

Qualitative analysis of antibiotic residue and antibiotic resistance testing of *E.coli* isolated from traditionally heat-treated fresh milk sold in local restaurants of Bujumbura City, Burundi

IRIBAGIZA Albert^{1*}, GWIMILE Elina Simon^{2*}, NGANGA Peter Mathias⁴, NKUNDWANAYO Canésius³, NTAKIRUTIMANA Désiré³, NTIRANDEKURA Jean Bosco¹, NISHEMEZWE Gérard¹, NTUNZWENIMANA Mélance^{1,2}, NIYOKWIZERA Pascal³

¹University of Burundi, Faculty of Agronomy and Bio-Engineering, Department of Animal Health and Productions, research center : CRAVE; B.P. 2940. Bujumbura, Burundi.

²University of Burundi, East Africa Nutritional Sciences Institute

³Ministère de l'Agriculture, Elevage et de l'Environnement, Direction Général de l'Elevage, Direction de la Santé Animale, Laboratoire National Vétérinaire (Burundi)

⁴Sokoine University of Agriculture.

*Corresponding Author

IRIBAGIZA Albert &
GWIMILE Elina Simon

University of Burundi,
Faculty of Agronomy and
Bio-Engineering,
Department of Animal
Health and Productions,
research center: CRAVE;
B.P. 2940. Bujumbura,
Burundi.

Article History

Received: 02.10.2024

Accepted: 19.11.2024

Published: 17.12.2024

Summary: Cow milk could be used to reduce malnutrition since it is highly nutritious, and many people in Bujumbura city for survival. However, the risk of food contamination by antibiotic residues is one of the significant problems facing public health, and is a result of the irresponsible use of veterinary drugs. This study evaluated antibiotic residues and resistance in milk samples to assess contamination levels and resistance patterns among *E. coli* isolates. Cross section study design and purposive sampling method was used in selection of area and sample collection. Thirty samples from traditionally heat-treated fresh milk sold in local restaurants were collected each 250mls and transported to National Veterinary Laboratory of Burundi. The detection of antibiotic residue was done by using SNAP duo Test Plus test (IDEXX) which detects beta lactams and tetracycline residue including cephalexin at or below established maximum residue limits. Out of 30 milk samples tested, 16.7% were positive for antibiotic residues, with beta-lactam detected in one sample and tetracyclines in four. Although contamination levels were relatively low, the findings raise concerns about improper veterinary drug management at the farm level. Antibiotic resistance analysis revealed high resistance among *E. coli* isolates to Ampicillin and Tetracycline, commonly used in the dairy industry and human medicine. Frequent usage of these antibiotics creates selective pressure, promoting the survival of resistant strains. The study underscores the critical need for stricter regulations and improved antibiotic management practices in dairy farming to reduce contamination risks and curb the spread of antibiotic-resistant bacteria. Enhanced monitoring systems and responsible handling of veterinary drugs are essential to ensure food safety and protect public health.

Keywords: Antibiotic residue, Antibiotic resistance, *E.coli*, Heat-treated fresh milk, Local restaurant, Bujumbura city, Burundi.

Cite this article:

IRIBAGIZA, A., GWIMILE, E. S., NGANGA, P. M., NKUNDWANAYO, C., NTAKIRUTIMANA, D., NTIRANDEKURA, J. B., NISHEMEZWE, G., NTUNZWENIMANA, M., NIYOKWIZERA, P., (2024). Qualitative analysis of antibiotic residue and antibiotic resistance testing of *E.coli* isolated from traditionally heat-treated fresh milk sold in local restaurants of Bujumbura City, Burundi. *ISAR Journal of Agriculture and Biology*, 2(12), 5-9.

1.0 Introduction

European Union (EU) and the Centre for Veterinary Medicine, an agency under the Food and Drug Administration (FDA/CVM) in the United States, defines Antibiotic residue as “pharmacologically active substances (whether active principles, recipients or degradation products) and their metabolites which remain in

foodstuffs obtained from animals to which the drugs in question have been administered” (FAO/WHO, 2021).

Veterinary drugs include various classes of therapeutic medicines used to treat a variety of diseases occurring in food-producing animals, including the control of endoparasites and ectoparasites. The most commonly used antimicrobial drugs are antibiotics used

to control mastitis and rickettsia diseases in dairy cattle these includes group of tetracycline's and group of beta lactams (Hansen, 1974).

Contamination of milk products with antibiotic residue is significantly influenced by the general public's ignorance of antimicrobial resistance (AMR), which is caused by the buildup of antibiotic residues in food of animal origin and their ignorance of the adverse health consequences of AMR (Paludetti et al., 2019; Virto et al., 2022). The activities includes increased uncontrolled use of antibiotics without a prescription from a veterinary doctor and less attention to following safety regulations during animal treatment and lack of appropriate medical monitoring of dairy farms with no prophylaxis or surveillance program for tuberculosis, brucellosis and mastitis(Albert et al., 2024; Rahman et al., 2021).

Antimicrobial resistance is a major threat to human development as it affects our ability to treat a range of infections (WHO, 2018). The existence of resistant bacterial strains produces severe health consequences on the human body, these reasons make it important to effectively control antibiotic residues in animal-derived food products (Getahun et al., 2023). The efficacy of commonly used antimicrobials is threatened because of AMR among pathogens; for example, *Acinetobacter species*, *Pseudomonas species*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Staphylococcus aureus*, *Streptococcus pneumoniae* have become resistant to key antimicrobials(WHO, 2018).

In Burundi, despite the socio-economic significance and health benefits of milk and dairy products, there is a lack of interest among researchers in this sector and monitoring and inspection focusing on antibiotic residue by the regulation body is poor (Albert et al., 2024; Kang et al., 2021). Most existing studies focus primarily on enhancing the productivity of dairy cows as mentioned (Bitama & Ndayishimiye, 2023; Michael, 2016; Ouma & Godfrey, 2020). Hence, this study will focus on qualitative analysis antibiotic residue and Isolate *E.coli* and test its antibiotic susceptibility profile as it is often used as an indicator of fecal contamination in food and water.

The findings will also inform best practices in dairy farming and support public health initiatives against antibiotic resistance, contributing to food safety, regulatory standards, and consumer trust.

2.0 Material and Methods

2.1 Study area

This study was carried out in Bujumbura city located around 3°23'S 29°22'E. Bujumbura as one of provinces of Burundi, is divided three communes (as of 2014), which are sub-divided into 13 neighborhoods (per Ministerial Order No. 530/1279 of 22 September 2005). These communes includes Muha, Ntahangwa and Mukaza.

Bujumbura is the economic capital, largest city and main port of Burundi. Bujumbura is on the north-eastern shore of Lake Tanganyika, the second deepest lake in the world after Lake Baikal. The city also lies at the mouth of the Ruzizi River and the smaller Mutimbuzi, Ntahangwa, Muha and Kanyosha Rivers. From 2023 data the urban population ranges 1,143,202 and density of 9,000/km² (23,000/sq mi).



Fig 1: Map of Burundi, With Bujumbura city highlighted in red (www.istockphoto.com)

2.2 Study and sample design.

Cross section study design and purposive sampling method was used in selection of area and sample collection. Thirty samples were collected each 250mls of heat-treated fresh milk and transported to National Veterinary Laboratory of Burundi, the temperature condition was maintained at 4°C to 8°C, and analysis was carried out with 6 to 8 hours after the arrival. This study was carried out from month of July to October 2024.

2.3 Analysis of Antibiotic residue.

The detection of antibiotic residue was done by using SNAPduo Test Plus test (IDEXX) which detects beta lactams and tetracycline residue including cephalixin at or below established maximum residue limits (Alves et al., 2020).

The procedure was carried out according to manufacturer provided the kit manual (Laboratories, 2016). Approximately 1 milliliter of milk was collected using a pipette from the test kit and transferred into a tube provided with the kit, where it was swirled to homogenize mixture. The sample was then poured into the test device, and the active circle was observed until it turned half-blue, at which point it was snapped. After waiting for 6 to 8 minutes, the results were compared with the provided reference table.

It should be noted that; when every test was conducted, oxytetracycline was used in parallel as control to ensure proper functioning of the kit.



Figure 2: Antibiotic residue testing

Result Interpretation: If the sample spot was lighter than the control spot, the sample was positive. If the sample spot was equal to or darker than the control spot, the sample was negative.

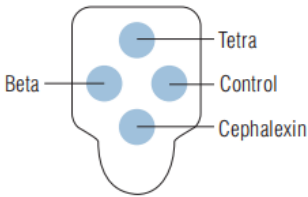


Figure 3: The detection site of beta lactams and tetracycline’s residue

2.4 Isolation of E.coli

Trypton Bile X- glucuronide (TBX) medium, Eosin Methylene Blue (EMB) Agar and MacConkey agar were used to isolate and identify *E.coli* After incubated at 44 °C for 18 h to 24 h. Biochemical tests including KIA, Indole, Citrate Utilization, MR - VP test, Catalase test, Motility and Gram staining were used to confirm the isolated colonies. All procedure was carried out according to Method for Detection, Isolation and Identification of Pathogenic *E. coli* in Food based on ISO14397 (FSSAI, 2023).

Escherichia coli appears as blue green colonies in TBX, dark purple colonies with metallic sheen in EMB and dry colonies lactose fermenting with violet/pink color. The isolated and identified colonies of *E.coli* were transferred to nutrient agar slant for antibiotic resistance testing.



Figure 4: Colonies of Isolated *E.coli* in nutrient agar slant

2.5 Antibiotic resistance testing

E.coli colonies isolated and identified from 12 samples were subjected to antibiotic resistance test by using Kirby-Bauer disk diffusion technique as referred to (Rinky et al., 2023). Where Mueller Hinton agar (Liofilchem, Italy) at 37°C for 24 h was used.

Antibiotic discs (Liofilchem s.r.l , Italy) of Tetracyclines (TE):30µg, Ciproflaxin (CIP):5µg, Ampicillin (AMP): 10µg, Chloramphenical (C) : 30µg, Sulfa/Trimethoprim (SXT): 30µg, and Gentamicin (CN):30µg were used.

Inhibition zones were measured and results recorded in excel and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guideline (Liofilchem, 2024; Weinstein & Lewis, 2020).

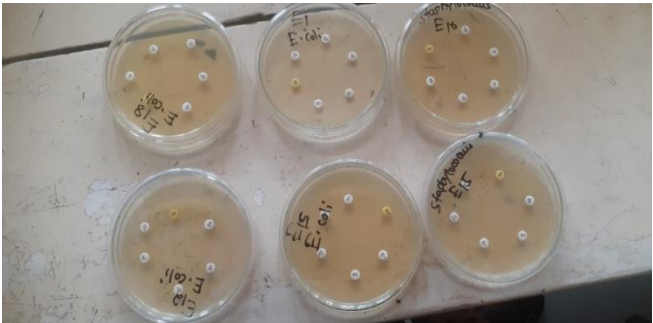


Figure 4: Antibiotic resistance testing

2.6. Statistical analysis

The data were recorded in excel and analyzed in R4.2.2. The proportion of antibiotic residue and Antibiotic Resistant test of *E.coli* were determined.

3.0 Results

Table 1: Qualitative results of antibiotic residue

Status	Number of samples	Percentage (%)
Positive	5	16.7
Negative	25	83.3

Five (5) samples were detected positive were one (1) sample with beta lactam and four (4 samples with tetracycline’s.

Table 2: Antibiotic Resistant test results of *E.coli*

Status / Antibiotic tested	TE : 30 µL	CIP : 5 µL	C : 30 µL	CN : 30 µl	SXT : 30 µL	AMP : 10 µL
Resistance	9	1	2	1	4	12
Intermediate	3	0	0	0	0	0
Sensitive	0	11	10	11	8	0

Among the 12 *E. coli* isolates tested against 6 antibiotics, all isolates demonstrated high resistance to Ampicillin. Resistance to Tetracycline was observed in 9 isolates, while 3 showed intermediate resistance. For Ciprofloxacin (CIP), Chloramphenicol (C), Sulfamethoxazole/Trimethoprim (SXT), and Gentamicin (CN), a significant portion of the isolates were sensitive, with 11, 10, 11, and 8 isolates showing sensitivity, respectively.

4.0 Discussion

4.1 Prevalence of antibiotic residue

The qualitative results of this study indicate that 16.7% of the 30 tested samples showed positive for antibiotic residues, with 1 sample detected for beta-lactam and 4 for tetracyclines. While this level of contamination is relatively low, it raises concerns about improper handling of veterinary drugs at the farm level. A recent study in Burundi found that 9 out of 84 raw milk samples tested positive for antibiotic residues, suggesting contamination is not at a high level but highlighting the need for improved practices among farmers (Albert et al., 2024). Other studies have reported higher contamination rates in different regions (Pogurschi et al., 2015; Rinky et al., 2023), while lower levels were found in the study by Rahman et al. (2021), which reported prevalence of 7%, 8%, and 4% across various sample groups. According to the ongoing debate of whether heat treatment help to eliminate residue (Makoni et al., 2014). The study of (Anika et al., 2019) has revealed that even the heat treatment does not destroy antibiotics and/or their metabolites residues in milk however proper maintaining of withdrawal period after antibiotic treatment in lactating cows guarantees absence of residues in milk as no antibiotic residue is detected in raw milk samples tested.

4.2 Antibiotic resistance among the isolated *E.coli*

Among thirty samples, twelve samples were *E.coli* positive, the presence of *E. coli* in heat-treated milk can be result from direct improper temperature to eliminate the *E.coli* and indirect contamination due to improper handling after treatment or poor storage condition (Ali et al., 2011). *E.coli* frequently contaminates food and it is a good indicator of fecal pollution hence it's presence in milk products indicates the presence of other enteropathogenic microorganisms which constitute a public health hazard (Bagré et al., 2014; Cohen, 2006). The study in South Sudan reported 63% of the samples were *E. coli* positive (Ali et al., 2011). The study in Burkina Faso reported *E. coli* contaminated 38% of raw milk samples (Bagré et al., 2014). While the study from Morocco reported 10% of samples were *E.coli* positive, the 5% of it comes from dairy products (Benkerroum et al., 2004). The highest prevalence of *E.coli* was observed in Arusha, Tanzania, where 68/75 (90.67%) of raw milk, samples were detected *E.coli* positive (Lubote et al., 2014). All these studies indicated that contamination of milk and milk products, with pathogenic bacteria is largely due to processing, handling, poor storage and unhygienic conditions. The twelve isolated *E.coli* tested with six antibiotics including Ampicillins, Tetracyclines, Gentamicin, Ciprofloxins, Sulfa/Trimethoprim and Chloramphenicol. The isolates of *E.coli* exhibited higher resistance with Ampicillins and Tetracycline compared to other tested discs. This is can be explained by the fact that two groups of drugs are most used groups in dairy industry and in human treatment, the intervention of these drugs explains the causes of resistance (Rinky et al., 2023; Virto et al., 2022).

Comparing to the study which was conducted in milk supply chain in Bangladesh, the isolated of *E.coli* from raw milk, shows that 46% of the antibiotics showed sensitivity, 45.26% of resistance, and 8.75% demonstrated intermediate resistance, the highest resistant was seen AMP (86.3%) which was followed by TC (76%) (Rinky et al., 2023). To which is similar to this study where Ampicillin exhibited total resistant followed by Tetracycline, this is due to the frequent use of Ampicillin and Tetracyclines in agriculture, veterinary and human medicines creates selective pressure, favoring the survival and proliferation of resistant *E. coli* strains.

Conclusion

The study found that 16.7% of the tested milk samples contained antibiotic residues, with specific concerns about the handling of veterinary drugs on farms. Additionally, *E. coli* isolates showed high resistance to Ampicillin and Tetracycline, likely due to the frequent use of these antibiotics in veterinary medicine. This highlights the need for improved practices in antibiotic use to reduce contamination and resistance, ensuring safer milk products for consumers.

References

1. Albert, I., Gérard, N., Napoleon, M., & Ntunzwenimana, M. (2024). Knowledge of Production Conditions and the Quality of Raw Milk Produced in Burundi. 12(3), 127–137. <https://doi.org/10.11648/j.jfms.20241203.11>
2. Ali, A. A., Abdelgadir, W. S., Box, P. O., & North, K. (2011). Incidence of *Escherichia coli* in Raw Cow 's Milk in Khartoum State. 2(1), 23–26.
3. Alves, J. F., Paula, G. H., Silva, R. C. F., Leão, P. V. T., Leão, K. M., Nicolau, E. S., & Silva, M. A. P. (2020). Residues of antibiotics in milk: Persistence and quality interference. *Canadian Journal of Animal Science*, 100(1), 93–101. <https://doi.org/10.1139/cjas-2018-0224>
4. Anika, T. T., Noman, Z. Al, Rifat, M., Ferdous, A., Khan, S. H., Mukta, M. A., Islam, S., Hossain, T., & Rafiq, K. (2019). Time dependent screening of antibiotic residues in milk of antibiotics treated cows. 7710(December), 4–8.
5. Bagré, T. S., Kagambèga, A., Bawa, H. I., & Bsadjo, G. (2014). Antibiotic susceptibility of *Escherichia coli* and *Salmonella* strains isolated from raw and curds milk consumed in Ouagadougou and Ziniaré, Burkina Faso. April 2015. <https://doi.org/10.5897/AJMR2014.6632>
6. Benkerroum, N., Bouhlal, Y., Attar, A. E. L., & Marhaben, A. (2004). Occurrence of Shiga Toxin – Producing *Escherichia coli* O157 in Selected Dairy and Meat Products Marketed in the City of Rabat, Morocco. *Journal of Food Protection*, 67(6), 1234–1237. <https://doi.org/10.4315/0362-028X-67.6.1234>
7. Bitama, P. C., & Ndayishimiye, A. (2023). Economic study of the milk value chain through the Kiganda-Bujumbura Mairie circuit in Burundi. *East African Journal of Science, Technology and Innovation*, 4(4), 1–13. <https://doi.org/10.37425/eajsti.v4i4.657>

8. Cohen, N. (2006). *Risque hygiénique lié à la présence des Escherichia coli dans les viandes et les produits carnés : Un réel problème de santé publique ?*
9. FSSAI. (2023). *Manual of Methods of Analysis-Microbiological Examination of Food and Water*.
10. Getahun, M., Abebe, R. B., Sendekie, A. K., Woldeyohanis, A. E., & Kasahun, A. E. (2023). Evaluation of Antibiotics Residues in Milk and Meat Using Different Analytical Methods. In *International Journal of Analytical Chemistry* (Vol. 2023). Hindawi Limited. <https://doi.org/10.1155/2023/4380261>
11. Hansen, R. G. (1974). Milk in Human Nutrition. In *Nutrition and Biochemistry of Milk/maintenance*. <https://doi.org/10.1016/b978-0-12-436703-6.50013-2>
12. Kang, E. K., Mutua, F., Roesel, K., Ntawubizi, M., Kankya, C., & Niragira, S. (2021). A review of the food safety architecture in the East African Community:Animal-source foods, fruits and vegetables. *ILRI Discussion PAPER A*, 3, 1–23. <https://creativecommons.org/licenses/by/4.0>. Unless
13. Laboratories, I. (2016). *IDEXX SNAPduo* ST Plus Test*. 81900.
14. Liofilchem. (2024). © Lio lchem ® - Antibiotic Disc Interpretative Criteria and Quality Control - Rev.19 / 30.09.2024 •.
15. Lubote, R., Shahada, F., & Matem, A. (2014). Prevalence of Salmonella spp . and Escherichia coli in raw milk value chain in. *American Journal of Research Communication*, 2(9), 1–13.
16. Makoni, N., Mwai, R., Redda, T., van der Zijpp, A., & van der Lee, J. (2014). White Gold: Opportunities for Dairy Sector Development Collaboration in East Africa.Centre for Development Innovation, Wageningen UR (University & Research centre). CDI report CDI-14-006. Wageningen. In *Centre for Development Innovation* (Issue November). www.wageningenUR.nl/cdi%0Ahttp://library.wur.nl/WebQue
17. Michael, N. I. L. (2016). Overview of dairy processing and marketing in East African dairy value chains: Opportunities and challenges. *African Journal of Food Science*, 10(11), 254–262. <https://doi.org/10.5897/ajfs2016.1465>
18. Ouma, E., & Godfrey, M. (2020). *Opportunities and constraints to smallholder dairy market development in Burundi*.
19. Paludetti, L. F., Kelly, A. L., O'brien, B., & Gleeson, D. (2019). Monitoring residue concentrations in milk from farm and throughout a milk powder manufacturing process. *Journal of Dairy Research*, 86(3), 341–346. <https://doi.org/10.1017/S0022029919000578>
20. Rahman, M. S., Hassan, M. M., & Chowdhury, S. (2021). Determination of antibiotic residues in milk and assessment of human health risk in Bangladesh. *Heliyon*, 7(8). <https://doi.org/10.1016/j.heliyon.2021.e07739>
21. Rinky, F., Reza, S., Nowar, A., Ghosh, S., Rahman, A., & Alim, S. R. (2023). Analysis of Microbiological Quality and Antibiotic Resistance Patterns in Milk Supply Chain. *Bioresearch Communications*, 10(1), 1462–1473. <https://doi.org/10.3329/brc.v10i1.70686>
22. Virto, M., Santamarina-García, G., Amores, G., & Hernández, I. (2022). Antibiotics in Dairy Production: Where Is the Problem? In *Dairy* (Vol. 3, Issue 3, pp. 541–564). MDPI. <https://doi.org/10.3390/dairy3030039>
23. Weinstein, M. P., & Lewis, J. S. (2020). The clinical and laboratory standards institute subcommittee on Antimicrobial susceptibility testing: Background, organization, functions, and processes. In *Journal of Clinical Microbiology* (Vol. 58, Issue 3). <https://doi.org/10.1128/JCM.01864-19>
24. WHO. (2018). WHO Report on Surveillance of Antibiotic Consumption. In *Who*. <http://apps.who.int/iris%0Ahttps://apps.who.int/iris/bitstream/handle/10665/277359/9789241514880-eng.pdf>