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Detection of Antimicrobial Drug Residues in Raw Cow Milk samples in Logar and Maidan Wardak Province, Afghanistan

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ABSTRACT

Background: Nowadays, various types of antibiotics are being used worldwide in the veterinary sector particularly, for the promotion of growth and treatment of livestock. Significant portions of antibiotics are released through the milk of dairy animals inviolate and exert serious harmful effects on human health. Milk that utilizes by human consumption should also comply with safety criteria regarding antibiotic residues. The objective of this study was to determine the level of antibiotic residues in raw milk conferred by the milk producer's cooperatives of Logar and Maidan Wardak provinces to Guzargah dairy union for further processing and distribution.

Materials and Methods: In this research 110, raw cow milk samples for a one-year period were collected and analyzed (n=110). 55 samples were collected from Logar province and 55 samples were collected from Maidan Wardak province. Milk samples were tested by Betastar Combo Test Kit. All samples considering the laboratory procedures were transported to the laboratory and tested for the presence of Tetracycline and Beta lactams as these antibiotics are widely used for the treatment of bovine mastitis.

Finding: Out of 110 milk samples, 12 samples were positive for the presence of Tetracycline residues and 7 samples were positive for the presence of Beta lactams. For further accuracy of the result, all the positive samples were tested by Microbial Inhibition Assay (MIA), and the same result is obtained.

Conclusion: The study results show and indicate that antibiotic residues are present in the samples which paves the way for the contamination of human food chains. Considering that milk is mostly consumed by the elderly and kids, hence, it can be the main concern for public health.

Keywords: Cow, Milk, Antibiotic residues, Human health.

INTRODUCTION

Food is necessary for humans to perform a number of tasks throughout their lives, including growth, reproduction, energy production, maintaining their health, and recovering from illnesses. There are various food sources that fulfill these purposes. Milk is one of the healthiest naturally occurring foods available to humans (Farzana et al., 2009). The natural secretion of milk is obtained from the mammary glands of healthy milk-producing animals; no additional materials or extractions are used (Sharma et al., 2012). Colostrum should not be present in the milk, and it is crucial to make sure that milk from different classifications and labels meets the requirements of Codex Alimentarius Commision (Asfaw et al., 2023). The milk's overall urea content shouldn't

be higher than 700 ppm (Chebel, 2021). The process of making milk and products is supported scientifically by the chemical composition of milk and its physicochemical behavior (Zhang et al., 2020).

Antibiotics are one of the many chemical, physical, and microbiological contaminants that can contaminate milk and dairy products. One of the most frequent pollutants found in milk and dairy products has the ability to kill bacteria and stop their growth even at lower quantities (Hossain et al., 2021). Antibiotic residues are the results of certain antibiotics that build up in the body when animals get topical or oral prescriptions. Antibiotic residues of this type have been detected in bodily fluids such milk, muscle tissue, and blood (Oliver et al., 2020).

Antibiotics are commonly employed in the treatment of many ailments, including mastitis, which is a prevalent ailment among dairy cows Beta-lactam, tetracycline, sulfonamides, macrolides, and aminoglycosides are the primary classes of antibiotics (Dong et al., 2021). These chemicals are utilized widely as food supplements, growth enhancers, and to improve the quality of food (instead of using antibiotics) (Tanzin et al., 2016). The presence of antibiotic residues in milk and animal meat and their transfer to the human body have negative repercussions that include hygienic, industrial, and economic issues due to rising of resistant strains to these antibiotics (Straley et al., 2006).

The sterile effects of antibiotics include the rising and spread of resistant bacteria(Pant et al., 2013). the body's inability to respond to prescribed dosages, a variety of allergic reactions, particularly in those who are susceptible to them, the inability to detect pathogenic microorganisms in the lab, the disruption of the body's natural microflora, the effects of carcinogenesis, human mutation, and the inability to synthesize certain vitamins (Hansson et al., 2020). Among the industrial side effects of antibiotics, it is possible to mention preventing the action of yeasts or yeasts that are used to produce dairy products such as yougurt and cheese, the destruction of useful bacteria that are used in the production of yogurt, cheese, and other fermented products, inhibition of lactic acid producing bacteria and the negative effects of fermented dairy products (Reuben et al., 2020). From an economic point of view, the presence of antibiotic residues in food products from animals causes the imbalance of these types of products (Yang et al., 2021). In addition to causing pessimism in consumers, it also affects the market for selling animal products and health concerns.

Drug resistance, according to the Centers for Disease Control and Prevention (CDC), is one of the most significant health issues. According to recent research, a number of bacteria that are resistant to antibiotics is rising and certain bacterial infections are not improving when antibiotic therapy is used (Bartkiene et al., 2020).

Since there is unclear low of health issue assesment of dairy products in Afghanistan specifically for antibiotics usage. This study conducted to address this challenge and to identify the antibiotic residues including Tetracycline and Beta-lactam in raw cow’s milk collected from the Kabul Guzargah Dairy Union from Logar and Maidan Wardak provinces. As a significant part of Kabul market dairy products are originated of these neighbor provinces. This study conducted based on the two main hypothses H0: There is no significant availability and impact of the antibiotics in the milk samples H1: There is a significant availability and impact of antibiotics in the milk samples. To address the problems the following objectives have been attempted to: **a)** identification and determination of Tetracycline and Beta-lactam antibiotic residues in the raw milk of cows. **b)** Information about the misuse of antibiotics (Tetracycline and Beta-lactam antibiotic residues in the raw milk of cows).

MATERIALS AND METHODS

Sample collection

The duration of the current study was one year (August 23, 2021– August 06, 2022). Samples were collected from two provinces Logar and Maidan Wardak. There are dairy farmer cooperatives in both provinces and the union supported and linked these farmers to the MCCs (Milk Collection Centers). The collected milks then conferring to the union in transportation containers each container may consist of milk coming from different farmers, therefore each collected milk sample not related to individual dairy cow. The samples were collected 3 times in a season within four seasons (12 time/year with a total sample 110 (n=110)). The place collection samples were Guzargah Dairy Union to identify the presence of Beta-lactam and Tetracycline antibiotic residues.

Antimicrobial Drug Residues test

All the samples have been collected in a sterilized and separated container and have been transferred to the animal health diagnosis and research laboratory located in Darul Aman of Kabul. The milk samples were tested according to the procedure given by the kit manufacturer company (BALLYA). Beta-lactam + Tetracycline Combo Test Kit is a two-sensor kit, it's rapid to detect two types of antibiotic residues in milk and dairy products by using colloidal gold immunochromatography technology. two kits have been used and each kit had 96 tests. As soon as 7-10 minutes to result the samples categorized as negative and positive by the observation. When the samples were completely mixed with the reagent the reagents were left to stand for 5 minutes. After the mentioned time, the strips were carefully removed from their box and placed into the sample and reagent mixture. Five minutes are given again for the expected reactions to occur. To see the results, the red lines are read considering the intensity of the lines compared to the control line. If the intensity of the antibiotic test lines (beta-lactam and tetracycline) were darker or equal to the control line, the milk sample is recorded as negative or free of antibiotic residues. In the same way, if there was no line in the strip, the milk sample is positive or contains antibiotic residues (Zanella et al., 2010) and is considered positive. The data were analyzed by Cochran's Q test to determine the efficacy and significance of the antibiotic residues in Milk samples.

Statistical Analysis

Analysis of variance (ANOVA) was performed through statistics 10 and IBM SPSS statistics 21 to test the Cochran's Q test for all groups. Beta-lactam + Tetracycline Combo Test Kit is used to find the level and presence of antibiotic residues in milk and dairy products, and data analyzed based on F-ratio and P-value (α=0.05, P<0.05).

RESULTS

Beta-lactam and Tetracycline detection

To test the hypothesis, the Cochran's Q test applied to determine the efficacy and significance of the antibiotics in the milk samples collected from two random areas. A total of 110 samples of milk have been collected from the sites, and accordingly, all the samples were tested in the lab to find out the condition of the availability of the antibiotics in the samples. Two of the essential categories of antibiotics were applied: Beta-lactam and Tetracycline. Table 1 depicts the general descriptive statistics of the 110 samples categorized into three parts: Beta-lactam, Tetracycline, and the number of positive samples was crosschecked by the Microbial Indication Assay (MIA). As a confirmatory test in this study we have used the Microbial Inhibition Assay

method for the rapid screening of antibiotics in milk. Only the positive samples retested by the MIA. However, the result of data in Table 1, demonstrates that out of 110 samples, a total of 103 did not have the Beta-lactam antibiotics indicating a value of 1.

Overall, only 7 of the samples contained the mentioned antibiotic residues in the milk samples. while, Tetracycline consists of 98 samples that indicate a negative sign of the antibiotics, and only 12 of the samples had a positive sign. It is worth mentioning that among the 19 positive samples 4 samples contained both mentioned groups of antibiotics, therefore, 15 contaminated samples crosschecked by Microbial Inhibition Assay depicts that 15 of the samples had the positive sign and were tested with the antibiotics of either category.

Table 1: Frequency of the milk collected samples for two antimicrobial drug assay Beta-lactam and Tetracycline against Microbial Inhibition Assay.

Frequencies	Value	
	1 (-)	2 (+)
Beta-lactam	103	7
Tetracycline	98	12
Microbial Inhibition Assay	95	15

Data analysis in Table 2 depicts that the N value is 110, meaning there are 110 samples in the test. Cochran's Q test value is 8.909, having a degree of freedom value of 2 (i.e., n-1 = 2). Considering the above decision criteria, the Asymp. The significance value, a test statistics value at 95% L.O.S., is only 0.012. This means that this 0.012<=0.05.

Table 2. depicts the test statistics value using Cochran's Q non-parametric test. The hypothesis for the testing of the antibiotic's availability and efficacy.

N	110
Cochran's Q	8.909a
Df	2
Asymp. Sig.	.012
Exact Sig.	.010
Point Probability	.006

The numeric state that the null hypothesis of having no significant availability and impact of the antibiotics in the milk samples is rejected, and the alternative hypothesis of having a significant availability and impact of the antibiotics in the milk samples is retained. In a nutshell, the random samples randomly collected from the two sites demonstrate that the milk has the two mentioned antibiotics and is showing a significant value. Meanwhile, the summary of the hypothesis testing under Cochran's Q test is depicted in Table 3, 4 and 5, which indicates that the null hypothesis at a 0.012 significance value is rejected.

Table 3. Hypothesis Test Summary that the samples are significant to address the second hypothesis (H1).

Null Hypothesis	Test	Sig.	Decision
The distributions of Beta-lactam and Tetracycline are the same.	Related-Samples Cochran's Q Test	.012	Reject the Null Hypothesis

Table 4. number, percentage, and frequency of Beta-lactam antimicrobial drug in 110 collected samples

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative	103*	93.6	93.6*	93.6
	Positive	7	6.4	6.4	100.0
	Total	110	100.0	100.0	

Table 5. number, percentage, and frequency of antimicrobial drug Tetracycline in 110 samplse of the two province

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative	98*	89.1	89.1*	89.1
	Positive	12	10.9	10.9	100.0
	Total	110	100.0	100.0	

The result of kit test shows the importance of the rapid detection of antibiotic residues in milk by using the Betastar combo kits with excellent sensitivity (the kit used in this study is used worldwide). This study shows that it is possible to identify antibiotic residues with this method easily and in a short time. In this current study, 110 samples of fresh cow's milk were evaluated to identify the residues of tetracycline and beta-lactam antibiotics, as a result, 19 samples were found positive. Among the positive samples, 12 samples (10.9%) contained tetracycline residues, and 7 samples (6.4%) contained beta-lactam antibiotic residues (Table 6). It should be mentioned that among the 19 positive samples, 4 samples were determined to contain the residues of both common antibiotics (Tetracyclines and Beta-lactams).

Table 6. Number and percentage of contamination of milk samples with residues of tetracycline and beta-lactam antibiotics.

Province	Samples	The number and percentage of positive samples in each province.			
		Tetracycline	%	Beta-lactam	%
Logar	55	8	14.5	3	5.4
Maidan Wardak	55	4	7.2	4	7.2

Out of the collected samples of raw cow's milk from the Guzargah Dairy Association were analyzed for the presence of tetracycline and Beta-lactam antibiotic residues. From the total of 55 samples of Logar Province, 11 samples were evaluated positive for the presence of tetracycline antibiotic residues and 3 samples for the existence of Beta-lactam antibiotic residues. Meanwhile, out of 55 samples from Maidan Wardak province, 8

samples were positive, of which 4 samples contained tetracycline antibiotic residues and 4 samples contaminated with Beta-lactam antibiotic residues were evaluated as positive.

DISCUSSION

Unfortunately, in Afghanistan there have been no studies that can detect the presence of residues of a specific group of antibiotics in milk, but there is a facility to identify antibiotics using the Microbiological Inhibition Assay (MIA) method at the Animal Disease Research and Diagnosis Institute located in Darul Aman, Kabul. But in this research, the above-mentioned method was used only to confirm positive samples. The difference and advantage of this research is the possibility of the mention group of antibiotics identification, and the same research conducted in other countries.

This study conducted to investigate the residues of tetracycline and Beta-lactam antibiotics in the central regions of Afghanistan mainly wardak and logar states cow's milk. Using the combo test kit method, over the component of milk, the 14 milk samples of cow's milk, 3 (21.43%) and 2 samples (40%) of 5 samples of goat's milk were detected positive for the presence of residues of Beta-lactam antibiotics and at the same time 2 samples (14.2%) of cow's milk and 1 sample (20%) goat milk has been identified positive for tetracycline antibiotics. Meanwhile, my findings include 6.4% Beta-lactam antibiotic residues and 10.9% tetracycline residues and show a lower percentage of contamination with the residues of both antibiotics.

In the same way, in another research that was conducted in Romania in Bucharest in 2015, raw milk samples were collected from cattle farms and milk processing factories, so 210 raw milk samples were collected during 2015 from January to December (Bakshy et al., 2021). From the total 210 samples, 114 samples were collected from milk processing factories and 96 samples were collected from cattle farms and were evaluated by the Beta Star Combo test to detect the residues of Beta-lactam and tetracycline antibiotics. In this test, 66 samples (31.42%) were evaluated positive for the residues of Beta-lactam and tetracycline antibiotics.

While in the research I conducted, the percentage of contamination with both groups of Beta-lactam and tetracycline antibiotics was evaluated (17.3%) and this shows that the percentage of contamination with the residues of the mentioned antibiotics in his study is relatively higher. Similarely the study was conducted in 2018 in Thrissur, India, on 165 milk samples (Khosravi et al., 2021). All the samples have been evaluated by Microbial Inhibition Assay (MIA) and Charm MRLBLTET (Beta Star Kambokit). Contamination with the residues of tetracycline and Beta-lactam antibiotics in the milk of cows in Logar and Maidan Wardak provinces has been evaluated much more.

In addition the same study conducted in the Gilan region of Mashhad, Iran (Hassani et al., 2022), to check the residues of Beta-lactam group antibiotics, a total of tested 114 samples of raw milk and found the antibiotic residues (20.17%) positive. Furthermore, the study conducted by Shitandi and his colleagues in 2001 in Kenya to investigate the residues of Beta-lactam antibiotics in raw milk, he tested 100 milk samples and reported the percentage of antibiotic residues (21%) as positive (M El-Zubeir & El Owni, 2009). He states that in developed countries antibiotic residues are seen in the milk of animals that have been treated with excessive amounts of antibiotics. The difference between previous research and this study on both Beta-lactam and tetracycline antibiotics the percentage of positive cases of Beta-lactam antibiotic residues in this study is lower (6.4%). It can be positively eualuated by the ministry of poblic health in Afghanistan results getting down the spread level of resistant pathogenes.

CONCLUSION

Tetracycline and beta-lactam antibiotic residues were found to be significantly high in the milk of both of the provinces that were the focus of this investigation. This finding underscores the significance of the problem for public health due to rising of resistance pathogenic microorganisms. The most significant risks associated with consuming these products include the development of microbial resistance against antibiotics. The misuse of antibiotics by livestock farmers and animal breeders is on the rise, and the presence of antibiotic residues in milk and other products can have a negative impact on consumers.

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REFERENCES

Asfaw, T., Genetu, D., Shenkute, D., Shenkutie, T. T., Amare, Y. E., Habteweld, H. A., & Yitayew, B. (2023). Pathogenic Bacteria and Their Antibiotic Resistance Patterns in Milk, Yoghurt and Milk Contact Surfaces in Debre Berhan Town, Ethiopia. *Infection and Drug Resistance*, *16*, 4297–4309. https://doi.org/10.2147/IDR.S418793

Bakshy, K., Heimeier, D., Schwartz, J. C., Glass, E. J., Wilkinson, S., Skuce, R. A., Allen, A. R., Young, J., McClure, J. C., Cole, J. B., Null, D. J., Hammond, J. A., Smith, T. P. L., & Bickhart, D. M. (2021). Development of polymorphic markers in the immune gene complex loci of cattle. *Journal of Dairy Science*, *104*(6), 6897–6908. https://doi.org/10.3168/jds.2020-19809

Bartkiene, E., Lele, V., Sakiene, V., Zavistanaviciute, P., Ruzauskas, M., Stankevicius, A., Grigas, J., Pautienius, A., Bernatoniene, J., Jakstas, V., Zadeike, D., Viskelis, P., & Juodeikiene, G. (2020). Fermented, ultrasonicated, and dehydrated bovine colostrum: Changes in antimicrobial properties and immunoglobulin content. *Journal of Dairy Science*, *103*(2), 1315–1323. https://doi.org/10.3168/jds.2019-16357

Chebel, R. C. (2021). Associations between days on close-up diets and immune responses prepartum, metabolites peripartum, and risk of postpartum diseases in Jersey cows. *Journal of Dairy Science*, *104*(6), 7135–7153. https://doi.org/10.3168/jds.2020-19700

Dong, L., Meng, L., Liu, H., Wu, H., Hu, H., Zheng, N., Wang, J., & Schroyen, M. (2021). Effect of therapeutic administration of β-lactam antibiotics on the bacterial community and antibiotic resistance patterns in milk. *Journal of Dairy Science*, *104*(6), 7018–7025. https://doi.org/10.3168/jds.2020-20025

Farzana, K., Akhtar, S., & Jabeen, F. (2009). Prevalence and antibiotic resistance of bacteria in two ethnic milk based products. *Pakistan Journal of Botany*, *41*(2), 935–943.

Hansson, P., Holven, K. B., Øyri, L. K. L., Brekke, H. K., Gjevestad, G. O., Rehfeld, J. F., Raza, G. S., Herzig, K. H., & Ulven, S. M. (2020). Dairy products influence gut hormone secretion and appetite differently: A randomized controlled crossover trial. *Journal of Dairy Science*, *103*(2), 1100–1109. https://doi.org/10.3168/jds.2019-16863

Hassani, S., Moosavy, M. H., Gharajalar, S. N., Khatibi, S. A., Hajibemani, A., & Barabadi, Z. (2022). High prevalence of antibiotic resistance in pathogenic foodborne bacteria isolated from bovine milk. *Scientific Reports*, *12*(1), 1–10. https://doi.org/10.1038/s41598-022-07845-6

Hossain, M. I., Kim, K., Rahaman Mizan, M. F., Toushik, S. H., Ashrafudoulla, M., Roy, P. K., Nahar, S.,

Jahid, I. K., Choi, C., Park, S. H., & Ha, S. Do. (2021). Comprehensive molecular, probiotic, and quorum-sensing characterization of anti-listerial lactic acid bacteria, and application as bioprotective in a food (milk) model. *Journal of Dairy Science*, *104*(6), 6516–6534. https://doi.org/10.3168/jds.2020-19034

Huang, M. S., Cheng, C. C., Tseng, S. Y., Lin, Y. L., Lo, H. min, & Chen, P. W. (2019). Most commensally bacterial strains in human milk of healthy mothers display multiple antibiotic resistance. *MicrobiologyOpen*, *8*(1), 1–11. https://doi.org/10.1002/mbo3.618

Khosravi, M., Nouri, M., Mohammadi, A., Mosavari, N., & Constable, P. D. (2021). Preparation of immunomagnetic beads coupled with a rhodamine hydrazine immunosensor for the detection of Mycobacterium avium subspecies paratuberculosis in bovine feces, milk, and colostrum. *Journal of Dairy Science*, *104*(6), 6944–6960. https://doi.org/10.3168/jds.2020-18819

M El-Zubeir, I. E., & El Owni, O. (2009). *Antimicrobial Resistance of Bacteria Associated with Raw Milk Contaminated by Chemical Preservatives Value chain and processing of camel milk functional food View project Risk Assessments in Food Safety and Health (RAFaH) View project*. *December*. https://www.researchgate.net/publication/265192183

Oliver, J. P., Gooch, C. A., Lansing, S., Schueler, J., Hurst, J. J., Sassoubre, L., Crossette, E. M., & Aga, D. S. (2020). Invited review: Fate of antibiotic residues, antibiotic-resistant bacteria, and antibiotic resistance genes in US dairy manure management systems. *Journal of Dairy Science*, *103*(2), 1051–1071. https://doi.org/10.3168/jds.2019-16778

Pant, R., Nirwal, S., & Rai, N. (2013). Prevalence of antibiotic resistant bacteria and analysis of microbial quality of raw milk samples collected from different regions of Dehradun. *International Journal of PharmTech Research*, *5*(2), 804–810.

Reuben, R. C., Roy, P. C., Sarkar, S. L., Rubayet Ul Alam, A. S. M., & Jahid, I. K. (2020). Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *Journal of Dairy Science*, *103*(2), 1223–1237. https://doi.org/10.3168/jds.2019-17092

Sharma, D., Kumar Sharma, P., Singh Saharan, B., Malik, A., & Saharan, B. S. (2012). Isolation, Identification and Antibiotic Susceptibility Profiling of Antimicrobial Resistant Listeria monocytogenes from Dairy Milk Sustainable development by bioplastics and biofuels production View project Biomolecules production View project Isolation, Identification and Antibiotic Susceptibility Profiling of Antimicrobial Resistant Listeria monocytogenes from Dairy Milk. *International Journal of Microbial Resource Technology*, *1*(1), 1–4. https://www.researchgate.net/publication/268060973

Straley, B. A., Donaldson, S. C., Hedge, N. V., Sawant, A. A., Srinivasan, V., Oliver, S. P., & Jayarao, B. M. (2006). Public health significance of antimicrobial-resistant gram-negative bacteria in raw bulk tank milk. *Foodborne Pathogens and Disease*, *3*(3), 222–233. https://doi.org/10.1089/fpd.2006.3.222

Tanzin, T., Nazir, K. H. M. N. H., Zahan, M. N., Parvej, M. S., Zesmin, K., & Rahman, M. T. (2016). Antibiotic resistance profile of bacteria isolated from raw milk samples of cattle and buffaloes. *Journal of Advanced Veterinary and Animal Research*, *3*(1), 62–67. https://doi.org/10.5455/javar.2016.c133

Yang, X., Wang, Z., Zhang, C., Wang, L., Pang, L., Zhang, D., Man, C., & Jiang, Y. (2021). Assessment of the production of Bacillus cereus protease and its effect on the quality of ultra-high temperature-sterilized whole milk. *Journal of Dairy Science*, *104*(6), 6577–6587. https://doi.org/10.3168/jds.2020-19818

Zanella, G. N., Mikcha, J. M. G., Bando, E., Siqueira, V. L. D., & MacHinski, M. (2010). Occurrence and antibiotic resistance of coliform bacteria and antimicrobial residues in pasteurized cow's milk from Brazil. *Journal of Food Protection*, *73*(9), 1684–1687. https://doi.org/10.4315/0362-028X-73.9.1684

Zhang, J., Yang, M., Cai, D., Hao, Y., Zhao, X., Zhu, Y., Zhu, H., & Yang, Z. (2020). Composition, coagulation characteristics, and cheese making capacity of yak milk. *Journal of Dairy Science*, *103*(2), 1276–1288. https://doi.org/10.3168/jds.2019-17231