Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [11] October 2018 : 20-25

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Screening of Antibiotic Residues in Cattle Milk by Paper Strip Assay

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ABSTRACT
The aim of this study for the screening of the antibiotic residues in cattle milk in Hassan taluk in Hassan district of Karnataka. The qualitative screening of antibiotic residues was carried out using paper strip assay kit procured from ICAR – National Dairy Research Institute, Karnal. Haryana, India. In this present study, the milk samples were collected from the various milk collecting points in and around Hassan taluk and were subjected to assay by using paper strip assay kit method. Out of 538 samples analysed, 24 samples tested positive for antibiotic residues. This study confirms had confirms the presence of the antibiotic residue in the milk samples and further the identification and quantification of the antibiotic residues by by analytical technique.
Keywords: Antibiotic residues, qualitative, paper strip assay, cattle milk

Received 21.06.2018 Revised 22.07.2018 Accepted 02.10.2018

INTRODUCTION

Milk is one of the most nutritious and complete food. It is rich in high quality protein providing all ten essential amino acids, fat especially essential fatty acids, most of the mineral and vitamins. Meanwhile milk as a nutrient has the main role in human diet, especially for children [1]. India stands first in the global milk production, with 165.4 million tonnes produced in 2016-17 from its 304 million strong dairy herd. Animals are exposed to antimicrobials to treat and prevent diseases. Many of these antimicrobials are identical or closely resemble drugs used in humans which can lead to adverse reactions and emergence of antibiotic resistance [2]. Concerns about food safety, with animal source foods are increasing in developing countries where urbanization, increasing incomes and changing of life-styles are associated with greater dependence on marketed foods by an increasing number of people [3]. The safety of food is threatened by various agents including pathogenic microorganisms, aflatoxins, pesticides and antimicrobial agents. Pathogenic microorganisms constitute the most important food related to threat public health. Little information about level of the antimicrobial residues in food is found in developing countries. While pasteurization and other forms of heat treatment eliminate pathogenic microorganisms from animal source food.

Common management practice for dairy animals worldwide includes antibiotic therapy. Residues of these antibiotics—whether infused, injected, or added to the diet—may enter the milk supply from the treated animals. Regulations for use of antibiotics require that milk from treated animals withdrawn from sale for a prescribed time. When proper procedures for use of a drug and withdrawal of the milk are not followed, milk containing drug residues may be sent to the marketplace. Antibiotics have been used in the dairy industry for more than five decades in dairy cattle production to treat or prevent disease and to increase milk production or improve feed efficiency [4]. Residual antibiotics in milk can seriously affect

consumers' health causing allergic reactions and developing resistant strains. Antibiotic contamination in milk can also cause significant economic losses for producers and manufacturers of milk and milk products.

Although the use of antimicrobial drugs has significantly improved the health and production efficiency of food-producing animals, antimicrobial resistance is of concern. The continuous presence of the antimicrobial drugs in animals has led to the evolution of antimicrobial resistant microbes that are pathogenic to animals and humans. Moreover, antimicrobial residues in food products of animal origin may potentially cause allergic reactions and alter the dynamics of the microflora in the intestinal tracts of humans [5]

Although antimicrobial drugs are useful for treatment of human infections, their occurrence in milk causes adverse public health effects such as drug resistance and hypersensitivity that could be life threatening [6, 7]. The use of antibiotics therapy to treat and prevent udder infections in cows is a key component of mastitis control in many countries. Due to the widespread use of antibiotic for treatment of mastitis in dairy cows, much effort and concerns have been directed towards the proper management and monitoring of antibiotics usage in treatments in order to prevent contamination of raw milk. As widespread use of antibiotics has created potential residue problems in milk and milk products that are consumed by the general public. Because of the public health significance, milk and milk products contaminated with antibiotics beyond a given residue levels, are considered unfit for human consumption [8]. The good quality of milk must contain no harmful or toxic residues, such as antimicrobial drugs. The extra-label use of these antimicrobial treatments, insufficient withdrawal period and lack of records are the most common causes of theses residue in milk, which lead to the increase of these residues in milk above the acceptable maximum residue limits (MRLs). The (MRL) is defined as the maximum concentration of a residue, resulting from the registered use of an agricultural or veterinary chemical that is recommended to be legally permitted or recognized as acceptable in or on a food, agricultural commodity, or animal feed. The concentration is expressed in mg/kg of the commodity or mg/L in the case of a liquid commodity or ppm/ppb [9]. The MRL is based on the Acceptable Daily Intake (ADI) for a given compound, which is the amount of a substance that can be ingested daily over a life time without appreciable health risk. MRLs are fixed on the basis of relevant toxicological data including information on absorption, distribution, metabolism and excretion [10]. In addition the lack of good veterinary practice and illegal use of veterinary drugs by farmers will increase this problem [11-13]. Various analytical methods have been described to determine antibiotic residues in milk, such as microbiological, chromatographic, immunochemical, receptor and enzyme-based tests. Microbiological tests are commonly applied in dairy and in survey studies[14]

Material and Methods

Milk Sample Collection:

A total of 538 milk samples were randomly collected from the dairy farmers in the Hassan taluk of Hassan district. Karnataka. The 2ml of the milk samples collected in the Effendroff tube from each farmers stored in the refrigeration temperature until the analysis.

The test was carried out by paper strip assay kit for detection of antibiotic residues in milk procured from ICAR – National Dairy Research Institute, Karnal.

Antibiotic residue analysis

The test was carried out by paper strip assay kit for detection of antibiotic residues in milk procured from ICAR – National Dairy Research Institute, Karnal.

Procedure:

300 µL of distilled water (pH 7.0) was added to the tube containing nutrient disc and was vortexed for 25 seconds. The paper strip was dipped into the milk sample and inserted into the tube containing reconstituted nutrient media.This was incubated at 64^{o} C for 45 minutes in the dry block incubator (Fig 1). Results were interpreted based on the colour change. Appearance of blue colour indicated the absence of antibiotic residues whereas no colour development indicated the presence of antibiotic residues in the sample (Fig 2).

Fig. 1. DRY BLOCK INCUBATOR

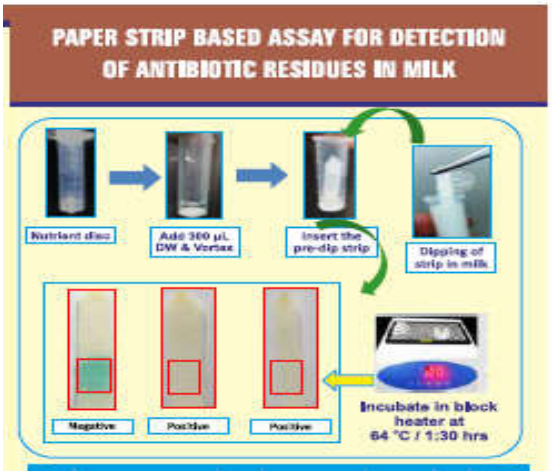

Fig. 2. Procedure of detection of antibiotic residue

RESULTS AND DISCUSSION

A total of 538 milk samples were collected from 21 villages of Hassan Taluk, Hassan District, Karnataka and analyzed for the presence of the antibiotic residue in the milk samples by paper strip assay kit procured from ICAR – National Dairy Research Institute, Karnal. The 24 samples tested positive for the presence of antibiotic residues. The Seven milk samples from the Sankanhalli (Fig.3) and Rajagere tested positive presence of the antibiotic. The Sappanahalli village had four positive samples (Fig 4), Chikkakadalur village followed with two positive samples. Kabbati, Somanahalli, Tattehalli and Koravangala had one positive sample each. Thirteen villages milk samples showed negative for the presence of the antibiotic residues in the milk samples (Table 1). This method only showed the presence of the antibiotics or not. By using the analytical methods like High Performance Liquid Chromatography (HPLC) further quantification of the antibiotic residues in the milk samples.

Table 1: Thirteen villages milk samples showed negative for the presence of the antibiotic residues in the milk samples

Sl.no.	Place	No. of samples tested	No. of positive samples
1	Chikkagondanalli	24	-
2	Kabbati	24	1
3	Somanahalli	48	1
4	Doddakondagulla	24	-
5	BT Koppal	24	-
6	Sankanhalli	24	7
7	Rajagere	24	7
8	Tattehalli	24	1
9	Bhuvanahalli	24	-
10	Koravangala	24	1
11	Jakkenahalli	24	-
12	Sappanahalli	24	4

13	Kattaya	24	-
14	Kallarakoppalu	24	-
15	Chengahalli	24	-
16	Chikkakadalur	24	2
17	Halekoppalu	24	-
18	Hullikoppalu	24	-
19	Dasarakoppalu	24	-
20	Thamlapura	34	-
21	Haralahalli	24	-

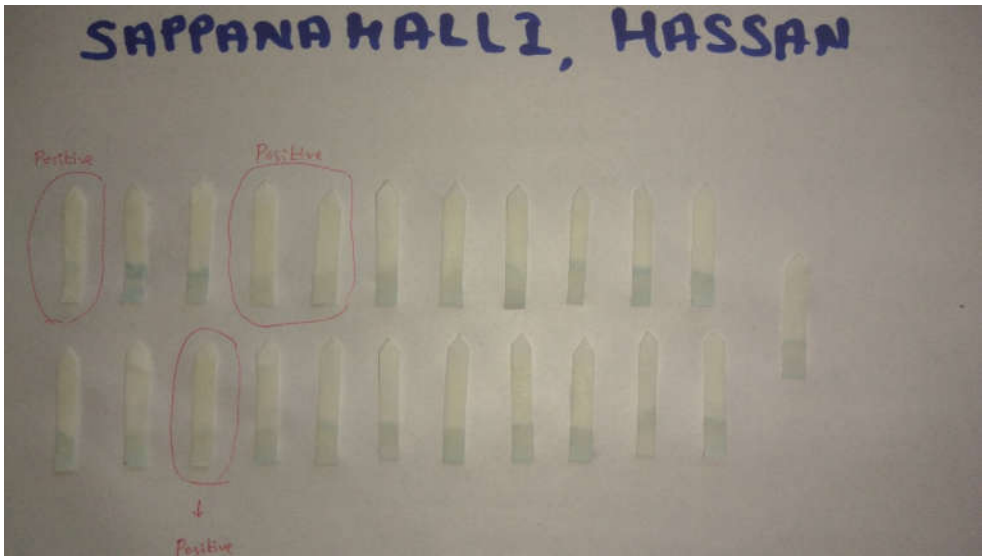

Fig. 3. Positive samples at Sappanahalli, Hassan

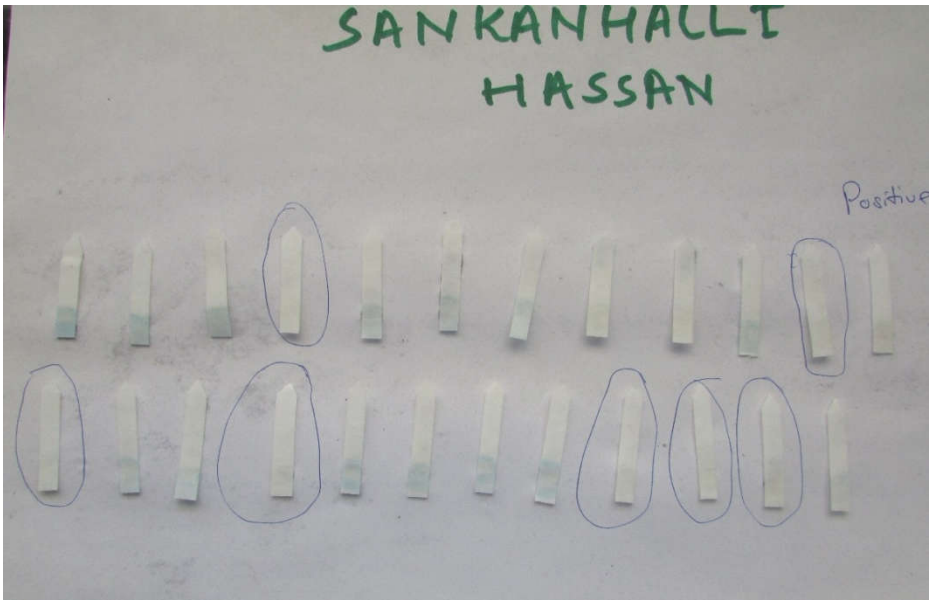

Fig. 4. Positive samples at Sankenahalli, Hassan

A qualitative receptor assay for antibiotic and antimicrobial residues in milk was used in a survey of commercial milk samples obtained in the eastern Pennsylvania, Central New Jersey, New York City area.. Sixty-three percent of milk samples contained one or more residues; 27% contained 2 residues; 11% contained 3 or more residues. Tetracyclines and sulfonamides were the most predominant residues detected [15]. Evaluate the relationship between antibiotic residues value in raw cow milk by Copan Milk Test is inhibition assays. The milk samples (n = 200) were randomly collected during summer and winter seasons from dairy farms in Tabriz. Out of 115 samples were positive for antibiotic residues. Antibiotic

residue in winter season was significantly higher than in summer season ($P < 0.05$) [16]. The antibiotic residues in raw milk and pasteurized milk products were analyzed in terms of penicillin G, oxytetracycline, gentamicin, streptomycin and neomycin by using TLC (Thin Layer Chromatography)/ Bioautographic method, in which Bacillus subtilis ATCC 6633 was used as test microorganism. The raw milk samples found positive for the oxytetracycline and 33.5 g/L penicillin G and 7688.4 µg/L of neomycin antibiotics [17]. The 71 milk samples out of 91 samples from individual animals and 9 samples out of 101 market samples contained oxytetracycline residues in Hyderabad, India were detected flour metric method [18] . Chung *et al.,* (2009) reported that in the microbial assays, 21 of 269 samples were screened to have possible antibiotic residues and further HPLC analysis revealed that 4 samples were detected to contain sulfamerazine and ciprofloxacin [19]. The milk samples contained residues of oxytetracycline, penicillin G and neomycin more than the maximum residue levels permitted. 18 samples out of 133 milk samples tested were found to have tetracycline residues in the milk smaples of Punjab, India [20]. The qualitative and semi-quantitative analysis with rapid screening kits revealed that 23% samples were positive for antibiotic residues in the fresh milk for penicillin and sulphonamide and further HPLC analyses detected 81% samples positive for amoxicillin, 41% for sulfadimethoxine, 27% for penicillin G and 12% for ampicillin out of 140 milk samples [21].

CONCLUSION

The present study was conducted to screen for the presence of the antibiotic residue in the cattle milk of Hassan taluk in Hassan district, Karnataka . The milk samples procured from seven villages found positive for presence of the antibiotic residues in Hassan taluk. The farmers mix the milk from two or three animals this will lead to the dilution of the milk and interfere with presence of antibiotics residue in the milk samples. The antibiotic residues, which can be considered an important factor threatening human health, development of antibiotic resistance and numerous losses that would caused in the milk industry. This study will further help for the quantitative estimation for the presence of the antibiotic residues in the milk samples by analytical technique.

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CITATION OF THIS ARTICLE

Ravi Kumar.C, Ranjith. J, Pavankumar.K.N, Prakash.N, Abbas. M. H, and Sahitya. C.P. Screening of Antibiotic Residues in Cattle Milk by Paper Strip Assay. Bull. Env. Pharmacol. Life Sci., Vol 7 [11] October 2018: 20-25