World Journal of Dairy & Food Sciences 12 (1): 66-70, 2017
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2017.66.70

Study on Antimicrobial Residues in Bovine Bulk Milk from Bishoftu Dairy Farms, Central Ethiopia

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Abstract: A cross-sectional study was conducted to determine the prevalence and assess the level of awareness of dairy farm owners to avoid antimicrobial residues in bulk milk of cattle at Bishoftu town dairy farms. A total of 260 bulk milk samples were randomly collected in the respective study dairy farms. Questionnaire survey was conducted in all the study 26 dairy farm owners.The questionnaire was designed to obtain information on dairy farmers' knowledge and awareness that may lead to increased milk-borne risk of drug residue. Each milk sample was qualitatively screened for antimicrobial residues (regardless of its type) using the microbial inhibition plat test. A sample was positive if the zone of inhibition was 1cm and above. Out of 260 milk samples analyzed for antimicrobial residue 17(6.5%) were positive and the mean zone of inhibition level was 1.5cm. More than 85.5% of the respondents were not aware of the major managemental activities vital to avoid antimicrobial residues. The presence of drug residues in milk above tolerance level and lack of awareness and/or practical activity on avoidance of antimicrobial residual in the milk attained for human consumption is a public health hazard. Therefore all drugs given to dairy animals must be used for specific disease indications according to label recommendations and withdrawal periods. Giving awareness, teaching and training programs for farm owners and workers can reduce the public health risk of drug residue. Continuous surveillance should also be practiced by authorized governmental body.

Key words: Antimicrobial · Milk · Residue · Dairy · Bishoftu · Ethiopia

INTRODUCTION

Antibiotics have been used in the dairy industry for more than five decades. They are used in dairy cattle production primarily to treat or prevent disease and to a lesser extent to increase milk production or improve feed efficiency. Antibiotics used as growth promoters are administered at low doses for extended periods [1]. The therapeutic regimen is dictated by label instructions by the manufacturer or in accordance with extra-label instructions by a veterinarian. Antibiotics are administered to animals through injections (e.g., intramuscular, intravenous, or subcutaneous), orally, topically, or via intramammary or intrauterine infusion. Several types of antibiotics are commonly used in food animals [2].

The use of antibiotic 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. However, 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 [2].

Antibiotic residues are small amounts of drugs or their active metabolites which remain in milk after treating the cows. Problems associated with antibiotic residues in

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milk include the risk of allergic reactions after consumption by penicillin-sensitized persons, increased resistance of pathogens towards antibiotics, transfer of antibiotic resistant bacteria to human, carcinogenicity (Sulphamethazine, Oxytetracycline, Furazolidone), Mutagenicity, nephropathy(Gentamicin), hepatotoxicity, Bone marrow toxicity (Chloramphenicol) and inhibition of bacterial starter cultures used in dairy production [2].

The concerns arise mainly from the possibility that antibiotic-resistant bacteria transferred from animals to humans, through contact and the environment (e.g., water, manure) or through contaminated milk products [3]. Even though, different studies have been undertaken the Ethiopian dairy industry has not adopted any control programs to ensure the safety of the milk as expected. The drug residue limits, which apply to both the parent drug and its metabolites, need to be forced at all levels in Ethiopia dairy industry in order to protect the health of consumer. Therefore, the objectives of this study were:

- To determine the prevalence of antimicrobial residues in milk samples destined for human consumption.
- To assess the level of knowledge of the dairy farm owners about drug residues in milk and its public health hazard.

MATERIALS AND METHODS

Study Area: The study was conducted in dairy farms from Bishoftu town. The town is located at 9 ° N and 40 ° E It is 47 km south east of Addis Ababa, the Capital city of Ethiopia.

Study Population: The study populations were 30 intensively managed dairy farms in Bishoftu town, which have average herd size of 56 cattle and milk production per day per cow was ranging from 11-25 litres.

Study Design: The study design was cross-sectional and conducted from October, 2013 to May, 2014.

Sample Size Determination and Sample Collection: The sample size was calculated using the formula given by Thrusfield [4] for simple random sampling technique. An expected prevalence of 8.5% [6] 95% confidence interval and 5% absolute precision were considered during sample size determination. Accordingly the calculated sample size was 119 but to increase the precision the sample size was increased to 260 and equally divided to each study dairy farms.

Study Methodology: Milk samples were collected in each study dairy farm from their bulk milk. Each sample was labelled and accompanied by necessary identification information, which included date of sampling and identification code. All milk samples were transported under chilled conditions to the laboratory and stored at –20°C until laboratory analysis was conducted. Milk samplewas screened for antibiotic residues using the microbial inhibition plat test. The test was detected the presence of antimicrobial in milk attained for consumption [7].

Briefly, using *Bacillus subtilis* BGA strain as the test organism, 0.5% McFarland's standard suspension of the organism was prepared. This was used to inoculate the surface of Mueller-Hinton agar plates prepared at pH ¼ 7.0 and containing 0.2 mg/ml of trimethoprim. A sterile disc shaped paper was prepared of 2 mm thickness, which was immersed to milk sample which was applied on the surface of the agar medium.Positive control was set up with 1 mg/ml of tetracycline, while negative control set up with distilled water. The agar plates were incubated at 37°C for 18 to 24hr. After incubation a zone of inhibition of 1cm or more was considered a positive case of the milk sample containing drug residues. A positive control was expected to have zones of inhibition while the negative control was not expected to have any zone of inhibition [7].

Questionnaire Survey: The survey was included owners in all the study dairy farms who were interviewed using semi-structured closed and open-ended questions. Information included was mainly related to knowledge and awareness of dairy farms owner on milk drug residues and their public health hazards. Questions on major management practices used to avoid drug residue in milk also were included in the questionnaire.

Statistical Analysis: ?

RESULTS

Among the total 260 milk samples collected and tested for antimicrobial residues 17(6.5%) were found positive and the mean zone of inhibition was 1.5cm.

Table 1: Response to managemental aspects to avoid antimicrobial residues in milk

Questions	No. contacted	Respondents	Response status	
			Yes(%)	No(%)
Establish a valid veterinarian-client-patient relationship	30	26	2(7.7)	24(92.3)
Use only prescription (Rx) or drugs with veterinarian's guidance	30	26	6(23.1)	20(76.9)
Administer all drugs properly and identify all treated animals	30	26	4(15.4)	22(84.6)
Maintain and use proper treatment records on all treated animals	30	26	2(7.7)	24(92.3)
Implement employee/family training of proper drug use to avoid marketing adulterated milk and milk products	30	26	3(11.5)	23(88.5)
Use Drug Residue Screening Tests	30	26	2(7.7)	24(92.3)
Mean			3(11.5)	23(88.5)

Revise the highlighted figures

More than 88.5% of the respondents did not practice the major managemental activities that are important to avoid antimicrobial residues in milk in their farms. The summary of the questionnaire is indicated in Table 1.

DISCUSSION

A total of 260 milk sampleswascollected from 26 intensively managed dairy farms. Of which 17 (6.5%) were found positive for antimicrobial residue in milk attained for consumption. The finding of this study ishigher than 0.08-0.26% reported in Sweden by Sternesjo and Johnsson [8], 4.3% in Brazil by Borges *et al.* [9] and 4% in Kenya by Ekuttan *et al.* [10]. It isn't a slight difference in this critical point (Antibiotic residues & human health??) However our finding lower than 8.5% reported in Ethiopia by Abebew [6] and much lower than 25.5% reported in Iran by Babapour and Fartashmehr [10]. The higher prevalence of antibiotic residue reported by the previous studies in Ethiopia and Iran could be associated with their larger sample size and use of highly sensitive drug residue detection methods.

The questionnaire survey used to assess the knowledge of dairy farm owners in this study included issues that were helpful to gain insights into farm management practices associated with antimicrobial usage. The proportions reported for each managemental practices indicated in table 1 were much higher than the findings of studies conducted in Ethiopia and other countries by Abebew [6], Kaneene and Ahl's [12], Workineh *et al*. [13] and Tesfaye [14].

As it was indicated in this study 92.3% of dairy owners did not establish a valid veterinarian-client-patient relationship so thataround 76.9 % dairy farms hadno knowledge about the drug they have been used and did not use prescribed drugs. This finding ishigher than the finding of Tesfaye [14] who reported 3.9%. In this study 92.3% of dairy farms hadno proper treatment records on all treated animals and hence the owners have no information about the effect of antibiotic residue on human health. Only 7.7 % of the respondents were keeping with drawl period of drug when they were thought by drug administered. Nearly around 10.4% respondents reported that they have Knowledge of effect of antibiotic residue on human being. The use of antibiotics continues to be a predominant in the treatment and control of disease [15].

Health services were given mostly by the practitioners coming to the farms or sometimes by taking the animals to veterinary clinics. Regular health programs by professionals were not practiced, but in general, it can be hypothesized that all farms had access to health services provided by professionals when needed [6]. 23.1% of the farmers used only prescribed drug. Other than the veterinarian, 76.9% of dairy farms administer drug primarily by owners or herdsmen.

Only 7.7% of the farms surveyed kept records of antibiotic treatment that could be verified. According to Kaneene and Ahl's [12] survey of dairy producers in Michigan, USA indicated that insufficient record keeping and poor knowledge about drug withdrawal periods among producers were important factors leading to drug residues in milk.

About 7.8% of dairy farms had joinedImplement training of proper drug use to avoid marketing adulterated milk and milk products and according to Mercer *et al.* [16] the presence of antibiotic residues in milk is strongly associated with several variables Such as milk production at time of treatment, type and amount of antibiotic used, type of vehicle used in antibiotic formulations and the disease state of the animal.

Around 7.7% of dairy farms used drug residue screening tests before distribution milk attained for human consumption. The use of antibiotics in food animals has caused concern regarding the impact of them on human health. Although the public health risks are difficult to

define, it is accepted that antibiotic residues may induce allergic reactions in sensitized individuals and may have negative effects on the composition of the human intestinal flora [17].

Approximately 92.3% of dairy farms did not test milk attained for human consumption before distribution which, may led to the development of multi-drug resistance in human pathogens [17]. Furthermore, milk contaminated with even low concentrations of antibiotics residues may also create problems in the production of fermented milk by products, because such compounds inhibit the growth of the starter culture. Therefore, drug residues remain very significant from the prospective of international trade and consumer confidence because it results in international trade barrier [18].

To increase considerable foreign currency, milk and milk products need to be fit the requirement by World Trade Organization (WTO) and Codex Alimentarius Commission (CAC) should be adhered. One of the Requirements is that antibiotics residues in food should be below MRLs (Maximum residue limits). But, the indiscriminate use of veterinary drugs can hinder the country's interest to fulfill the need to export to those WTO Member countries. Therefore, attempts should be made to reduce the magnitude of the problem at various levels through the prudent use of antibiotics. Awareness need to be created at different levels including controlling authorities, concerned organizations and the consumers.

One basic limitation to conduct risk analysis in this method is inability to detect the exact amount of chemical residues in dairy cow products, because without accurate detection, exact risk is impossible to assess. Despite limitation of method used in this study it is recommended that this process needs highly qualified expertise, sensitive instruments and modern analytical techniques. High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) and Mass Spectrometry (MS) are sensitive instruments while Solid Phase Micro-extraction (SPME) and Microdialysis are modern analytical techniques used for residue analysis.

CONCLUSIONS

This study showed high prevalence of antimicrobial residues in Bishoftu town dairy farms and more than 88.5% of the farm owners' lacked proper management practices or awareness to avoid drug residues in milk and to prevent human health hazards.

Therefore, based on the above conclusions the following recommendations are forwarded.

- Rapid screening procedures of antibiotic residues and instant grading and prohibition of food containing antibiotics more than maximum residual level should be applied to ensure the production of antimicrobial residue-free milk.
- Veterinarians should follow strict regulation concerning the use of antibiotics in livestock industry, inspect cattle for residues prior and after animal products are prepared for human consumptions and ensure that these are implemented at all level.
- Concerned authorities should undertake appropriate education program for milk producers, extension workers and consumers to create awareness of the problem of antimicrobial residues caused by misuse of veterinary drugs.
- In depth and wide area covering study should be carried out to determine the magnitude of the problem in order to safeguard the consumer from various effects of antimicrobial residues.

Competing Interests: The authors declare that they have no competing interests.

ACKNOWLEDGMENT

The authors would like to thanks all the Addis Ababa University college of veterinary medicine and agriculture staff members and Institute of biodiversity for their kind cooperation, liberal help in provision of material and generous permission to use their laboratory at any time and all dairy owners for their collaboration.

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