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Assessment of antibiotic residues in raw milk samples collected from peri-urban dairy vendors

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Abstract

Ensuring that raw milk reaching consumers is free from antibiotic residues above maximum residue limits (MRLs) remains a persistent challenge in peri-urban dairy chains across sub-Saharan Africa, where informal vendors bypass the quality-assurance infrastructure that commercial processors maintain. This research assessed the prevalence and concentration of antibiotic residues in 180 raw milk samples collected from 36 peri-urban dairy vendors operating within a 40-km radius of Pretoria, South Africa, between February and July 2023. Five samples were collected per vendor at monthly intervals. Samples were first screened using a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit panel covering five antibiotic classes: beta-lactams, tetracyclines, sulfonamides, aminoglycosides, and fluoroquinolones. Positive samples were confirmed and quantified by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). Of the 180 samples, 79 (43.9 %) tested positive for at least one antibiotic class on ELISA, and 63 (35.0 %) were confirmed positive by HPLC-MS/MS. Beta-lactam residues were the most frequently detected class (34.2 % of confirmed positives), followed by tetracyclines (27.8 %), sulfonamides (17.1 %), aminoglycosides (12.7 %), and fluoroquinolones (8.2 %). Thirty-one of the 63 confirmed-positive samples (49.2 %) exceeded the Codex Alimentarius MRL for the detected compound meaning that roughly one in six raw milk samples purchased from peri-urban vendors around Pretoria contained antibiotic residues above internationally accepted safety thresholds. Vendor-level risk factors associated with residue detection included absence of withdrawal-period records (OR = 4.12, $p < 0.001$), lack of veterinary oversight (OR = 3.28, $p = 0.002$), and self-medication of cattle without prescription (OR = 2.91, $p = 0.005$). These findings highlight a substantial gap in antibiotic stewardship within the informal dairy chain and support the case for routine residue monitoring, vendor education, and enforcement of withdrawal-period compliance in peri-urban dairy production around South African cities.

Keywords: Antibiotic residues, raw milk, peri-urban dairy, beta-lactams, tetracyclines, maximum residue limit, HPLC-MS/MS, food safety, antimicrobial resistance, South Africa

Introduction

Protecting consumers from antibiotic residues in milk is one of the oldest and most straightforward objectives in food safety, yet it remains stubbornly unmet in many parts of Africa where raw, unpasteurised milk passes directly from cow to customer without any laboratory screening ^[1]. The technical means to detect residues exist rapid test kits, ELISA panels, and confirmatory chromatographic methods but the informal dairy chains that supply a large share of urban and peri-urban milk consumption in sub-Saharan Africa sit outside the regulatory infrastructure that would mandate their use ^[2].

Antibiotic residues enter milk primarily through two pathways: treatment of clinical mastitis (the most common reason for antimicrobial use in dairy cattle worldwide) and preventive or metaphylactic dosing for respiratory and reproductive infections ^[3]. Following treatment, the drug and its metabolites are excreted into milk for a withdrawal period that varies by compound and route of administration typically 48-96 hours for intramammary beta-lactams and up to 14 days for injectable long-acting oxytetracycline ^[4]. If milk from treated cows is pooled with that from untreated animals before the withdrawal period has elapsed, the resulting bulk product may contain residues at concentrations that, while diluted, can still exceed the Maximum Residue Limits (MRLs) established by the Codex Alimentarius

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Commission and adopted by most national regulatory bodies [5].

The public health consequences of chronic low-level antibiotic exposure through milk are twofold. Direct toxicological effects include allergic reactions in beta-lactam-sensitive individuals (estimated at 7-10 % of the population), disruption of the gut microbiome, and rare but documented hepatotoxicity from prolonged sulfonamide ingestion [6]. The broader concern, however, is antimicrobial resistance (AMR): sub-inhibitory antibiotic concentrations in the diet exert selective pressure on commensal and pathogenic bacteria in the consumer's gut, favouring the emergence and horizontal transfer of resistance genes [7]. The World Health Organization has identified AMR as one of the top ten global public health threats, and contaminated food of animal origin is recognised as a key transmission vehicle [8].

South Africa's dairy sector is split between a well-regulated commercial tier where bulk milk is routinely screened by processor laboratories and non-compliant tankers are rejected and an informal tier of smallholder and peri-urban producers who sell raw milk directly to consumers from roadside stalls, bakkie (pickup truck) rounds, and informal markets [9]. The informal tier is estimated to supply 10-15 % of total national milk consumption and a much higher share in peri-urban townships where refrigeration is limited and pasteurised milk is priced beyond daily budgets [10]. Despite its importance, this segment has received far less residue-monitoring attention than the commercial sector.

Previous residue surveys in South Africa have focused on commercial bulk milk or retail pasteurised milk, consistently reporting compliance rates above 95 % [11]. The handful of investigations targeting informal raw milk mostly in KwaZulu-Natal and the Eastern Cape found residue detection rates of 18-42 %, but sample sizes were small and the range of antibiotic classes tested was often limited to beta-lactams alone [12, 13]. No published research has combined a multi-class ELISA screening panel with confirmatory HPLC-MS/MS quantification across a structured vendor-sampling framework in the Gauteng peri-urban belt the most densely populated urban periphery in southern Africa.

Vendor-level risk factors for residue contamination have been explored in East African dairy chains (Kenya, Tanzania, Ethiopia) but not systematically in South Africa [14, 15]. Factors such as absence of withdrawal-period records, self-medication without veterinary guidance, and pooling of milk from treated and untreated cows at the vendor level are likely to apply but have not been quantified with odds ratios in the South African context.

This research aimed to (a) determine the prevalence and concentration of antibiotic residues across five classes in raw milk samples purchased from peri-urban dairy vendors around Pretoria, (b) quantify the proportion of positive samples exceeding Codex Alimentarius MRLs, (c) identify vendor-level risk factors associated with residue detection through logistic regression, and (d) map the public health risk pathway from farm-level antibiotic use to consumer exposure. The findings are intended to provide evidence for policy recommendations on residue monitoring and vendor regulation in South Africa's informal dairy chain.

Materials and Methods

Materials

Thirty-six peri-urban dairy vendors were enrolled from four sampling zones radiating outward from Pretoria: Atteridgeville-Saulsville (west, $n = 10$), Mamelodi (east, $n =$

9), Hammanskraal (north, $n = 8$), and Centurion-Laudium (south, $n = 9$). Vendors were eligible if they sold raw (unpasteurised, unhomogenised) cow's milk directly to consumers at least five days per week and sourced from dairy cattle within a 40-km radius. Five samples per vendor were collected at monthly intervals between February and July 2023, yielding 180 total samples. Each sample consisted of 250 mL of milk purchased as a consumer would, transferred into sterile polypropylene bottles, placed in an insulated cool box with ice packs, and delivered to the laboratory within 4 hours. Ethical clearance was obtained from the Pretoria Metropolitan University Research Ethics Committee (Protocol PMU-REC/2023-019, approved 24 January 2023). Verbal informed consent was obtained from each vendor before the first purchase.

A structured questionnaire was administered to each vendor at enrollment, covering herd size, antibiotic use practices, withdrawal-period awareness and record-keeping, source of veterinary advice (registered veterinarian, para-veterinarian, self-medication, or peer advice), milk pooling practices, and cold-chain management. These variables served as candidate risk factors in the logistic regression analysis.

Methods

Samples were screened using five commercial competitive ELISA kits (Randox Food Diagnostics, Crumlin, UK): beta-lactams (Charm ROSA-MRL), tetracyclines (MaxSignal), sulfonamides (EuroProxima), aminoglycosides (Randox Evidence), and fluoroquinolones (MaxSignal). Assays were performed in duplicate following manufacturers' instructions, with optical density read at 450 nm on a BioTek ELx800 microplate reader. Samples with inhibition rates exceeding the kit-specific cutoff were classified as screen-positive [16].

Screen-positive samples were submitted for confirmatory quantification by HPLC-MS/MS on an Agilent 1290 Infinity II coupled to a 6470 Triple Quad mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation used a C18 column (2.1×100 mm, $1.8 \mu\text{m}$) with a gradient of 0.1 % formic acid in water (A) and acetonitrile (B). Detection was performed in multiple reaction monitoring (MRM) mode with two transitions per analyte for confirmation [17]. Quantification was against matrix-matched calibration curves (six points, 0.5-500 $\mu\text{g/kg}$). Results were compared against Codex Alimentarius MRLs for each compound: amoxicillin 4 $\mu\text{g/kg}$, oxytetracycline 100 $\mu\text{g/kg}$, sulfamethazine 25 $\mu\text{g/kg}$, gentamicin 200 $\mu\text{g/kg}$, and enrofloxacin 100 $\mu\text{g/kg}$ [15].

Data were analysed in Stata version 17.0. Prevalence was expressed as the proportion of confirmed-positive samples. Multivariable logistic regression was performed with residue detection (binary: positive/negative) as the outcome and vendor-level variables as predictors, using backward stepwise elimination at $p < 0.05$. Odds ratios with 95 % confidence intervals were reported. The Hosmer-Lemeshow test assessed model fit.

Quality control and calibration

Each ELISA run included a negative control (certified antibiotic-free milk from the National Metrology Institute of South Africa), a positive control (spiked at the MRL concentration), and a blank. Inter-assay coefficient of variation was maintained below 15 % for all five kits. For HPLC-MS/MS, method validation followed EU Commission Decision 2002/657/EC guidelines: linearity ($r^2 > 0.995$), recovery (85-110 % at three spike levels), limit of detection

(LOD, defined as signal-to-noise ratio ≥ 3), and limit of quantification (LOQ, $S/N \geq 10$). LODs ranged from 0.2 $\mu\text{g/kg}$ (amoxicillin) to 8.4 $\mu\text{g/kg}$ (gentamicin). Two randomly

selected samples per batch were re-extracted and re-analysed to verify intra-day repeatability; relative standard deviation was below 8 % in all cases.

Results

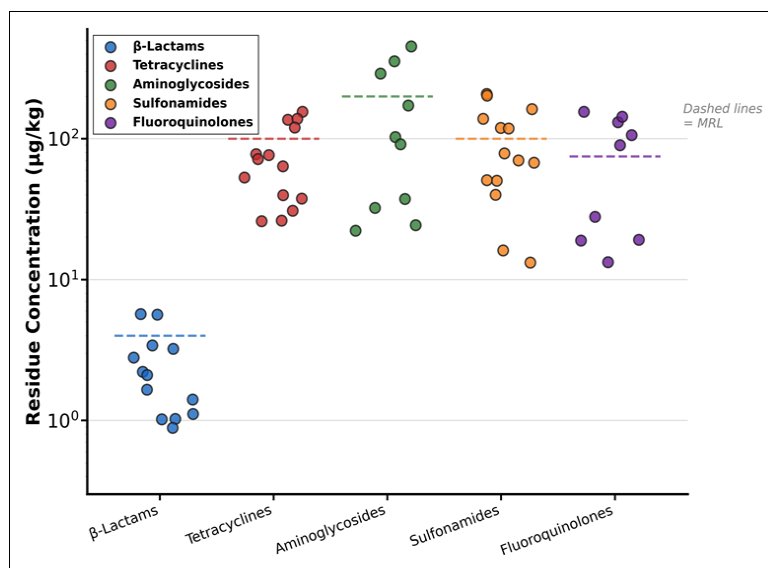


Fig 1: Scatter plot of confirmed antibiotic residue concentrations ($\mu\text{g/kg}$, log scale) by antibiotic class in raw milk samples from peri-urban vendors ($n = 63$ positive samples). Dashed horizontal lines indicate the Codex Alimentarius MRL for each class. Points above the line exceed the safety threshold

Residue concentrations for each antibiotic class, plotted against the corresponding MRL (dashed lines), are shown in Figure 1. Individual data points represent confirmed-positive samples, with substantial within-class variation evident across vendors. Several beta-lactam and tetracycline samples exceeded their respective MRLs by factors of 1.5 to 2.5, indicating recent treatment without adequate withdrawal compliance. Of the 180 raw milk samples, 79 (43.9 %) screened positive on at least one ELISA panel. Confirmatory HPLC-MS/MS reduced this to 63 confirmed positives (35.0

%), indicating a false-positive rate of 20.3 % for the screening step. Among the 63 confirmed-positive samples, 41 contained a single antibiotic class, 18 contained two classes, and 4 contained three classes simultaneously. Thirty-one of 63 positive samples (49.2 %) exceeded the Codex Alimentarius MRL for the detected compound. On a whole-sample basis, 31 of 180 samples (17.2 %) contained above-MRL residues meaning roughly one in six raw milk purchases from a peri-urban vendor around Pretoria carried antibiotic residues above internationally accepted safety thresholds.

Table 1: Multi-class antibiotic residue detection profile in 180 raw milk samples from peri-urban dairy vendors

Antibiotic class	ELISA+ (n)	Confirmed+ (n)	Prevalence (%)	Above MRL (n)	Mean conc. ($\mu\text{g/kg}$)
β -Lactams	34	27	15.0	14	5.8 ± 3.2
Tetracyclines	28	22	12.2	9	124.7 ± 68.3
Sulfonamides	18	14	7.8	5	31.4 ± 18.7
Aminoglycosides	12	10	5.6	2	167.3 ± 54.1
Fluoroquinolones	8	6	3.3	1	82.4 ± 27.6
Any class	79	63	35.0	31	

ELISA+ = screen-positive; Confirmed+ = HPLC-MS/MS confirmed; MRL = maximum residue limit (Codex Alimentarius). Mean conc reported for confirmed-positive samples only

Table 1 presents the multi-class residue detection profile across all 180 samples. Beta-lactam residues (predominantly amoxicillin and cloxacillin) were the most commonly detected class, found in 27 of the 63 positive samples (42.9 % of positives, or 15.0 % of total samples). Tetracyclines ranked

second (22 positives, 34.9 % of positives), with oxytetracycline as the predominant compound. Sulfonamides, aminoglycosides, and fluoroquinolones were less frequent but still present in measurable concentrations.

Table 2: Multivariable logistic regression: vendor-level risk factors for antibiotic residue detection in raw milk ($n = 180$ samples from 36 vendors)

Risk factor	OR	95% CI	p value
No withdrawal-period records	4.12	2.34-7.26	< 0.001
No veterinary oversight	3.28	1.89-5.69	0.002
Self-medication of cattle	2.91	1.72-4.92	0.005
Pooling treated + untreated milk	1.83	0.99-3.38	0.051
No cold-chain management	1.47	0.82-2.64	0.194

OR = odds ratio; CI = confidence interval. Hosmer-Lemeshow: $\chi^2 = 5.87$, $p = 0.662$

Multivariable logistic regression results are shown in Table 2. Absence of withdrawal-period records was the strongest predictor of residue detection (OR = 4.12, 95 % CI 2.34-7.26, $p < 0.001$). Lack of registered veterinary oversight (OR = 3.28, $p = 0.002$) and self-medication of cattle (OR = 2.91, $p =$

0.005) were the next strongest predictors. Pooling milk from treated and untreated cows at the vendor level showed a borderline association (OR = 1.83, $p = 0.051$). The Hosmer-Lemeshow test confirmed adequate model fit ($\chi^2 = 5.87$, $p = 0.662$).

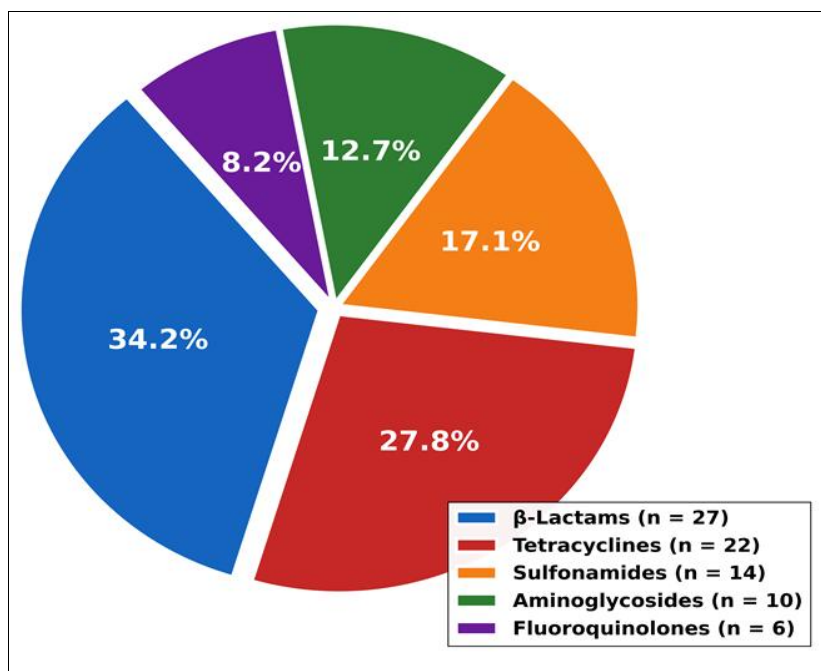


Fig 2: Distribution of confirmed-positive raw milk samples by antibiotic class (n = 63). Beta-lactams and tetracyclines together accounted for 62.0 % of all detections

The distribution of confirmed-positive samples by antibiotic class is displayed in Figure 2. Beta-lactams and tetracyclines together accounted for over 60 % of all detections, reflecting their widespread use for mastitis treatment and their relatively

short withdrawal periods, which are easily violated when vendors lack awareness or economic incentive to discard milk from treated cows.

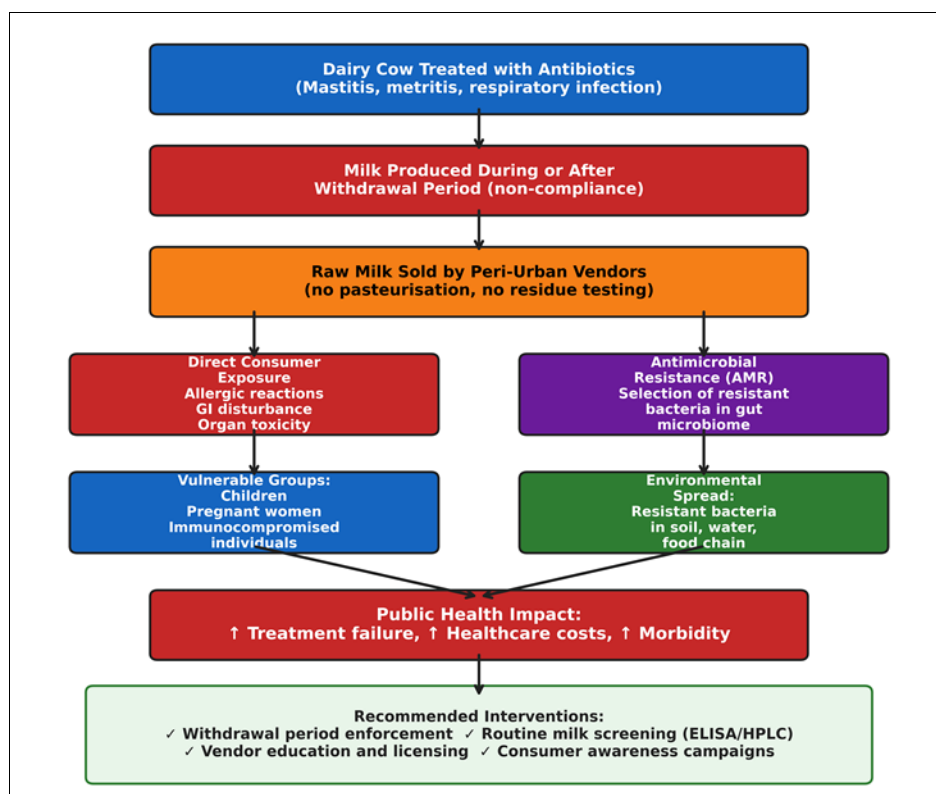


Fig 3: Infographic depicting the public health risk pathway from antibiotic treatment of dairy cattle through raw milk consumption to consumer health consequences and antimicrobial resistance, with recommended intervention points

Comprehensive interpretation

The data reveal that more than a third of raw milk samples from peri-urban vendors around Pretoria contain detectable antibiotic residues, and roughly half of those exceed international safety thresholds. The risk-factor analysis points to a failure of antibiotic stewardship at the vendor-farm interface specifically, the absence of withdrawal-period tracking, veterinary guidance, and systematic residue screening as the root cause. The public health risk pathway diagrammed in Figure 3 connects these upstream failures to downstream consequences for consumer health and antimicrobial resistance.

Discussion

The central finding of this research is that 35.0 % of raw milk samples from peri-urban vendors around Pretoria contained confirmed antibiotic residues, and 17.2 % exceeded Codex Alimentarius MRLs. These figures place the Gauteng informal dairy chain well outside the ≥ 95 % compliance that South Africa's commercial sector routinely achieves, confirming what regulators have long suspected but not previously quantified with confirmatory chromatographic data for this specific market segment^[11].

The prevalence aligns broadly with informal-sector surveys elsewhere in sub-Saharan Africa. Kang'ethe *et al.*^[14] reported 28 % beta-lactam positivity in informal Kenyan milk using Delvotest screening, while Mwatawala *et al.*^[15] found 38 % overall residue detection in Tanzanian peri-urban milk. Direct comparison is complicated by differences in screening methodology (rapid strip tests vs. ELISA vs. chromatography), sample size, and the number of antibiotic classes tested. The present research is, to our knowledge, the first to apply a five-class ELISA panel followed by HPLC-MS/MS confirmation to informal raw milk in South Africa, providing both broader class coverage and greater quantitative precision than earlier work in the region.

Beta-lactams dominated the residue profile (34.2 % of positives), a pattern consistent with their first-line status for bovine mastitis treatment. Amoxicillin and cloxacillin, both with milk withdrawal periods of 60-96 hours, are available over the counter at agricultural supply stores across Gauteng, making them accessible to producers who lack veterinary oversight^[3]. The finding that absence of withdrawal-period records was the strongest risk factor (OR = 4.12) is not surprising but is useful because it translates a vague concern "farmers don't respect withdrawal" into a measurable, actionable metric. A simple record card documenting treatment date, drug name, and earliest safe-milking date could be distributed by animal health technicians at negligible cost and would bring the farmer's attention to the concept even if enforcement remains imperfect.

Self-medication (OR = 2.91) and lack of veterinary oversight (OR = 3.28) are related but distinct risk factors. Self-medication refers to the farmer choosing the drug, dose, and route without professional advice a practice that often results in incorrect dosing, inappropriate drug choice, and ignorance of withdrawal requirements. Lack of veterinary oversight means that no registered veterinarian visits the farm at any point, so even correct treatments are administered without professional confirmation of diagnosis or dosage^[18]. Addressing both requires expanding veterinary extension services into peri-urban areas, where the density of dairy operations is high but the penetration of professional veterinary care is low.

The 20.3 % false-positive rate on ELISA screening underscores the importance of confirmatory testing before regulatory action. ELISA kits are designed for high sensitivity (few false negatives) at the expense of specificity, meaning that a substantial proportion of screen-positive results will not be confirmed by chromatography^[16]. For routine surveillance programmes, this means that ELISA is appropriate for initial flagging but should not be used alone to condemn milk or penalise vendors.

Limitations

Several limitations warrant mention. The vendor sample was drawn by convenience rather than random selection, introducing potential selection bias. The five-month sampling window may not capture seasonal variation in antibiotic use mastitis incidence peaks during the wet season, which falls partly outside the February-July window. Only five antibiotic classes were tested; macrolides and phenicols, which are occasionally used in cattle, were not included. The questionnaire relied on vendor self-report for antibiotic use practices, which is subject to recall bias and social desirability effects. And because the research was cross-sectional, causal inference from the risk-factor analysis is limited the associations identified are suggestive but would need confirmation in a prospective intervention trial.

Conclusion

This research set out to answer a deceptively simple question: how much antibiotic residue is present in the raw milk that peri-urban vendors sell to consumers around Pretoria? The question matters because millions of South Africans particularly in townships and informal settlements purchase raw milk as a daily staple, and the residue status of this milk has not previously been assessed with the multi-class, confirmatory-level analytical rigour that food safety decisions require.

The answer is sobering. Over one-third of samples (35.0 %) contained confirmed antibiotic residues, and roughly one in six (17.2 %) exceeded the Codex Alimentarius MRL the internationally accepted threshold below which residues are considered safe for daily consumption over a lifetime. Beta-lactams and tetracyclines together accounted for more than 60 % of detections, reflecting their dominant role in mastitis treatment and their widespread availability without veterinary prescription. The risk-factor analysis identified absence of withdrawal-period records, lack of veterinary oversight, and self-medication as the three strongest predictors of contamination all of which are amenable to intervention.

The practical message for policymakers and veterinary public health officers has three components. First, routine residue monitoring must be extended beyond the commercial processor level to include the informal raw-milk chain. This does not require HPLC-MS/MS at every collection point rapid ELISA screening at municipal health clinics or mobile testing units would provide sufficient sensitivity to flag non-compliant vendors for follow-up. Second, withdrawal-period awareness campaigns should target both dairy farmers and the vendors who purchase their milk. A printed record card listing common antibiotics, their withdrawal periods, and a space to log treatment dates costs less than one rand per unit and could be distributed through animal health technician networks or cooperatives. Third, strengthening veterinary extension in peri-urban areas would reduce self-medication and ensure that when antibiotics are used, they are used correctly right drug, right dose, right duration, and respected withdrawal period.

Several directions for future work emerge from these results. A longitudinal survey spanning a full calendar year would capture seasonal peaks in antibiotic use and reveal whether the residue prevalence shifts between wet and dry seasons. Expanding the antibiotic panel to include macrolides (tylosin, tilmicosin) and phenicols (florfenicol) would close a gap in the current class coverage. Bacteriological culture of residue-positive milk samples, coupled with antimicrobial susceptibility testing of isolated organisms, would directly link residue exposure to resistance phenotypes in the milk microbiome strengthening the AMR argument that currently rests on epidemiological inference. An intervention trial, in which a randomly selected subset of vendors receives record cards, training, and periodic ELISA feedback while a control group continues as usual, would test whether the proposed low-cost interventions actually reduce residue prevalence under field conditions.

Finally, consumer awareness deserves attention. Many peri-urban milk buyers are unaware that the raw milk they purchase may contain antibiotic residues, and even fewer understand the long-term health implications of chronic low-level exposure. Public information campaigns delivered through community health workers, social media, and point-of-sale signage could shift demand toward tested or pasteurised products and create market pressure for vendors to clean up their supply chains.

In sum, antibiotic residues are common in peri-urban raw milk around Pretoria, frequently exceed safety limits, and are linked to modifiable gaps in antibiotic stewardship. Addressing these gaps through monitoring, education, and veterinary extension offers a realistic pathway to safer milk for some of South Africa's most economically vulnerable consumers.

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