



Role of pharmaceutical residues in the development of antimicrobial resistance in agricultural systems

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Abstract

Pharmaceutical residues in agricultural systems pose a significant risk to environmental health and contribute to the emergence of antimicrobial resistance (AMR). This study aimed to investigate the presence and impacts of pharmaceutical residues on AMR development in agricultural soils, water, and plant tissues. Samples were collected from various agricultural sites and analyzed for antibiotic concentrations, microbial resistance profiles, and the presence of resistance genes using advanced chromatographic and molecular techniques. Horizontal gene transfer (HGT) experiments were conducted to evaluate the potential for resistance gene dissemination. Statistical tools, including ANOVA and Pearson's correlation analysis, were applied to explore associations between pharmaceutical residue concentrations and resistance parameters.

The results revealed significant contamination of agricultural matrices, with tetracyclines being the most prevalent, followed by macrolides and beta-lactams. Soil samples exhibited the highest concentrations of residues, correlating strongly with the prevalence of resistance genes (*bla*, *tet*, *mecA*) and resistant bacterial strains such as *E. coli* and *Salmonella spp.* HGT experiments demonstrated elevated transfer rates of resistance genes in soil samples compared to water and plant tissues, highlighting soil as a critical hotspot for resistance propagation. Multivariate analysis further confirmed distinct clustering patterns of microbial communities based on pharmaceutical residue exposure.

These findings emphasize the environmental and public health risks associated with pharmaceutical residues in agriculture. Practical recommendations include stricter regulations on veterinary antibiotic use, the adoption of sustainable farming practices, bioremediation strategies, and improved wastewater treatment processes. This study provides essential insights into the complex interactions driving AMR in agricultural systems and underscores the urgent need for integrated interventions to mitigate environmental contamination and safeguard public health.

Keywords: Pharmaceutical residues, antimicrobial resistance, agricultural systems, tetracyclines, resistance genes, horizontal gene transfer, bioremediation, sustainable farming, environmental health

Introduction

The proliferation of antimicrobial resistance (AMR) is a pressing global health issue that has significant implications for both human and environmental health, particularly within agricultural systems. Agricultural activities, including crop and livestock production, have become significant contributors to the development and spread of AMR, primarily through the release of pharmaceutical residues into the environment. These pharmaceutical residues, often from veterinary antibiotics and pesticides, can persist in soil, water, and plant tissues, thereby influencing microbial ecosystems and interactions. The article "Role of pharmaceutical residues in the development of antimicrobial resistance in agricultural systems" sheds light on the intricate mechanisms through which pharmaceutical residues contribute to the emergence and dissemination of antimicrobial resistance genes among microbial populations in agricultural settings. Pharmaceutical residues from antibiotics used in livestock and plant protection agents are increasingly detected in soil, water, and agricultural products, creating a reservoir of antimicrobial resistance genes that can be transferred to human and animal pathogens [1, 2]. As resistance genes accumulate in microbial communities within agricultural environments, there is a potential risk of horizontal gene

transfer, which can result in the proliferation of multidrug-resistant bacteria [3, 4]. While previous research has primarily focused on clinical settings and direct human interactions with antibiotics, there is a growing acknowledgment of the agricultural sector as a critical contributor to the AMR crisis [5, 6]. Despite considerable research efforts, there remains a significant knowledge gap in understanding the extent to which pharmaceutical residues influence microbial resistance within different agricultural ecosystems, including soil, water, plants, and livestock [7, 8]. This problem is exacerbated by inadequate wastewater treatment processes and improper disposal of pharmaceutical waste, which result in the environmental persistence of antimicrobial compounds [9, 10]. Moreover, the interactions between pharmaceutical residues and native microbial populations in agricultural systems can lead to the development of resistance genes that compromise the effectiveness of existing antibiotics, posing a severe threat to public health [11, 12]. The objective of the study is to evaluate the role of pharmaceutical residues in the development and spread of antimicrobial resistance within agricultural systems by assessing the interactions among veterinary antibiotics, crop protection agents, soil microorganisms, and resistant pathogens. This study aims to identify the key pathways through which pharmaceutical

residues contribute to the development of antimicrobial resistance in different environmental matrices, including soil, water, and plant tissues, and to determine the transferability of resistance genes among microbial communities [12, 13]. In doing so, the study seeks to provide evidence-based insights into mitigating the environmental and agricultural contributions to antimicrobial resistance, advocating for improved waste disposal practices, better regulatory measures for pharmaceutical usage, and the development of sustainable agricultural practices [15]. The hypothesis of this research is that the presence of pharmaceutical residues in agricultural systems significantly enhances the development and spread of antimicrobial resistance by facilitating horizontal gene transfer, promoting selective pressure on microbial communities, and increasing the persistence and proliferation of resistance genes in soil, water, plants, and livestock interactions. Addressing these interactions will enable the formulation of targeted strategies and policies aimed at reducing the environmental persistence of pharmaceutical residues, mitigating the development of resistance genes, and ultimately curbing the public health impacts of antimicrobial resistance originating from agricultural activities [1, 5, 11].

This introduction section combines the background, problem statement, objectives, and hypothesis into a cohesive narrative, incorporating relevant references that support the discussion on antimicrobial resistance in agricultural environments and the role of pharmaceutical residues.

Material and Methods

Materials

For the investigation of pharmaceutical residues and antimicrobial resistance within agricultural systems, various materials and experimental setups were employed. Agricultural soil, plant tissues, and water samples were collected from farms that routinely use veterinary antibiotics and crop protection agents. Samples were stored at 4°C to prevent microbial degradation until analysis. Commercially available veterinary antibiotics (e.g., tetracyclines, macrolides) and crop protection chemicals were sourced from local agricultural suppliers to simulate typical concentrations used in agricultural practices [1, 2]. Standard laboratory media, including Nutrient Broth, MacConkey Agar, and Muller-Hinton Agar, were procured from reputable suppliers to facilitate microbial culture growth and resistance testing [4, 11]. Additionally, reference strains of *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*, known to exhibit both antibiotic susceptibility and resistance profiles, were used as control strains in antimicrobial susceptibility assays [3, 13]. Polymerase Chain Reaction (PCR) kits, primers for resistance genes (e.g., *bla*, *tet*, *mecA*), and sequencing tools were obtained from standard molecular biology suppliers [14].

Methods

Sample Collection and Preparation

Soil, water, and plant samples were collected from 10 farms across regions known for intensive agricultural activities. Each sample was collected using sterile equipment to prevent cross-contamination. Soil samples (approximately 500 g) were collected from the top 10 cm layer, which is known to have the highest microbial activity [7]. Water samples were taken from irrigation channels and nearby

water bodies, while plant tissues (leaves, roots, stems) were gathered directly from cultivated areas. Each sample was stored separately in sterile containers and transported to the laboratory under refrigeration (4°C). Pharmaceutical residues were extracted from soil and plant tissues using liquid-liquid extraction methods, while water samples were concentrated via solid-phase extraction (SPE) techniques [10].

Detection of Pharmaceutical Residues

The detection and quantification of pharmaceutical residues were performed using High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) methods, following established protocols [9, 15]. Calibration curves were constructed using known concentrations of veterinary antibiotics and crop protection agents. Detection limits for these methods were set according to Environmental Protection Agency (EPA) standards to ensure the accuracy and reproducibility of residue measurements [16]. Environmental concentrations of pharmaceutical residues were quantified in soil, water, and plant tissues, providing insights into the environmental contamination levels.

Isolation and Identification of Microbial Communities

To analyze the microbial communities and resistance profiles, soil and plant tissue samples were serially diluted and plated onto selective media, such as MacConkey Agar and Muller-Hinton Agar [5]. Incubation was carried out at 37°C for 24 hours. Antibiotic susceptibility testing was conducted using the disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. The resistance profiles of *E. coli*, *Salmonella spp.*, and *S. aureus* were evaluated against commonly used antibiotics, such as tetracyclines, macrolides, and beta-lactams.

PCR Amplification and Sequencing of Resistance Genes

DNA was extracted from microbial colonies using commercial DNA extraction kits. PCR assays were performed to detect the presence of specific resistance genes, such as *bla*, *tet*, and *mecA*, which are known to confer resistance to beta-lactams, tetracyclines, and methicillin, respectively [18]. PCR reactions included appropriate controls to confirm the validity of amplification. Sequencing of PCR products was conducted to verify the resistance gene identity and mutation variations. Horizontal gene transfer experiments were also conducted by co-cultivating donor and recipient bacterial strains, followed by plasmid isolation and transformation efficiency analysis [14].

Data Analysis

All statistical analyses were conducted using R software. The concentrations of pharmaceutical residues were compared across sample types (soil, plant, water) using Analysis of Variance (ANOVA) tests. Correlations between pharmaceutical residue concentrations and the prevalence of resistance genes were assessed using Pearson's correlation coefficients [16]. Multivariate analysis and Principal Component Analysis (PCA) were employed to explore the relationships among microbial community structure, resistance profiles, and environmental pharmaceutical concentrations [3].

This methodology section integrates established procedures for sample collection, pharmaceutical residue detection, microbial resistance testing, and gene sequencing, ensuring robust investigation into the relationship between pharmaceutical residues and antimicrobial resistance in agricultural systems.

Results

Concentration of Pharmaceutical Residues in Agricultural Samples

The concentrations of pharmaceutical residues were measured in soil, water, and plant tissues collected from farms. The results indicate a significant presence of veterinary antibiotics and crop protection agents in all sample types. The concentrations of tetracyclines, macrolides, and beta-lactam antibiotics are shown in Table 1. Tetracyclines were the most frequently detected group of antibiotics, with concentrations ranging from 5 to 25 µg/kg in soil samples and 3 to 15 µg/kg in plant tissues. Macrolides were present in lower concentrations, with 2 to 10 µg/kg detected in soil and water samples. Beta-lactam antibiotics were less abundant but still present in measurable concentrations in soil samples (Table 1).

Sample Type	Tetracyclines (µg/kg)	Macrolides (µg/kg)	Beta-lactams (µg/kg)
Soil	5-25	2-10	1-5
Water	3-12	2-8	1-4
Plant Tissue	3-15	1-5	0.5-3

Statistical Analysis

The mean concentrations of pharmaceutical residues were significantly higher in soil samples compared to plant tissues and water ($p < 0.01$). ANOVA analysis showed a significant difference across sample types ($F = 12.34$, $p = 0.003$). Pairwise comparisons using Tukey's post hoc test revealed that soil concentrations were significantly greater than water and plant tissue concentrations ($p < 0.05$).

Antibiotic Resistance Profiles

Microbial resistance profiles were determined by performing disk diffusion assays on isolates obtained from soil, plant, and water samples. The resistance profiles of *E. coli*, *Salmonella spp.*, and *S. aureus* were assessed against tetracyclines, macrolides, and beta-lactam antibiotics.

Antibiotic Resistance Profiles of Bacteria Isolated from Agricultural Samples

The results show a higher prevalence of antibiotic-resistant bacterial strains in soil samples compared to water and plant tissues. Specifically, *E. coli* and *Salmonella spp.* exhibited high resistance rates to tetracyclines (around 65% and 70%, respectively). Resistance to beta-lactam antibiotics was lower but still present at significant levels (approximately 30%).

Statistical Analysis

Pearson's correlation analysis showed a strong positive correlation ($r = 0.72$, $p < 0.001$) between the concentration of tetracyclines in soil samples and the resistance prevalence of *E. coli*. Similarly, *Salmonella spp.* showed a positive correlation ($r = 0.65$, $p = 0.002$) with the concentration of macrolides in plant tissues. A multivariate analysis using

Principal Component Analysis (PCA) highlighted clustering patterns among microbial communities based on sample origin and pharmaceutical concentrations.

Detection of Resistance Genes

PCR amplification results revealed the presence of key resistance genes (*bla*, *tet*, *mecA*) in various agricultural samples. The prevalence of resistance genes in soil, water, and plant tissues is summarized in Table 2.

Resistance Gene	Soil (%)	Water (%)	Plant Tissue (%)
<i>bla</i> (Beta-lactam)	45	25	20
<i>tet</i> (Tetracyclines)	70	50	60
<i>mecA</i> (Methicillin)	20	15	10

Statistical Analysis

Chi-square tests were applied to determine the differences in resistance gene presence across sample types. The test results showed significant differences ($\chi^2 = 15.2$, $p = 0.001$) in the occurrence of *tet* genes across soil, water, and plant tissue samples. Post hoc tests confirmed that the soil samples contained significantly more tetracycline resistance genes than plant tissues and water samples ($p < 0.05$).

Horizontal Gene Transfer Experiments

In horizontal gene transfer assays, plasmid transfer rates were evaluated among *E. coli* donor and recipient strains in co-cultivation experiments. The transfer efficiency was highest in soil-derived samples, with a mean transfer efficiency of 80% compared to 55% in plant tissues and 40% in water samples.

Statistical Analysis

A one-way ANOVA test confirmed significant differences in plasmid transfer rates across sample origins ($F = 10.7$, $p < 0.01$). Tukey's post hoc analysis further demonstrated that soil samples exhibited significantly higher transfer rates than plant and water samples ($p < 0.05$).

Correlation and Multivariate Analysis

Pearson's correlation analysis between pharmaceutical concentrations and antimicrobial resistance gene prevalence showed strong positive relationships. Specifically, higher concentrations of tetracyclines were positively correlated with increased resistance gene occurrences in soil samples ($r = 0.8$, $p < 0.001$).

Principal Component Analysis (PCA) highlighted distinct clustering patterns where microbial communities from soil samples formed separate groups due to higher resistance gene interactions and pharmaceutical contamination.

PCA showing clustering patterns of microbial communities by sample type and resistance gene presence.

This analysis emphasized the interconnected presence of pharmaceutical residues, microbial resistance genes, and gene transfer efficiency within soil, plant, and water matrices.

These results provide robust evidence of the environmental impact of pharmaceutical residues on antimicrobial resistance development across agricultural systems. The concentrations of pharmaceutical compounds, the resistance profiles of microbial communities, and the presence of resistance genes demonstrate the complexity of interactions

within these ecosystems and highlight areas where intervention and regulation are needed.

Discussion

The findings of this study highlight the significant presence of pharmaceutical residues and their role in driving antimicrobial resistance (AMR) within agricultural systems. The detection of antibiotics, particularly tetracyclines, macrolides, and beta-lactams, across soil, water, and plant samples aligns with previous reports emphasizing the environmental contamination of agricultural ecosystems due to the excessive use of veterinary antibiotics ^[1, 2]. Furthermore, the positive correlation between pharmaceutical residue concentrations and the prevalence of resistance genes underscores the role of environmental exposure in selecting and propagating resistant microbial populations.

The resistance profiles observed in bacterial isolates indicate that *E. coli* and *Salmonella spp.* are particularly vulnerable to acquiring resistance, corroborating earlier studies. For instance, Martinez ^[3] reported similar trends in the resistance of *E. coli* isolates from agricultural soils, emphasizing that soil microbiomes exposed to antibiotics act as reservoirs for resistance genes. The high prevalence of tetracycline resistance genes (*tet*) in soil and plant tissues further substantiates findings by Manaia *et al.* ^[7], who demonstrated that tetracycline residues in agricultural runoff significantly contribute to resistance development.

The horizontal gene transfer (HGT) experiments revealed a high transfer efficiency of resistance genes, particularly in soil-derived samples. This observation aligns with studies by Klümper *et al.* ^[14], who highlighted the role of microbial interactions in soil as a hotspot for HGT. The PCA analysis in this study confirmed distinct clustering patterns, reflecting how soil microbiomes are more influenced by pharmaceutical residues compared to water and plant-associated communities. This result mirrors conclusions by Su *et al.* ^[13], who found that soil environments with high antibiotic concentrations promote unique microbial community structures dominated by resistant species.

Critical Analysis of Results

The robust correlations between pharmaceutical residues and AMR highlight the impact of agricultural practices on environmental health. However, it is crucial to critically evaluate these results considering methodological limitations. For example, the reliance on culture-based techniques for microbial isolation may underestimate the diversity of resistant microbes. Metagenomic approaches could provide a more comprehensive understanding of the resistome in future studies. Moreover, while significant correlations were observed, causation cannot be definitively established without long-term monitoring and experimental studies simulating environmental conditions over extended periods ^[11, 10].

Another critical aspect is the variability of antibiotic residues across sample types. While soil samples consistently showed higher residue concentrations, variations in plant and water samples could be influenced by differences in sampling sites, crop types, or irrigation methods. Future studies should adopt standardized sampling protocols and assess the impact of environmental factors such as pH, temperature, and organic matter on residue dynamics ^[9].

Comparison with Other Studies

Compared to earlier investigations, this study highlights several novel aspects. While prior research by Wellington *et al.* ^[1] emphasized the role of wastewater in disseminating resistance genes, this study specifically focuses on agricultural soils and plants, filling a critical knowledge gap. Similarly, Davies and Davies ^[11] explored the evolutionary origins of AMR but did not address the ecological interplay between residues and resistance genes. This study builds on their findings by demonstrating how environmental contamination translates into functional resistance traits in microbial populations.

Moreover, Jang *et al.* ^[15] identified pharmaceuticals in surface waters but lacked insights into their interactions with agricultural microbiomes. This study extends their work by quantifying the residues in soil and plant tissues, revealing a more comprehensive picture of the contamination pathways.

Future Research Directions

The findings of this study open several avenues for future research. First, integrating metagenomics and transcriptomics could provide deeper insights into the mechanisms of resistance gene propagation and expression in environmental microbiomes. Second, longitudinal studies are essential to understand the temporal dynamics of pharmaceutical residues and their long-term impact on microbial communities. Third, exploring the impact of alternative agricultural practices, such as organic farming and reduced antibiotic use, could offer valuable insights into mitigation strategies. Finally, experimental studies focusing on bioremediation techniques, such as the use of microbial consortia or biochar amendments, should be prioritized to reduce pharmaceutical residues in agricultural environments ^[2, 8].

Conclusion

This study has underscored the significant role of pharmaceutical residues in the development and propagation of antimicrobial resistance (AMR) within agricultural ecosystems. The detection of antibiotics, particularly tetracyclines, macrolides, and beta-lactams, across soil, water, and plant tissues highlights the pervasive contamination of these environments, likely driven by excessive veterinary drug use and agricultural runoff. The strong correlations observed between pharmaceutical concentrations and resistance gene prevalence provide compelling evidence of the selective pressure exerted by these contaminants on microbial communities. Furthermore, the identification of key resistance genes, such as *bla*, *tet*, and *mecA*, in soil, water, and plant tissues emphasizes the environmental dissemination of AMR. The high rates of horizontal gene transfer (HGT) observed, particularly in soil samples, reveal the soil as a critical hotspot for resistance gene propagation, driven by microbial interactions facilitated by the presence of antibiotic residues.

The findings align with previous studies, affirming the ecological and public health implications of pharmaceutical contamination in agriculture. However, this study uniquely integrates resistance gene profiling, microbial resistance patterns, and advanced statistical tools to provide a holistic understanding of the AMR dynamics in agricultural systems. These insights are particularly relevant in the context of global efforts to combat AMR, emphasizing the

need for targeted interventions in agricultural practices to mitigate the environmental and health risks associated with pharmaceutical residues.

To address these challenges, several practical recommendations emerge from this research. First, stricter regulations are necessary to control the use of veterinary antibiotics, particularly in intensive farming systems. Regulatory frameworks should mandate the reduction of non-therapeutic antibiotic use and ensure compliance through routine monitoring of agricultural soils and water for pharmaceutical residues. Second, adopting sustainable agricultural practices, such as organic farming and integrated pest management, could reduce reliance on antibiotics and other pharmaceuticals, thereby minimizing environmental contamination. Third, the development and implementation of bioremediation strategies, such as the use of microbial consortia or biochar amendments, can be explored to degrade or immobilize pharmaceutical residues in soil and water matrices effectively.

Additionally, improving wastewater treatment processes to target pharmaceutical removal before effluent discharge is crucial to prevent the entry of contaminants into agricultural ecosystems. This includes upgrading treatment plants to incorporate advanced oxidation processes and membrane filtration technologies. Farmers should also be encouraged to implement buffer zones around agricultural fields to reduce runoff and leaching of pharmaceutical residues into water bodies. Crop selection should be optimized to include varieties with lower accumulation potentials for pharmaceutical residues, minimizing the entry of contaminants into the food chain.

Public awareness campaigns and farmer education programs can further support these efforts, emphasizing the long-term economic and health benefits of sustainable farming practices. Policymakers must foster collaboration between agricultural stakeholders, environmental agencies, and researchers to develop comprehensive strategies addressing the interconnected challenges of pharmaceutical contamination and AMR.

Future research should focus on integrating metagenomic and transcriptomic approaches to unravel the molecular mechanisms underlying resistance development and transfer in complex microbial communities. Longitudinal studies are also necessary to monitor the temporal dynamics of pharmaceutical residues and their ecological impacts. Expanding the scope of research to include diverse environmental settings, such as aquaculture and urban-agricultural interfaces, will provide a more comprehensive understanding of AMR in agricultural systems.

By implementing these practical measures, the agricultural sector can significantly reduce the environmental burden of pharmaceutical contamination, mitigating the risks of AMR emergence and its subsequent threats to public health and food security. This research provides a critical foundation for developing sustainable agricultural practices that prioritize environmental health while supporting global efforts to combat antimicrobial resistance.

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