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# ASSESSMENT OF ANTIBIOTIC CONTAMINATION IN MILK AND ITS IMPACT ON CONSUMER HEALTH

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## Abstract

*Antibiotic residues in bovine milk remain a globally relevant food-safety and public-health concern. This expanded review synthesizes evidence on sources and pathways of contamination; occurrence patterns; analytical screening and confirmatory workflows; regulatory standards and maximum residue limits (MRLs); and the spectrum of health implications, including antimicrobial resistance (AMR), hypersensitivity reactions, microbiome perturbation, and toxicological effects. It further integrates case studies of surveillance and control, formulates risk assessment approaches, and proposes layered mitigation strategies aligned with a One Health framework. The paper emphasizes harmonization of analytical performance criteria, risk-based monitoring, and stewardship policies that reduce residues at source while preserving animal welfare and dairy-sector efficiency (Codex Alimentarius Commission, 2021; European Commission, 2021; U.S. Food and Drug Administration [FDA], 2023; World Health Organization [WHO], 2022).*

## INTRODUCTION

Milk and dairy products contribute essential macronutrients and micronutrients to the human diet. Ensuring their safety requires stringent controls along the farm-to-table continuum. Antibiotics are indispensable tools in modern dairy practice for treating clinical infections especially mastitis and, in some settings, for metaphylaxis and prophylaxis. Misuse, dosing errors, or failure to observe labeled withdrawal periods can lead to residues persisting in milk beyond legally permitted thresholds (Codex Alimentarius Commission, 2021; FDA, 2023). Even subtherapeutic exposure may select for resistance in commensals, undermine fermentation-based processing, and provoke hypersensitivity in

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susceptible individuals (WHO, 2022). Globalized trade in dairy further elevates the need for harmonized standards and robust analytical capabilities to detect an expanding range of compounds and metabolites (European Commission, 2021).

This review consolidates evidence and practice guidance for assessing antibiotic contamination in milk, spanning etiological pathways, analytical technologies, regulatory frameworks, and health impacts. It proposes a pragmatic mitigation agenda tailored to producers, processors, laboratories, and policymakers. Although the focus is bovine milk, principles extend to other dairy species.

## 2. Sources and Pathways of Antibiotic Contamination in Milk

Antibiotic residues reach milk through many overlapping routes: direct therapeutic treatment of lactating animals, intramammary (dry-cow) therapy, group-level metaphylaxis or prophylaxis, off-label or extra-label use, dosing/administration errors, and cross-contamination from equipment, tanks or communal pipelines. Long-acting injectable or depot formulations and drugs with high lipophilicity or strong protein binding may produce prolonged excretion and low-level persistence in milk. Intrinsic factors (drug class, formulation, lipophilicity, protein binding, metabolism/excretion pathway) and extrinsic factors (animal health, stage of lactation, milk yield and milking frequency, hygiene and on-farm segregation, and quality of record keeping) all modulate residue kinetics. Good veterinary oversight, accurate diagnosis, correct dosing, clear labelling and decision-support that calculates and flags withdrawal periods (and prevents off-label usage) are critical to reduce inadvertent residue violations.

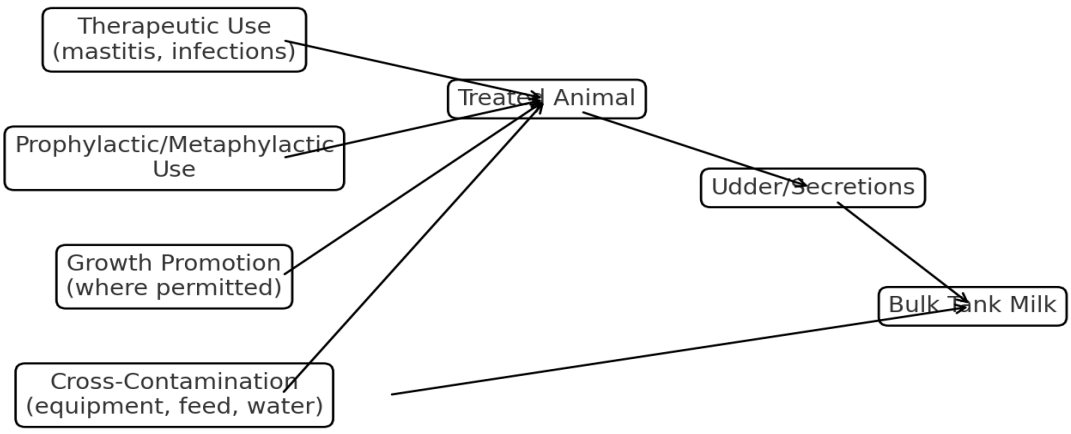


Figure 1. Conceptual pathways by which antibiotic residues can enter milk.

**Routes, Mechanisms, Risk Modifiers and Control Measures**

| Route / source | Short description | Mechanism creating residue in milk | Typical drug classes often implicated (examples) |
|---|---|---|---|
| **Therapeutic treatment of lactating animals** | Individual animals treated for systemic infection (IM, SC, oral). | Drug enters bloodstream → partitions into milk depending on lipophilicity, pKa, protein binding and mammary blood flow. | Penicillins, cephalosporins, macrolides, tetracyclines, sulfonamides, fluoroquinolones. |

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| **Intramammary / dry-cow therapy** | Antimicrobials infused into udder at drying-off or for mastitis. | Residual depot in teat canal/udder tissue leads to slow leaching into milk, especially during early lactation. | Beta-lactams, aminoglycosides, cloxacillin, cephalosporins. |
| **Metaphylactic / prophylactic group treatment** | Whole-herd or group dosing to prevent/treat an outbreak. | Large proportion of herd exposed → elevated risk of residues entering bulk tank. | Tetracyclines, sulfonamides, macrolides (depending on regulations). |
| **Off-label / extra-label use** | Use of a product by an unapproved route, species, or dose. | Unpredictable milk excretion; withdrawal periods unknown or longer than label. | Any antimicrobial when used extra-label (human fluoroquinolones, long-acting injectables). |
| **Administration errors** | Wrong dose, route, interval, or wrong animal. | Overdose or misapplied route increases systemic exposure and milk transfer. | Any class risk depends on drug. |
| **Cross-contamination (equipment, vessels, pipelines)** | Residues transfer from contaminated equipment or shared pipelines. | Residues remain on surfaces or in pooled milk → contaminate subsequent loads. | Highly soluble drugs; some cleaning-resistant compounds. |
| **Residual tissue depots / sustained-release formulations** | Depot following injection or slow-release product. | Slow release from tissue → prolonged low plasma and milk levels. | Long-acting macrolides, depot penicillins/cephalosporins. |

| Route / source | Key risk modifiers (intrinsic & extrinsic) | Typical detection / excretion pattern (general) | Practical mitigation & monitoring actions |
|---|---|---|---|
| **Therapeutic treatment of lactating animals** | Dose, route, frequency, product formulation (short vs long acting), health (mastitis increases transfer), milk yield. | .From hours to days; long-acting injectables extend window; metabolites may persist. | Follow label and vet prescription; record treatment; flag withdrawal; segregate milk from treated animals until clearance. |
| **Intramammary / dry-cow therapy** | Formulation (sustained-release salts/vehicles), incomplete treatment, premature calving, early milking post-treatment. | Extended tail in first days of next lactation; can exceed systemic drug excretion times. | Use approved dry-cow products; record dry-off date; withhold early milk post-calving; test milk if uncertain.. |
| **Metaphylactic / prophylactic group treatment** | Failure to segregate treated groups; variable compliance; errors in calves vs milking cows. | Widespread low-level residues; intermittent positives. | Avoid routine prophylaxis; treat targeted groups; segregate milk; label and track treated milk; test before reintroduction |
| **Off-label / extra-label use** | Lack of vet oversight, reliance on human drugs, incomplete knowledge of withdrawal. | Highly variable; often longer than expected. | Require vet prescription; consult regulatory guidance; document |

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| | | | justification; conservative withdrawal periods. |
| **Administration errors** | Human error, poor labeling, low training, poor record keeping. | Single-cow high residues → contaminate bulk milk. | Use SOPs; double-check identity; train staff; dosing charts; electronic records. |
| **Cross-contamination (equipment, vessels, pipelines)** | Inadequate cleaning, pooled containers, shared pipelines. | Low-level contamination; sporadic positives. | Validated CIP cleaning; dedicated waste containers; routine swab testing if recurrent. |
| **Residual tissue depots / sustained-release formulations** | Formulation type, injection site, fat stores, reduced clearance. | Weeks for some depots; long tail of excretion possible. | Avoid long-acting formulations in lactating cows unless permitted; document withdrawal periods. |

**3. Occurrence, Screening, and Monitoring**

Residue occurrence in milk is heterogeneous across regions and production systems, reflecting differences in disease burden, drug access, and enforcement rigor. Surveillance typically combines tiered approaches:

On-farm or intake rapid tests, cheap, fast screening to prevent bulk contamination.

Laboratory immunoassays, targeted screening for common drug classes.

Confirmatory analysis (HPLC, LC–MS/MS) – highly specific, quantitative confirmation for regulatory compliance.

Efficiency is improved with risk-based sampling, prioritizing farms/seasons with higher mastitis prevalence, prior residue violations, or greater antimicrobial use. Test method validation (specificity, sensitivity, accuracy, repeatability) is essential to ensure reliable results, and robust QA systems (internal standards, proficiency testing, ring trials) maintain laboratory.

**Detection**:

- Microbiological inhibition tests: cheap, broad-spectrum, but prone to false positives and lack specificity.
- Receptor/enzyme-based rapid tests (lateral-flow, ELISA): faster and more specific, but limited multi-class coverage.
- Chromatographic/mass spectrometry methods (HPLC, LC–MS/MS): gold standard, highly sensitive and specific, quantitative, but costly and requiring skilled staff and infrastructure.

**Surveillance and Detection Methods**

| Method / Tier | Principle | Typical use | Strengths | Limitations |
|---|---|---|---|---|
| **On-farm or intake screening tests (rapid tests)** | Immunoassay or receptor-binding lateral-flow devices; sometimes microbial inhibition kits | Immediate screening of cow milk or tanker intake | Very fast (minutes); Easy to use; Prevents contaminated milk entering supply | Limited to certain drug classes; Semi-quantitative only; Cross-reactivity may occur |
| **Microbiological inhibition tests** | Growth inhibition of indicator organism (e.g., Bacillus | Broad-spectrum screen; low- | Inexpensive; Detects wide range; Useful for | False positives (from natural inhibitors, detergents); Low |

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| | stearothermophilus) | cost settings | large-scale screening | specificity; Not quantitative |
| **Laboratory immunoassays (ELISA, enzyme-based)** | Antibody–antigen or enzyme substrate interaction | Screening bulk samples for drug classes | High throughput; More specific than microbial tests; Many commercial kits | Limited to class-specific detection; May miss off-target residues; Cross-reactivity possible |
| **Receptor/enzyme-based rapid tests** | Receptor proteins or enzymes binding antibiotics with visual readout | On-farm or intake checks for key antibiotics | Fast and simple; Good specificity to target class | Limited multi-class coverage; Possible false negatives near threshold |
| **Confirmatory chemical analysis (HPLC, LC–MS/MS)** | Chromatographic separation and UV/fluorescence or mass spectrometry | Regulatory compliance; quantitative confirmatory testing | Gold standard; High sensitivity and specificity; Quantitative; Multi-class capable | High cost; Requires skilled operators; Advanced infrastructure; Slower turnaround |
| **Risk-based sampling & QA measures** | Sampling weighted by risk factors; QA with internal standards, proficiency testing | National surveillance and industry monitoring | More efficient than random sampling; Ensures robustness and comparability | Requires reliable farm-level data; QA adds costs and training |


Figure 2. Typical multi-tier workflow from screening to confirmatory analysis. Sensitivity and specificity increase to the right.

**Figure 2. Typical multi-tier workflow from screening to confirmatory analysis.**

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**4. Analytical Methods: Principles, Performance, and Pitfalls**

Chromatography-based techniques remain the gold standard for confirmatory testing. HPLC with ultraviolet or fluorescence detection is suitable for certain analytes after sample cleanup (solid-phase extraction or liquid–liquid extraction). LC–MS/MS expands coverage to dozens of antibiotics and metabolites in a single run with sub-μg/L limits of detection when methods are optimized for matrix effects. Key challenges include matrix suppression, analyte instability (e.g., β-lactam hydrolysis), and achieving ruggedness across instruments (European Commission, 2021).

Method validation parameters should include selectivity, linearity, recovery, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and measurement uncertainty. Isotopically labeled internal standards are essential to correct extraction and ionization variability. Participation in external proficiency testing bolsters credibility (European Commission, 2021; FAO/WHO, 2020).

Non-targeted high-resolution mass spectrometry (HRMS) is emerging for suspect screening and elucidation of untargeted features, including metabolites and transformation products. Portable MS and biosensor platforms are being piloted for rapid on-site verification; however, standardized performance criteria, reference materials, and interlaboratory studies are needed before routine regulatory deployment (FAO/WHO, 2020).

**5. Regulatory Standards and Maximum Residue Limits (MRLs)**

Internationally, the Codex Alimentarius Commission establishes reference maximum residue limits for veterinary drugs in foods through the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluations. Regions maintain jurisdiction-specific tolerances and enforcement policies. In the European Union (EU), Regulation (EU) No 37/2010 lists permitted substances and their MRLs for food-producing animals, while Implementing Regulation (EU) 2021/808 defines performance criteria and validation for analytical methods used in official control of residues (Codex Alimentarius Commission, 2021; European Commission, 2021).

In the United States, the Pasteurized Milk Ordinance (PMO) and FDA tolerances guide control and enforcement, with mandatory routine screening at milk plants (FDA, 2023).

Differences in MRLs arise from risk-assessment paradigms, consumption patterns, and uncertainty factors. Harmonization efforts aim to reduce trade friction, but exporters must meet the strictest applicable standard in destination markets. Transparent communication to producers regarding label changes, prohibited lists, and surveillance priorities is crucial to reduce inadvertent violations (FAO/WHO, 2020).

**6. Public Health Impacts**

Antimicrobial resistance (AMR): Chronic, low-dose exposure to antibiotic residues may co-select for resistant bacteria in the human gut or transfer resistance determinants via the food chain. While direct causal links are challenging to prove epidemiologically, precautionary reduction of residues supports broader AMR stewardship objectives (WHO, 2022; World Organisation for Animal Health [WOAH], 2021).

Hypersensitivity reactions: β-lactam residues, even at trace levels, can trigger reactions in sensitized individuals, justifying strict tolerances and targeted screening for β-lactams (FDA, 2023).

Gut microbiota disruption: Subinhibitory exposures may alter microbial composition and function, with potential metabolic and immunomodulatory consequences. Research gaps include dose–response relationships and population susceptibility (FAO/WHO, 2020).

Toxicological effects: Certain drug classes exhibit organ-specific toxicities at elevated exposures; risk assessments incorporate acceptable daily intakes (ADIs) and uncertainty factors to derive MRLs protective for lifetime consumption (Codex Alimentarius Commission, 2021).

Technological/processing impacts: Residues can inhibit starter cultures in yogurt and cheese production, leading to fermentation failures, off-flavors, and yield losses—creating economic incentives for processors to enforce strict raw-milk testing (FAO/WHO, 2020).

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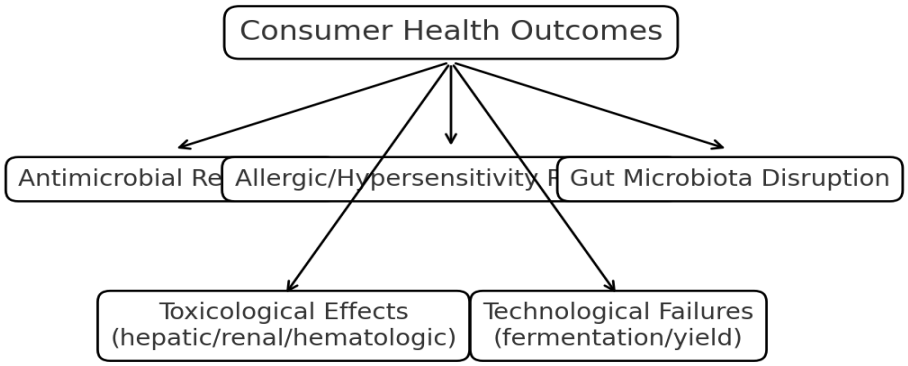


Figure 3. Conceptual map of consumer health outcomes linked to antibiotic residues in milk.

**7. Case Studies and Surveillance Lessons**

Case Study A Risk-based screening: A cooperative dairy introduced risk scoring that weighted mastitis incidence, prior residue violations, and antibiotic purchasing to prioritize farm visits and sampling frequencies. Noncompliance rates fell within a year as education and corrective actions targeted high-risk farms (FAO/WHO, 2020).

Case Study B Method harmonization: A national reference laboratory transitioned from HPLC to LC–MS/MS multi-residue panels covering >50 analytes. Implementation required method transfer, analyst training, and interlaboratory comparisons; detection capability improved and turnaround times declined (European Commission, 2021).

Case Study C Selective dry-cow therapy: Adoption of selective dry-cow therapy, guided by somatic cell counts and culture results, reduced blanket intramammary antibiotic use without compromising udder health, decreasing the probability of residue-positive milk post-calving (WOAH, 2021).

**8. Risk Assessment: From Exposure to Risk Characterization**

Risk assessment integrates residue occurrence (concentration distributions), consumption data, and toxicological endpoints. Deterministic screening compares high-end exposure against the acceptable daily intake (ADI). Probabilistic models account for variability in intake and residue levels, providing population-level risk metrics. Special attention is warranted for vulnerable groups such as children, pregnant individuals, and those with known drug allergies (Codex Alimentarius Commission, 2021; FAO/WHO, 2020).

Uncertainty analysis should address analytical measurement error, sampling design, inter-individual variability, and model assumptions. Transparent documentation strengthens policy decisions. Monte Carlo simulation and Bayesian hierarchical models are increasingly used to propagate uncertainty and quantify risk under various monitoring scenarios (FAO/WHO, 2020).

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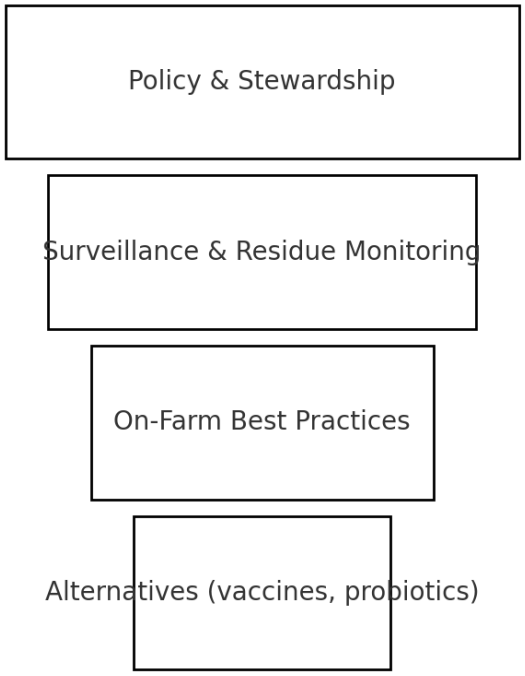


Figure 4. Hierarchy of mitigation strategies to reduce antibiotic residues in milk.

**9. Mitigation Strategies and Best Practices**

On-farm practices: Accurate diagnosis and targeted therapy; veterinary oversight; adherence to labeled doses and withdrawal periods; clear identification and segregation of treated animals; meticulous treatment records; and maintenance of milking equipment to prevent cross-contamination. Decision-support tools (e-prescriptions, mobile apps) that flag withdrawal periods and drug interactions can reduce human error (WOAH, 2021).

Processor controls: Supplier approval programs, routine intake screening, hold-and-test protocols, and traceability systems that enable rapid containment of suspect loads. Statistical process control (SPC) can detect shifts in residue nonconformance rates and guide targeted audits (FDA, 2023).

Surveillance and data use: Integration of laboratory findings with animal health and purchasing data helps detect emerging risks, inform training, and optimize sampling strategies. Dashboards that visualize nonconformances by farm and antibiotic class support data-driven stewardship (FAO/WHO, 2020).

Alternatives to antibiotics: Vaccination against prevalent mastitis pathogens, improved bedding and hygiene, selective dry-cow therapy, teat sealants, nutritional support (trace minerals, vitamins), and exploration of bacteriophages and probiotics are promising (WOAH, 2021; WHO, 2022).

Education and culture: Continuous training, feedback loops, and a food-safety culture that empowers staff to report near-misses and stop production when needed are essential components of sustained improvement (FDA, 2023).

**10. Future Perspectives and Research Priorities**

Analytical frontiers: Expansion of non-targeted screening (high-resolution MS) to capture emerging drugs and metabolites; portable MS and biosensor platforms for rapid on-site verification; standardization of reference materials; and cloud-

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based libraries for spectral matching (FAO/WHO, 2020; European Commission, 2021).

Data and digital: Digital treatment logs, e-prescriptions, and decision-support tools that flag withdrawal periods and drug interactions in real time can reduce error. Data-sharing frameworks among regulators, processors, and producers can support risk-based controls while preserving privacy (FDA, 2023).

Policy and economics: Incentive structures that reward low-residue performance, coupled with harmonized enforcement, can balance animal welfare with public health. Economic analyses are needed to evaluate the cost-effectiveness of alternative mastitis control strategies and enhanced surveillance (FAO/WHO, 2020).

One Health integration: Coordinated surveillance across farms, food, environment, and clinics is necessary to attribute AMR risks more precisely and to evaluate cross-sector interventions (WHO, 2022; WOAH, 2021).

## 11. Practical Toolkit for Stakeholders

Producers: Maintain a treatment log; color-code or isolate treated animals; schedule milk withholding with clear dates; conduct pre-harvest tests (FDA, 2023).

Veterinarians: Employ diagnostics to guide therapy; choose narrow-spectrum drugs when appropriate; set explicit withdrawal dates; educate on side effects (WOAH, 2021).

Processors: Verify supplier compliance; apply random and risk-based sampling; communicate violations transparently with corrective action plans (European Commission, 2021).

Regulators: Publish accessible MRLs and prohibited lists; invest in reference labs and proficiency testing; report surveillance outcomes to build trust (Codex Alimentarius Commission, 2021; FAO/WHO, 2020).

## 12. Conclusion

Antibiotic residues in milk are a preventable hazard, but preventing them requires coordinated action at multiple levels of the dairy value chain. At the farm level, responsible prescribing by veterinarians based on accurate diagnosis, evidence-based protocols, and strict adherence to withdrawal periods remains the cornerstone of prevention. Equally important are disciplined on-farm practices: robust record-keeping, effective training of farm staff, segregation of treated animals, and maintenance of clean milking equipment.

**A layered system of protection combines:** Prudent prescribing and stewardship: Ensuring antimicrobials are only used when clinically necessary, with preference for narrow-spectrum agents and adherence to guidelines. On-farm and intake screening: Rapid tests to detect suspect milk before it enters bulk supply, preventing costly recalls. Laboratory-based confirmatory testing: High-sensitivity and high-specificity techniques (LC–MS/MS) to quantify residues and provide defensible evidence for regulatory enforcement. Regulatory frameworks and enforcement: Clear maximum residue limits (MRLs), harmonized withdrawal periods, and transparent penalties for violations to create accountability. From an economic perspective, residue prevention protects farmer revenue by avoiding milk rejections, trade restrictions, and reputational damage, while also reducing waste. At the same time, protecting consumers from antimicrobial residues is integral to public health and trust in dairy products.

**Innovation is reshaping the field: Analytics:** Development of multi-residue, high-throughput methods; portable biosensors; and faster in-line detection systems. Data systems: Digital herd management platforms that auto-calculate withdrawal periods, flag treated animals, and integrate test results into supply chain traceability. Incentives and stewardship programs: Industry and government-led schemes that reward compliance, fund training, and support alternatives (vaccination, biosecurity, selective dry-cow therapy).Finally, alignment with the One Health framework ensures that milk residue control is not an isolated food safety issue but a component of the global fight against antimicrobial resistance (AMR). By minimizing unnecessary antimicrobial exposure in animals and preventing residues in food, dairy systems contribute to protecting the effectiveness of antimicrobials for human and veterinary medicine alike (WHO, 2022; WOAH, 2021).

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**Appendix A. Abbreviations and Glossary**

ADI – Acceptable Daily Intake
AMR – Antimicrobial Resistance
ELISA – Enzyme-Linked Immunosorbent Assay
HPLC – High-Performance Liquid Chromatography
LC–MS/MS – Liquid Chromatography–Tandem Mass Spectrometry
LOD/LOQ – Limit of Detection/Limit of Quantification
MRL – Maximum Residue Limit
SCC – Somatic Cell Count
SPE – Solid-Phase Extraction
TMR – Total Mixed Ration

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