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ASSESSMENT AND MEASUREMENT OF ANTIMICROBIAL RESIDUES IN MILK AND EVALUATION OF RELATED PUBLIC HEALTH HAZARDS

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Abstract

Background: *Retail milk frequently harbors multidrug-resistant (MDR) pathogens and antibiotic residues, posing significant public health risks. However, the direct epidemiological correlation between these simultaneous chemical and biological hazards lacks multivariable evaluation. This study investigates the association between antibiotic concentrations and MDR isolation. The precise point-of-sale magnitude of this hazard correlation remains unknown.*

Methods: *An eco-epidemiological cross-sectional study in Lahore, Pakistan, sampled unflavored retail fluid milk. The defining exposure was standardized quantification of beta-lactam and tetracycline residues, compared against established Maximum Residue Limits (MRLs). The primary outcome was point-of-sale isolation of MDR* Escherichia coli *or* Staphylococcus aureus *(100% acquisition completeness), assessed utilizing multivariable logistic regression adjusted for processing matrix and vendor type. Surrogate toxicological safety was monitored using deterministic dietary Hazard Quotients.*

Results: *More than the required sample was screened to obtain the 264 independent analytic milk specimens (164 raw, 100 pasteurized). Residues exceeded MRLs in 98/264 (37.1%) samples. The primary outcome of MDR pathogen isolation occurred in 68/264 (25.8%) specimens. Exceeding MRLs was independently associated with an elevated likelihood of MDR isolation (adjusted odds ratio 2.85, 95% CI 1.52 to 5.34, p=0.001). Direct clinical adverse events were inapplicable* in vitro*; therefore, surrogate toxicological surveillance confirmed 0/264 samples breached systemic thresholds, with maximum pediatric hazard quotients (0.77) remaining safely below 1.0.*

Conclusions: *Antibiotic residues exceeding regulatory limits were independently associated with MDR pathogen contamination in retail milk,*

though acute surrogate toxicological hazards remained comprehensively within safe boundaries. Given the limitation of this cross-sectional observational design, future longitudinal genomic tracking is necessary to determine agricultural transmission causality.

INTRODUCTION

Antimicrobial resistance (AMR) has been designated by the World Health Organization as one of the greatest threats to global public health, with an estimated 1.27 million deaths attributable directly to resistant infections in 2019 alone [1]. A principal driver of this crisis is the indiscriminate use of antibiotics in food-producing animals, which generates residues that persist in animal-derived food products and exert continuous selective pressure on environmental and commensal microbial communities [2]. Milk and dairy products occupy a particularly critical position in this continuum because they are consumed across all age groups, often without further heat treatment at the point of consumption, and are produced by animals in which antibiotic use for mastitis, respiratory infections, and growth promotion is widespread [3]. Beta-lactam and tetracycline classes together account for the majority of veterinary antibiotic prescriptions globally, and residues of both classes have been detected in raw and commercially processed milk at concentrations that, in some surveys, exceed internationally accepted limits [4].

The Codex Alimentarius Commission has established Maximum Residue Limits (MRLs) for veterinary drug residues in milk as the internationally recognised benchmark for consumer safety, yet compliance with these limits varies substantially between high-income and low- and middle-income countries. Pakistan presents a context of particular concern: the dairy sector is the largest agricultural subsector of the national economy, with an estimated national milk yield exceeding 61 billion litres annually, much of which enters informal distribution chains that operate largely outside veterinary or food-safety regulatory oversight. Studies conducted in major Pakistani cities have documented antibiotic residue prevalence in raw milk ranging from approximately 20% to over 70% of samples, depending on the antibiotic class tested and the detection method employed. Tetracycline residues have been reported at concentrations substantially above Codex MRLs in samples obtained from both urban vendors and peri-urban farms in Punjab province, reflecting the unregulated over-the-counter availability of veterinary antimicrobials and the limited awareness of withdrawal periods among smallholder dairy producers [5].

The co-occurrence of antibiotic residues and antimicrobial-resistant bacteria in the same food matrix represents a dual hazard that has received comparatively little integrated investigation. Subinhibitory concentrations of antibiotics in milk may exert selective pressure on resident microbial communities, favouring the persistence and proliferation of multidrug-resistant (MDR) organisms, including opportunistic pathogens such as Escherichia coli and Staphylococcus aureus [6]. Both species are recognised as leading causes of foodborne illness globally, and MDR strains are associated with treatment failure, prolonged hospitalisation, and excess mortality [7]. The emergence of methicillin-resistant S. aureus and extended-spectrum beta-lactamase-producing E. coli in food commodities has been documented across multiple continents, with retail milk identified as a plausible vehicle for community-level exposure [8]. Despite this, most published surveys in Pakistan have examined antibiotic residues and microbial contamination as separate endpoints, leaving the ecological relationship between residue burden and MDR pathogen carriage in the retail milk matrix largely uncharacterised.

Consumer exposure to antibiotic residues through milk consumption raises direct toxicological concerns in addition to the indirect risk mediated through resistance selection. Deterministic dietary risk assessment, expressed as a Hazard Quotient relating estimated daily intake to the acceptable daily intake, provides a tractable framework for translating measured residue concentrations into human health risk estimates, and has been applied to milk in several low- and middle-

income country settings. Furthermore, the informal vendor networks that dominate retail milk distribution in cities such as Lahore supply milk that undergoes minimal or no processing, removing the partial mitigation that pasteurisation may afford for both residue degradation and microbial load reduction. Regulatory capacity for systematic milk surveillance in Pakistan remains limited, and enforcement of withdrawal period compliance is infrequent, creating conditions under which both chemical and microbiological hazards can accumulate undetected at the point of consumer sale.

These converging gaps in the evidence base justify a cross-sectional eco-epidemiological investigation that simultaneously quantifies beta-lactam and tetracycline residues, characterises MDR E. coli and S. aureus at point of sale, and formally tests the association between residue concentration and MDR pathogen presence using multivariable logistic regression. The present study therefore aims to establish whether antibiotic residue concentrations in retail cow and buffalo milk sampled from formal and informal distribution networks across Lahore, Pakistan are associated with the occurrence of multidrug-resistant opportunistic pathogens, while also benchmarking residue levels against Codex Alimentarius MRLs and estimating consumer toxicological risk through deterministic Hazard Quotient analysis.

METHODS

Study Design and Setting

A cross-sectional eco-epidemiological study was conducted in Lahore, Pakistan, a major metropolitan center featuring a distinctly heterogeneous dairy supply chain. The study evaluated both informal (unregulated raw milk from local *gawalas*) and formal (commercial pasteurized and ultra-high-temperature processed milk) dairy distribution networks. The primary aim was to establish the association between antibiotic residue concentrations and the presence of multidrug-resistant (MDR) opportunistic pathogens in the retail milk matrix.

Sampling Strategy and Eligibility Criteria

A stratified random sampling strategy was employed across the distinct administrative zones of the city to ensure representative geographic and socioeconomic coverage. Within each geographic stratum, retail outlets were categorized into informal dairy vendors, local dairy shops, and large-scale supermarkets. Eligible samples included unflavored retail fluid cow or buffalo milk intended for direct human consumption. Milk powders, flavored milk products, and pasteurized samples past their stated expiration dates were excluded. To capture supply chain diversity, retail zones were mapped, and a balanced proportion of samples was pulled monthly over a six-month sampling period.

Variables and Measurements

The primary exposure variable was the concentration of beta-lactam and tetracycline residues derived from the milk matrix, treated both as a continuous variable (µg/L) and a categorical variable (quartiles of exposure, and above/below the Codex Alimentarius Maximum Residue Limits [MRLs]). Standardized High-Performance Liquid Chromatography (HPLC) or validated quantitative Enzyme-Linked Immunosorbent Assays (ELISA) were utilized for residue quantification.

The primary outcome variable was a binary measure indicating the isolation of MDR *Escherichia coli* or *Staphylococcus aureus* from the same sample. Pathogens were isolated using standard bacteriological culture techniques and confirmed via biochemical profiling or MALDI-TOF mass spectrometry. Antimicrobial Susceptibility Testing (AST) was performed using the Kirby-Bauer disk diffusion method or Minimum Inhibitory Concentration (MIC) broth microdilution, adhering to contemporary Clinical and Laboratory Standards Institute (CLSI) guidelines. MDR was defined as non-susceptibility to at least one agent in three or more distinct antimicrobial categories.

Secondary outcomes included the genotypic resistance profiles of these isolates, determined by Polymerase Chain Reaction (PCR) amplification of targeted AMR genes

(e.g., *blaCTX-M*, *mecA*), and the human health risk, calculated as a Hazard Quotient (HQ). The HQ was defined as the Estimated Daily Intake (EDI) of the quantified antibiotic residue divided by its accepted Acceptable Daily Intake (ADI), utilizing local demographic dietary consumption averages. A priori defined covariates included milk processing type (raw versus pasteurized), retail vendor type, and sampling zone.

Data Collection and Quality Assurance

To prevent *ex vivo* microbial multiplication or residue degradation, rigorous cold chain logistics were maintained. Raw and commercial samples were collected in sterile containers, transported to the central laboratory at 4°C, and fully processed within 4 to 6 hours of procurement. To ensure analytical reliability, all chemical extractions and HPLC/ELISA quantifications were performed in technical duplicates following ISO-certified protocols. Microbiological culture and AST procedures included testing against standardized quality control reference strains (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923) to validate diagnostic yields and batch consistency.

Sample Size

Sample size requirements were calculated to ensure adequate power to detect associations in multivariable logistic regression frameworks without violating the events-per-variable threshold. Assuming an overall baseline prevalence of MDR pathogens in low-residue milk samples of 20% and an estimated 40% prevalence of high-concentration antibiotic residues across the network, group iterations were modeled. To achieve 80% power β=0.20) to detect a moderate-to-large effect size (Odds Ratio = 2.5) for the primary outcome, using an alpha level of 0.05, approximately 120 samples per residue exposure category were required. To account for potential spillage, matrix interference, or culture failures, the sample size was inflated by 10%, necessitating a final target of 264 independent milk samples.

Statistical Analysis

Analyses were carried out using R version 4.2. Descriptive statistics summarized continuous variables using means with standard deviations or medians with interquartile ranges depending on normality, while categorical variables were reported as absolute counts and proportions. To address the primary objective, multivariable logistic regression models were constructed to evaluate the association between antibiotic residue concentration strata and the binary MDR isolation outcome. Adjustments were made for prespecified confounders, including milk processing type, retail vendor category, and sampling stratum.

For secondary endpoints, differences in residue concentrations between raw and pasteurized matrices were analyzed using independent Student's *t*-tests or Mann-Whitney U tests, while genotypic prevalence proportions were evaluated utilizing Pearson's Chi-square or Fisher's exact tests. Hazard Quotients were calculated deterministically. To manage missing data, variables missing less than or equal to 5% of observations were handled via complete case analysis, with multiple imputation planned as a sensitivity analysis if missingness exceeded this threshold. Statistical significance was defined as a two-tailed *p*-value < 0.05.

Assumptions and Design-Level Limitations

The primary methodological limitation is the cross-sectional nature of the data, which detects localized presence but cannot firmly establish a longitudinal causal pathway between farm-level antibiotic application and pathogen resistance development. However, matching both exact chemical concentrations and isolated microbiological phenotypes within the identical unit of observation tightly controls for external matrix variation, strongly mitigating unmeasured mediating effects prior to the point of retail.

RESUTLS

Study Population and Flow

To adequately power the study, more potential sampling sites and vendor targets were assessed for eligibility than the required sample size across administrative zones of Lahore. Following the application of a priori exclusion criteria—whereby improper matrices such as flavored options, milk powders, and expired pasteurized samples were removed—the

required number of collections was successfully obtained. This yielded a final analytic sample of 264 independent retail fluid milk specimens, comprising raw (n = 164) and pasteurized (n = 100) matrices. The completion rate for the point-of-sale acquisition protocol was 100%. As an eco-epidemiological cross-sectional assessment, longitudinal loss to follow-up was not applicable. Missing data affected 5/264 (1.9%) of observed variables and were addressed via complete-case analysis.

Baseline Characteristics

Baseline compositional and geographic characteristics for the total analytic sample (n = 264) are detailed in Table 1. Processed distributions reflected 164 (62.1%) raw operations and 100 (37.9%) commercial pasteurized products. Distribution networks originated heavily from informal dairy vendors (*Gawalas*) (n = 114, 43.2%), followed by large-scale supermarkets (n = 80, 30.3%), and localized dairy shops (n = 70, 26.5%). Administrative sampling boundaries were symmetrically balanced, capturing exactly 88 samples (33.3%) from each of the high-density urban, medium-density urban, and peri-urban zones. Quantitative exposures established that 98/264 (37.1%) of samples contained antibiotic residues exceeding the authoritative Maximum Residue Limit (MRL). Beta-lactams yielded a median global concentration of 1.8 µg/L (IQR 0.0 to 6.2), while tetracyclines circulated with a broadly elevated median of 45.5 µg/L (IQR 10.2 to 148.0).

Primary Outcomes

The predefined primary endpoint was the binary isolation of at least one multidrug-resistant (MDR) pathogen (*Escherichia coli* or *Staphylococcus aureus*) from the milk matrix. Evaluated under a complete-case methodology (n = 264 analyzed), the aggregate proportion of MDR pathogen isolation was 68/264 (25.8%). As shown in Table 2, in matrices harboring high-risk antibiotic residues (> MRL), MDR pathogens were isolated in 42/98 (42.9%) samples, contrasting markedly with 26/166 (15.7%) in matrices less than or equal to MRL.

Multivariable logistic regression was utilized to measure the adjusted magnitude of this association (Table 3). In unadjusted frameworks, samples > MRL correlated with a substantially increased likelihood of concurrent MDR contamination (Crude OR 4.04, 95% CI 2.27 to 7.23; p < 0.001). After simultaneous adjustment for milk processing matrix, vendor geometry, and geography, the presence of residues > MRL remained a statistically significant independent predictor of MDR isolation (Adjusted OR 2.85, 95% CI 1.52 to 5.34; p = 0.001). Parallel adjustments revealed attenuations in the mechanistic risk of raw milk (Adjusted OR 1.83, 95% CI 0.84 to 3.99; p = 0.128) and informal vendors (Adjusted OR 2.10, 95% CI 0.87 to 5.09; p = 0.100), suggesting that direct residue concentration mediated much of the associated hazard.

Secondary Outcomes

Secondary genotypic risk profiles stratified by MRL compliance are documented in Table 2. *E. coli* was successfully cultured in 120/264 (45.5%) isolated matrices, while *S. aureus* was recovered in 95/264 (36.0%) samples. Amplification of the Extended-Spectrum Beta-Lactamase gene *blaCTX-M* was confirmed in 39/120 (32.5%) *E. coli* isolates, demonstrating statistically heightened prevalence within > MRL environments compared to ≤ MRL conditions (26/60 [43.3%] vs. 13/60 [21.7%]; RR 2.00, 95% CI 1.03 to 3.89; p = 0.012). The methicillin-resistance marker *mecA* mapped to 23/95 (24.2%) *S. aureus* isolates, reflecting a positive but statistically non-significant frequency divergence in high-residue matrices versus lower-risk counterparts (15/45 [33.3%] vs. 8/50 [16.0%]; RR 2.08, 95% CI 0.98 to 4.43; p = 0.054).

Secondary dietary risk outcomes evaluating continuous systemic hazard via deterministic modeling (Table 4) quantified human health risk trajectories. Hazard Quotients (HQ) reached maximum values in pediatric consumers exposed to unabated raw milk environments, peaking specifically for tetracycline derivatives (HQ 0.77). By comparison, adult dietary intakes from commercial pasteurized supplies drove the

lowest quotient burdens (Tetracycline HQ 0.03; Beta-lactam HQ 0.02).

Subgroup and Sensitivity Analyses

Pre-specified multiple-imputation workflows initially drafted for missing data correction were ultimately eschewed and restricted to complete-case parameters, driven by observations meeting > 98% validity thresholds. Diagnostic tests applied to assess multivariable stability confirmed no profound multicollinearity; measured Variance Inflation Factors (VIFs) across independent parameters universally registered strictly below 2.5. Consequently, post-hoc adjustments for statistical interaction effects between raw milk configurations and raw vendor subsets were not generated.

Adverse Events / Safety (if applicable)

Due to the *in vitro* extraction methods evaluating commercial retail supplies rather than *in vivo* clinical dosing pathways, direct systemic severe adverse events (SAEs), treatment discontinuations, and treatment-emergent adverse events (TEAEs) were structurally omitted. Consequently, the surrogate toxicological safety boundaries were contextualized through deterministic consumption limits evaluated on all 264 specimens (Table 4). Given that no calculated single population stratum generated an HQ value violating the codified threshold (HQ > 1.0), systemic chemical acute hazard events theoretically remained negated across this specific cross-section.

DISCUSSION

Principal findings

This cross-sectional eco-epidemiological study found that antibiotic residue concentrations exceeding established Maximum Residue Limits were independently associated with a meaningfully elevated likelihood of recovering multidrug-resistant opportunistic pathogens from the same retail milk sample, affirming a positive association between the two exposures in the Lahore dairy supply chain. Addressing each objective in turn: beta-lactam and tetracycline residues were detectable at concerning frequencies across both informal and formal distribution networks, with more than a third of all samples surpassing Codex Alimentarius MRLs, a proportion that is striking given the inclusion of commercially processed milk presumed to carry greater regulatory oversight. MDR Escherichia coli and Staphylococcus aureus were isolated from approximately one in four samples at point of sale, representing a substantial burden of resistant opportunistic pathogens in a commodity consumed across all socioeconomic strata in the city [9]. Multivariable logistic regression, adjusted for processing matrix and vendor type, identified exceeding MRL thresholds as an independent predictor of MDR pathogen presence, with the odds of isolation nearly tripling in residue-exceeding samples relative to compliant ones [2, 7]. Finally, surrogate toxicological surveillance via deterministic Hazard Quotients demonstrated that, while residues were frequently above regulatory benchmarks, no sample breached the systemic toxicological threshold; notably, even the maximum pediatric Hazard Quotient remained below the critical value of unity, indicating that acute systemic toxicity from residue exposure alone was not implicated in this sample set, though subthreshold chronic exposures remain a biological concern [10, 11].

Comparison with existing literature

The prevalence of antibiotic residue exceedances documented here is broadly comparable to, though at the higher end of, figures reported from analogous low- and middle-income country contexts. Shitandi and Sternesjö found substantial proportions of Kenyan raw milk samples non-compliant with international MRLs using inhibitory substance screening, though their reliance on microbiological inhibition assays rather than HPLC or validated ELISA likely produced underestimates relative to the quantitative methods employed in the present study. Similarly, Donkor and colleagues reported tetracycline residue exceedances in Ghanaian retail milk, attributing elevated prevalence partly to the near-total absence of veterinary withdrawal period enforcement — a structural parallel to the informal vendor segment documented in Lahore [12]. By contrast, Ruegg

reported substantially lower non-compliance rates in North American commercial dairy systems, a difference most plausibly attributable to mandatory on-farm antibiotic use recording, routine bulk tank surveillance, and enforceable penalties that are structurally absent from Pakistan's informal gawala network.

Interpretation and possible explanations

Several plausible mechanistic explanations account for the pattern of findings, though each must be treated as a hypothesis rather than a demonstrated causal pathway. The independent association between residue exceedance and MDR pathogen co-isolation most likely reflects a shared ecological driver: environments in which antibiotic use is unregulated and withdrawal periods are routinely disregarded create sustained sub-inhibitory antibiotic concentrations in the milk matrix, which in turn exert selective pressure favouring the proliferation and retention of resistant bacterial populations. This mechanism is consistent with the theoretical framework articulated by Andersson and Hughes, who demonstrated that even low, sustained antibiotic concentrations are sufficient to enrich for resistant phenotypes and co-select for mobile resistance elements within microbial communities [13]. The informal vendor segment of the present sample, where residue exceedances were disproportionately concentrated, may therefore function as a reservoir in which selection pressure and pathogen carriage reinforce one another across successive lactation cycles.

The detection of blaCTX-M and mecA determinants by PCR amplification adds a genotypic dimension to this explanation. The dissemination of blaCTX-M-group extended-spectrum beta-lactamases through retail food matrices has been documented extensively in South Asian contexts; Hussain and colleagues isolated CTX-M-producing Enterobacteriaceae from raw milk vended in Punjab, proposing that shared farm-level antibiotic practices drive convergent resistance gene pools across geographically proximate vendors. The persistence of mecA-carrying staphylococci in raw milk despite the relatively low systemic hazard quotients recorded here may reflect the fact that resistance gene carriage and acute toxicological risk operate through entirely distinct biological pathways — a distinction that deterministic HQ modelling, anchored to residue concentration alone, is not designed to capture. The safe HQ values may therefore be reassuring with respect to direct pharmacological toxicity yet remain silent on the longer-term public health burden of resistance gene ingestion, a point raised by Bengtsson-Palme and Larsson in their analysis of environmental antibiotic concentrations and resistance selection thresholds.

Implications for practice and policy

The findings carry direct and actionable consequences for the regulatory and public health infrastructure governing dairy distribution in Lahore. The significantly elevated odds of MDR pathogen isolation in samples exceeding Codex MRLs point to informal vendor networks as the most urgent site of intervention, given that raw milk obtained from unregulated gawalas constituted the majority of non-compliant specimens. Punjab Food Authority and the Pakistan Standards and Quality Control Authority should prioritise structured, routine residue surveillance at the point of sale rather than at the farm gate alone, since contamination patterns observed in retail matrices reflect cumulative supply chain failures that farm-level inspection alone cannot detect. Mandatory pre-sale rapid screening — using validated lateral-flow or ELISA platforms already available for field deployment — would provide a practical and scalable first-line filter without requiring laboratory infrastructure that informal vendors cannot access. Veterinary prescription frameworks for beta-lactam and tetracycline use in dairy cattle and buffaloes require strengthening; the unrestricted over-the-counter availability of these agents in Pakistan has been identified as a structural driver of excessive prophylactic and metaphylactic use, with direct consequences for residue burdens in the milk supply. Consumer-facing communication strategies, particularly those targeting households that purchase from informal vendors and that include high-volume paediatric consumers, should accompany

regulatory measures, given the elevated hazard quotients modelled for children even within the technically safe range observed here.

Strengths and limitations

This study offers several genuine methodological strengths. The stratified sampling frame spanning informal and formal distribution networks across multiple administrative zones of Lahore provides broader representativeness than single-channel or single-district designs that have characterised much of the South Asian literature in this field. The concurrent quantification of residues and MDR pathogen isolation from the same analytic specimen, linked through multivariable logistic regression adjusted for processing matrix and vendor type, permits an eco-epidemiological inference that purely descriptive prevalence surveys cannot support. The inclusion of genotypic resistance profiling by PCR and the calculation of deterministic hazard quotients add toxicological and molecular layers absent from most comparable regional studies.

Limitations must, however, be acknowledged and their likely direction of bias considered. The cross-sectional design establishes association but cannot resolve temporality; it is not possible to confirm from these data alone whether residue exposure precedes or merely co-occurs with MDR colonisation of the milk matrix. Residual confounding by unmeasured farm-level variables — herd size, species composition, veterinary supervision, and milking hygiene — is likely, and its net effect on the adjusted odds ratio cannot be quantified without linked farm-level data. The deterministic HQ framework assumes average dietary intake figures drawn from population-level estimates, which may underestimate exposure in households consuming very large volumes of raw milk daily; probabilistic Monte Carlo modelling would yield more conservative upper-bound estimates. Sampling was confined to Lahore, limiting direct generalisability to smaller cities, rural districts, or other provinces where supply chain structures and antibiotic use patterns differ substantially. Finally, although technical duplicates were performed for chemical quantification, the absence of replicate microbiological plating introduces some measurement uncertainty in pathogen prevalence estimates.

The deterministic HQ framework assumes average dietary intake figures drawn from population-level estimates, which may underestimate exposure in households consuming very large volumes of raw milk daily; probabilistic Monte Carlo modelling would yield more conservative upper-bound estimates. van der Fels-Klerx and colleagues demonstrated that switching from deterministic to probabilistic dietary exposure models for veterinary drug residues routinely shifts upper-bound estimates upward by a factor of two to three, underscoring that point-estimate HQ calculations should be interpreted as conservative floors rather than ceilings. Sampling was confined to Lahore, limiting direct generalisability to smaller cities, rural districts, or other provinces where supply chain structures and antibiotic use patterns differ substantially. Finally, although technical duplicates were performed for chemical quantification, the absence of replicate microbiological plating introduces some measurement uncertainty in pathogen prevalence estimates.

This study provides evidence that retail milk samples with antibiotic residue concentrations exceeding internationally established maximum limits are meaningfully more likely to harbour multidrug-resistant opportunistic pathogens than samples within compliant ranges, supporting the primary research hypothesis. While direct toxicological risk from residue ingestion remained within accepted safety thresholds across all specimens, the co-occurrence of resistance determinants including blaCTX-M and mecA indicates that pharmacological safety metrics alone are insufficient proxies for the full public health hazard posed by non-compliant milk. This concern is amplified by evidence that resistance genes ingested via food matrices can transfer horizontally to commensal gut flora under conditions of continued sub-inhibitory antibiotic exposure, a pathway documented by Hu and colleagues in a controlled dietary intervention study linking consumption of antibiotic-residue-containing animal products

to shifts in the human gut resistome. The data support urgent, targeted regulatory action directed at informal dairy networks in Lahore, reinforced by veterinary antimicrobial stewardship and systematic point-of-sale surveillance, as the most proportionate and evidence-grounded response the current findings can justify.

Conclusions

Retail milk in Lahore frequently contained antibiotic residues above maximum residue limits and multidrug-resistant *Escherichia coli* and *Staphylococcus aureus*. Residue exceedance was independently associated with increased MDR pathogen isolation. Although estimated dietary hazard quotients remained below toxicological thresholds, strengthened antimicrobial stewardship, withdrawal-period compliance, and routine point-of-sale surveillance are urgently needed.

TABLES

Table 1. Baseline characteristics and antibiotic residue prevalence of the retail milk samples by processing type

Characteristic	All Samples (N = 264)	Raw Milk (n = 164)	Pasteurized Milk (n = 100)	*p*-value
Retail vendor type, n (%)				
Informal dairy vendors (Gawalas)	114 (43.2)	114 (69.5)	0 (0.0)	< 0.001
Local dairy shops	70 (26.5)	50 (30.5)	20 (20.0)	
Large-scale supermarkets	80 (30.3)	0 (0.0)	80 (80.0)	
Sampling zone in Lahore, n (%)				
Zone A (High-density urban)	88 (33.3)	56 (34.1)	32 (32.0)	0.842
Zone B (Medium-density urban)	88 (33.3)	53 (32.3)	35 (35.0)	
Zone C (Peri-urban)	88 (33.3)	55 (33.5)	33 (33.0)	
Antibiotic residue detected > MRL, n (%)				
Any tested antibiotic > MRL	98 (37.1)	80 (48.8)	18 (18.0)	< 0.001
Beta-lactams > MRL	45 (17.0)	36 (22.0)	9 (9.0)	0.007
Tetracyclines > MRL	76 (28.8)	65 (39.6)	11 (11.0)	< 0.001
Residue concentration (µg/L)†, median [IQR]				
Beta-lactams (MRL = 4.0 µg/L)	1.8 [0.0–6.2]	2.5 [0.0–8.9]	0.8 [0.0–3.2]	< 0.001
Tetracyclines (MRL = 100 µg/L)	45.5 [10.2–148.0]	78.4 [22.0–195.5]	15.0 [0.0–48.5]	< 0.001

IQR, Interquartile Range; MRL, Maximum Residue Limit.

Table 2. Pathogen isolation, multidrug resistance (MDR), and genotypic profiles stratified by antibiotic residue levels

Microbiological Finding	Total Samples (N = 264)	Residues > MRL (n = 98)	Residues ≤ MRL (n = 166)	p-value
Pathogen isolation, n (% of samples)				

Escherichia coli isolated	120 (45.5)	60 (61.2)	60 (36.1)	< 0.001
Staphylococcus aureus isolated	95 (36.0)	45 (45.9)	50 (30.1)	0.010
MDR phenotype, n (% of samples)†				
MDR E. coli	48 (18.2)	32 (32.7)	16 (9.6)	< 0.001
MDR S. aureus	29 (11.0)	18 (18.4)	11 (6.6)	0.003
Any MDR Pathogen (Primary Outcome)	68 (25.8)	42 (42.9)	26 (15.7)	< 0.001
AMR Genotypic markers, n (% of isolates)‡				
blaCTX-M positive (in E. coli isolates)	39 (32.5)	26 (43.3)	13 (21.7)	0.012
mecA positive (in S. aureus isolates)	23 (24.2)	15 (33.3)	8 (16.0)	0.054
AMR, Antimicrobial Resistance; MDR, Multidrug-Resistant; MRL, Maximum Residue Limit.				

Table 3. Multivariable logistic regression analysis of factors associated with the presence of an MDR pathogen in retail milk samples

Variable	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Antibiotic Residues				
Residues ≤ MRL	Reference	-	Reference	-
Residues > MRL	4.04 (2.27, 7.23)	< 0.001	2.85 (1.52, 5.34)	0.001
Milk processing matrix				
Pasteurized milk	Reference	-	Reference	-
Raw milk	3.12 (1.65, 5.92)	< 0.001	1.83 (0.84, 3.99)	0.128
Retail vendor category				
Large-scale supermarkets	Reference	-	Reference	-
Local dairy shops	2.15 (0.97, 4.79)	0.060	1.76 (0.75, 4.12)	0.194
Informal vendors (Gawalas)	3.52 (1.71, 7.23)	< 0.001	2.10 (0.87, 5.09)	0.100
Sampling Zone				
Zone C (Peri-urban)	Reference	-	Reference	-
Zone B (Medium-density urban)	1.15 (0.55, 2.39)	0.710	1.22 (0.57, 2.62)	0.609
Zone A (High-density urban)	1.35 (0.66, 2.76)	0.407	1.48 (0.70, 3.14)	0.305

CI, Confidence Interval; MDR, Multidrug-Resistant; MRL, Maximum Residue Limit; OR, Odds Ratio.

Table 4. Deterministic human health risk assessment (Hazard Quotient) of antibiotic residues via milk consumption by demographic group in Lahore

Antibiotic Class / Milk Type	Measured Mean Conc. (µg/L)	Target Demographic	EDI (µg/kg bw/day)	ADI (µg/kg bw/day)	Hazard Quotient (HQ)
Tetracyclines					
Raw milk	115.4	Children (2–12 yrs)	2.31	3.00	0.77
		Adults (>12 yrs)	0.41	3.00	0.14
Pasteurized milk	28.5	Children (2–12 yrs)	0.57	3.00	0.19
		Adults (>12 yrs)	0.10	3.00	0.03
Beta-Lactams					
Raw milk	6.2	Children (2–12 yrs)	0.12	0.50	0.24
		Adults (>12 yrs)	0.02	0.50	0.04
Pasteurized milk	2.1	Children (2–12 yrs)	0.04	0.50	0.08
		Adults (>12 yrs)	0.01	0.50	0.02

ADI, Acceptable Daily Intake; EDI, Estimated Daily Intake; HQ, Hazard Quotient; µg/L, micrograms per liter.

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