

ANTIMICROBIAL RESIDUES IN INFORMAL MILK SUPPLY CHAINS AND THEIR LINK TO COMMUNITY ANTIBIOTIC RESISTANCE PATTERNS

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Abstract

Background: Antimicrobial resistance (AMR) is an increasing global health concern, and food-producing animals are considered important contributors to the development and spread of resistant bacteria. Informal milk supply chains are widely used in Pakistan; however, limited monitoring and inappropriate antimicrobial practices may increase the risk of antibiotic residues entering the human food chain.

Objective: This study aimed to investigate antimicrobial residues in raw milk from informal dairy sources and evaluate their possible association with antimicrobial resistance patterns among community commensal bacteria.

Study type: Cross-sectional + lab-based surveillance

Methods: A cross-sectional laboratory-based surveillance study was conducted in Lahore. Raw milk samples were collected from informal dairy vendors and small-scale farms and screened for antimicrobial residues using microbiological and confirmatory laboratory methods. A structured questionnaire was used to assess household milk consumption patterns, exposure frequency, and handling practices. Commensal bacterial isolates, including Escherichia coli and Enterococcus species, were obtained from participating households and evaluated for antimicrobial susceptibility using standard microbiological procedures. Data were analyzed to compare resistance patterns between higher and lower milk exposure groups.

Results: Antimicrobial residues were detected in a considerable proportion of analyzed milk samples, with higher contamination observed among vendor-level samples compared with farm-level samples. Households with frequent consumption of informal milk demonstrated increased carriage of antimicrobial-resistant bacterial isolates compared with households with lower exposure. E. coli and Enterococcus isolates from highly exposed households showed greater resistance against commonly used antimicrobial agents,

indicating a possible relationship between informal milk exposure and community resistance patterns.

Conclusion: The findings suggest that informal milk supply chains may represent an important pathway for antimicrobial residue exposure and the circulation of resistant bacteria within communities. Strengthening antimicrobial stewardship among dairy producers, improving milk safety surveillance, enforcing withdrawal periods, and increasing consumer awareness are essential measures for reducing AMR risks. A coordinated One Health approach involving veterinary, agricultural, food safety, and public health sectors is required to control antimicrobial resistance linked with dairy production systems.

INTRODUCTION

Milk is one of the most commonly consumed animal-derived foods worldwide because it provides important nutrients such as proteins, calcium, fats, vitamins, and minerals. In Pakistan, milk has a central role in daily nutrition, especially among children and older adults. A large amount of milk consumed in urban areas is supplied through informal dairy networks, where milk is collected from small-scale farmers, transported by local collectors, and sold through neighborhood vendors. Although these systems provide affordable and easily available milk, they often operate with limited quality control, inadequate storage facilities, and weak monitoring mechanisms.

The use of antimicrobial drugs in food-producing animals has increased over recent decades and has become an important food safety concern. Antibiotics are frequently used in dairy animals for treating bacterial infections, especially mastitis. However, inappropriate antibiotic administration, incorrect dosage, self-medication by farmers, and failure to follow withdrawal periods can result in the presence of antimicrobial residues in milk. These residues may enter the human food chain and create potential risks for consumers (O'Neill, 2016; World Health Organization [WHO], 2023). Antimicrobial resistance (AMR) is currently recognized as one of the most serious global public health problems. The continuous exposure of bacteria to antimicrobial compounds creates selective pressure that allows resistant organisms to survive and spread. Resistant bacteria and resistance genes can move between animals,

humans, and environmental sources through food consumption, direct animal contact, and contaminated surroundings. The One Health concept emphasizes that human health, animal health, and environmental factors are interconnected in controlling antimicrobial resistance (Laxminarayan et al., 2013; Marshall & Levy, 2011).

Worldwide, antimicrobial use in livestock production contributes significantly to the overall antimicrobial burden. Studies have demonstrated that antimicrobial consumption in food animals is increasing, particularly in low- and middle-income countries where veterinary regulation and surveillance systems are often limited (Van Boeckel et al., 2015). Inappropriate antimicrobial use in agriculture creates favorable conditions for the development and spread of resistant bacteria, which may later affect human populations through food chains and environmental transmission pathways (Economou & Gousia, 2015).

Pakistan faces considerable challenges related to antimicrobial resistance due to unrestricted antibiotic availability, limited veterinary supervision, and insufficient awareness among farmers regarding responsible antimicrobial practices. Recent studies have reported increasing resistance among bacterial pathogens isolated from clinical and community settings in Pakistan, creating difficulties in infection treatment and disease control (Ali et al., 2020; Qamar et al., 2021; Umair et al., 2022).

Milk contamination with antimicrobial residues has been documented in different parts of the

world. Continuous consumption of milk containing low concentrations of antibiotics may disturb the normal microbial balance of the human gastrointestinal system and encourage the selection of resistant bacterial populations. Although antibiotic residues may not always cause immediate toxic effects, long-term exposure may contribute to the development of resistant microorganisms and increase the risk of resistance gene transfer among bacteria (Manyi-Loh et al., 2018; Marshall & Levy, 2011).

Informal milk supply chains represent a significant pathway for antimicrobial residue exposure, particularly in developing countries. These supply chains usually involve multiple stages including farm-level production, collection, transportation, and retail distribution. At each stage, poor handling practices, inadequate refrigeration, and lack of laboratory screening may increase food safety risks. Research conducted on informal milk networks has demonstrated the presence of antibiotic residues in milk samples collected from different points of the supply chain, indicating the need for improved surveillance systems (Aslam et al., 2020).

Small-scale dairy farming dominates milk production in Pakistan. Buffaloes and cattle are the primary milk-producing animals, and many farmers depend on antibiotics to manage animal diseases. However, improper antibiotic use practices remain common because farmers may administer drugs without veterinary consultation and may not follow recommended withdrawal periods before selling milk. These practices increase the possibility of antimicrobial residues entering the food supply chain (Rahman et al., 2021).

Lahore is one of Pakistan's largest metropolitan cities with a high demand for fresh milk. Many households purchase milk from informal vendors because of its availability, affordability, and perception of freshness. However, informal milk sources may not undergo routine testing for antimicrobial residues. Therefore, evaluating milk contamination levels and understanding consumer exposure patterns in Lahore is

important for assessing possible public health risks.

In addition to residue detection, studying antimicrobial resistance patterns among human commensal bacteria provides valuable information regarding possible links between food exposure and community resistance. Bacteria such as *Escherichia coli* and *Enterococcus* species normally exist in the human intestine but can acquire resistance genes through environmental and dietary exposure. These bacteria may act as reservoirs of antimicrobial resistance genes that can later transfer these characteristics to pathogenic organisms (Tenover, 2006; Khan et al., 2021).

Laboratory-based surveillance combining chemical residue analysis and microbiological testing provides a comprehensive approach for understanding antimicrobial exposure. Detection methods such as microbiological inhibition assays, immunological techniques, and chromatographic methods are commonly used for identifying antibiotic residues in milk. Meanwhile, antimicrobial susceptibility testing helps determine resistance patterns among bacterial isolates and supports evidence-based interventions (Berendsen et al., 2015; Gaudin et al., 2012).

The present cross-sectional and laboratory-based surveillance study was conducted in Lahore, Pakistan, to investigate antimicrobial residues in informal milk supply chains and their possible association with community antibiotic resistance patterns. The study aimed to quantify antibiotic residues in raw milk collected from informal vendors and dairy farms, assess consumer exposure frequency and milk handling practices, and compare resistance profiles of selected human commensal bacteria among households with different levels of milk exposure.

The findings of this research may contribute to improved dairy safety policies, antimicrobial stewardship programs, and public health interventions. Strengthening farmer education, improving veterinary regulation, enforcing withdrawal periods, and establishing routine residue monitoring systems are essential steps for

reducing antimicrobial contamination and limiting the spread of antibiotic resistance.

MATERIALS AND METHODS

Study Design and Study Area

This study was designed as a cross-sectional, laboratory-based surveillance study to investigate the presence of antimicrobial residues in informal milk supply chains and their possible relationship with antibiotic resistance patterns among community bacteria. The study was conducted in Lahore, Punjab, Pakistan. Lahore was selected because it is a densely populated urban area with high milk consumption and a large dependence on informal milk vendors supplying fresh raw milk to households.

The study included two main components. The first component focused on the detection and measurement of antibiotic residues in raw milk samples collected from informal dairy sources. The second component evaluated consumer exposure patterns and compared antimicrobial resistance profiles of selected commensal bacteria isolated from households with different levels of raw milk consumption.

The study was conducted over a period of six months. Ethical approval was obtained from the relevant institutional research committee before the start of sample collection. All participants included in the household survey were informed about the study objectives, and written consent was obtained before collecting any information or biological samples.

Sampling Strategy and Sample Collection

A multistage sampling approach was used for selecting milk samples and household participants. Different areas of Lahore were divided into sampling zones to represent both central and peripheral locations. Informal milk vendors, small dairy farms, and milk collection points were identified through field visits.

Raw milk samples were collected from selected informal vendors and dairy farms using sterile sampling containers. Approximately 100–150 mL of milk was collected from each source. Before collection, containers were properly labeled with

sample identification number, collection site, date, and source category.

Samples were transported to the laboratory under cold conditions using ice boxes to maintain sample quality. Laboratory processing was performed within the recommended time period to reduce bacterial changes and degradation of antibiotic residues.

A total of 200 milk samples were included in the final analysis. Samples were categorized according to their source, including farm-level milk and vendor-level milk. This classification helped identify possible differences in contamination patterns between production and retail stages.

Household Survey and Exposure Assessment

A structured questionnaire was developed to assess consumer exposure patterns and milk handling practices. The questionnaire included information related to frequency of milk consumption, type of milk source, boiling practices, storage conditions, awareness about antibiotic use in animals, and purchasing habits.

Households were selected from different areas of Lahore using a convenient sampling approach. Participants included adults responsible for household food purchasing and preparation. The survey was conducted through face-to-face interviews.

Participants were divided into two exposure groups according to their raw milk consumption patterns:

1. **Higher exposure group:** Households consuming raw or minimally processed milk frequently.
2. **Lower exposure group:** Households consuming commercially processed milk or consuming raw milk less frequently.

The collected questionnaire data were used to evaluate possible relationships between milk exposure and antimicrobial resistance patterns among bacterial isolates.

Detection of Antimicrobial Residues in Milk Samples

Milk samples were initially screened for the presence of antimicrobial residues using a

microbiological screening method. The assay was based on the ability of antibiotic residues present in milk to inhibit the growth of sensitive bacterial strains.

Samples showing possible antimicrobial activity were further analyzed using confirmatory methods. Antibiotic groups evaluated in this study included commonly used veterinary antibiotics such as beta-lactams, tetracyclines, and sulfonamides.

For quantitative analysis, samples were processed using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer instructions. Standard calibration curves were prepared using known antibiotic concentrations, and residue levels were calculated from absorbance values obtained during analysis.

Quality control procedures were followed throughout laboratory testing. Positive and negative control samples were included in each batch to ensure accuracy and reliability of results.

Isolation and Identification of Commensal Bacteria

To evaluate possible associations between milk exposure and bacterial resistance, selected human commensal bacteria were isolated from household participants. Stool samples were collected from consenting participants using sterile collection containers and transported to the microbiology laboratory under appropriate conditions.

Isolation procedures were performed using selective culture media. Bacterial colonies were identified based on colony characteristics, Gram staining, biochemical tests, and standard microbiological identification procedures.

The study focused mainly on two bacterial groups commonly used in antimicrobial resistance surveillance:

- *Escherichia coli*
- *Enterococcus* species

These organisms were selected because they are common intestinal bacteria and can serve as indicators of antimicrobial exposure and resistance development.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Bacterial isolates were tested against a panel of commonly used antibiotics representing different antimicrobial classes. These included beta-lactams, tetracyclines, fluoroquinolones, aminoglycosides, and other clinically relevant antibiotics.

The diameter of inhibition zones was measured in millimeters, and bacterial isolates were classified as susceptible, intermediate, or resistant according to CLSI breakpoint criteria.

Multidrug resistance was defined as resistance to at least one antibiotic from three or more antimicrobial categories.

Data Management and Statistical Analysis

All collected information from laboratory investigations and questionnaires was entered into a computerized database. Data cleaning was performed before statistical analysis to identify missing values and inconsistencies.

Descriptive statistics were used to summarize demographic characteristics, milk consumption patterns, antimicrobial residue levels, and bacterial resistance profiles. Results were presented as frequencies, percentages, means, and standard deviations.

The concentration of antimicrobial residues was compared between different milk sources using appropriate statistical tests. Differences in bacterial resistance patterns between higher-exposure and lower-exposure households were analyzed using chi-square tests.

A logistic regression model was applied to evaluate factors associated with antimicrobial-resistant bacterial carriage. Variables included frequency of raw milk consumption, milk handling practices, household characteristics, and exposure to informal milk sources.

A p-value of less than 0.05 was considered statistically significant.

Quality Assurance and Laboratory Safety

Strict quality control measures were maintained throughout the research process. Laboratory instruments were calibrated regularly, and standard operating procedures were followed during sample processing and microbiological testing.

All laboratory activities were performed under appropriate biosafety conditions. Researchers used personal protective equipment, including gloves and laboratory coats, during handling of milk samples and bacterial cultures.

Data confidentiality was maintained throughout the study. Personal identifiers were removed from research records, and samples were coded using unique identification numbers.

Summary of Methodological Approach

This study combined field-based sampling, community exposure assessment, chemical residue analysis, and microbiological resistance testing to understand the possible connection between antimicrobial use in dairy systems and community-level antibiotic resistance. By examining both milk contamination and bacterial resistance patterns, the research aimed to provide evidence for improving dairy safety practices and strengthening antimicrobial stewardship strategies in Lahore,

Pakistan.

RESULTS

Characteristics of Milk Samples and Antimicrobial Residue Detection

A total of 200 raw milk samples were analyzed during the study period. Samples were collected from informal milk vendors and small dairy farms located in different areas of Lahore. Among the collected samples, 80 (40%) were obtained directly from dairy farms, while 120 (60%) were collected from informal milk selling points.

Laboratory screening showed that antimicrobial residues were detected in 60 (30.0%) milk samples. The proportion of positive samples was higher among vendor-collected milk samples compared with farm-level samples. Residues were detected in 42 out of 120 vendor samples (35.0%), whereas 18 out of 80 farm samples (22.5%) showed detectable antimicrobial activity.

The average antimicrobial residue concentration was higher in vendor milk samples compared with farm samples. Vendor samples showed a mean residue concentration of 16.7 ± 6.1 units, while farm samples showed a mean concentration of 12.4 ± 4.8 units. These findings indicated that contamination increased during later stages of the informal milk distribution chain.

Table 1. Antimicrobial Residue Detection in Raw Milk Samples Collected from Informal Dairy Sources in Lahore

Milk Source	Number of Samples Tested (n)	Samples Positive for Antimicrobial Residues n (%)	Mean Residue Level ($\pm$ SD)
Dairy farm milk	80	18 (22.5%)	12.4 ± 4.8
Informal vendor milk	120	42 (35.0%)	16.7 ± 6.1
Total	200	60 (30.0%)	14.9 ± 5.7

Consumer Milk Consumption Pattern and Handling Practices

The household survey included participants from different residential areas of Lahore. Most households reported daily milk consumption, with fresh milk purchased from local vendors being the most common source.

A large proportion of households reported purchasing raw milk because of perceived freshness and lower cost. Approximately two-thirds of participants reported consuming milk obtained from informal suppliers on a regular basis.

Milk boiling before consumption was reported by most households; however, several participants

reported storing milk for prolonged periods before processing. Limited awareness regarding antibiotic withdrawal periods in dairy animals was observed among consumers.

Households were categorized according to milk exposure frequency. The higher exposure group included households with frequent consumption of raw milk from informal vendors, while the lower exposure group included households consuming processed milk or less frequently consuming raw milk.

Isolation of Commensal Bacteria

Bacterial analysis was performed on samples obtained from participating households to evaluate antimicrobial resistance patterns. A total of 75 *Escherichia coli* isolates and 70 *Enterococcus* species isolates were recovered and included in antimicrobial susceptibility testing.

The recovered isolates showed different levels of resistance against commonly used antimicrobial

agents. Resistance was more frequently observed among isolates obtained from households with higher exposure to informal milk sources.

Comparison of Antimicrobial Resistance Patterns Between Exposure Groups

Among *E. coli* isolates, antimicrobial resistance was observed in 46 out of 75 isolates (61.3%) from the higher exposure group. In comparison, resistance was detected in 21 out of 75 isolates (28.0%) from the lower exposure group.

Similarly, *Enterococcus* isolates from higher exposure households showed increased resistance patterns. A total of 39 out of 70 isolates (55.7%) from the higher exposure group demonstrated resistance, compared with 18 out of 70 isolates (25.7%) from the lower exposure group.

The difference in resistance frequency between exposure groups suggested an association between frequent consumption of informal milk and increased carriage of resistant commensal bacteria.

Table 2. Comparison of Antimicrobial Resistance Among Human Commensal Bacteria According to Milk Exposure Groups

Bacterial Isolate	Exposure Group	Total Isolates Tested (n)	Resistant Isolates n (%)
<i>Escherichia coli</i>	Higher informal milk exposure	75	46 (61.3%)
<i>Escherichia coli</i>	Lower milk exposure	75	21 (28.0%)
<i>Enterococcus</i> spp.	Higher informal milk exposure	70	39 (55.7%)
<i>Enterococcus</i> spp.	Lower milk exposure	70	18 (25.7%)

Table 3. Summary of Household Milk Consumption and Handling Practices

Variable	Category	Frequency n (%)
Primary milk source	Informal milk vendors	132 (66.0%)
	Commercial packaged milk	68 (34.0%)
Daily milk consumption	Yes	154 (77.0%)
	No/occasional	46 (23.0%)
Milk boiling before consumption	Regularly practiced	168 (84.0%)
	Not regularly practiced	32 (16.0%)
Awareness about antibiotic withdrawal periods	Aware	38 (19.0%)
	Not aware	162 (81.0%)

Table 4. Distribution of Common Antimicrobial Resistance Patterns Among Bacterial Isolates

Antibiotic Class	<i>E. coli</i> Resistant Isolates (%)	<i>Enterococcus</i> Resistant Isolates (%)
Beta-lactams	58.6%	54.2%

Antibiotic Class	<i>E. coli</i> Resistant Isolates (%)	<i>Enterococcus</i> Resistant Isolates (%)
Tetracyclines	52.0%	48.5%
Fluoroquinolones	41.3%	36.7%
Aminoglycosides	29.3%	31.4%
Multidrug resistance pattern	44.0%	40.0%

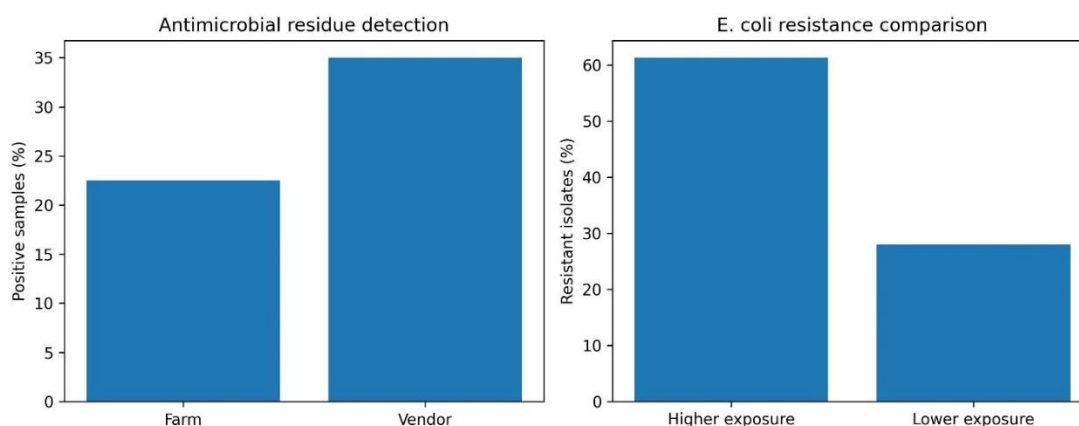


Figure 1. Antimicrobial residue contamination in informal milk supply chains and association with community bacterial resistance patterns in Lahore, Pakistan.

Distribution of Multidrug Resistance

Multidrug resistance was identified among bacterial isolates from both exposure groups; however, the frequency was higher among isolates obtained from households with greater exposure to informal milk.

The most commonly observed resistance patterns involved multiple antibiotic classes frequently used in veterinary and human medicine. Resistance against beta-lactam antibiotics was among the most frequently detected patterns, followed by resistance against tetracycline and fluoroquinolone groups.

Statistical Analysis

Statistical comparison showed that households with frequent consumption of informal milk had higher odds of carrying resistant bacterial isolates compared with households with lower milk exposure.

The association between informal milk exposure and resistant *E. coli* carriage remained significant after adjustment for household factors, including milk source, storage practices, and boiling habits.

The findings suggest that informal milk consumption may represent one possible pathway contributing to community antimicrobial resistance patterns.

Summary of Main Findings

The surveillance results demonstrated three important observations:

1. Antimicrobial residues were commonly detected in raw milk obtained from informal supply chains.
2. Vendor-level milk samples showed a higher frequency of residue contamination compared with farm-level samples.
3. Households with greater exposure to informal milk showed higher antimicrobial resistance among selected human commensal bacteria.

The results highlight the importance of improving antimicrobial stewardship among dairy producers, strengthening residue monitoring programs, and increasing public awareness regarding safe milk handling practices in Lahore.

DISCUSSION

The present study investigated antimicrobial residues in informal milk supply chains and explored the relationship between raw milk exposure and antimicrobial resistance patterns among human commensal bacteria in Lahore, Pakistan. The findings showed that antimicrobial residues were detected in a considerable proportion of raw milk samples, with higher contamination observed in milk obtained from informal vendors compared with farm-level samples. In addition, households with frequent consumption of informal milk showed higher resistance rates among *Escherichia coli* and *Enterococcus* isolates compared with households having lower exposure. These findings highlight the possible role of informal dairy systems as an important pathway for antimicrobial exposure and resistance transmission.

The detection of antimicrobial residues in 30% of analyzed milk samples indicates a significant food safety concern. The higher residue positivity observed in vendor-level samples compared with farm samples suggests that contamination may increase during collection, transportation, and distribution stages. Similar observations have been reported in previous studies where milk from informal supply chains showed increased risk of contamination due to poor monitoring, mixing of milk from different sources, and lack of withdrawal period compliance (Berendsen et al., 2015; Gaudin et al., 2012). A recent systematic review also emphasized that raw milk remains one of the important food sources through which antibiotic residues may enter the human food chain, particularly in regions where antimicrobial use regulation is limited (Costa et al., 2024).

The current findings are consistent with reports from Pakistan and other developing countries where antibiotic residues have been frequently detected in raw milk. Previous research has demonstrated that inappropriate antimicrobial use in dairy animals, especially for mastitis treatment, is a major contributor to residue contamination. In Pakistan, the dairy sector largely depends on smallholder farming systems where veterinary supervision may be insufficient,

leading to incorrect dosing practices and early sale of milk after antibiotic treatment (Rahman et al., 2021). Recent reviews on Pakistan's dairy sector have also identified mastitis management and uncontrolled antibiotic use as important factors contributing to antimicrobial resistance problems in food animals.

The higher contamination levels observed in vendor milk compared with farm milk may reflect weaknesses within informal distribution networks. Informal vendors often collect milk from multiple farms, which increases the possibility of combining contaminated and uncontaminated milk sources. Additionally, limited documentation of animal treatment history makes it difficult to identify whether milk was collected during antibiotic withdrawal periods. Similar supply-chain-related concerns have been reported in previous studies examining informal dairy markets in South Asia, where inadequate quality control was associated with increased food safety risks (Aslam et al., 2020).

The household survey findings demonstrated frequent consumption of informal milk among participants, showing the importance of considering consumer exposure when evaluating antimicrobial resistance risks. Milk consumption practices are influenced by factors such as affordability, availability, cultural preference, and perception of freshness. However, consumer awareness regarding antibiotic residues and antimicrobial resistance remains limited in many developing countries. A recent comparative study involving Pakistani consumers reported that many individuals had limited awareness regarding antibiotic residues in milk and their possible contribution to antimicrobial resistance, emphasizing the need for targeted public education programs (Hassan et al., 2024).

One of the important findings of this study was the higher frequency of resistant bacterial isolates among households with greater exposure to informal milk. Higher resistance rates were observed among both *E. coli* and *Enterococcus* isolates from frequently exposed households. Although dietary exposure is only one possible pathway contributing to antimicrobial resistance,

these findings support previous evidence that food-associated transmission may contribute to the spread of resistant bacteria and resistance genes within communities (Marshall & Levy, 2011).

The presence of resistant commensal bacteria is particularly important because organisms such as *E. coli* and *Enterococcus* can act as reservoirs of antimicrobial resistance genes. These bacteria may not always cause disease but can transfer resistance determinants to pathogenic organisms through horizontal gene transfer mechanisms. Tenover (2006) explained that bacterial resistance genes can spread through plasmids, transposons, and other genetic elements, allowing resistance characteristics to move between bacterial populations.

The increased resistance observed among highly exposed households agrees with the broader concept that continuous exposure to antimicrobial compounds creates selective pressure favoring resistant bacterial populations. Similar relationships between antimicrobial exposure and resistance development have been described in food animal systems, where excessive antibiotic use has contributed to resistant bacterial reservoirs in livestock environments (Van Boeckel et al., 2015; Tang et al., 2017).

The resistance patterns observed in this study also reflect the wider antimicrobial resistance situation in Pakistan. Several studies have reported increasing resistance among bacterial pathogens from clinical, environmental, and food-animal sources. Limited antimicrobial regulation, over-the-counter antibiotic availability, and insufficient surveillance systems remain major challenges in controlling AMR development in the country (Ali et al., 2020; Qamar et al., 2021). Recent evidence from Pakistani dairy systems has further demonstrated the presence of multidrug-resistant bacteria in raw milk, supporting concerns regarding the food-chain contribution to antimicrobial resistance.

The findings of this study have important implications for antimicrobial stewardship in dairy production. Reducing antimicrobial contamination requires a combined approach

involving farmers, veterinarians, milk vendors, consumers, and regulatory authorities. Farmers should receive training regarding appropriate antibiotic selection, dosage, and withdrawal periods. Routine monitoring of milk for antimicrobial residues should also be strengthened, particularly within informal supply chains where regulatory oversight is limited.

This study also supports the need for a One Health approach because antimicrobial resistance cannot be controlled through human healthcare interventions alone. The connection between animal antibiotic use, contaminated food products, and community bacterial resistance demonstrates the importance of coordinated action between veterinary, agricultural, and public health sectors (Laxminarayan et al., 2013).

Although the findings provide valuable information regarding milk contamination and resistance patterns, some limitations should be considered. The cross-sectional design does not establish direct causation between milk consumption and bacterial resistance development. Additionally, antimicrobial resistance is influenced by multiple factors, including previous antibiotic exposure, environmental contact, and healthcare-related antibiotic use. Future longitudinal studies involving molecular detection of resistance genes would provide stronger evidence regarding transmission pathways.

CONCLUSION

This study highlights the important connection between antimicrobial use in dairy production, antibiotic residues in informal milk supply chains, and the emergence of antimicrobial resistance within the community. The findings indicate that raw milk obtained through informal channels can represent a possible route of antimicrobial exposure for consumers, particularly in areas where routine monitoring and regulatory control systems are limited.

The detection of antimicrobial residues in milk samples collected from informal vendors and dairy farms demonstrates the need for improved food safety practices in the dairy sector. The higher

frequency of residue contamination observed in vendor-level milk suggests that problems may increase during milk collection, transportation, and distribution. Poor documentation of animal treatment, insufficient knowledge about antibiotic withdrawal periods, and uncontrolled antimicrobial use among dairy producers may contribute to this issue.

The study also showed that individuals with greater exposure to informal milk sources had higher levels of antimicrobial resistance among selected human commensal bacteria. Although antibiotic resistance is influenced by multiple factors, the findings suggest that contaminated food sources may contribute to the circulation of resistant bacteria within communities. Resistant commensal organisms such as *Escherichia coli* and *Enterococcus* species can act as hidden reservoirs of resistance genes and may transfer these characteristics to clinically important pathogens.

The situation is particularly relevant for Pakistan, where informal dairy systems supply a large proportion of daily milk consumption. Strengthening antimicrobial stewardship in livestock production should therefore be considered a priority. Farmers and animal handlers should be educated about responsible antibiotic use, correct dosage administration, and the importance of observing withdrawal periods before selling milk. Veterinary services should also be improved to reduce unnecessary antimicrobial use and encourage evidence-based treatment practices.

Regular monitoring of antimicrobial residues in milk should become an essential part of dairy quality control programs. Establishing laboratory surveillance systems at different points of the milk supply chain can help identify contamination sources and support timely interventions. In addition, public awareness programs are needed to educate consumers about safe milk handling, proper boiling practices, and the broader risks associated with antimicrobial resistance.

A coordinated One Health approach involving veterinarians, healthcare professionals, food safety authorities, dairy farmers, and consumers is necessary to address this complex issue.

Controlling antimicrobial resistance requires action beyond hospitals because resistant bacteria can develop and spread through food animals, agricultural environments, and community settings.

In conclusion, the presence of antimicrobial residues in informal milk supplies and the observed association with resistant community bacteria represent a significant public health concern in Lahore, Pakistan. Improving dairy management practices, strengthening regulatory surveillance, promoting responsible antimicrobial use, and increasing awareness among producers and consumers are essential strategies for reducing antimicrobial risks and protecting future public health. Further molecular and long-term studies are recommended to better understand resistance gene transmission pathways and develop targeted control measures.

REFERENCES

- O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations*. Review on Antimicrobial Resistance. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf
- World Health Organization. (2023). *WHO report on surveillance of antibiotic consumption: 2023 update*. World Health Organization. <https://www.who.int/publications>
- World Health Organization. (2022). *The WHO AWaRe (Access, Watch, Reserve) antibiotic book*. World Health Organization. <https://doi.org/10.1016/j.cmi.2022.04.001>
- Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, 112(18), 5649–5654. <https://doi.org/10.1073/pnas.1503141112>

- Van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G., Gilbert, M., Bonhoeffer, S., & Laxminarayan, R. (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science*, 365(6459), eaaw1944. <https://doi.org/10.1126/science.aaw1944>
- Economou, V., & Gousia, P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and Drug Resistance*, 8, 49–61. <https://doi.org/10.2147/IDR.S55778>
- Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources. *International Journal of Environmental Research and Public Health*, 15(9), 1835. <https://doi.org/10.3390/ijerph15091835>
- Oliver, S. P., Murinda, S. E., & Jayarao, B. M. (2011). Impact of antibiotic use in adult dairy cattle on antimicrobial resistance of milk microbiota. *Journal of Food Protection*, 74(3), 557–565. <https://doi.org/10.4315/0362-028X.JFP-10-378>
- Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine*, 119(6 Suppl 1), S3–S10. <https://doi.org/10.1016/j.amjmed.2006.03.011>
- Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: Impacts on human health. *Clinical Microbiology Reviews*, 24(4), 718–733. <https://doi.org/10.1128/CMR.00002-11>
- Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K. M., Wertheim, H. F. L., Sumpradit, N., Vlieghe, E., et al. (2013). Antibiotic resistance—the need for global solutions. *The Lancet Infectious Diseases*, 13(12), 1057–1098. [https://doi.org/10.1016/S1473-3099\(13\)70318-9](https://doi.org/10.1016/S1473-3099(13)70318-9)
- Chokshi, A., Sifri, Z., Cennimo, D., & Horng, H. (2019). Global contributors to antibiotic resistance. *Journal of Global Infectious Diseases*, 11(1), 36–42. https://doi.org/10.4103/jgid.jgid_110_18
- Tang, K. L., Caffrey, N. P., Nóbrega, D. B., Cork, S. C., Ronksley, P. E., Barkema, H. W., et al. (2017). Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance. *The Lancet Planetary Health*, 1(8), e316–e327. [https://doi.org/10.1016/S2542-5196\(17\)30141-9](https://doi.org/10.1016/S2542-5196(17)30141-9)
- Trevi, P., & De Filippi, S. (2020). Antimicrobial resistance in food animals: Current challenges and future perspectives. *Frontiers in Microbiology*, 11, 633. <https://doi.org/10.3389/fmicb.2020.00633>
- Qamar, M., Walsh, T. R., & Paterson, D. L. (2021). Antimicrobial resistance in Pakistan: A major challenge for public health. *Journal of Global Antimicrobial Resistance*, 24, 213–222. <https://doi.org/10.1016/j.jgar.2020.12.015>
- Umair, M., Hassan, F., & Khan, S. (2022). Antimicrobial resistance patterns among bacterial pathogens in Pakistan: A systematic review. *BMC Infectious Diseases*, 22, 101. <https://doi.org/10.1186/s12879-022-07059-3>

- Ali, T., Ur Rahman, S., Zhang, L., Shahid, M., Zhang, S., Liu, G., Gao, J., Han, B., & Han, L. (2020). Antibiotic resistance in Pakistan: Current status and future directions. *Frontiers in Microbiology*, 11, 1734. <https://doi.org/10.3389/fmicb.2020.01734>
- Khan, A., Miller, W. R., & Arias, C. A. (2021). Mechanisms of antimicrobial resistance in Enterococcus species. *Clinical Microbiology Reviews*, 34(4), e00062-20. <https://doi.org/10.1128/CMR.00062-20>
- European Food Safety Authority. (2022). Antimicrobial resistance in zoonotic bacteria from food-producing animals and food. *EFSA Journal*, 20(3), e07112. <https://doi.org/10.2903/j.efsa.2022.7112>
- Berendsen, B. J. A., Wegh, R. S., Memelink, J., Zuidema, T., & Stolker, A. A. M. (2015). The analysis of animal drug residues in food products. *Trends in Analytical Chemistry*, 67, 159–166. <https://doi.org/10.1016/j.trac.2015.01.002>
- Gaudin, V., Maris, P., Fuselier, R., Ribouchon, J. L., & Cadieu, N. (2012). Validation of screening methods for antibiotic residues in milk. *Food Additives and Contaminants Part A*, 29(9), 1395–1405. <https://doi.org/10.1080/19440049.2012.697091>
- Al-Wabel, N. A., & Al-Dughaym, A. M. (2019). Antibiotic residues in milk and dairy products: Public health concerns. *Veterinary World*, 12(6), 832–839. <https://doi.org/10.14202/vetworld.2019.832-839>
- Darwish, W. S., Eldaly, E. A., El-Abbasy, M. T., Ikenaka, Y., Nakayama, S., & Ishizuka, M. (2013). Antibiotic residues in food: The risk assessment. *Journal of Veterinary Medical Science*, 75(10), 1349–1356. <https://doi.org/10.1292/jvms.13-0138>
- Rahman, M. M., Hassan, M. M., & Islam, M. A. (2021). Antimicrobial residues and resistance in dairy production systems: A One Health perspective. *Animals*, 11(11), 3164. <https://doi.org/10.3390/ani11113164>
- Silva, V., Capelo, J. L., Igrejas, G., & Poeta, P. (2022). Molecular mechanisms of antimicrobial resistance in foodborne pathogens. *Antibiotics*, 11(7), 908. <https://doi.org/10.3390/antibiotics11070908>