Quantification and risk assessment of antibiotic residues among milk and fermented milk products vended around Zaria and Sabon-gari Local Government Areas, Kaduna State, Nigeria.

Muhammad Muhsin Fathuddin[1*], Saleh Alhaji Ado[1], Muhammad Bashir Tijjani[1], Haruna Makonjuola Kazeem[4], Rafeedah Fathuddin[2], John Solomon Obidah[3]

[1] Ahmadu Bello University, (Department of Microbiology), Zaria – 810107, (Kaduna State), Nigeria
[2] B. S. Abdur Rahman Crescent Institute of Science & Technology, (School of Life Sciences (SLS)), Chennai 600048, (Tamil Nādu), India
[3] Modibbo Adama University, (Department of Microbiology), Yola – 640231, (Adamawa State), Nigeria
[4] Ahmadu Bello University, (Department of Veterinary Microbiology), Zaria – 810107, (Kaduna State), Nigeria

*Corresponding author: muhsinfathuddin@gmail.com

https://orcid.org/0000-0001-9948-4081

Received: 27.06.2024 Revised: 07.09.2024 Accepted: 13.09.2024 Online: 15.12.2024

Abstract This study detected and determined the hazard quotient of residue levels associated with ampicillin, ciprofloxacin, gentamicin, and tetracycline in milk and milk products vended in Zaria and Sabon-gari Local Government Areas. Overall, 400 milk and fermented milk products were gathered randomly. After screening via a microbial inhibition assay, sixty-five (65) samples showed the presence of antagonistic agents. The residues of ampicillin, ciprofloxacin, gentamicin, and tetracycline were quantified using high-performance liquid chromatography and were detected in 29 (44.62%), 21 (32.31%), 21 (32.31%), and 34 (52.31%) samples respectively. The residual levels in all the positive samples for ampicillin and ciprofloxacin exceeded the Codex Alimentarius Commission's recommended limits. In contrast, residue levels exceeded the Codex limits in 15 (71.43%) gentamicin samples and 32 (94.12%) tetracycline samples. The mean residue levels of ampicillin, ciprofloxacin, gentamicin, and tetracycline were 429.70±297.12, 374.28±262.74, 411.52±226.73, and 407.60±262.69 µg/L, respectively. In Nigeria, the average per capita milk consumption contributed to a mean estimated daily intake (EDI) of 0.14 for adults and 0.55 for children for ampicillin, while EDI for ciprofloxacin, gentamicin, and tetracycline were at 0.13 and 0.52; 0.14 and 0.57; and 0.14 and 0.54, for adult and children respectively. The hazard quotient (HQ) calculated using the Nigerian average data was less than one (1) for adults and children. Based on the global average per capita milk consumption, the overall EDI values for tetracycline were 1.42 and 5.69, gentamicin was 1.50 and 6.01, ciprofloxacin was 1.37 and 5.47, and ampicillin was 1.44 and 5.75 for adult and children respectively. The HQ (Global Average) for all antibiotics was less than one in adults, but only ampicillin and ciprofloxacin exceeded one in children. The novelty of this study lies in the comprehensive assessment of the hazard quotient of antibiotic residues in milk and milk products sold in Zaria and Sabon-gari Local Government Areas.

Keywords: Ampicillin, Antibiotic residue, Ciprofloxacin, Gentamicin, *Kindirimo*, UV-HPLC

Introduction

Antibiotic residues refer to the remnants of antibiotics in food products, such as meat, milk, and eggs, after the animals have been treated with these medications (Adegbeye et al., 2024). These residues can pose significant health risks to consumers, particularly if they are not properly removed or degraded before consumption (Khalifa et al., 2024). Antibiotics are widely used in animal husbandry to treat diseases and promote growth (Rware et al., 2024). However, these medications can accumulate residues in animal tissues and products (Khalifa et al., 2024). These residues can be harmful if ingested (Mouiche et al., 2024), as they can cause allergic reactions (Londoño-Carmona et al., 2024), antibiotic resistance (Massaccesi et al., 2024), and other adverse effects. The presence of antibiotic residues in food products is a significant public health concern (Almashhadany et al., 2024). A study published in the journal "Food Additives & Contaminants" in

2024 found that antibiotic residues were present in all the milk samples collected in Dhaka, Bangladesh (Rahman et al., 2024). Another study published in 2024 found that among 90 Lebanese samples of milk and labneh (concentrated yogurt), 71% had antimicrobial residues, with quinolones and macrolides being the most detected (Ismail et al., 2024).

Regulatory organizations and food manufacturers have implemented several methods to mitigate the risks associated with antibiotic residues. For instance, the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) has set maximum residue levels (MRLs) for antibiotics in food products in their Codex Alimentarius Commission (CAC). Food manufacturers are required to guarantee that their goods comply with these restrictions. Furthermore, many countries have implemented programs to supervise and regulate the use of antibiotics in the context of animal farming. To summarize, antibiotic residues pose a significant public health risk to consumers. Various methods have been developed to detect and quantify these leftover substances, and regulatory agencies and food manufacturers are working to reduce these risks by implementing strict procedures and monitoring programs.

Despite the global recognition of this issue, there remains a notable gap in the literature regarding the quantification and risk assessment of antibiotic residues in dairy products retailed in specific regions of Nigeria, such as Zaria and Sabon-gari Local Government Areas (LGAs) in Kaduna State. This study's main objective was to quantify and assess the risk associated with antibiotic residue in milk and fermented milk products (FMPs) widely consumed in the region. Through this investigation, we aim to provide valuable insights into the extent of antibiotic residues in these products, which can contribute to developing effective regulatory measures to ensure the safety and quality of dairy products in the region. We aimed to assess the presence and quantify the antibiotic residues in milk and FMPs around Zaria and Sabon-gari LGAs and assess the risk associated with antibiotic residue in these products.

Materials and Methods

Antibiotics, Chemicals, and Reagents required for High-Performance Liquid Chromatography

The following antibiotics standard (CAS number): Ampicillin trihydrate (7177-48-2; AMP), Ciprofloxacin (85721-33-1; CIP), Gentamicin sulfate (1405-41-0; GEN) and Tetracycline (60-54-8; TET), were purchased from Zayo-Sigma (Jos, Plateau State, Nigeria), all with purity ≥ 99%. PTFE Lab Chemical Nylon Syringe Filter (MPNN2502) of 0.2µm (Pore Size) and 25mm (Diameter) was purchased from Vital Science Industry (Chennai, Tamil Nādu**,** India). All HPLC-grade reagents with a purity higher than 99.8%, including acetonitrile (ACN), water, and acetic acid, were purchased from Joe Chemicals (Zaria, Kaduna State, Nigeria). The detection and analysis of antibiotic residues were done using the Agilent 1200 Infinity LC Series, carried out at the Chemistry Analytical Laboratory, Department of Chemistry, Faculty of Science, Yobe State University, Damaturu, Yobe State, Nigeria.

Sample Collection

Four hundred (400) samples were used in the study, including 100 samples of each sort of milk and Fermented Milk Products (FMPs) [*Kindirimo*, *Nono*, and Yoghurt] vendor in Sabon Gari and Zaria (Figure 1). A sterile, transparent zip-lock bag collected 50 mL of milk and FMPs from several sample points. The bag was labeled and promptly submitted to Ahmadu Bello University's Industrial Microbiology Laboratory for examination.

Before being tested, the milk and milk product samples were brought to room temperature (25-30 ^{0}C) (Chauhan & Jindal, 2020; Patel et al., 2020).

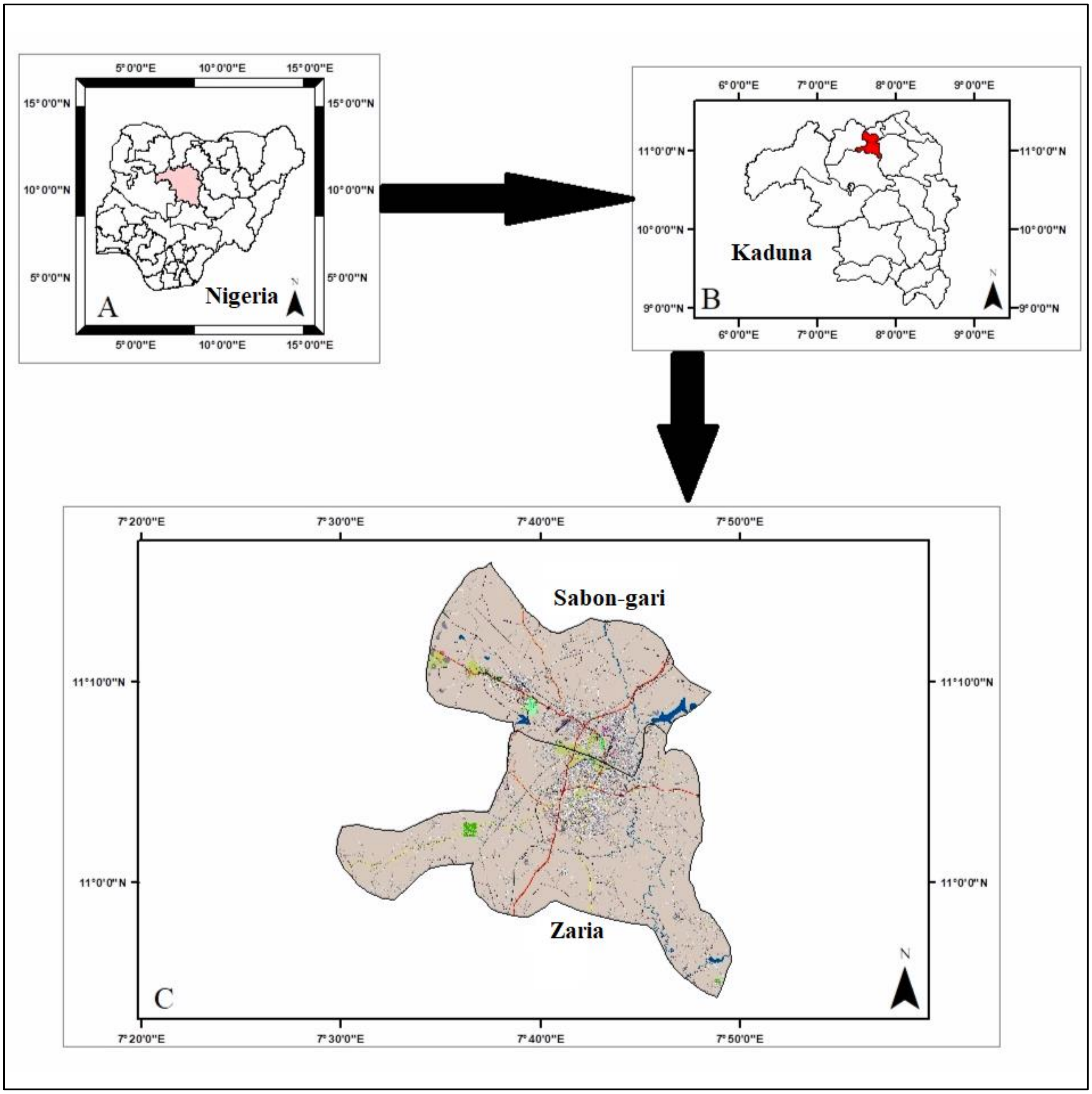

Figure 1: Map of the study area

Screening of milk and fermented milk products via Microbial Inhibition Assay (MIA)

The procedure is as described by Ezenduka (2019), Kurjogi et al. (2019), Hebbal et al. (2020) and Almashhadany (2021) with modifications. A culture of *Bacillus subtilis* was obtained from the Department of Public Health, Faculty of Veterinary Medicine, Ahmadu Bello University-Zaria, and was grown in a Nutrient broth medium at 37 ^{0}C. After 24 h of growth, the broth was standardized to 0.5 McFarland standard. Then, 0.1 mL of 0.5 McFarland standardized broth was spread on the surface of Mueller-Hinton agar plates and was bored four times using a sterile Cork borer. About one hundred µL of the Milk and FMPs were then inoculated in four holes, each representing each sample. Plates were incubated at 37 ± 2 ^{0}C for 24 hours, and a zone showing inhibition of the bacterial growth was measured with an annular diameter ≥ 2 mm, indicating a positive result for antimicrobial residues. Each test was performed in duplicate. The positive samples were selected for further HPLC analysis.

Preparation of milk samples and quantification of antibiotic residues by HPLC

The procedure follows Moudgil et al. (2018), Kurjogi et al. (2019), Moudgil et al. (2019), and Alnassrallah et al. (2022), with modifications. The four main steps will evaluate the precipitation of milk proteins, extraction of antimicrobials from the milk matrix, clean-up, and concentration eluting. The precipitation of the milk proteins was carried out by mixing 8 mL of 10% acetic acid with 100 mL of the cold sample (either milk, kindirimo, nono, or yoghurt), and vortexed for 2 minutes. Then, the extraction of antimicrobials from the milk matrix was achieved by centrifuging the mixture at 3500 rpm for 10 minutes at 4 ^{0}C. The cleanup involved using a syringe nano-filter, and the supernatant from the centrifugation was collected using a syringe and then passed through the syringe nano-filter into an HPLC vial (1 mL). The concentration eluting involved using the mobile phase with an aqueous phase of 10% acetic acid and an organic phase of acetonitrile: water (60:40, vol/vol). The antibiotics to be targeted in the present study will be ampicillin (AMP), tetracycline (TET), gentamicin (GEN), and ciprofloxacin (CIP). The estimates of the number of antibiotic residues in the positive sample were computed. The calibration curve for each antibiotic standard was a plot by least-square linear regression analysis of the peak area ratio of spiked concentration (20, 40, 80, 160, and 320 ng/mL) to the internal standard (Y-axis) against the corresponding spiked concentration [X-axis]. The quantification was performed through internal standardization at 260 nm (AMP), 230 nm (CIP), 225 nm (GEN), and 355 nm (TET). The residues detected in the analyzed samples were identified by comparing the retention time with the reference standard peaks. An overlap of the sample peak at the desired retention time indicated correct identification.

Dietary Exposure to Antibiotic Residues: A Risk Assessment

The risk assessment of dietary intake of antibiotic residues was carried out based on the procedure followed by Aalipour et al. (2015), Patel et al. (2020), Rahman et al. (2021), and Kumar et al. (2021). The mean level of antibiotic concentration in milk and milk product samples was evaluated. Average daily consumption of milk and fermented milk products (FMPs) based on 60 kg body weight for an adult and 15 kg body weight for children was considered (CAC, 2014). A risk analysis was done using the hazard quotient (HQ) [Equation 1].

Hazard Quotient (HQ) = Estimated daily intake (EDI)/Acceptable daily intake (ADI)…. (1)

Where: Estimated Daily Intake (EDI) for antibiotic residues was calculated using [Equation 2], and Acceptable daily intake (ADI) for antibiotic residues was obtained from the established values in the JECFA's CAC (2023).

EDI= [(Mean of µg of antibiotic per kg of food) × (Daily intake of food)]/Bodyweight (kg)….(2)

The assessment of potential health risks associated with consuming milk containing antibiotic residues was evaluated by considering the residue analysis results from the present study. The per capita consumption of milk and FMPs (kg/day) in Nigeria was obtained from The Food and Agriculture Organization of the United Nations (2024) - with major processing by Our World in Data. In the year 2021, the Global Average Per Capita Milk and FMPs Consumption was 87.03 kg/Year (i.e., 0.2384 Kg/day), while the Nigerian Average Per Capita Milk and FMPs Consumption was 8.31 kg/Year (i.e., 0.0228 Kg/day). Statistical analysis was carried out on the data obtained using Excel spreadsheets.

Results and Discussion

In the current study, the analytical determination of four antimicrobials (AMP, CIP, GEN, and TET) in milk and milk product samples was carried out, and then, based on the existing and representative data on the average milk consumption for adults, the risk of dietary exposure to the residual quantity via milk consumption was evaluated for the population of Nigeria and the world.

The Presence of Antibiotic Residues in the milk and Fermented Milk Product samples

Table 1 shows the results of the Microbial Inhibition Assay (MIA). Out of one hundred (100) milk samples, twenty-one (21) samples showed the presence of antibiotic/antagonistic agents as they gave a Zone of Inhibition (ZOI) of ≥2 mm. For *Kindirimo*, out of one hundred, fifteen (15) samples tested showed the presence of antibiotic/antagonistic agents. For *Nono*, out of one hundred, seventeen (17) samples showed the presence of antibiotic/antagonistic agents. For Yoghurt, out of one hundred, twelve (12) samples tested showed the presence of antibiotic/antagonistic agents. A total of sixty-five (65) samples showed the presence of antibiotic/antagonistic agents out of the four hundred (400) samples tested.

A study conducted in Palakkad, India, by Hebbal et al. (2021) screened 215 raw milk samples for antibiotic residues via MIA; 22 (10.23%) were positive. Almashhadany (2021) reported that he screened 320 raw milk samples from Erbil City, Iraq, and detected antibiotic residues via the agar well diffusion (13.4%) and the disk diffusion assay (11.9%). Kurjogi et al. (2019) screened and detected antibiotic residue in 2 samples out of the 13 tested from Dharwad district, India. In Himachal Pradesh, India, Kumar et al. (2021) detected antibiotic residues in 16 (9.2%) samples from 173. These findings are lower than our study (16.25%). There is a paucity of papers using the MIA to detect antibiotic residue in Nigeria. Most researchers use commercial antibiotic residue kits such as Premi® (Olatoye et al., 2016; Yusuf et al., 2017) or the Delvotest test (Oluwafemi et al., 2018) or other methods such as the four-plate method (Stella et al., 2020).

Table 1. Microbial Inhibition in milk and fermented milk products

Sample Type	Sample Size	Number of positive samples (≥2 mm)	Number of negative samples (<2 mm)	Mean Percentage positive samples
Milk	100	21	79	5.25
Kindirmo	100	15	85	3.75
Nono	100	17	83	4.25
Yogurt	100	12	88	3.00
Total	400	65	335	16.25

Key: ≥2 mm = inhibition zones more than or equal to 2 mm; < 2 mm = inhibition zones less than 2 mm.

Detection of the Presence of Antibiotic Residue (HPLC)

Table 2 shows the results of the detection of the antibiotic residues. Out of the total 65 (100.00%) milk and FMP samples, 32 (49.23%) detected residue of AMP, 26 (40.00%) detected CIP residue, 29 (44.62%) detected GEN residue and 52 (80.00%) detected TET residue. Out of the 21 (32.31%) Milk samples, 14 (66.67%) detected AMP, 08 (38.10%) detected CIP, 11 (52.38%) detected GEN and 18 (85.71%) detected TET. For *Kindirimo* [15 (23.08%)], *Nono* [17 (26.15%)] and Yoghurt [12 (18.46%)], AMP was detected in 04 (26.67%), 10 (58.82%) and 04 (33.33%) samples, respectively. CIP residues were detected in 04 (26.67%), 08 (47.06%), and 06 (50.00%), respectively. GEN residues were detected in 06 (40.00%), 04 (23.53%), and 08 (66.67%), respectively. TET residues were detected in 08 (53.33%), 15 (88.24%), and 11 (91.67%), respectively.

Table 2. The overall detection of different antibiotic residues in milk and fermented milk products

Sample	Number of Samples Tested* [n (%)]	Antibiotics: Ampicillin [n (%)]	Ciprofloxacin [n (%)]	Gentamicin [n (%)]	Tetracycline [n (%)]
Milk	21 (32.31)	11 (16.92)	6 (09.23)	6 (09.23)	15 (23.08)
Kindirimo	15 (23.08)	6 (09.23)	4 (06.15)	7 (10.77)	8 (12.31)
Nono	17 (26.15)	8 (12.31)	6 (09.23)	5 (07.69)	7 (10.77)
Yogurt	12 (18.46)	4 (06.15)	5 (07.69)	3 (04.62)	4 (06.15)
Total	65 (100.00)	29 (44.62)	21 (32.31)	21 (32.31)	34 (52.31)

*Samples after MIA Screening | Percentage values are given in parentheses.

According to Hebbal et al. (2021), out of the 22 samples tested after MIA Screening, only five (2.33%) were positive for oxytetracycline. Kumar et al. (2021) reported oxytetracycline in 14 (8.1%) samples and amoxicillin in 2 (1.6%) samples (out of 173 samples) in Himachal Pradesh, India, after MIA Screening. Also, Kurjogi et al. (2019) detected azithromycin and tetracycline residues from 2 samples. Oluwafemi et al. (2018) found that out of 20 samples collected from Abeokuta, Ogun State, Nigeria, 10 (50%) contained tetracycline antibiotic residue.

Yusuf et al. (2018) found that out of 313 samples examined, 74 (23.64%) confirmed positive for tetracycline residues in Kano State, Nigeria. Olatoye et al. (2016) found that in Oyo state, Nigeria, out of 328 samples of fresh milk, 180 local cheese (*Wara*), and 90 fermented milk (*Nono*) samples detected Penicillin-G residue in 41.1% fresh milk, 40.2% *Nono* and 24.4% *Wara*, respectively. These figures are less than our findings (44.62%, 32.31%, 32.31%, and 52.31%).

Quantification of the antibiotic residues in milk and fermented milk products

Table 3 shows the results of quantifying the mean residue level found in milk and FMPs. Based on the CAC (2023) specifies that the Maximum Residue Limit (MRL) values for the antibiotics AMP, CIP, GEN, and TET in milk and FMPs are 4.00, 5.00, 200.00 and 100.00 µg/L respectively. Our study's mean residue values were higher than the recommended MRL values. Milk had a mean residue level for AMP, CIP, GEN, and TET of 410.4±264.24, 347.1±357.54, 357.7±218.34 and 436.8±309.16 µg/L respectively. Kindirimo had 473.4±360.74, 304.3±240.49, 316.2±166.71 and 340.0±206.29 µg/L respectively. *Nono* had 465.4±270.63, 445.8±240.97, 519.8±396.68 and 417.1±273.10 µg/L respectively. Yoghurt had 353.3±478.41, 357.3±220.20, 502.9±147.14, and 396.9±227.00 µg/L respectively. Oluwafemi et al. (2018) found that the concentration of antibiotic residues detected in samples ranged between 5ng/L and 1569ng/L. While Olatoye et al. (2016) found the mean concentration for Penicillin-G residue in fresh milk, *Nono* and *Wara* to be 15.22±0.61, 8.24±0.50 and 7.6±0.60 µg/L, respectively

Table 3. Estimation of the antibiotic residues in the milk and fermented milk products

Sample	Antibiotic	Mean ± SD (µg/L)	MRL value (µg/L)
Milk	Ampicillin	377.67 ± 226.79	4.00
	Ciprofloxacin	228.00 ± 209.16	5.00
	Gentamicin	372.50 ± 204.37	200.00
	Tetracycline	371.80 ± 256.34	100.00
Kindirimo	Ampicillin	271.50 ± 210.22	4.00
	Ciprofloxacin	304.25 ± 298.21	5.00
	Gentamicin	294.14 ± 169.81	200.00
	Tetracycline	290.75 ± 235.75	100.00
Nono	Ampicillin	410.63 ± 270.18	4.00
	Ciprofloxacin	444.17 ± 316.98	5.00
	Gentamicin	374.40 ± 233.41	200.00
	Tetracycline	411.57 ± 204.71	100.00
Yogurt	Ampicillin	353.25 ± 508.83	4.00
	Ciprofloxacin	394.40 ± 161.84	5.00
	Gentamicin	592.67 ± 278.86	200.00
	Tetracycline	345.50 ± 350.56	100.00
Mean (Combined)	Ampicillin	361.97 ± 230.37	4.00
	Ciprofloxacin	343.90 ± 217.42	5.00
	Gentamicin	378.29 ± 197.37	200.00
	Tetracycline	357.82 ± 244.79	100.00

Key: SD – Standard deviation, MRL – Maximum Residue Limit, ∩ - Codex Alimentarius Commission (2023)

Kumar et al. (2021) reported that samples above the MRL limit were obtained: 3 out of 12 samples had oxytetracycline residues, and 2 2 samples had amoxicillin. At the same time, Patel et al. (2020) received a mean residue value for fluoroquinolones (47.92 µg/kg), gentamicin (80.44 µg/kg), and tetracycline (667.09 µg/kg) at levels above the defined MRL. Rahman et al. (2021) got a mean residue value for amoxicillin (124 µg/L) and oxytetracycline (61.29 µg/L), with amoxicillin being above the MRL limit. Rahman et al. (2024) got only one sample that had enrofloxacin, levofloxacin, and ciprofloxacin residues (121 μgL^{-1}) above the MRL limit, with none of the samples with oxytetracycline residue exceeding the MRL limit. Olatoye et al. (2016) found the number of samples (fresh milk, *Nono,* and *Wara*) to be 39.3%, 36.7%, and 21.1%, respectively, with penicillin residue above the recommended Codex MRL of 4.0 µg/L.

Evaluation of daily intake Assessing Risk with Hazard Quotient Estimation based on the Nigerian Average

Table 4 shows the results of EDI based on the Nigerian Average Per Capita Milk Consumption for the antibiotics found in milk and FMPs. Based on the CAC (2023) specifies that the ADI values for the antibiotics AMP, CIP, GEN, and TET in milk and FMPs are 03, 03, 20, and 30 µg/kg/day, respectively. The mean EDI values for AMP, CIP, GEN, and TET of 0.14, 0.13, 0.14, and 0.14 µg/kg/day for adults and 0.55, 0.52, 0.57, and 0.54 µg/kg/day for children respectively. All the obtained EDI values were less than the recommended ADI value. The mean HQ values for AMP, CIP, GEN, and TET were 0.05, 0.06, 0.01, and 0.00 for adults and 0.18, 0.26, 0.03, and 0.02 for children. The HQ values obtained were all less than one (<1), indicating they are safe for consumption.

Table 4. Assessment of estimated daily intake and risk based on the Nigerian average[ఔ]

Sample	Antibiotic	ADI[○] (µg/kg/day)	EDI_A (µg/kg/day)	EDI_C (µg/kg/day)	HQ_A	HQ_C
Milk	Ampicillin	03	0.14	0.57	0.05	0.19
	Ciprofloxacin	02	0.09	0.34	0.07	0.17
	Gentamicin	20	0.14	0.56	0.01	0.03
	Tetracycline	30	0.14	0.56	0.01	0.02
Kindirimo	Ampicillin	03	0.10	0.41	0.06	0.14
	Ciprofloxacin	02	0.11	0.46	0.06	0.23
	Gentamicin	20	0.11	0.44	0.01	0.02
	Tetracycline	30	0.11	0.44	0.00	0.01
Nono	Ampicillin	03	0.16	0.62	0.06	0.21
	Ciprofloxacin	02	0.17	0.67	0.08	0.34
	Gentamicin	20	0.14	0.57	0.01	0.03
	Tetracycline	30	0.16	0.62	0.01	0.02
Yoghurt	Ampicillin	03	0.13	0.53	0.04	0.18
	Ciprofloxacin	02	0.15	0.60	0.07	0.30
	Gentamicin	20	0.22	0.90	0.01	0.04
	Tetracycline	30	0.13	0.52	0.01	0.02
Overall (Combined)	Ampicillin	03	0.14	0.55	0.05	0.18
	Ciprofloxacin	02	0.13	0.52	0.06	0.26
	Gentamicin	20	0.14	0.57	0.01	0.03
	Tetracycline	30	0.14	0.54	0.00	0.02

ఔ - Food and Agriculture Organization of the United Nations (2024) with major processing by Our World in Data. ADI - Acceptable Daily Intake, ○ - Codex Alimentarius Commission (2023), EDI – Estimated Daily Intake. $_A$ – Adult, $_C$ – Children, HQ – Hazard Quotient

Evaluation of daily intake Assessing Risk with Hazard Quotient Estimation based on the Global Average

Table 5 shows the results of EDI based on the Global Average Per Capita Milk Consumption for the antibiotics found in milk and FMPs. The mean EDI values for AMP, CIP, GEN, and TET were 1.44, 1.37, 1.50, and 1.42 µg/kg/day for adults and 5.75, 5.47, 6.01, and 5.69 µg/kg/day for children respectively. The EDI values were less than the recommended ADI value among adults, but AMP and CIP were higher for children. The mean HQ values for AMP, CIP, GEN, and TET were 0.48, 0.68, 0.08, and 0.05 for adults and 1.92, 0.36, 0.04, and 0.02 for children. The HQ values obtained were the same as those obtained for EDI, i.e., in the children category, AMP and CIP, which were greater than one (>1) among children.

Table 5. Assessment of estimated daily intake and risk based on the global average[ஐ]

Sample	Antibiotic	ADI[◠] (µg/kg/day)	EDI_A (µg/kg/day)	EDI_C (µg/kg/day)	HQ_A	HQ_C
Milk	Ampicillin	03	1.50	6.00	0.50	2.00
	Ciprofloxacin	02	0.91	3.62	0.45	1.81
	Gentamicin	20	1.48	5.92	0.07	0.30
	Tetracycline	30	1.48	5.91	0.05	0.20
Kindirimo	Ampicillin	03	1.08	4.32	0.36	1.44
	Ciprofloxacin	02	1.21	4.84	0.60	2.42
	Gentamicin	20	1.17	4.67	0.06	0.23
	Tetracycline	30	1.16	4.62	0.04	0.15
Nono	Ampicillin	03	1.63	6.53	0.54	2.18
	Ciprofloxacin	02	1.76	7.06	0.88	3.53
	Gentamicin	20	1.49	5.95	0.07	0.30
	Tetracycline	30	1.64	6.54	0.05	0.22
Yogurt	Ampicillin	03	1.40	5.61	0.47	1.87
	Ciprofloxacin	02	1.57	6.27	0.78	3.13
	Gentamicin	20	2.35	9.42	0.12	0.47
	Tetracycline	30	1.37	5.49	0.05	0.18
Overall (Combined)	Ampicillin	03	1.44	5.75	0.48	1.92
	Ciprofloxacin	02	1.37	5.47	0.68	0.36
	Gentamicin	20	1.50	6.01	0.08	0.04
	Tetracycline	30	1.42	5.69	0.05	0.02

[ஐ] – Food and Agriculture Organization of the United Nations (2024) with major processing by Our World in Data. ADI – Acceptable Daily Intake, [◠] - Codex Alimentarius Commission (2023), EDI – Estimated Daily Intake. $_A$ – Adult, $_C$ – Children, HQ – Hazard Quotient

There is a paucity of papers using the EDI and HQ to establish the safety of these detect antibiotic residue in milk and FMPs in Nigeria. Rahman et al. (2021) obtained for oxytetracycline an EDI and HQ of 0.168 (µg/kg/day) and 0.0056, and for amoxicillin, 0.341 (µg/kg/day) and 0.0017, respectively, among adults. While Aalipour et al. (2015) got an EDI for tetracycline for the adult group at 0.96 ($\mu g/Kg_{bw/d}$) and the children group at 4.43 ($\mu g/Kg_{bw/d}$). Patel et al. (2020) got the EDI (µg/kg b.wt /day) in the Gujarat population for Tetracycline (0.66), fluoroquinolones (0.80), and Gentamicin (4.20), and in PAN India as 0.36, 0.49, and 0.24 respectively. The HQ in the Gujarat population was 0.022, 0.130, and 0.210; in PAN India, it was 0.012, 0.079, and 0.012, respectively.

Tables 4 and 5 show the HQ and EDI based on the Nigerian and Global averages, respectively. The Nigerian EDI values were less than the ADI values, while the Global EDI values were higher for the ampicillin and ciprofloxacin among the children group. The same pattern can also be seen in the HQ. This change in the Global EDI and HQ is due to the higher per capita consumption of milk and FMPs (kg/day) than the Nigerian average, which is about 10x higher. The Nigerian population is safe from these residues due to the low per capita consumption of milk and FMPs (kg/day).

Conclusions

Food tainted with antibiotics is a significant economic and public health problem that affects people all around the world, including Nigeria. This study's data demonstrates that milk and FMPs in Sabon-gari and Zaria LGAs, Nigeria, contained residues of ampicillin, ciprofloxacin, gentamicin, and tetracycline, found in a significant portion of the analyzed samples. The overall HQ for the antibiotic residues in these milk and FMPs shows that the Nigerian population can safely consume these products due to the low per capita consumption. Residue accumulation in the body might cause detrimental effects at any point, even though the amounts are below the maximum residue limit. Consequently, regulatory bodies in Nigeria should monitor antibiotic residues in human-drink milk to combat this worldwide issue. Probiotics and other antimicrobials generated from plants offer a potential alternative to antibiotics. Veterinarians should refrain from the illogical use of antibiotics unless necessary.

Acknowledgements

The authors wish to acknowledge Dr. Umaru Abdullahi, Modibbo Adama University, Adamawa State, for his assistance during the HPLC. Dr. S. Awwalu and Mal. Balarati (Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, ABU-Zaria) assisted in designing the work for antibiotic residue analysis. Mal. Y.M. Suleiman (Department of Veterinary Public Health and Preventative Medicine, Faculty of Veterinary Medicine, ABU-Zaria) facilitated the procurement of consumables in this study. I sincerely appreciate the Technical Staff: Mallam A.M. Shittu, Mal. D. Dalhatu, Mal. B. Abdullahi, Mal. A. Tabari, Mal. M. Yusuf, and Mal. I. Adamu, for their expertise, troubleshooting skills, and dedication, were instrumental in overcoming obstacles during the research and experimentation phases.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Review and Editing: Muhammad Muhsin Fathuddin, Rafeedah Fathuddin, and John Solomon Obidah. Original Draft Preparation: Muhammad Muhsin Fathuddin. Visualization: Muhammad Muhsin Fathuddin, Rafeedah Fathuddin, and John Solomon Obidah. Validation: Muhammad Muhsin Fathuddin, Rafeedah Fathuddin, John Solomon Obidah, Saleh Alhaji Ado, Muhammad Bashir Tijjani, Haruna Makonjuola Kazeem. Resources: Muhammad Muhsin Fathuddin and Rafeedah Fathuddin. Methodology: Muhammad Muhsin Fathuddin, Rafeedah Fathuddin, John Solomon Obidah, Saleh Alhaji Ado, Muhammad Bashir Tijjani, Haruna Makonjuola Kazeem. Investigation: Muhammad Muhsin Fathuddin and Rafeedah Fathuddin, Saleh Alhaji Ado, Muhammad Bashir Tijjani, Haruna Makonjuola Kazeem. Funding acquisition: Muhammad Muhsin Fathuddin and Rafeedah Fathuddin Formal Analysis: Muhammad Muhsin Fathuddin, Rafeedah Fathuddin, and John Solomon Obidah. Data Curation: Muhammad Muhsin Fathuddin, Rafeedah Fathuddin, and John Solomon Obidah. Conceptualization: Muhammad Muhsin Fathuddin (lead), Rafeedah Fathuddin, John Solomon Obidah, Saleh Alhaji Ado, Muhammad Bashir Tijjani, and Haruna Makonjuola Kazeem. Project Administration: Muhammad Muhsin Fathuddin. Supervision: Saleh Alhaji Ado, Muhammad Bashir Tijjani, Haruna Makonjuola Kazeem

References

Aalipour, F., Mirlohi, M., Jalali, M., & Azadbakht, L. (2015). Dietary exposure to tetracycline residues through milk consumption in Iran. *Journal of Environmental Health Science and Engineering*, pp. 13, 1–7. https://doi.org/10.1186/s402 01-015-0235-6

Adegbeye, M. J., Adetuyi, B. O., Igirigi, A., Adisa, A., Palangi, V., Aiyedun, S., Alvarado-Ramírez E. R., Elghandour M. M. M. Y., Molina O. M., Abiodun A. Oladipo & Salem, A. Z. M. (2024). Comprehensive insights into antibiotic residues in livestock products: Distribution, factors, challenges, opportunities, and implications for food safety and public health. *Food Control*, *163*(2024), 110545. https://doi.org/10.1016/j.foodcont.202 4.110545Almashhadany, D. A. (2021). Screening of antibiotic residues in raw milk of cows and buffaloes by diffusion assays. *Italian Journal of Food Safety,* 10(3), 9034. https://doi.org/10.4081/ijfs.2021.9034

Almashhadany, D. A., Hassan, A. A., Rashid, R. F., Abdulmawjood, A., & Khan, I. U. (2024). Assessment and Assay Comparison for Detection of Antimicrobial Residues in Freshwater Aquaculture Fish in Erbil Governorate, Iraq. *Antibiotics*, 13(3), 225. https://doi.org/10.339 0/antibiotics13030225

Alnassrallah, M. N., Alzoman, N. Z., & Almomen, A. (2022). Qualitative immunoassay for the determination of tetracycline antibiotic residues in milk samples followed by a quantitative improved HPLC-DAD method. *Scientific Reports,* 12(1), 14502. https://doi.org/10.1038/s41598-022-18886-2

Chauhan, A., and Jindal, T. (2020). *Microbiological Methods for Environment, Food and Pharmaceutical Analysis*. Springer Nature. https://doi.org/10.1007/978-3-030-52024-3

Codex Alimentarius Commission, *Guidelines for The Simple Evaluation of Dietary Exposure to Food Additives* [CAC/GL 3-1989] | CODEX ALIMENTARIUS FAO/WHO 2014. From: https://www.fao.org/input/download/standards /6/cxg_003e.pdf (Accessed 04 September 2024).

Codex Alimentarius Commission, *Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for Residues of Veterinary Drugs in Foods* [CX/MRL 2-2021] | CODEX ALIMENTARIUS FAO/WHO 2021. From: https://www.fao.org/fao-who-codex alimentarius/sh-roxy/en/?lnk=1&url =https%253A%252F%252Fworkspace.fao.org %252Fsites%252Fcodex%252FStandards %252FCXM %2B2 %252FMRL2e.pdf (Accessed 06 April 2024).

Ezenduka, E. V. (2019). Screening of antimicrobial residues in poultry meat in Enugu metropolis, Enugu State, South East Nigeria. *Veterinaria Italiana,* 55(2), 143-148. https://doi.org/10. 12834/VetIt.395.1870.

Food and Agriculture Organization of the United Nations (2024) – with major processing by Our World in Data. “Per capita consumption of milk, excluding butter – FAO” [dataset]. Food and Agriculture Organization of the United Nations, “Food Balances: Food Balances (2013, old methodology and population)”; Food and Agriculture Organization of the United Nations, “Food Balances: Food Balances (2010-)” [original data]. Retrieved June 3, 2024, from https://ourworldindata.org/grapher/per-capita-milk-consumption

Hebbal, M. A., Latha, C., Menon, K. V., & Deepa, J. (2020). Occurrence of oxytetracycline residues in milk samples from Palakkad, Kerala, India. *Veterinary World,* 13*(*6), 1056. www.doi.org/ 10.14202/vetworld.2020.1056-1064

Ismail, G., El Hawari, K., Jaber, F., Verdon, E., & Al Iskandarani, M. (2024). Occurrence of antimicrobial residues in milk and labneh consumed in Lebanon. *Food Additives & Contaminants: Part B,* 17(1), 87-99. https://doi.org/10.1080/19393210.2023.2298478

Khalifa, H. O., Shikoray, L., Mohamed, M. Y. I., Habib, I., & Matsumoto, T. (2024). Veterinary Drug Residues in the Food Chain as an Emerging Public Health Threat: Sources, Analytical Methods, Health Impacts, and Preventive Measures. *Foods,* 13(11), 1629. https://doi.org/10.3390/foods1 3111629

Kumar, A., Panda, A. K., & Sharma, N. (2022). Determination of antibiotic residues in bovine milk by HPLC-DAD and assessment of human health risks in Northwestern Himalayan region, India. *Journal of Food Science and Technology*, 59(1), 95–104. https://doi.org/10.10 07/s13197-021-04988-8

Kurjogi, M., Issa Mohammad, Y. H., Alghamdi, S., Abdelrahman, M., Satapute, P., & Jogaiah, S. (2019). Detection and determination of stability of the antibiotic residues in cow’s milk. *PloS one,* 14(10), e0223475 https://doi.org/10.1371/journal. pone.0223475

Londoño-Carmona, J., Blandón-Escobar, S., Montoya-Zuluaga, J., Betancourt-Chaves, P., Castillo-Moreno, S., Arboleda-Múnera, C., & Vallejo-Timarán, D. (2024). Antibiotic residues and microbial contamination in pasteurized whole milk intended for human consumption. *Veterinary World*, 17(3), 720. https://doi.org/10.14202% 2Fvetworld.2024. 720-727

Massaccesi, L., Albini, E., Massacci, F. R., Giusepponi, D., Paoletti, F., Sdogati, S., Morena F., Agnelli A., Leccese A., Magistrali C. F. & Galarini, R. (2024). Impact of Soil Fertilization with Pig Slurry on Antibiotic Residues and Resistance Genes: A Longitudinal Study. *Antibiotics,* 13(6), 486. https://doi.org/10.3390/antibiotics13060486

Moudgil, P., Bedi, J. S., Aulakh, R. S., Gill, J. P. S., and Kumar, A. (2018). Validation Of HPLC Multi-Residue Method for Determination of Fluoroquinolones, Tetracycline, Sulphonamides, and Chloramphenicol Residues in Bovine Milk. *Food Analytical Methods*, 12(2), 338-346. https://doi.org/10.1007/s12161-018-1365-0

Moudgil, P., Bedi, J. S., Aulakh, R. S., and Gill, J. P. (2019). Analysis of antibiotic residues in raw and commercial milk in Punjab, India vis-à-vis human health risk assessment. *Journal of Food Safety*, 39(4), e12643. https://doi.org /10.1111/jfs.12643

Mouiche, M. M. M., Okah-Nnane, N. H., Moffo, F., Djibo, I., Mapiefou, N. P., Mpouam, S. E., Mfopit Y. M., Mingoas J. K., Tebug S. F. & Ndukum, J. A. (2024). Antibiotic Residues in Foods of Animal Origin in Cameroon: Prevalence, Consumers' Risk Perceptions and Attitudes. *Journal of Food Protection*, 87(4), 100237. https://doi.org/10.1016 /j.jfp.2024. 100237

Olatoye, I. O., Daniel, O. F., & Ishola, S. A. (2016). Screening of antibiotics and chemical analysis of penicillin residue in fresh milk and traditional dairy products in Oyo state, Nigeria. *Veterinary World*, 9(9), 948. https://doi.org/10.14202/vetwo rld.2016.948-954

Oluwafemi, F., Oluwabamiwo, B. F., Oluwatosin, O. O., Akinrinade, O. R., & Kolapo, A. L. (2018). Assessment of microbiological and residual antibiotics status in milk sold in Abeokuta, Ogun State. Nigeria. *Turkish Journal of Agriculture-Food Science and Technology*, 6(6), 642-651. https://doi.org/10.2 4925/turjaf.v6i6.642-651.1415

Patel, N. M., Kumar, R., Savalia, C. V., Desai, D. N., & Kalyani, I. H. (2020). Dietary exposure and risk assessment of antibiotics residues in marketed bovine raw milk. *Journal of Entomology and Zoology Studies*, 8(4), 1823-1827.

Rahman, A., Paul, P., Sarkar, M. R., Sikdar, K. Y. K., Esti, I. Z., Abid, N. M., Bari L. & Faroque, A. B. M. (2024). Antibiotic residues in pasteurised and Raw Cow's milk in Dhaka, Bangladesh. *Food Additives & Contaminants: Part B*, 1–9. https://doi.org/10.10 80/193932 10.2024.2335234

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/10.101 6/j.heliyon.2021.e07739

Rware, H., Monica, K. K., Idah, M., Fernadis, M., Davis, I., Buke, W., Solveig D., Daniel K., Duncan C., Morten B. & Keith, H. (2024). Examining antibiotic use in Kenya: farmers' knowledge and practices in addressing antibiotic resistance. *CABI Agriculture and Bioscience,* 5(1), 21. https://doi. org/10.1186/s 43170-024-00223-4

Stella, O. I. O., Ezenduka, E. V., & Anaelom, N. J. (2020). Screening for tylosin and other antimicrobial residues in fresh and fermented (nono) cow milk in Delta state, South-South, Nigeria. *Veterinary World*, 13(3), 458. https://doi. org/10.14202/vetworld.2020.458-464

Yusuf, M. S., Kabir, J., Bello, M., & Babashani, M. (2017). Occurrence of tetracycline residues in raw milk from dairy farms in Kano state, Nigeria. In *The Proceedings of the 54th Annual Congress of the Nigerian Veterinary Medical Association*