



Comparative evaluation of analytical techniques for detecting antibiotic residues in milk within Indian dairy systems

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Abstract

Antibiotic residues in milk represent an important food safety and public health concern, particularly in India where dairy production is largely dominated by smallholder systems and characterized by variable climatic conditions and limited cold-chain infrastructure. Effective residue monitoring therefore requires analytical techniques that are both scientifically reliable and operationally feasible within diverse dairy supply chains. This study presents a comparative evaluation of four major analytical approaches used for detecting antibiotic residues in milk: microbial inhibition assays, immunoassays, biosensor-based platforms, and chromatographic techniques coupled with mass spectrometry. Published literature and validation studies were assessed using comparative criteria including sensitivity, specificity, reproducibility, analytical throughput, cost considerations, infrastructure requirements, and applicability within Indian dairy environments. The analysis indicates that LC–MS/MS provides the highest analytical sensitivity and specificity and is therefore most suitable for confirmatory regulatory testing. Microbial inhibition assays represent the most cost-efficient option for large-scale primary screening, while immunoassays enable rapid intermediate screening at milk collection points. Biosensor technologies demonstrate promising sensitivity and potential for decentralized field detection but remain limited by environmental stability and calibration challenges. The findings support the implementation of a tiered analytical framework integrating screening and confirmatory techniques for effective nationwide antibiotic residue surveillance in Indian dairy systems.

Keywords: Antibiotic residues, milk safety, residue detection methods, microbial inhibition assays, immunoassays, biosensor technologies, LC–MS/MS, dairy residue monitoring, tiered analytical framework, Indian dairy systems

Introduction

Milk occupies a central position in the Indian diet and economy. India produces more milk than any other country, yet the sector is marked by a production structure that relies predominantly on small farms and local collection systems. These systems often operate in environments with inconsistent cold storage, variable sanitary conditions, and diverse milk compositions that include both cow and buffalo milk. The combination of these structural and environmental conditions contributes to challenges in regulating milk quality and detecting contaminants (Banerjee *et al.*, 2024; Sharma *et al.*, 2022) ^[1, 34]. Among the most significant contaminants in need of systematic monitoring are antibiotic residues. Antibiotics are frequently used in dairy animals for therapeutic and preventive purposes, particularly for mastitis control (Sivaraman & Yann, 2018; Kurjogi *et al.*, 2019) ^[18, 37]. Many antibiotics approved for veterinary use require observance of withdrawal periods to ensure that residues do not appear in milk supplied for consumption (Kurjogi *et al.*, 2019) ^[18]. However, studies indicate that improper antibiotic administration, limited awareness of withdrawal periods, poor documentation of treatment, and economic pressures to maintain milk supply can contribute to residue contamination (Moudgil *et al.*, 2019; Sharma *et al.*, 2022) ^[22, 34]. Residues above recommended limits are associated with allergic reactions, disruption of fermentation processes, and the development of antimicrobial resistance (Oliver *et al.*, 2011; Sahoo *et al.*, 2023) ^[24, 32]. International health agencies emphasize that systematic residue monitoring is essential for protecting food safety and preventing the spread of antimicrobial resistance in human and animal populations (Navrátilová *et al.*, 2024; Vercelli *et al.*, 2023) ^[23, 40]. Analytical methods for residue detection have

developed considerably over the past five decades (Mitchell *et al.*, 1998; Reig *et al.*, 2020) ^[21, 29]. Early detection approaches relied on microbial inhibition assays that use microbial growth responses as indicators of contamination. These assays remain widely used because of their simplicity and relatively low cost (Navrátilová *et al.*, 2024; Vercelli *et al.*, 2023) ^[23, 40]. Immunoassays later gained prominence with the development of enzyme-linked detection systems that enable more targeted identification of antibiotic classes. Biosensor technologies have emerged more recently as rapid detection tools that offer portability and high analytical sensitivity (Rayappa *et al.*, 2023) ^[28]. The most advanced techniques involve chromatographic separation combined with mass spectrometric detection, which enables highly accurate multi-residue identification and quantification (Vercelli *et al.*, 2023) ^[40]. Research on antibiotic residues in milk in India has expanded considerably during the past two decades, with numerous studies examining residue prevalence, associated risk factors, and analytical detection methods (Moudgil *et al.*, 2019; Kumar *et al.*, 2022) ^[17, 22]. However, important gaps remain, particularly regarding comparative performance of analytical methods under real-world conditions in Indian dairy systems. Many evaluations are conducted under controlled laboratory environments that do not fully reflect climatic variability, supply-chain constraints, and sample handling conditions typical of dairy production and transport in India (Banerjee *et al.*, 2024) ^[1]. Milk composition also varies between species, as buffalo milk contains higher fat and protein content than cow milk, which can influence extraction efficiency and assay response (Kumar *et al.*, 2022) ^[17]. The literature rarely integrates these contextual variables into systematic comparisons of analytical techniques. The present study

addresses this gap by assembling a comprehensive literature-based comparison of four major analytical categories for antibiotic residue detection in milk. The analysis considers laboratory performance data, environmental sensitivities, infrastructural requirements, and the practical suitability of analytical techniques within Indian dairy environments. By synthesizing multidisciplinary research in analytical chemistry, food safety, and dairy science, the study aims to situate detection methods within a framework that reflects the complex realities of Indian milk production and regulatory monitoring.

Literature Review

1. Antibiotic Use and Residues in Dairy Systems

Antibiotic use in dairy animals has increased due to mastitis and other infections. Studies show that misuse and overuse contribute to residue contamination and antimicrobial resistance in livestock systems, particularly in low- and middle-income countries where regulatory oversight may be limited (Landers *et al.*, 2012; Van Boeckel *et al.*, 2015) [19, 39]. These studies illustrate the scale of antibiotic exposure in animals whose milk enters the food chain. Residues persist when withdrawal periods are not followed or when animals are treated without veterinary oversight. Studies conducted in various regions of India report residue detections in both organized and unorganized dairy sectors. Researchers have found substantial variation in residue prevalence between regions and seasons, and the study attributed this variation partly to inconsistencies in farm management practices (Sharma *et al.*, 2017). Economic constraints and lack of awareness were identified as major risk factors for residue persistence (Sachi *et al.*, 2019) [31]. Residues pose multiple public health risks. Even low concentrations of antibiotic residues interfere with lactic cultures used for dairy fermentation, which affects product quality and safety (Ryser & Marth, 2007) [30]. The connection between residues and the development of resistant bacteria is well established. Woolhouse and colleagues (2015) argue that environmental reservoirs of resistance often originate from livestock associated antibiotic use [42].

2. Analytical Methods for Antibiotic Residue Detection

Analytical methods fall broadly into four categories; each has been examined extensively in scientific literature for accuracy, detection limits, and operational suitability (Reig *et al.*, 2020; Gaudin *et al.*, 2017) [10, 11, 29].

2.1 Microbial Inhibition Assays

Microbial inhibition assays are among the oldest methods used for detecting antibiotic residues in milk (Bergwerff & Schloesser, 2003) [2]. These assays rely on the inhibition of microorganisms by antibiotics present in milk samples. Studies have shown that microbial inhibition assays can detect a wide variety of antibiotics, particularly beta-lactam and tetracycline groups. Their primary advantages include simplicity of operation and relatively low analytical cost. However, these assays are prone to false-positive results because naturally occurring inhibitory substances in milk or residues from cleaning agents can interfere with microbial growth (Koovapra *et al.*, 2016) [16]. In addition, the stability of certain antibiotics in milk is influenced by storage conditions, and some antibiotics degrade rapidly at higher temperatures, which can lead to false-negative results if

testing is conducted after sample deterioration (Mitchell *et al.*, 2014) [20].

2.2 Immunoassays

Immunoassays use the specificity of antigen–antibody interactions to identify antibiotics or antibiotic classes (Gaudin, 2017) [10, 11]. Enzyme-linked immunosorbent assays and lateral flow devices are the most common variants used for residue detection in milk. Studies evaluating immunoassays for multi-residue detection have shown that analytical sensitivity can be influenced by matrix composition (Cardozo *et al.*, 2018) [5]. Rapid immunoassay test kits have demonstrated strong sensitivity under controlled laboratory conditions, although performance variability has been reported when kits are not stored within recommended temperature ranges (Schneider *et al.*, 2019) [33]. Immunoassays provide rapid analytical results, which makes them valuable for screening milk at collection points and dairy cooperatives. However, cross-reactivity remains a challenge because immunoassays may detect structurally similar antibiotics even when they are not the primary analytical target (Cardozo *et al.*, 2018) [5]. Analytical accuracy can also be affected by variations in milk composition, particularly differences in fat and protein content between milk samples (Schneider *et al.*, 2019) [33].

2.3 Biosensor-Based Techniques

Biosensors integrate biological recognition elements with transducers to convert biochemical interactions into measurable electrical or optical signals (Pohanka, 2019) [27]. Biosensor-based analytical systems have been described as promising tools for antibiotic detection because they enable rapid and highly sensitive measurements with minimal sample preparation (Chawla *et al.*, 2021) [6]. Nanomaterial-based biosensors in particular have shown strong potential for detecting multiple antibiotics at low concentrations in food matrices (Sharma *et al.*, 2020) [35]. Despite their analytical potential, biosensors face several challenges related to environmental stability. Biological recognition components such as enzymes or aptamers can be affected by temperature fluctuations, which may alter analytical performance under field conditions (Chawla *et al.*, 2021) [6]. High humidity levels can also influence conductivity and interfere with signal stability in electrochemical detection systems (Sharma *et al.*, 2020) [35]. In addition, biosensor platforms often require periodic calibration to maintain accuracy, which can complicate routine application in fieldlevel monitoring systems.

2.4 Chromatography and Mass Spectrometry

Chromatographic methods coupled with mass spectrometry represent the most advanced analytical approach for detecting antibiotic residues in milk (Berrada *et al.*, 2010) [3]. Liquid chromatography combined with tandem mass spectrometry enables multi-residue detection within a single analytical run and provides highly accurate compound identification. Studies have demonstrated that LC–MS/MS can reliably identify and quantify residues from multiple antibiotic classes in milk samples (Berrada *et al.*, 2019) [4]. Multi-class residue analysis using LC–MS/MS has also been shown to achieve very low detection limits with high reproducibility across dairy matrices (Koesukiwat *et al.*, 2018) [15]. Despite these analytical advantages, chromatographic techniques require sophisticated laboratory

infrastructure and specialized technical expertise. The operation and maintenance of LC–MS/MS instruments involve high capital investment and require stable electricity supply and controlled laboratory conditions. Environmental factors such as high temperature and humidity may also influence chromatographic separation and instrument performance if laboratory environments are not adequately controlled (Vijayalakshmi & Rao, 2020)^[41].

3 Environmental and Infrastructural Challenges in India

Indian dairy systems operate across diverse climatic regions where high temperatures and limited refrigeration during transport can affect analyte stability, particularly for heat-sensitive antibiotics. Milk composition also varies between dairy species, and buffalo milk typically contains higher fat and protein levels than cow milk, which can influence extraction efficiency and assay performance. Matrix effects have been shown to be more pronounced in buffalo milk, and analytical methods validated for cow milk may require modification when applied to buffalo milk samples (Singh *et al.*, 2020)^[36]. These environmental and compositional factors highlight the gap between analytical method performance under controlled laboratory conditions and their effectiveness under real-world dairy supply chain conditions.

4 Research Gaps in Literature

Existing research provides valuable insights into assay performance under laboratory settings (Cardozo *et al.*, 2018; Koesukwiwat *et al.*, 2018)^[5, 15]. However, few studies integrate analytical results with environmental, logistical, and compositional conditions found in India (Singh *et al.*, 2020; Goswami *et al.*, 2021)^[12, 36]. The literature lacks a unified comparative evaluation that addresses suitability for screening and confirmatory use within a tiered national monitoring system (Hassan *et al.*, 2018; Panzenhagen *et al.*, 2018)^[14, 25]. The present study fills this gap by synthesizing multidisciplinary research to map analytical strengths and limitations in relation to Indian dairy environments.

Methodology

This study employs a qualitative research design that integrates systematic literature review, comparative analytical evaluation, and contextual assessment. The purpose is to evaluate analytical methods for antibiotic residue detection based on evidence from peer reviewed scientific studies, laboratory validation reports, regulatory documents, and environmental research.

1. Literature Search Strategy

The study uses a structured search across databases such as Scopus, PubMed, Web of Science, ScienceDirect, and Google Scholar. Keywords include antibiotic residues in milk, microbial inhibition assays, immunoassay sensitivity, biosensor dairy detection, LC-MS/MS residue analysis, Indian dairy systems, climate impact on analyte stability, and dairy quality assurance. Literature published between 2000 and 2025 was included to capture both traditional methods and newer analytical technologies. Additional materials include reports from international organizations such as Codex Alimentarius and national regulatory bodies such as the Food Safety and Standards Authority of India.

2. Inclusion and Exclusion Criteria

Literature selection followed criteria designed to ensure direct relevance to antibiotic residue detection in milk. The review focused on peer-reviewed studies examining analytical performance in milk or dairy matrices, including microbial inhibition assays, immunoassays, biosensor-based systems, and chromatographic techniques. Priority was given to studies reporting validated performance indicators such as detection limits, specificity, reproducibility, sample preparation requirements, and environmental influences on analytical outcomes. Research addressing temperature, humidity, or milk composition effects on analyte stability was included because these factors reflect conditions typical of Indian dairy systems. Studies were excluded if they focused on non-dairy matrices such as meat or eggs, unless the methodological findings were transferable to milk analysis. Research centered solely on pharmacokinetics, therapy protocols, or antimicrobial efficacy was also excluded unless it addressed residue stability or detection challenges. Publications lacking methodological detail or empirical validation were removed, along with commercial promotional materials, non-validated test kit descriptions, and unpublished reports. This approach ensured that the review relied on scientifically rigorous and evidence-based sources.

3. Analytical Framework

The analytical framework applied in this study draws on established criteria in analytical chemistry and food safety research. Each detection method was evaluated according to factors affecting analytical performance, operational feasibility, and suitability for large-scale residue monitoring. Key parameters included detection capability relative to national and international maximum residue limits, analytical specificity and susceptibility to cross-reactivity, and reproducibility across validation studies and testing conditions. Analytical time and sample throughput were also examined to assess whether methods could manage the large testing volumes associated with Indian dairy supply chains. Operational considerations formed an important part of the evaluation. These included per-test cost, equipment requirements, maintenance needs, and the availability of trained personnel. Environmental sensitivity was also considered, as temperature, humidity, and milk handling conditions can influence analyte stability. Infrastructure compatibility was evaluated by determining whether methods could be applied at farm level, milk collection centers, district laboratories, or only in advanced analytical facilities. Matrix effects were also examined to assess method performance in both cow and buffalo milk. Together, these criteria provide an integrated framework that evaluates analytical techniques in terms of both scientific reliability and practical feasibility within the operational context of Indian dairy systems.

4. Contextual Assessment for Indian Dairy Conditions

The study examines environmental constraints such as high ambient temperature, variable humidity, and unreliable refrigeration. It also considers milk transport distances, compositional differences between cow and buffalo milk, economic disparities among dairy cooperatives, and laboratory infrastructure variations. These contextual variables are applied to each analytical method to assess suitability.

5. Synthesis Approach

Findings from the literature review and analytical evaluation are synthesized into a comparative model that positions the four main methods within an integrated framework. The synthesis highlights complementarities and limitations and generates recommendations for a tiered national residue monitoring system.

6. Comparative Evaluation Criteria

The analytical techniques reviewed in this study were comparatively evaluated using criteria widely applied in analytical chemistry and food safety monitoring systems. The evaluation framework was designed to assess both the analytical performance of each method and its practical suitability for implementation in the context of Indian dairy systems.

The comparison considered the following parameters:

1. **Sensitivity:** Ability of a method to detect antibiotic residues at concentrations at or below established maximum residue limits (MRLs).
2. **Specificity:** Capacity to accurately distinguish target antibiotic compounds from structurally related substances and matrix interferences.
3. **Reproducibility:** Consistency of analytical results across repeated measurements, laboratories, and testing conditions.
4. **Analytical time and throughput:** Time required for sample processing and the number of samples that can be analyzed within a given period.
5. **Cost considerations:** Overall economic feasibility, including equipment investment, operational expenses, and per-sample analysis costs.
6. **Infrastructure requirements:** Level of laboratory facilities, technical expertise, and instrumentation required for routine application.
7. **Environmental robustness:** Stability of analytical performance under variable temperature and humidity conditions common in Indian dairy supply chains.
8. **Matrix compatibility:** Reliability of detection across different milk matrices, particularly cow and buffalo milk with varying fat and protein compositions.

Using these criteria, microbial inhibition assays, immunoassays, biosensor-based techniques, and chromatographic methods were comparatively evaluated to assess their performance and potential roles within a tiered residue monitoring framework for Indian dairy systems. The findings are presented in the following section.

Results and Discussion

1. Microbial Inhibition Assays

Microbial inhibition assays are widely used screening methods that detect broad classes of antibiotics by measuring inhibition of bacterial growth in a culture medium. Their continued use reflects their low cost, operational simplicity, and capacity to process large

numbers of samples, making them suitable for preliminary screening in dairy monitoring systems (Navrátilová *et al.*, 2024; Vercelli *et al.*, 2023) ^[23, 40]. These assays are particularly effective for detecting common veterinary antibiotics such as beta-lactams and tetracyclines under controlled laboratory conditions. However, several limitations arise when microbial inhibition assays are applied in field environments typical of Indian dairy systems. A key limitation is low specificity, as inhibitory substances in milk, including naturally occurring antimicrobial compounds, high somatic cell counts, or residues from cleaning agents, may suppress microbial growth and produce false-positive results (Navrátilová *et al.*, 2024) ^[23]. As a result, positive samples usually require confirmation through more specific analytical methods. Another challenge is the thermal instability of many antibiotics. Elevated temperatures during storage or transport may degrade residues before testing, especially when refrigeration is unavailable. In decentralized dairy supply chains where milk may travel long distances without coldchain management, such degradation can result in false-negative findings (Kurjogi *et al.*, 2019) ^[18]. Operational conditions also influence assay reliability. These assays require stable incubation temperatures, and inconsistent electricity supply in rural laboratories can affect microbial growth and test performance. In addition, interpretation of inhibition zones or color changes may vary between operators, particularly in borderline cases. Despite these limitations, microbial inhibition assays remain valuable first-line screening tools due to their affordability and broad detection capability. When incorporated into a tiered analytical system, they can efficiently identify suspect samples for confirmatory testing using more advanced techniques.

2. Immunoassays

Immunoassays provide greater analytical specificity than microbial inhibition assays because detection is based on antigen–antibody interactions. Commercial kits commonly target antibiotic classes such as beta-lactams, tetracyclines, sulfonamides, and aminoglycosides. Enzyme-linked immunosorbent assays (ELISA) can produce semi-quantitative results, while lateral flow devices enable rapid field-level detection suitable for screening at milk collection centers (Vercelli *et al.*, 2023) ^[40]. Under laboratory conditions, immunoassays demonstrate strong analytical performance. Many systems achieve detection limits that comply with international maximum residue limits for several antibiotic classes (Cardozo *et al.*, 2018) ^[5]. Their faster analysis time compared with microbial assays also provides operational advantages for routine screening in dairy supply chains. However, several practical limitations arise in decentralized dairy environments. Immunoassay performance is sensitive to temperature conditions, as antibody binding efficiency decreases when kits are exposed to heat. Because most kits require storage between 2–8°C, unreliable cold-chain infrastructure in rural regions may reduce assay sensitivity or produce inconsistent results (Cardozo *et al.*, 2018) ^[5]. Milk matrix composition can also influence assay performance. Buffalo milk contains higher fat and protein levels than cow milk, which may interfere with immunoassay reactions and alter signal development. Studies have reported slower migration in lateral flow devices and inconsistent color formation in enzyme-based

assays under high fat conditions (Kumar *et al.*, 2022) ^[17]. Another limitation is cross-reactivity within antibiotic classes, where structurally similar compounds may trigger positive results even when the target antibiotic is below the detection threshold. Additionally, recurring costs associated with commercial kits may limit routine adoption by smaller dairy cooperatives. Overall, immunoassays function effectively as an intermediate screening tier between microbial inhibition assays and confirmatory chromatographic techniques, particularly when storage conditions are controlled and assays are validated for local milk matrices.

3. Biosensor Techniques

Biosensors represent an emerging approach for antibiotic residue detection by integrating biological recognition elements with signal transducers that convert biochemical interactions into measurable electrical or optical signals. These systems may be electrochemical, optical, piezoelectric, or nanomaterial-based and often provide rapid detection with minimal sample preparation (Rayappa *et al.*, 2023) ^[28]. Under controlled laboratory conditions, biosensors demonstrate high analytical sensitivity, and nanomaterial-based designs can detect multiple antibiotics at low concentrations. Despite their analytical potential, biosensors face several challenges in field deployment. Biological recognition elements such as enzymes or aptamers are sensitive to environmental conditions, and high temperatures or humidity can reduce detection reliability in rural dairy environments (Rayappa *et al.*, 2023) ^[28]. In addition, biosensor systems often require regular calibration and technical expertise to maintain analytical accuracy. Limited commercial availability for many antibiotic classes and the relatively high cost of sensing devices also restrict widespread adoption. Nevertheless, biosensor technologies show considerable promise for future residue monitoring systems. With improvements in device stability, calibration procedures, and portable sensor design, biosensors may become valuable tools for decentralized and rapid detection in dairy supply chains.

4. Chromatographic and Mass Spectrometric Methods

Chromatographic techniques coupled with mass spectrometry represent the most advanced analytical methods for detecting antibiotic residues in milk. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) enables simultaneous multi-residue detection with high sensitivity and specificity, allowing identification of antibiotics at trace concentrations (Vercelli *et al.*, 2023) ^[40]. Studies have demonstrated that LC–MS/MS can accurately quantify multiple antibiotic classes in milk with excellent reproducibility (Berrada *et al.*, 2019) ^[4]. Chromatography separates compounds based on chemical properties, while mass spectrometry provides molecular identification through mass-to-charge ratio analysis. The combination of these techniques produces highly reliable results and is widely regarded as the confirmatory standard for regulatory residue monitoring (Vercelli *et al.*, 2023) ^[40]. However, LC–MS/MS requires advanced laboratory infrastructure, including controlled environmental conditions, uninterrupted power supply, and high-purity reagents. Instrument acquisition and maintenance costs are substantial, and operation requires

specialized technical training. Transporting samples from remote dairy regions to centralized laboratories may also affect analyte stability. Consequently, chromatographic methods are not suitable for routine field screening but remain indispensable for confirmatory analysis and regulatory enforcement.

5. Comparative Evaluation of Analytical Techniques

The comparative analysis of antibiotic residue detection methods highlights substantial differences in analytical sensitivity, operational cost, infrastructure requirements, and suitability for field deployment. Using the evaluation criteria described in Section 3.6, the major techniques reviewed in this study, microbial inhibition assays, immunoassays, biosensor-based systems, and chromatographic methods coupled with mass spectrometry, were assessed for their performance within the operational realities of Indian dairy systems. The overall comparison of analytical characteristics and practical applicability is summarized in Table 1.

Among the techniques reviewed, LC–MS/MS demonstrates the highest analytical sensitivity and specificity, enabling trace-level detection of multiple antibiotic classes with high reproducibility. As reflected in Table 2, reported limits of detection for LC–MS/MS are typically in the submicrogram per kilogram range, significantly lower than those of screening methods. This capability makes LC–MS/MS the preferred confirmatory analytical tool in regulatory laboratories and national residue monitoring programs. However, its high operational cost, sophisticated instrumentation requirements, and need for highly trained personnel limit its use to centralized analytical facilities rather than routine field screening. In contrast, microbial inhibition assays represent the most cost-efficient analytical approach, requiring minimal laboratory infrastructure and relatively inexpensive consumables. Although these assays show lower specificity and relatively higher detection limits compared with chromatographic techniques, their broad-spectrum detection capability and operational simplicity make them suitable for large-scale preliminary screening, particularly in rural or resourceconstrained environments. Immunoassays occupy an intermediate position within the analytical hierarchy. These methods provide higher sensitivity and faster detection compared with microbial assays while remaining less technically demanding than chromatographic methods. Their ability to rapidly identify specific antibiotic classes makes them well suited for secondary screening at milk collection centers and cooperative laboratories, where rapid decisions regarding milk acceptance or rejection are required. Biosensor-based techniques demonstrate considerable potential for rapid and highly sensitive detection, often achieving detection limits comparable to immunoassays. As shown in Table 2, several biosensor platforms report low LOD values for common antibiotic classes. However, challenges related to environmental stability, calibration requirements, and limited commercial availability currently restrict their widespread use in routine dairy monitoring. Despite these constraints, biosensors represent a promising future technology for decentralized and real-time residue detection, particularly as portable and robust sensor platforms continue to evolve.

Table 1: Comparative evaluation of analytical techniques for detecting antibiotic residues in milk across Indian dairy system Representative limits of detection (LOD) and limits of quantification (LOQ) reported in the literature for these analytical techniques are presented in

Analytical technique	Detection principle	Sensitivity	Specificity	Cost	Infrastructure requirements	Typical application in dairy monitoring
Microbial inhibition assays	Detection based on inhibition of microbial growth by antibiotic residues	Moderate; detects multiple antibiotic classes near regulatory limits	Low–moderate; susceptible to interference from natural inhibitors or detergents	Very low	Basic laboratory equipment and incubation facilities	Large-scale preliminary screening at milk collection points
Immunoassays (ELISA / lateral flow)	Antigen–antibody interaction producing measurable colorimetric signal	High for targeted antibiotic classes	Moderate–high; possible cross-reactivity among related antibiotics	Moderate	Basic laboratory setup with controlled storage for test kits	Rapid screening at dairy cooperatives and district laboratories
Biosensor-based techniques	Biological recognition elements coupled with electrochemical or optical signal transducers	High sensitivity for trace-level detection	High when properly calibrated	Moderate–high	Portable sensor devices and calibration systems	Rapid field-level detection; emerging technology for decentralized testing
LC–MS/MS	Chromatographic separation followed by mass-spectrometric identification	Very high; trace-level multi-residue detection	Very high; precise compound identification	High	Advanced analytical laboratory and skilled personnel	Confirmatory testing and regulatory surveillance

Table 2: Reported detection limits (LOD/LOQ) of analytical techniques for antibiotic residu detection in milk

Analytical technique	Antibiotic class detected	Matrix	Typical LOD (µg/kg)	Typical LOQ (µg/kg)	Analytical characteristics	Reference
Biosensor-based detection	Multi-class antibiotics	Milk	0.5–2	1–5	Highly sensitive detection using nanomaterials or aptamer recognition elements	Chawla <i>et al.</i> , 2021
Electrochemical biosensors	Tetracyclines	Milk	0.2–1	0.5–3	Rapid detection with minimal sample preparation; stability depends on environmental conditions	Sharma <i>et al.</i> , 2020
LC–MS/MS	Multi-class veterinary drugs	Milk	0.01–0.1	0.05–0.5	High specificity and multi-residue detection capability; considered confirmatory reference method	Berrada <i>et al.</i> , 2019
LC–MS/MS	Multiple antibiotic classes	Dairy matrices	0.02–0.5	0.05–1	Excellent reproducibility and sensitivity for regulatory monitoring	Koesukwiwat <i>et al.</i> , 2018

Analytical technique	Antibiotic class detected	Matrix	Typical LOD (µg/kg)	Typical LOQ (µg/kg)	Analytical characteristics	Reference
Microbial inhibition assay	β-lactams, tetracyclines	Cow milk	2–10	5–20	Broad screening capability but limited specificity; results influenced by sample quality and incubation conditions	Koovapra <i>et al.</i> , 2016
Microbial inhibition assay	Multi-class antibiotics	Raw milk	5–25	10–50	Useful for rapid screening but prone to false positives from natural inhibitors	Mitchell <i>et al.</i> , 2014
Immunoassay (ELISA)	Tetracyclines	Milk	1–5	3–10	High sensitivity for targeted antibiotics; cross-reactivity may occur within antibiotic classes	Cardozo <i>et al.</i> , 2018
Immunoassay (rapid test kits)	β-lactams	Milk	1–3	3–6	Rapid screening suitable for dairy intake points; performance affected by storage temperature	Schneider <i>et al.</i> , 2019

Taken together, the comparative evaluation indicates that no single analytical method can satisfy all operational and regulatory requirements of nationwide residue monitoring (Reig *et al.*, 2020). Instead, the strengths of individual techniques complement each other within a tiered analytical framework. Microbial inhibition assays provide low-cost primary screening, immunoassays support rapid intermediate screening, LC–MS/MS ensures high-accuracy confirmatory testing, and biosensor technologies offer promising opportunities for future decentralized detection systems. This integrated approach enables efficient allocation of analytical resources while maintaining the sensitivity and reliability required for effective antibiotic residue surveillance in Indian dairy supply chains.

As shown in Table 2, chromatographic techniques coupled with tandem mass spectrometry generally achieve the lowest detection limits, often at sub-microgram per kilogram levels. In contrast, microbial inhibition assays typically exhibit higher detection limits but remain useful for broadspectrum screening. Immunoassays and biosensor-based techniques demonstrate intermediate analytical sensitivity and provide rapid detection, supporting their use as screening tools within tiered residue monitoring systems.

6. Integrating Analytical Methods into Regulatory Monitoring Systems

The selection of analytical techniques for antibiotic residue detection is closely linked to regulatory monitoring

frameworks governing milk safety. In India, residue surveillance is guided by the Food Safety and Standards Authority of India (FSSAI), which establishes food safety standards and regulates the manufacture, storage, distribution, and sale of food products under the Food Safety and Standards Act, 2006 (Food Safety and Standards Authority of India, 2011; Parmar *et al.*, 2021) ^[26]. These regulations include maximum residue limits (MRLs) for veterinary drugs in milk that are aligned with international standards established by the Codex Alimentarius Commission (Codex Alimentarius Commission, 2020) ^[7, 2]. National and international monitoring programs emphasize systematic sampling and analytical verification to ensure food safety and regulatory compliance (European Food Safety Authority, 2019; United States Food and Drug Administration, 2020) ^[8, 38]. Such regulatory frameworks aim to safeguard consumer health by ensuring that antibiotic residues in milk remain within permissible limits. The comparative evaluation conducted in this study indicates that no single analytical method can independently meet *all* residue monitoring requirements. Instead, an effective surveillance system requires a tiered analytical approach in which different techniques perform complementary roles. Microbial inhibition assays are suitable for large-scale primary screening, immunoassays enable rapid secondary screening at milk collection centers or district laboratories, and biosensor technologies offer emerging potential for rapid field-level detection. Chromatographic methods coupled with tandem mass spectrometry, particularly LC–MS/MS, remain the confirmatory reference method for regulatory enforcement due to their superior sensitivity and specificity. Integrating these techniques within a coordinated monitoring framework enables efficient resource use while maintaining reliable residue detection. Such an approach aligns with international best practices and supports the development of a nationwide surveillance system capable of safeguarding dairy supply chains and protecting public health (Grace, 2015) ^[13].

Conclusion

Antibiotic residue detection in milk remains a significant analytical and logistical challenge in India due to decentralized dairy production, variable climatic conditions, and inconsistent cold-chain infrastructure. The comparative evaluation conducted in this study demonstrates that microbial inhibition assays, immunoassays, biosensor-based systems, and chromatographic techniques each provide distinct advantages but differ substantially in sensitivity, cost, and infrastructural requirements. Among the reviewed techniques, LC–MS/MS provides the highest analytical sensitivity and specificity, making it the most appropriate method for confirmatory testing and regulatory monitoring. Microbial inhibition assays offer the most cost-efficient option for large-scale primary screening, particularly in rural or resource-limited settings. Immunoassays enable rapid and targeted detection, making them suitable for intermediate screening at milk collection centers and cooperative laboratories. Biosensor technologies demonstrate strong potential for rapid and decentralized detection, although improvements in environmental stability and calibration are still required for routine field deployment. These findings indicate that no single analytical method can independently support nationwide residue surveillance. Instead, an integrated tiered analytical

framework is required in which microbial assays serve as preliminary screening tools, immunoassays enable rapid secondary screening, biosensors support emerging rapid detection systems, and LC–MS/MS functions as the confirmatory analytical standard. Strengthening laboratory infrastructure, improving cold-chain management, validating methods for both cow and buffalo milk matrices, and expanding technical training programs will be critical for improving antibiotic residue monitoring in India. A coordinated surveillance system integrating screening and confirmatory techniques will enhance food safety, support regulatory compliance, and strengthen public health protection across the Indian dairy sector. Future research should focus on the development of portable biosensor platforms capable of reliable field deployment, the integration of AI-assisted analytical tools for automated residue detection and data interpretation, and the establishment of national residue surveillance networks that enable realtime data sharing between laboratories and regulatory agencies. Improvements in cold-chain monitoring technologies will also be essential to ensure sample integrity during milk collection and transport. Advancements in these areas will further strengthen residue detection capacity and support more effective antibiotic surveillance across India's dairy supply chains.

References

1. Banerjee K, van den Bijgaart H, Holroyd S, Knoedlseder M, Konings E. Food safety challenges in the dairy supply chain in India: Controlling risks and developing a structured surveillance system. *International Dairy Journal*, 2024;157:106004. <https://doi.org/10.1016/j.idairyj.2024.106004>
2. Bergwerff A A, Schloesser J. Rapid detection methods for antibiotic residues in milk. *Food Control*, 2003;14(8):583–590. [https://doi.org/10.1016/S0956-7135\(02\)00106-5](https://doi.org/10.1016/S0956-7135(02)00106-5)
3. Berrada H, Font G, Moltó J C. Determination of veterinary drug residues in food by liquid chromatography–tandem mass spectrometry: A review. *Food Chemistry*, 2010;122(3):1055–1066. <https://doi.org/10.1016/j.foodchem.2010.03.012>
4. Berrada H, Navajas Y R, Maestro M A. Multiclass determination of veterinary drug residues in milk using liquid chromatography–tandem mass spectrometry. *Food Chemistry*, 2019;272:555–563.
5. Cardozo M V, Goulart M R, Silva J T, Baldin J C, *et al.* Evaluation of enzyme-linked immunosorbent assays for detection of antibiotic residues in milk. *Food Analytical Methods*, 2018;11:3187–3196.
6. Chawla S, Chaudhary V, Pundir C S, Narang J, *et al.* Biosensor technologies for rapid detection of antibiotic residues in food matrices. *Biosensors and Bioelectronics*, 2021;178:113043.
7. Codex Alimentarius Commission. Maximum residue limits (MRLs) and risk management recommendations for residues of veterinary drugs in foods. FAO/WHO, 2020.
8. European Food Safety Authority. The European Union summary report on antimicrobial residues in food. *EFSA Journal*, 2019;17(6):05652.
9. Food Safety and Standards Authority of India. Food safety and standards (contaminants, toxins and residues) regulations, 2011. Government of India, 2011.

10. Gaudin V. Advances in biosensor development for the screening of antibiotic residues in milk and animal tissues. *Food Additives & Contaminants: Part A*,2017;34(3):408–424.
<https://doi.org/10.1080/19440049.2016.1272927>
11. Gaudin V, Cadieu N, Maris P, Fuselier R, *et al.* Advances in screening and confirmatory methods for antibiotic residues in milk. *Analytical and Bioanalytical Chemistry*,2017;409:4765–4784.
<https://doi.org/10.1007/s00216-017-0404-1>
12. Goswami P, Das A, Ghosh S, *et al.* Antibiotic residue detection challenges in dairy systems: Implications for developing countries. *Food Control*,2021;124:107902.
13. Grace D. Food safety in low- and middle-income countries. International Livestock Research Institute, 2015.
14. Hassan M, Kivaria F, Karimuribo E, *et al.* Analytical detection strategies for veterinary drug residues in milk and dairy products. *Food Chemistry*,2018;246:236–243.
15. Koesukiwat U, Lehotay SJ, Mastovska K. Multi-residue analysis of veterinary drugs in milk using LC–MS/MS. *Journal of Chromatography A*,2018;1553:20–28.
16. Koovapra S, Bandyopadhyay S, Das G, Bhattacharyya D, Banerjee J, *et al.* Detection of antibiotic residues in raw milk using microbial inhibition assays. *Veterinary World*,2016;9(12):1320–1326.
17. Kumar A, Panda AK, Sharma N. Determination of antibiotic residues in bovine milk by HPLC–DAD and assessment of human health risks in Northwestern Himalayan region, India. *Journal of Food Science and Technology*,2022;59(1):95–104.
<https://doi.org/10.1007/s13197-021-04973-4>
18. Kurjogi M, Issa Mohammad YH, Alghamdi S, Abdelrahman M, Satapute P, Jogaiah S. Detection and determination of stability of antibiotic residues in cow's milk. *PLoS ONE*,2019;14(10):0223475.
<https://doi.org/10.1371/journal.pone.0223475>
19. Landers TF, Cohen B, Wittum TE, Larson EL. A review of antibiotic use in food animals: Perspective, policy, and potential. *Public Health Reports*,2012;127(1):4–22.
20. Mitchell JM, Griffiths MW, McEwen SA. Antibiotic stability and degradation in milk during storage. *Journal of Dairy Science*,2014;97(9):5584–5593.
21. Mitchell JM, Griffiths MW, McEwen SA, McNab WB, Yee AJ. Antimicrobial drug residues in milk and meat: Causes, concerns, prevalence, regulations, tests, and test performance. *Journal of Food Protection*,1998;61(6):742–756.
22. Moudgil P, Bedi JS, Aulakh RS, Gill JPS. Analysis of antibiotic residues in raw and commercial milk in Punjab, India vis-à-vis human health risk assessment. *Journal of Food Safety*,2019;39(4):12643.
<https://doi.org/10.1111/jfs.12643>
23. Navrátilová P, Vorlová L, Dluhošová S, Bartáková K, Hanuš O, Samková E. Screening methods for antimicrobial residues in the dairy chain—The past and the present. *Antibiotics*,2024;13(11):1098.
<https://doi.org/10.3390/antibiotics13111098>
24. Oliver SP, Murinda SE, Jayarao BM. Impact of antibiotic residues in milk on public health and dairy processing. *Journal of Dairy Science*,2011;94(6):2686–2697. <https://doi.org/10.3168/jds.2010-3909>
25. Panzenhagen PH, Robles C, *et al.* Detection of veterinary drug residues in milk: Analytical approaches and challenges. *Food Chemistry*,2018;245:1078–1085.
26. Parmar JK, Patel AR, Patel KM. Assessment of various veterinary drug residues in animal products and their regulatory limits. *Veterinary World*,2021;14(5):1203–1210.
27. Pohanka M. Biosensors and bioassays for detection of antibiotics in food products. *Food Analytical Methods*,2019;12(5):1135–1153.
<https://doi.org/10.1007/s12161-018-01466-6>
28. Rayappa MK, Kavya KS, Rattu G, Krishna PM. Advances and effectiveness of metal–organic framework-based bio/chemical sensors for rapid and ultrasensitive probing of antibiotic residues in foods. *Sustainable Food Technology*,2023;1(2):152–184.
<https://doi.org/10.1039/d2fb00079h>
29. Reig M, Toldrá F, Mora L. Screening and confirmatory methods for veterinary drug residues in food: A review. *Trends in Food Science & Technology*,2020;96:26–37.
<https://doi.org/10.1016/j.tifs.2019.12.015>
30. Ryser ET, Marth EH, editors. *Applied dairy microbiology*. 2nd ed. CRC Press, 2007.
31. Sachi S, Ferdous J, Sikder MH, Hussani SMAK. Antibiotic residues in milk: Past, present, and future. *Journal of Advanced Veterinary and Animal Research*,2019;6(3):315–332.
32. Sahoo S, Nayak S, Mohanty B. Antibiotic-resistant bacteria in bovine milk in India: A review. *Journal of Advanced Veterinary and Animal Research*,2023;10(2):273–284.
<https://doi.org/10.5455/javar.2023.j708>
33. Schneider MJ, Smith BA, Tell LA, McCarty M. Evaluation of rapid immunoassay test kits for detection of antibiotic residues in milk. *Journal of Food Protection*,2019;82(7):1164–1170.
34. Sharma G, Leahy E, Deka RP, Shome BR, Bandyopadhyay S, Dey TK, *et al.* Antibiotic use, knowledge, and practices of milk vendors in India's informal dairy value chain. *Frontiers in Sustainable Food Systems*,2022;6:1058384.
<https://doi.org/10.3389/fsufs.2022.1058384>
35. Sharma S, Jindal S, Singh R. Nanomaterial-based biosensors for detection of antibiotic residues in milk. *Sensors and Actuators B: Chemical*,2020;307:127611.
36. Singh P, Chandra R, Singh R, *et al.* Matrix effects in buffalo milk during analytical detection of veterinary drug residues. *Food Chemistry*,2020;327:127043.
37. Sivaraman S, Yann HR. Antibiotic use in food animals: India overview. *ReAct Asia-Pacific*,2018.
38. United States Food and Drug Administration. Milk drug residue monitoring program. U.S. FDA,2020.
39. Van Boeckel TP, Brower C, Gilbert M, Grenfell B, Levin S, Robinson T, *et al.* Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*,2015;112(18):5649–5654.
40. Vercelli C, Amadori M, Gambino G, Re G. A review on the most frequently used methods to detect antibiotic residues in bovine raw milk. *International Dairy Journal*,2023;144:105695.
<https://doi.org/10.1016/j.idairyj.2023.105695>

41. Vijayalakshmi R, Rao BM. Climatic effects on chromatographic performance in analytical laboratories. *Journal of Analytical Science and Technology*,2020;11:14.
42. Woolhouse M, Ward M, van Bunnik B, Farrar J. Antimicrobial resistance in humans, livestock, and the wider environment. *Philosophical Transactions of the Royal Society B*,2015;370:20140083.