacillus stearothermophilus*. All the samples were found to be negative for antibiotic residues. Before field testing, the LOD of the commonly used antibiotics namely Enrofloxacin, Ciprofloxacin, Neomycin and Oxytetracycline was determined to be 50, 25, 100 and 25 ppb respectively. This was much lower than the MRLs for each antibiotic indicating that it is a sensitive assay. The assay would be useful for screening bulk samples as a qualitative test before the confirmatory test using HPLC.

Keywords: Antibiotic residues, MIA, screening, milk

Introduction

The most important and frequently used group of veterinary drugs is antimicrobial agents (Fischer *et al.*, 2003). Antibiotics are administered at therapeutic doses for curative or preventive purposes against infectious diseases in animals. At sub-therapeutic doses, these agents are used as feed supplements. The use of antimicrobial therapy to treat and prevent udder infections in cow is a key component of mastitis control. Due to the widespread use of antimicrobials for treatment of mastitis in dairy cow, much effort and concern has been directed towards proper management and monitoring of antimicrobials used in such treatment to prevent contamination of raw milk. However, wide spread use of antimicrobial has created potential residue problems in dairy products (Hillerton *et al.*, 1999) [2].

The presence of residues may result from failure to observe the mandatory withdrawal periods, illegal or extra-label use of drugs and incorrect dosage (Ivona and Mat, 2000) [3]. Therefore, the maximum residue limits (MRL) set for each antimicrobial agent should be respected. Problems associated with antimicrobial residues in milk include the risk of allergic reactions after consumption by penicillin sensitized persons and increased resistance of pathogenic bacteria towards antimicrobials. The concerns arise mainly from the possibility that antimicrobial resistant bacteria that may be transferred from livestock to humans, through animal to human contact, through environment or in contaminated food products (CAC, 1998) [5]. Conducting appropriate screening tests to determine residue status can aid in maintaining a safe milk supply (Asredie and Engdaw, 2015) [4]. Various sophisticated, quantitative tests are available for detecting antibiotics in milk and are used by research institutes and pharmaceutical companies. The routine screening of bulk milk samples by dairies is done preferably by simple qualitative tests. The most common screening methods for antimicrobial drug residues are microbiological tests, based on the growth inhibition of microorganism (e.g. *Bacillus stearothermophilus*). There are on farm screening tests devised for rapid detection of low concentrations of antimicrobial residues in milk (Tyler, 1992) [7]. A well-known assay in this category includes the charm inhibition assay and the Delvotest (Bishop and White, 1999) [6]. A microbial assay using freeze dried vegetative form of *Geobacillus stearothermophilus* was developed and used for screening meat samples collected from broiler chicken (Ruth *et al.*, 2018) [9]. In the present study, the same assay was applied for detection of antibiotic residue in milk samples.

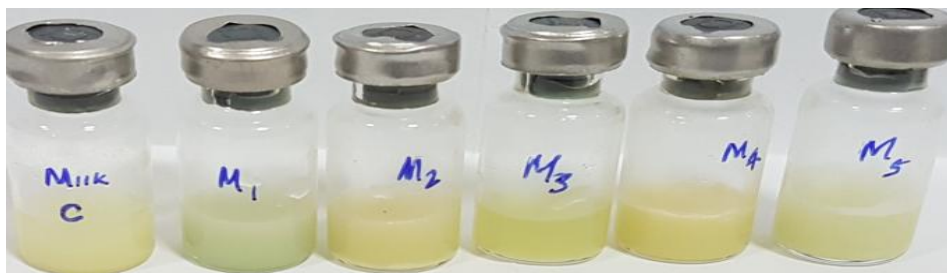
A total of 55 milk samples collected in Chennai from milk pooling points, commercial outlets and farms were tested for antibiotic residue and all samples were found negative for antibiotic residue which is indicated by the growth of *G. stearothermophilus* with change in color from blue to colourless (Fig. 1). Before field testing the limit of detection (LOD) of commonly used

antibiotics namely Enrofloxacin, Ciprofloxacin, Neomycin and Oxytetracycline for mastitis treatment was checked in the developed assay and the results are shown in Table 1 and representative photograph in Fig. 2. It was seen that the LOD was much lower than the MRLs for each antibiotic indicating that it is a sensitive assay and would be useful for ‘ruling in’ samples for further confirmatory test using high performance

layer chromatography (HPLC). Although the number of samples screened was limited the absence of detected residues is promising. The availability of a easy-to-perform field assay for residue detection in milk would act as a deterrent for animal owners to use the milk before the recommended withdrawal periods or to use antibiotics indiscriminately.



A- Samples before incubation



B - Samples after incubation at 37 °C overnight

Fig 1: The detection of antibiotic residues in milk samples collected from a farm by MIA

The change in colour from blue to yellow indicates absence of any antibiotic residue

Table 1: LOD of antibiotic-spiked milk samples detected by growth inhibition of *G. stearothersophilus*.

Antibiotic	Milk	
	MRL (ppb) as per European Union Regulation No. 2377/90	LOD (ppb)
Enrofloxacin	100	50
Ciprofloxacin	100	25
Neomycin	1500	100
Oxytetracycline	100	25



Oxytetracycline

OTC – Oxytetracycline; MC – Medium control; C – Control Concentration used for detection - 5 to 150 ppb



Ciprofloxacin

Cip - Ciprofloxacin; MC – Medium control; C – Control Concentration used for detection - 5 to 150 ppb
Note: change in colour from blue to yellow at 10 ppb concentration for both OTC and ciprofloxacin

Fig 2: The LOD of OTC and Ciprofloxacin spiked milk samples by MIA using *G. stearothersophilus*

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