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Quality and detection of antibiotic residues in informal raw milk markets of northern (Carchi and Imbabura) Ecuador

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ABSTRACT

In Ecuador, one of the most serious problems in dairy cattle production is that more than half of milk production is sold in informal markets, without proper quality and sanitary control. The aim of this study was to determine the quality and detect antibiotic residues in raw milk sold in informal markets located in the Imbabura and Carchi provinces of Ecuador. Quality parameters such as the percentages of fat, protein, lactose, non-fat solids, and total solids, as well as density and added water, were determined using the LACTOSCAN instrument; pH and instrument-reported acidity (°D) were measured using a potentiometer. Additionally, the parameter protein stability was analyzed using the 68% alcohol test, and antibiotic residues were detected using a test kit. A total of 271 raw milk samples were analyzed, including 129 from Carchi and 142 from Imbabura. Based on the applicable requirements of NTE INEN 9:2012, 39 samples (14.39%) complied with all evaluated requirements, whereas 232 (85.61%) did not comply with at least one requirement. The highest non-compliance rates were observed for protein stability (72.32%), titratable acidity (58.30%), and relative density (35.42%). Sixteen samples (5.90%) screened positive for antibiotic residues; among the positive samples, 68.75% (11/16) screened positive for tetracyclines and 31.25% (5/16) screened positive for beta-lactams, with a significantly higher frequency in Carchi (9.30%; 12/129) than in Imbabura (2.82%; 4/142) (Pearson's $\chi^2 = 5.118$; $p = 0.024$). The findings indicate a high frequency of deviations from the applicable physicochemical, hygienic-sanitary, and residue-related requirements among informally marketed raw milk samples in the two provinces.

1. INTRODUCTION

Globally, the consumption of milk and milk products is large due to its vital source of nutrients such as essential macronutrients (fats, proteins, and carbohydrates) and micronutrients such as vitamins (riboflavin, vitamins B5, and B12) and minerals (calcium, magnesium, and selenium) [1–3]. In Ecuador, the dairy industry is a key part of the Ecuadorian economy that represents 14% of the Gross Domestic Product of agri-food and provides approximately 10% of employment at the national level; in total, 5.6 million liters of raw milk are produced on a daily basis in Ecuador, with a per capita consumption of 105 l per year [4,5]. Milk production is mainly concentrated in the Highlands area of Ecuador (Inter-Andean region), with 80% of the total national production [6]. The provinces of Carchi and Imbabura, located in the north of Ecuador, contribute with 5.11% and 1.93% of the total milk production, respectively, that represents approximately a milk production of 285,074 l per day in Carchi and 107,704 l per day in Imbabura [4]. In Ecuador, informal milk commercialization represents an important component of the dairy supply chain. Recent information indicates that approximately 48.83% of milk production was destined for informal dairy products in 2024; the informal sale of milk results from several socio-economic factors such as local culture and affordable prices [6,7]. Previous studies in Ecuador have shown that raw milk sold informally may contain antibiotic residues, representing a potential food-safety concern and highlighting the need for continued monitoring of antimicrobial use in dairy production systems [8–12]. Additionally, the informal sale of raw milk harms the milk products industry, as, in informal markets, milk products are sold at lower prices compared to formal markets, leading to unfair competition [6,13]. A previous study done in Pichincha, a province of the highlands of Ecuador, showed that 94.31% of raw milk samples sold informally did not comply with the standards of the Ecuadorian Technical Standard INEN 9 (NTE INEN 9) [10]. Similarly, studies done in Manabí and Santo Domingo de los Tsáchilas, provinces located in the coastal region of Ecuador, showed that 87.68% of the raw milk samples did not comply with NTE INEN 9 [9]; meanwhile, in the southern region of Ecuador (Cañar, Azuay, and

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Loja), 96.09% of the samples did not meet national quality standards [12]. These results suggest that the quality of raw milk sold in informal markets is compromised. Considering that Carchi and Imbabura provinces contribute approximately 7% of the total milk production in the country, and it is known that there are informal sales points, the aim of this study was to determine the quality of and detect antibiotic residues in raw bovine milk sold informally in the provinces of Carchi and Imbabura, Ecuador.

2. MATERIALS AND METHODS

2.1. Study Design and Sampling

This was a cross-sectional study conducted from April 2023 to August 2024. Raw milk samples were collected from informal sales points located in all cantons of the provinces of Carchi (Bolívar, Espejo, Mira, Montufar, San Pedro de Huaca, and Tulcán) and Imbabura (Ibarra, Cotacachi, Otavalo, Pimampiro, Urcuquí, and Antonio Ante).

Although this was not a qualitative study, informal local inquiries and field reconnaissance were used to identify informal raw milk sales points within each canton. The search process continued until no additional sales locations were identified. This approach was used solely to improve the geographic coverage of sampling sites and was not intended for qualitative data collection or analysis. Informal sales points included open and closed/open-air markets, restaurants, neighborhood convenience stores, butcheries, artisanal cheese stores, milk collection centers, bakeries, private homes, specific public roads, vehicles, and street vendors.

Raw milk samples were collected according to the NTE INEN-ISO 707 standard [14], from informal sales points in Carchi and Imbabura during four sampling rounds, yielding 271 samples in total (129 from Carchi and 142 from Imbabura). No vendor was intentionally sampled more than once. Sampling covered the six cantons of each province, providing broad geographic coverage of informal raw-milk commercialization in the study area. Samples were collected at approximately 15-day intervals according to the field sampling schedule. Because the study was designed to characterize samples available at informal sales points rather than to estimate a probability-based population prevalence, the sampling coverage should not be interpreted as statistically representative of all informal milk vendors in the two provinces.

Milk samples were placed in containers with and without a preservative (2-bromo-2-nitropropane-1,3-diol) to prevent bacterial growth. Containers were properly labeled and transported to the Milk Quality Control Laboratory of the Uyumbicho Experimental Center of the Veterinary Medicine and Zootechnics Faculty of the Universidad Central del Ecuador, located in Pichincha province.

Milk samples were transported under refrigeration and maintained at 4°C ± 1°C until laboratory analysis. All analyses were performed within 48 hours after sample collection, following the recommendations established for raw milk quality assessment.

2.2. Milk Samples Laboratory Analysis

Prior to sample analysis, the Lactoscan, EKOMILK SCAN, and pH meter were calibrated according to the manufacturers' instructions.

Milk quality analyses were performed according to the applicable Ecuadorian Technical Standards. Compliance with NTE INEN 9:2012 [15] was assessed only for parameters explicitly included in this standard, including relative density, fat, titratable acidity, total solids, non-fat solids, crude protein, protein stability, somatic cell count, and other applicable quality requirements. pH and lactose were measured as descriptive milk-quality parameters but were not classified as compliant or non-compliant with NTE INEN 9:2012 because these parameters are not specified in the published 2012 edition of the standard. The physicochemical parameters fat (%), crude protein (%), lactose (%), total solids—TS (%), relative density (g/ml), for somatic cell count, the EKOMILK SCAN® equipment milk analyzer (Boeckel+Co, GmbH+Co, series SAP-CC-028608, Nova Zagora, Bulgaria); for this, we used a sample with a preservative, and it was placed in the equipment reader. Non-fat solids—SNF (%) were calculated for each sample as the difference between total solids and fat (SNF = TS − fat), in accordance with the definition established by NTE INEN 9:2012.

The pH and titratable acidity (°D) were determined using a calibrated PHT004TA pH meter (EON TRADING, Model PHT 004TA, Series SN H00601659, Stara Zagora Industrial Area, Promishlena Str. 18, Bulgaria) equipped with a suitable electrode for milk analysis. Acidity values (°D) were transcribed directly from the instrument display, not calculated from pH by the authors. No NaOH titration was performed, and these values were not validated against a reference titration method.

Protein stability was assessed using the 68% alcohol test, and antibiotic residues were determined using the TriTest BTS Test Kit (Ring Biotechnology Co., Ltd., China) following the manufacturer's instructions.

For somatic cell count, the EKOMILK SCAN® equipment (EON Trading INC, 6006, Stara Zagora Industrial Area, Promishlena Str. 18, Bulgaria) was used. For this, 5 ml of Ekoprim surfactant (EON Trading INC, 6006, Stara Zagora Industrial Area, Promishlena Str. 18, Bulgaria) was added to the milk samples without preservative to measure their viscosity, which is proportional to the number of somatic cells in the sample [16].

Antibiotic residues were screened using TriTest BTS Test Kit strips (Beta & Tetra & Sulfa; Ring Biotechnology Co. Ltd., Beijing, China) following the manufacturer's instructions. Milk samples were first brought to room temperature and thoroughly homogenized. Subsequently, 200 µl of milk was transferred into the reaction tube and mixed five times to ensure proper homogenization. The procedure followed the kit instructions used at the time of analysis. The kit lot and packaging insert are no longer available; therefore, the exact lot/version cannot be confirmed. Upon completion of the incubation period, the results were visually interpreted by comparing the intensity of the test lines with that of the control line. Samples were considered positive when the corresponding test line was lighter than the control line or absent, indicating antibiotic residues exceeded the assay detection limit. Conversely, samples were considered negative when the test line was equal to or darker than the control line. The assay simultaneously detects residues of β-lactams, tetracyclines, and sulfonamides in raw milk. Results were interpreted visually by comparing the intensity of the test line with that of the control line after the prescribed incubation period [17].

The TriTest BTS Test Kit is a qualitative lateral-flow immunoassay for the detection of β-lactams, tetracyclines, and sulfonamides in raw milk. According to the manufacturer, the assay is designed to detect antibiotic residues at concentrations corresponding to or below internationally established maximum residue limits (MRLs). Reported limits of detection include 2–4 µg/l for penicillin G, 3–4 µg/l for ampicillin, 3–4 µg/l for amoxicillin, 40–100 µg/l for tetracyclines, and 15–100 µg/l for sulfonamides, depending on the specific compound. Therefore, positive samples were interpreted as containing detectable antibiotic residues according to the assay specifications. Positive screening results do not confirm concentrations exceeding maximum residue limits or regulatory non-compliance.[17].

Finally, protein stability was measured using the 68% w/w alcohol test based on the NTE INEN 1500 [18]. For this test, 2 ml of milk without preservative was mixed with 2 ml of alcohol at 68% w/w, giving a 1:1 milk-to-alcohol volume ratio.

2.3. Statistical Analysis

Sample size calculation and laboratory results were recorded in a Microsoft Excel spreadsheet, version 2024. For statistical analysis, we used R software and RStudio version 1.2.5019 (RStudio Inc., Boston, MA, USA). In order to determine the percentage of compliance with NTE INEN 9 quality standards, the number of milk samples meeting the reference values was divided by the total number of samples collected. This calculation was done for each quality parameter (including the presence of antibiotic residues) and by province. These results were summarized in frequency tables.

For the assessment of overall compliance, only the requirements applicable under NTE INEN 9:2012 were considered: relative density, titratable acidity, fat, crude protein, total solids, non-fat solids, added water, somatic cell count, protein stability, Antibiotic screening results were reported separately and were not used to determine overall regulatory compliance. pH and lactose were analyzed descriptively but were not included in the regulatory compliance assessment because they are not specified as requirements in NTE INEN 9:2012. A sample was considered compliant only when it met all applicable requirements simultaneously.

In addition to describing the general quality of raw milk samples in both provinces, we calculated the mean, minimum, and maximum values for fat (%), crude protein (%), total solids (%), non-fat solids (%), lactose (%), somatic cell count, relative density (g/ml), and titratable acidity (°D) by province. For qualitative parameters such as presence of antibiotic residues (negative/positive) and protein stability (negative/positive), results were expressed in percentages.

Differences between provinces were evaluated using the Kruskal–Wallis test. When significant differences were detected, pairwise comparisons were performed using Dunn's post hoc test with Bonferroni correction to adjust for multiple comparisons, as the Kolmogorov–Smirnov test indicated that all variables were not normally distributed ($p < 0.05$). For qualitative analysis, a chi-square test was used. Statistical significance was established at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Raw Milk Quality in Imbabura and Carchi Provinces

From the total raw milk samples collected ($n = 271$) in informal sales points in these provinces of Ecuador, based on the applicable requirements of NTE INEN 9:2012, 39 samples (14.4%) complied with all evaluated requirements, whereas 232 samples (85.6%) did not comply with at least one requirement. Several similar studies done in Ecuador showed high variation in raw milk sold in informal markets and in compliance with milk quality standards [8]. The proportion of non-compliant milk samples observed in the present study (85.6%) was different than that previously reported in Rumiñahui canton (74%), Pichincha province (94.3%) [10], Manabí and Santo Domingo de los Tsáchilas (87.68%) [9], and the southern region of Ecuador (Cañar, Azuay, and Loja), with 96.09% of the samples that did not comply with Ecuadorian legislation [12].

Based on NTE INEN 9 standards, the parameters with the highest percentage of compliance were protein with 85.2% (231/271), total solids with 84.5% (229/271), and non-fat solids with 76.75% (208/271). In contrast, the parameters protein stability, titratable acidity, and relative density showed the highest percentages of non-compliance with 72.3% (196/271), 58.3% (158/271), and 35.42% (96/271), respectively (Table 1). The pH and lactose results were interpreted descriptively because these parameters are not specified as requirements in NTE INEN 9:2012.

3.2. Differences in Raw Milk Quality Between Provinces

There was a difference in the total percentage of non-compliance with NTE INEN 9 quality standards between provinces, with 92.25% (131/142) of the samples from Imbabura and 78.29% (101/129) from Carchi failing to comply with at least one applicable quality requirement. This difference was statistically significant (Pearson's $\chi^2 = 10.691$; $p = 0.001$).

No significant differences were found between the provinces for the majority of quality parameters (p values > 0.05), although higher percentages of non-compliance were found for Imbabura. For instance, in Imbabura, a range of 15.49%–76.76% of milk samples did not comply with the reference values for water added, relative density, pH, titratable acidity, protein stability, and somatic cell count parameters, while in Carchi, these percentages ranged from 17.83% to 72.87% (Table 2). The difference between provinces in protein-

Table 1. General description of milk quality of raw milk samples and percentage of compliance and non-compliance with the milk quality standard NTE INEN 9. Samples were collected from informal sale points in Imbabura and Carchi provinces in Ecuador ($N = 271$).

Quality parameters	Reference value NTE INEN 9	Minimum	Maximum	Mean	Compliance *n* (%)	Non-compliance *n* (%)
Relative density (g/ml)	1.028 – 1.032 at 20 °C	1.010	1.040	1.029	175 (64.6)	96 (35.4)
Titratable acid (°D)	13–17	6	85	22.50	113 (41.7)	158 (58.3)
Fat (%)	Min 3.0	0.21	9.00	3.83	212 (78.2)	59 (21.8)
Crude protein (%)	Min 2.9	1.39	5.75	3.16	231 (85.2)	40 (14.8)
Total solids (%)	Min 11.2	5.2	22.19	12.41	229 (84.5)	42 (15.5)
Non-fat solids (%)	Min 8.2	2,87	15.85	8.58	208 (76.8)	63 (23.2)
Water added (%)	0	0	56.73	1.75	226 (83.4)	45 (16.6)
Somatic cell count (SCC/ml)	Max 700,000	17030	2782000	479268	210 (77.5)	61 (22.5)
Protein stability	Negative	-	-	-	75 (27.7)	196 (72.3)
Antibiotic residues*	MRL	-	-	-	255 (94.1)	16 (5.9)

*MRL: Maximum Residue Limit established by the Codex Alimentarius Commission.

stability outcomes was not statistically significant. Carchi was the province with a higher percentage of milk samples with antibiotic residues (9.3%) compared to Imbabura (2.8%). For antibiotic residues specifically, the difference between provinces was statistically significant (Pearson's $\chi^2 = 5.118$; $p = 0.024$).

Significant differences were found for total solids, non-fat solids, crude protein, and lactose (p values < 0.05), although differences were small between provinces except for total solids, with a mean percentage of 12.6% for Carchi (11.6% of non-compliance) and 12.2% for Imbabura (19.0% of non-compliance) (Table 2). Kruskal–Wallis testing identified significant differences between provinces in crude protein ($p = 0.0374$), total solids ($p = 0.0247$), non-fat solids ($p = 0.0166$), and lactose ($p = 0.0136$). These p values compare distributions of measured values, not compliance proportions.

3.3. Nutritional, Hygienic, Physicochemical Quality, and Antibiotic Residues

Of the milk samples, 16.6% (45/271) were adulterated with water, affecting the percentage of fat, as 21.8% (59/271) of the samples did not comply with the reference value. Of note, 35.4% (96/271) of milk samples did not comply with the reference value for relative density, with a mean of 1.029 g/ml (Table 1). Additionally, 22.5% (61/271) of samples had a somatic cell count above 700,000 CS/ml (mean of 479,269 CS/ml).

Regarding pH, 70.5% (191/271) of the milk samples had values outside the descriptive range of 6.6–6.8 used in the original analysis. However, because pH is not specified as a requirement in NTE INEN 9:2012, these results were not considered regulatory non-compliance. Among the samples outside this descriptive range**,** 92.15% (176/191) had pH values below the lower descriptive value**,** whereas 7.85% (15/191) had pH values above the upper descriptive value. The overall mean pH was 6.21, indicating that most of the samples outside this descriptive range presented acidic values. The titratable acidity, 58.3% (158/271) of the samples did not comply with the reference value (mean of 22.50 °D), of which 89.2% (141/158) had values above the maximum limit of 17 °D and 10.8% (17/158) presented values below the minimum allowed. As for protein stability, 72.3% (196/271) of the milk samples did not meet the standard (Table 1). Sixteen of the 271 samples (5.90%) screened positive for antibiotic residues. Positivity was higher in Carchi (12/129; 9.30%) than in Imbabura (4/142; 2.82%), and this difference was statistically significant (Pearson's $\chi^2 = 5.118$; $p = 0.024$). From the positive samples, 68.75% (11/16) screened positive for tetracyclines, and 31.25% (5/16) screened positive for beta-lactams.

In this study, the parameters with the highest percentages of non-compliance were protein stability (72.3%) and titratable acidity (58.30%). The non-compliance of titratable acidity was similar to the one reported in Rumiñahui canton, where approximately 54% of samples complied with the acidity requirement, corresponding to approximately 46% non-compliance [19], but it was lower than the percentage reported in Pichincha, where 72.77% of the milk samples did not comply with the reference value [10]. Similarly, in the provinces of Manabí and Santo Domingo de los Tsáchilas, the percentage of non-compliance was observed at 68.7% for acidity [9].

Besides, we found a range of titratable acidity from 6 °D to 85 °D, which varied from the reference value ranges. These milk quality parameters are associated with each other. On the one hand, elevated

Table 2. Description of milk quality of raw milk samples and percentage of compliance and non-compliance with the milk quality standard NTE INEN 9 according to province. Samples were collected at informal sale points in Imbabura and Carchi provinces from Ecuador ($N = 271$).

Parameters	Reference value NTE INEN 9	Province	Minimum	Maximum	Mean	Compliance %	Non-compliance %	*p* value
Relative density (g/ml)	1.028 – 1.032 at 20 °C	Carchi	1.010	1.036	1.028	67.44	32.56	0.11
		Imbabura	1.013	1.040	1.029	61.97	38.03	
Titratable acid (°D)	13 – 17	Carchi	12.00	85.00	24.60	48.84	51.16	0.81
		Imbabura	6.00	58.00	20.60	35.21	64.79	
Fat (%)	Min 3.0	Carchi	0.59	7.51	3.82	80.62	19.38	0.60
		Imbabura	0.21	9.00	3.84	76.06	23.94	
Crude protein (%)	Min 2.9	Carchi	1.93	4.37	3.13	84.50	15.50	0.04
		Imbabura	1.39	5.75	3.18	85.92	14.08	
Total solids (%)	Min 11.2	Carchi	10.11	19.95	12.61	88.37	11.63	0.0247
		Imbabura	5.20	22.19	12.24	80.99	19.01	
Non-fat solids (%)	Min 8.2	Carchi	6.37	15.80	8.79	82.17	17.83	0.0166
		Imbabura	2.87	15.85	8.40	71.83	28.17	
Water added (%)	0	Carchi	0.00	30.19	1.79	82.17	17.83	0.57
		Imbabura	0.00	56.73	1.72	84.51	15.49	
Somatic cell count (SCC/ml)	Max 700.000	Carchi	17,030	1,670,000	433,556	77.52	22.48	0.82
		Imbabura	18,670	2,782,000	520,795	77.46	22.54	
Protein stability	Negative	Carchi	-	-	-	27.13	72.87	1.00
		Imbabura				27.46	72.54	
Antibiotic residues	MRL*	Carchi	-	-	-	90.70	9.30	0.024
		Imbabura				97,18	2.82	

*MRL: Maximum Residue Limit established by the Codex Alimentarius Commission.
For continuous variables, p values compare distributions of measured values, not compliance proportions; for categorical outcomes, p values compare outcome frequencies between provinces.

acidity and reduced pH have frequently been associated with microbial activity, inadequate hygienic practices during milking, and inadequate temperature control during milk storage, conditions that may promote lactose fermentation and lactic acid production [20,21]. However, as microbiological analyses were not performed in the present study, direct microbial contamination cannot be confirmed. On the other hand, low acidity and altered pH values may result from milk adulteration with alkaline products or health problems in the udder (i.e., mastitis) [2,21–24]. These alterations may also affect protein stability, an important technological property of milk, particularly during industrial processing where milk is subjected to thermal treatments such as pasteurization and cheese manufacturing [25–27]. Therefore, the physicochemical alterations observed in the present study may suggest deficiencies in handling, storage, or commercialization practices, although further microbiological analyses are required to determine their underlying causes [25–27].

Regarding somatic cell count, 22.51% of milk samples did not comply with the maximum reference value of 700,000 cells/ml. A previous study in Pichincha reported 22.77% non-compliance [10], whereas approximately 21% was reported for Rumiñahui [19]. However, our result was lower than that observed in Manabí and Santo Domingo de los Tsáchilas, where a 32.51% non-compliance with the NTE INEN 9 standard was found [9]. In general, these results suggest poor management practices of dairy cattle, especially during milking; good hygiene and milking practices are known to be closely related to udder health. Thus, poor milking management leads to udder health problems, such as mastitis, and higher somatic cell counts as a result of a natural immune response of the udder. High somatic cell counts affect the organoleptic characteristics of the milk and, therefore, its quality in the milk industry [28–33].

Regarding relative density, we found a non-compliance percentage of 35.42%, which is higher than that found throughout the province of Pichincha (22.0% non-compliance), and lower than the non-compliance rate observed in Manabí and Santo Domingo de los Tsáchilas (43.4%), as well as in the Rumiñahui canton of Pichincha (26.0%) [9,10,19]. The relative density of milk increases when fat is removed, while it decreases when fat is added to the milk or whey residues from the cheese industry [1,34–39].

In fat, in our study, 21.8% of milk samples did not comply with the fat reference value, having a minimum value of 0.21, far lower than the NTE INEN 9 reference value of 3.0, and 16.6% of samples had water added, with a maximum percentage of water added of 56.7.

It is important to note that 5.90% of the raw milk samples analyzed in this study screened positive for detectable antibiotic residues according to the test used. Antibiotic-residue positivity has also been reported in other Ecuadorian studies, including approximately 7% in Rumiñahui and 6.4% in Manabí and Santo Domingo [9,19]. In contrast, our result was lower compared to the one reported in the whole of Pichincha, where 28.5% of milk samples had antibiotic residues [10]. The presence of antibiotic residues in raw milk sold informally warrants attention because these compounds may persist in milk and dairy products and may affect product quality and consumer safety [40–42]. Factors contributing to this problem include inappropriate antibiotic use, failure to respect withdrawal periods, inadequate disease prevention and control measures, lack of veterinary supervision, and easy access to antimicrobials [41,43]. Antibiotic residues in milk have been associated with adverse effects such as hypersensitivity reactions, alterations of intestinal microbiota, and interference with dairy processing technologies [41,43].

From a One Health perspective, the detection of antibiotic residues highlights the importance of responsible antimicrobial use in livestock production systems. Although antimicrobial resistance, resistant bacteria, resistance genes, and human exposure pathways were not evaluated in the present study, the presence of antibiotic residues indicates the need for continued surveillance and further investigations integrating animal, environmental, and public-health components [44–46]. Beyond the direct health effects associated with antibiotic residues, the presence of these compounds in informally marketed raw milk should also be considered within the One Health framework. Informal food supply chains may facilitate human exposure to antimicrobial residues and antimicrobial-resistant microorganisms through products that are frequently commercialized with limited sanitary oversight. Continuous exposure to low concentrations of antimicrobial agents may contribute to the selection and maintenance of resistant bacterial populations, representing a potential link between antimicrobial use in livestock and the dissemination of antimicrobial resistance among humans, animals, and the environment. Consequently, monitoring antibiotic residues in raw milk is not only relevant for food safety but also for mitigating the broader public health risks associated with antimicrobial resistance. Antimicrobial resistance is recognized by the World Health Organization as one of the top global public health and development threats facing humanity [47].

Regarding nutritional parameters, crude protein, total solids, non-fat solids, and fat percentage were the least problematic when assessing compliance with NTE INEN 9 standards. Over 79% of milk samples complied with the NTE INEN 9 reference values. This result was similar to those reported in Pichincha, Manabí, and Santo Domingo de los Tsáchilas provinces [9,10].

This study has a number of strengths but also some limitations. This is the first study conducted in Imbabura and Carchi provinces; thus, the results provide an overview of the quality of raw milk that is sold informally at different points. Additionally, milk samples were collected from different informal selling points from all cantons in each province, providing broad geographic coverage of the study areas but not ensuring statistical representativeness because the sampling was not probability-based. One limitation in our study was, however, the lack of information regarding the informal markets where raw milk is sold. Nevertheless, information on the location of informal sales points was gathered from local knowledge and community-based identification of selling locations until no additional points were identified. This approach was used solely to map and locate informal milk sales points and did not involve interviews, questionnaires, surveys, or the collection of personal information from individuals. Although our study was not qualitative, this strategy helped identify sampling points and to ensure adequate geographic coverage in both provinces. Another limitation is that it was not possible to collect extra variables that could help explain the obtained results. Extra variables such as previous use of antibiotics, time of withdrawal, farm origin, and dairy cattle management practices could be useful to interpret results. Since we focus on informal sales points, this information is not available; however, our results give a general idea of the quality of milk sold at these points. Additionally, microbiological analyses and antimicrobial resistance assessments were beyond the scope of this study; therefore, the presence of microbial contamination, antimicrobial-resistant bacteria, or resistance genes could not be evaluated.

4. CONCLUSION

More than 80% of the raw milk samples collected from informal sales points in Imbabura and Carchi provinces did not comply with at least one

quality parameter established by NTE INEN 9. The observed alterations in acidity, protein stability, and other physicochemical indicators suggest deficiencies in handling, storage, or commercialization conditions. However, because microbiological analyses were not performed, the microbiological status of the milk cannot be determined from the present study. Additional investigations incorporating microbiological assessments are required to provide a comprehensive evaluation of the safety of informally marketed raw milk.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study did not involve human subjects, human data collection, or animal experimentation. Informal milk sales points were identified through field reconnaissance and publicly available local information. No interviews, surveys, questionnaires, or collection of personal information were conducted. The study exclusively involved the purchase and laboratory analysis of commercially available raw milk samples obtained from informal retail points.

10. PUBLISHER'S NOTE

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11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI) tools for writing and editing of the manuscript, and no images were manipulated using AI.

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