



Prevalence of antibiotic residues in milk samples of small-scale dairy households in Bengaluru, India

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ABSTRACT

This study aimed to estimate the burden of subclinical mastitis in community setting, animal handlers' knowledge, attitude and practices on mastitis and antibiotic use, and to detect antibiotic residues in milk samples. A total of 211 households belonging to four clusters of Karnataka state in Southern province of India were selected through 30-cluster approach. A total of 380 milk samples were analyzed for Subclinical Mastitis (SCM) by California Mastitis Test (CMT) and antibiotic residues by spore-based and Charm Rosa tests. Animal handler's basic knowledge, attitude and practices (KAP) on antibiotics use were recorded. SCM prevalence was found 42% in the study site; antibiotic residues were detected in 21% (79/380) of milk samples; 7.1% and 5.52% of samples had maximum residual limits (MRL) and Multi Drug Residues (MDR), respectively. Antibiotic residues were detected in 32.22% of the households. Majority of animal handlers heard about antibiotics (83.31%), 51% of handlers treated the animals on their own and 23% of them bought antibiotics without prescription. High prevalence of SCM in the study setting and antibiotic load in milk samples raise food safety concern. Regulatory measures for prudent antibiotic use and knowledge dissemination among animal handlers for clean milk production are the measures required to tackle the problem.

Keywords: Antibiotic residues, Households, India, Milk, Subclinical Mastitis

India is one of the largest producer and consumer of milk in the world. As per the National Dairy Development Board (NDDB) of India, milk production has increased from 55 to 198 million tons during 1991-2020. The demand for milk and milk products eventually led to intensified dairy activities in the last two decades (Nisha 2008, Sachi *et al.* 2019). Mastitis is the major disease which affects the milk quality and quantity. With the high burden of clinical mastitis (18%) and subclinical mastitis (45%) and growing demand for treatment of mastitis is resulting in the use of large quantities of antibiotics in dairy farming (Krishnamoorthy *et al.* 2021). This is leading to a vicious cycle of antibiotic use, presence of residues in animal food products and the emergence of antimicrobial resistance (Hedge *et al.* 2013, Kurjogi *et al.* 2014, Ambika *et al.* 2021). India is one of the top five countries next to China,

USA and Brazil in the antimicrobial consumption in animal production (Van Boeckel *et al.* 2015) and it is expected to increase 312% by 2030 (Vishnuraj 2016). Significant presence of antimicrobial residues in milk (Padol *et al.* 2015), beef (Basulira *et al.* 2019), hospital waste water (Lien *et al.* 2017) and aquatic products (Uchida *et al.* 2016) have already been reported. Consumption of adulterated milk leads to hypersensitivity reactions and alteration of gut microflora which are detrimental to the health of consumers (Kumar *et al.* 2013, Montero *et al.* 2018). Comprehensive community-based studies on antibiotic use and antibiotic residues in milk are limited and seldom published. Hence, this study aimed to assess the knowledge and practice of animal handlers in managing mastitis, antimicrobial usage, subclinical mastitis and detecting antimicrobial residues in milk.

MATERIALS AND METHODS

Study site and study design: The study was conducted in Bengaluru North district which has 52,768 bovine population and is significantly contributing to milk production and supply to Bengaluru city, Karnataka, India [<https://www.kmfnandini.coop/>]. A multistage stratified 30-cluster sampling technique (WHO 2015) was used.

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In the first strata, three administrative blocks also called taluks (Doddaballapur, Devanahalli, and Nelamangala) from Bengaluru rural district were selected based on high, medium, and low cattle population. These study sites were conveniently selected so that samples could be transported to the laboratory within 2 h. Second strata included a total of randomly selected 30 villages (clusters) from 939 villages. In third strata, a list of households with more than three cattle was prepared and simple random sampling method was used to select 7 or 8 households (HH) from each village resulting in selection of 211 HH in the study areas.

Data collection: The research team collected the data and samples from September 2018 to August 2019. A structured questionnaire was used to collect household data such as age, sex, village, taluk, district and animal data included number of livestock, milking cattle, breeds, number of calvings and clinical history of mastitis. Additionally, a pre-tested semi-structured questionnaire was used to interview animal handlers to assess the knowledge, attitude and practices (KAP) related to mastitis and antibiotics usage such as use of antibiotics, attitude on withdrawal period, practices in discarding the milk under treatment, over the counter purchase of antibiotics, etc. A written informed consent was obtained from all animal handlers willing to participate in the study and to publish the data.

SCM detection: California Mastitis Test (CMT) was conducted as per the manufacturer's protocol (DeLaval, Kansas City, Missouri, USA) by adding 2 ml each of milk and CMT reagent into the mastoid paddles containing four shallow cups labeled as LF (left fore), LH (left hind), RF (right fore) and RH (right hind) and mixed well. The results were considered positive if the milk in any one quarter resulted in thickening and/or clot/gel within 10 to 20 sec and the animal was identified to have SCM. Milk was collected from all the SCM positive and 50% of SCM negative cows were transported to laboratory at ICAR-NIVEDI, Bengaluru.

Antibiotic residue analysis: Milk samples were transported on cold chain to ICAR-National Dairy Research Institute (NDRI), Karnal, India for antibiotic residue analysis. The samples were primarily screened by Dipicolinic acid (DPA) test which is a spore-based test as previously reported (Kumar *et al.* 2013) to determine the complete absence or presence of antibiotic residues. The positive milk samples were quantified by Charm ROSA test kit (Charm Sciences, Inc, MA, USA) for detection of six antibiotic groups namely sulfa drugs, β -lactam, tetracycline, chloramphenicol, enrofloxacin and streptomycin as these groups are commonly used for the treatment purpose in cattle (Fig. 1). As per Codex Alimentarius Commission (CAC) guidelines by World Health Organization (WHO), the residual limits were characterized as Maximum Residual Limits (MRL) and if more than one class of antibiotics are present the samples were represented as Multiple Drug Residue (MDR).

Data analysis and mapping: The data entered and

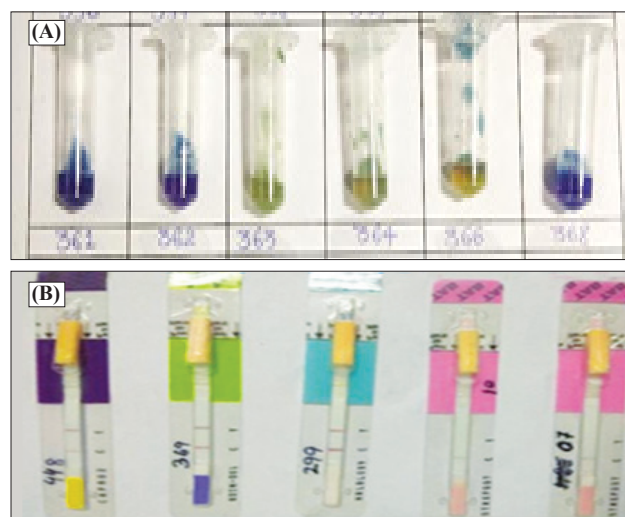


Fig. 1. (A) Spore based test for detection of antibiotic residues in milk samples-purple colour indicates presence of antibiotic residue; (B) Charm ROSA test results – first three samples indicate antibiotic positivity in the test line.

analyzed using Epi Info version 7.2.2.6, data quality was assured by random checks on 10% entries and was re-entered on identification of errors. Data is presented as frequency and proportions and QGIS software (version 3.14) was used for generating maps of the study area.

RESULTS AND DISCUSSION

SCM detection: A total of 1138 cattle were identified in 211 HH comprising Holstein Friesian-HF (56%), calves (20%) and heifers (11%). Of 1138 cattle, 47% (533) milking cattle were screened for CMT, 3% (18) cows were detected as clinical mastitis and 42% (226) were found to be SCM (Table 1).

Table 1. Prevalence of subclinical mastitis in the study area

Taluk	Cows screened	CMT test result (%)
Devanahalli	186	79 (42)
Doddaballapur	102	44 (43)
Nelamangala	156	66 (42)
Bengaluru North	89	55 (62)
Total	533	244 (46)

KAP of animal handlers: A total of 211 animal handlers were interviewed, majority of the respondents were male (65%). Among respondents, 87% were educated and 74% had education above secondary grade. Around 49% were milking cows and 61% of them were stall fed. Only 37% of the farmers used disinfectant for cleaning the shed though 98% of them practiced cleaning the shed at least once a day. Ninety eight per cent of the people had heard about clinical mastitis whereas 89% did not know about SCM. Majority of farmers (83%) had heard about antibiotics and they knew that antibiotics are used for the treatment purpose. Few of them stated (5%) that antibiotics can be used for increasing the milk production. With respect to withdrawal period after the use of antibiotics, 81% of them

Table 2. Animal handler's response on knowledge and practical usage of antibiotics

Animal handler's knowledge and practice related to mastitis in cattle (n=211)	Response n (%)	
	Yes	No
<i>Knowledge parameters</i>		
Heard about mastitis in dairy cows	207 (98.1)	4 (1.9)
Heard of subclinical mastitis	24 (11.37)	187 (88.63)
Heard of antibiotics	176 (83.41)	35 (16.58)
Antibiotics are used to treat infection	169 (80.09)	42 (19.91)
Antibiotics can be used to increase milk production in cows?	10 (4.74)	201 (95.26)
Antibiotics can be used to promote growth in cattle	4 (1.9)	207 (98.1)
Withdrawal period after the use of antibiotics in milking cows	41 (19.43)	170 (80.57)
<i>Attitude parameters</i>		
Do you allow cows to lie down immediately after milking?	73 (34.6)	138 (65.4)
<i>Practice parameters</i>		
Do you treat animals on your own?	108 (51.18)	103 (48.82)
Do you buy antibiotics without prescription?	49 (23.22)	162 (76.78)

Values in parenthesis represents percentage.

were not aware of withdrawal period and 7% said that the duration of the withdrawal was one day. 51% of the animal handlers said that they will treat their animals on their own and 23% of them bought antibiotics without prescription (Table 2).

Antibiotic residues: Of 380 samples tested, 24% (92) of milk samples were positive for DPA test and 21% (79) for Charm Rosa test. Antibiotic residues were detected in both CMT positive (25%) and negative (14%) milk samples (Table 3). Among the six antibiotic classes tested, β -lactam was found highest (5.52%) followed by chloramphenicol (4.21%), enrofloxacin (3.16%), sulpha drugs (2%) and streptomycin (0.5%). High proportions (3.95%) of β -lactam residues were detected in Devanahalli block.

The presence of more than one antibiotic was found in 21 (5.52%) milk samples with six different combinations of Multi Drug Residues (MDR). Twelve samples had the combination of chloramphenicol and enrofloxacin, two samples had enrofloxacin and β -lactam combination and four samples had three antibiotics combination. As per Codex Alimentarius, MRL was calculated, 7.1% of the milk samples were found to have residual limits above the recommended; two milk samples with β -lactam residues of 7.5 ppb. Out of 21 MDR milk samples, 9 were found exceeding the MRL limits.

In this community-based study, household cattle were screened for mastitis, antibiotic residues in milk samples and assessed KAP among animal handlers in four blocks of Bengaluru North. The prevalence of SCM was found to be 42% (226) and 21% had antibiotic residues. The

knowledge levels about antibiotics were good, whereas the practices related to management of mastitis and antibiotic use were found to be poor.

The prevalence of SCM across 21 states in the country varied from 9% to 86% (Kumari *et al.* 2018) and reports estimated SCM burden in the study state to be 22-40% (Padol *et al.* 2015, Ambika *et al.* 2021, Kurjogi *et al.* 2014). In these reported studies, samples were drawn from either organized or unorganized cattle or buffalo farms at various time points. In the present study, through cluster sampling approach, 42% SCM was recorded from community setting at single point of time. The data from animal handlers support the findings that 35% of respondents allowed the animals to lie down immediately after milking and this practice tend to increase the chance of udder infection thereby leading to SCM cases. Majority (98%) of animal handlers were aware of clinical mastitis and this is quite obvious due to the physical changes in milk and udder observed during clinical mastitis. Whereas, the knowledge on SCM was poor (11%) as there were no clinical symptoms to interpret by the animal handlers. Prolonged unnoticed SCM condition will reduce milk production. So, creating awareness on SCM and training for the continuous monitoring by simple tests like CMT need to be implemented for clean milk production. Cleanliness is an important measure for the prevention of mastitis in the dairy sheds. Majority of the animal handlers had practice of cleaning the shed on day today basis, however many of them ignored disinfectant usage (63.51%). Simple management practices such as use of disinfectant need to

Table 3. Antibiotic residues in CMT positive and negative cattle

	Devanahalli		Doddaballapur		Nelamangala		Bengaluru North		Total	
	CMT									
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
DPA test n (%)	21(27)	11(21)	16(36)	8(26)	18(27)	2(5)	13(24)	3(19)	92(38)	24(18)
Charm Rosa test positive n (%)	20(25)	9(17)	12(27)	7(15)	15(22)	2(5)	13(24)	1(6)	60(25)	19(14)

CMT, California mastitis test; values in parenthesis represents percentage.

be inculcated to control SCM which has indirect bearing on reduced antibiotic use (Moghadam *et al.* 2016).

Currently, antimicrobial treatment is indispensable to keep animal welfare and economic aspects in balance. Previous studies from Guwahati region of Assam reported 23.3% of residues and almost same percentage was reported from unorganized dairy farms of Indore, Madhya Pradesh (Dinki *et al.* 2013, Priyanka *et al.* 2017). The prevalence of individual antibiotic groups such as beta lactams, streptomycin, erythromycin and sulpha drugs were found in rural clusters. Animal handlers had the knowledge on antibiotics and its use and 23.22% farmers stated direct purchase of antibiotics for treating animals on their own without consulting clinicians. Over the counter access to antibiotics was the major reason for the widespread use of antibiotics causing residues in milk and dissemination of antimicrobial resistant pathogens in India (Arora *et al.* 2004). In fact, majority of the animal handlers were educated but, still the awareness about the SCM, antibiotic residues, withdrawal period and management of animal were lacking. The study revealed that 80.57% were not aware of antibiotic withdrawal period and handling. Previous studies have reported consumption of residue loaded milk leading to health complications (Kebede *et al.* 2014, Mutua *et al.* 2020).

MDR prevalence was found in urban and peri-urban clusters which clearly depicts the usage pattern of antibiotics in the urban cluster. As per regulatory bodies, chloramphenicol was not permitted in foods of animal origin and its presence was observed 4.2% (16/380) in the study site. This represents the underlying problems of use of antibiotic for treating the diseases including mastitis and its withdrawal period. In order to regulate drug usage in animal production and to tackle the persistence of antimicrobial residues in foods of animal origin, organizations such as the Food and Agricultural Organization (FAO), World Health Organization (WHO), Codex Alimentarius Commission and European Economic Community (CAC & EEC) have defined MRL for livestock products (Priyanka *et al.* 2017). In spite of several organizations involved in regulating drug usage, the current study reported 7.1% (27/380) of milk samples showing residues above permissible limits (MRL), there is still lack of implementation and practices to follow the regulations and guidelines in veterinary healthcare as reported by Basulira *et al.* (2019).

The presence of drug residues more than residual limit is a concern in these clusters which eventually may lead to emergence of MDR pathogens. The antibiotic residues in animals and their food products possess multidimensional problems to humans, animals and environment as these residues are resultants of improperly metabolized antibiotics orally administered to livestock are excreted into the environment (Dinki *et al.* 2013). The unmetabolized residues remain in manure, soil and water and gain entry into the food chain resulting in antimicrobial resistant pathogen dissemination (Kumar *et al.* 2005, Van *et al.* 2016).

Capacity building, public awareness program on food safety and antimicrobial stewardship in livestock are very low in developing countries (Garcia *et al.* 2019). Indian government has initiated National Action Plan on AMR, nationwide surveillance on AMR and policies to address the problem (Singh *et al.* 2014, Moghadam *et al.* 2016). The present study identified the knowledge gaps of farmers, high prevalence of SCM (42%) from community setting, and antibiotic residues in milk samples to the tune of 21%.

As antibiotics are used for other infectious diseases too, a holistic survey on both the presence of other common infectious diseases and the antibiotics used in those circumstances will bring out further clarity on the presence of antibiotic residues in milk. The other factors such as veterinarians' preferences over different antibiotics in different regions, their advisories, and promptness of farmers in following those advisories strictly, non-scientific use of antibiotics by the para-veterinarians, etc. also play a role for the presence of residues in milk.

The study mapped high prevalence of subclinical mastitis, unawareness about drug withdrawal periods among animal handlers, and significantly higher prevalence of MDR residue in Bengaluru districts of Karnataka, India. To ensure milk safety, monitoring of residues at village level cooperatives by performing rapid tests is the preliminary step to reduce the burden of residues. Management of livestock with proper sanitation measures is especially important for the sustainable production without resorting to antibiotic use. Animal handlers, veterinarians and other non-qualified staff called quacks play a major role in animal production and raising awareness about the proper usage, disposal and withdrawal of antibiotics among all stakeholders. One Health approach is needed to ensure food safety which starts from farm to table through regular surveillance, enforcement of laws for drug prescription and selling, and continuous monitoring by regulating authorities.

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