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Antibiotic Resistance in Wastewater: Contamination Sources, Multidrug-Resistant Bacteria, and Treatment Impact in Southern Vietnam

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ABSTRACT

Antimicrobial resistance (AMR) poses a critical global challenge, and wastewater is a significant reservoir for antibiotic-resistant bacteria and resistance genes. This study investigates bacterial contamination and antibiotic resistance in wastewater from Southern Vietnam, specifically from the regions of Dong Nai, Binh Duong, Tay Ninh, and Ho Chi Minh City, by analyzing 105 samples of treated and untreated wastewater from healthcare, industrial, pharmaceutical, livestock, food production, and residential sources. Bacterial contamination was observed in 33.3% of samples, with untreated wastewater exhibiting higher contamination rates (38.6%) than treated wastewater (29.5%). The pharmaceutical and industrial wastewaters exhibited the highest contamination rates of 100% and 39.4%, respectively. Among the 51 bacterial isolates, *Pseudomonas* (27.5%), *Burkholderia* (23.5%), and *Enterobacter* (21.6%) were the predominant genera. Antibiotic susceptibility testing revealed high resistance rates, with 66.7% of isolates resistant to tetracycline, 33.3% resistant to sulfamethoxazole/trimethoprime, and 28.2% resistant to ciprofloxacin. Multidrug resistance (MDR) was observed in 25.5% of the isolates, with the highest MDR rates recorded in pharmaceutical (100%) and industrial (68.3%) wastewaters. These findings emphasize the need for stricter regulations on wastewater discharge, particularly from the pharmaceutical and industrial sectors, alongside the implementation of advanced wastewater treatment technologies and routine surveillance programs to mitigate the spread of AMR. This study highlights the regional dynamics of AMR in Vietnam and contributes to the global understanding of wastewater's role in AMR dissemination, providing a foundation for targeted interventions to protect public health and the environment.

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INTRODUCTION

The rapid emergence of antimicrobial resistance (AMR) has become a critical global concern, threatening the efficacy of antibiotics and the management of infectious diseases (Quang et al., 2024). Multidrug-resistant bacteria (MDRB) represent an escalating challenge, as the rate of new antibiotic development lags behind the speed at which bacteria acquire resistance (Theuretzbacher et al., 2023). In addition to clinical misuse and overprescription, untreated wastewater and poorly managed effluent discharge significantly contribute to the proliferation of antibiotic-resistant genes (ARGs) and their dissemination across ecosystems (Kalli et al., 2023; Le et al., 2023). Wastewater from healthcare, agricultural, and industrial sources serves as a reservoir for resistant bacteria, offering conditions that facilitate survival, exchange, and amplification of resistance determinants (Rozman et al., 2020; Zhao et al., 2023).

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Globally, extensive research has documented the presence of ARGs and MDRBs in wastewater. Studies have reported strong correlations between antibiotic residues, ARGs, and the environmental dissemination of resistance genes (Alhazmi et al., 2024). For instance, previous studies have indicated a linear relationship between the concentration of antibiotics in wastewater and the emergence of antibiotic-resistant bacteria (ARB) and ARGs. This correlation highlights the impact of antibiotic contamination on resistance (Noor et al., 2021). In Nigeria, effluents from pharmaceutical manufacturing have been identified as reservoirs for diverse ARGs, raising public health concerns due to potential zoonotic transmission. The presence of high antibiotic concentrations in these effluents contributes to the selection and proliferation of resistant bacteria, posing risks to both environmental and human health (Leiva et al., 2021). Despite these global findings, investigations into wastewater contamination and AMR in developing countries are limited. In Vietnam, although research on antibiotic residues in food is regulated and well-documented, studies on wastewater contamination and its role in AMR propagation are scarce. This gap is particularly critical given the rapid industrialization and healthcare expansion in the region, which increases the risk of resistant bacteria spreading through untreated wastewater.

To address these gaps, this study investigated the prevalence and distribution of ARBs in wastewater samples collected in Southern Vietnam. By isolating and identifying bacterial species, evaluating their antibiotic susceptibility, and examining resistance patterns, this research provides insights into the current status of AMR in wastewater. Furthermore, this study correlates resistance levels with treatment processes and source types to elucidate the ecological and public health implications of wastewater contamination in Vietnam. The findings contribute to the global discourse on AMR by offering region-specific insights and identifying opportunities for targeted interventions to mitigate the spread of resistance in wastewater systems.

MATERIALS AND METHODS

Study design and sampling

This cross-sectional descriptive study evaluated the prevalence and distribution of ARBs in wastewater from various locations in Southern Vietnam, including Dong Nai, Binh Duong, Tay Ninh, and Ho Chi Minh City. A total of 105 wastewater samples were collected between September 2022 and March 2023, representing treated and untreated wastewater from diverse sources, such as healthcare facilities, industrial plants, pharmaceutical factories, livestock farms, food production facilities, and residential areas. The collection process adhered to the Vietnamese National Standard TCVN 8880:2011 (available at https://tieuchuan.vsqi.gov.vn) for the microbiological analysis of water. Independent sampling teams collected samples, which were coded with unique identifiers based on the date and sequence of collection (e.g., YYMMDD.NT.xxx). Samples were stored in sterile containers at 2–8°C to preserve bacterial viability and transported to the laboratory within 24 hours. Samples that failed to meet these criteria were excluded.

Ethical considerations

This study did not involve direct human or animal experiments, therefore, no formal ethics approval was required. Sampling procedures were conducted in compliance with national and local environmental regulations to minimize environmental impact. Laboratory analyses adhere to biosafety guidelines to protect researchers and the environment.

Bacterial isolation and identification

Wastewater samples were cultured on selective media (MacConkey agar, Thermo Scientific^{TM}, Cat. No. CM0007B, and Mannitol salt agar, Thermo Scientific^{TM}, Cat. No. CM0085B) to promote the growth of specific bacterial species. The bacterial colonies were then purified via subculturing on nutrient agar (Merck, Darmstadt, Germany, Cat. No. 1054500500) to obtain pure isolates for identification. We used the NK-IDS 14 GNR identification system (Nam Khoa Biotech, Vietnam, Catalog Ref. NKIDS14), a biochemical method for accurately identifying bacterial genera and species.

Antibiotic susceptibility testing

Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100, 30th edition) (Weinstein & Lewis, 2020). A panel of eight antibiotics from various pharmacological classes was tested, including azithromycin, tetracycline, sulfamethoxazole/trimethoprim, ceftazidime, gentamicin, piperacillin/tazobactam, ciprofloxacin, and meropenem. These antibiotics were selected based on their common use in clinical settings and widespread use in agriculture within Vietnam, particularly tetracycline and sulfamethoxazole/trimethoprim, which are frequently used in both healthcare and veterinary practices in Vietnam (Phu et al., 2022). These antibiotics are particularly important in studies on AMR because they significantly contribute to the development of resistant bacteria (Conde-Cid et al., 2020).

The standardized bacterial suspensions were adjusted to a turbidity equivalent to the 0.5 McFarland standard. Mueller-Hinton agar plates (Thermo Scientific™, Cat. No. CM0337B) were inoculated with the adjusted bacterial suspensions, and antibiotic disks Thermo Scientific™) were aseptically placed on the agar surface. The plates were incubated at 35°C for 16–18 h under controlled conditions. Post-incubation, the diameters of the inhibition zones around the disks were measured using a sliding caliper. Results were categorized as sensitive, intermediate, or resistant according to the CLSI interpretative criteria.

Data analysis

The data, including bacterial contamination rates, antibiotic resistance profiles, and multidrug resistance (MDR) patterns, were analyzed using Python. The following libraries were used for data manipulation, statistical testing, and visualization: Pandas for data manipulation, NumPy for numerical operations, Matplotlib for visualization generation, and SciPy for statistical testing. Descriptive statistics were used to determine the frequencies and proportions of bacterial contamination and resistance across the samples. Comparative analyses were performed to evaluate differences in resistance levels between treated and untreated wastewater samples, as well as among wastewater sources, including healthcare, industrial, agricultural, and residential effluents. This approach ensured comprehensive and reproducible results with robust quality control measures throughout the study.

RESULTS AND DISCUSSIONS

Bacterial contamination of wastewater

A total of 105 wastewater samples were analyzed, of which 35 (33.3%) were contaminated with bacteria. Contamination was more prevalent in untreated wastewater samples, with 17 out of 44 samples (38.6%) testing positive, compared to 18 out of 61 samples (29.5%) for treated wastewater. These findings highlight the crucial role of wastewater treatment in mitigating bacterial contamination. A detailed breakdown of the bacterial contamination rates according to treatment status is provided in Table 1.

Bacterial distribution by source

The analysis of bacterial contamination across various sources revealed significant variations in contamination rates (Figure 1). Pharmaceutical sources exhibited the highest contamination

Table 1. Bacterial contamination in treated and untreated wastewater

Sample type	Total samples	Bacterial contamination	Percentage contamination (%)
Untreated wastewater	44	17	38.6
Treated wastewater	61	18	29.5
Total	105	35	33.3

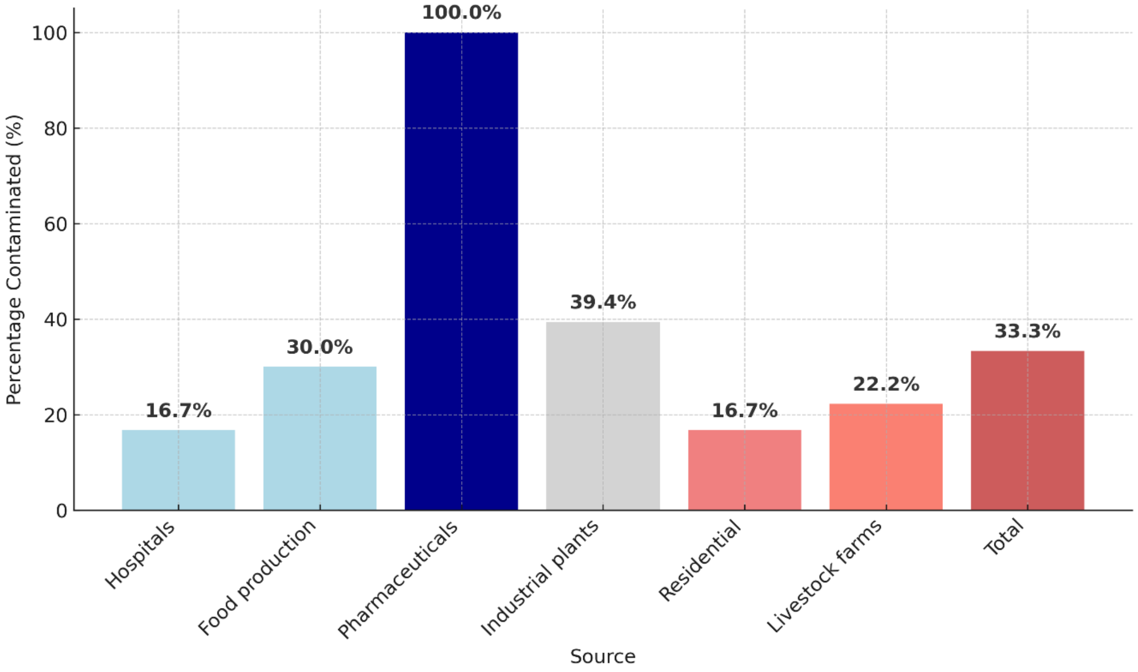

Fig. 1. Percentage of bacterial contamination from various sources

rate, with 100% of the samples tested positive for bacterial presence. Industrial plants exhibited a contamination rate of 39.4%, indicating considerable bacterial contamination. Food production sites and livestock farms had moderate contamination levels of 30.0% and 22.2%, respectively. Hospitals and residential areas had the lowest contamination rates (16.7%), reflecting relatively lower bacterial presence. The overall contamination rate was 33.3% across all sources, highlighting the variability in bacterial contamination across environmental and industrial settings.

Bacterial genera distribution

Among the 51 isolated bacterial strains, the most prevalent genera were *Pseudomonas* (27.5%), *Burkholderia* (23.5%), and *Enterobacter* (21.6%). The other detected genera included *Staphylococci*, *Escherichia*, *Shigella*, and *Vibrio*, each contributing smaller proportions to the overall bacterial population. This distribution indicates the predominance of certain genera, particularly *Pseudomonas*, *Burkholderia*, and *Enterobacter*, in wastewater samples. A detailed breakdown of the bacterial genera is presented in Figure 2, illustrating their relative prevalence and diversity.

Antibiotic resistance patterns

Susceptibility testing of 51 bacterial strains isolated from 35 wastewater samples revealed significant variations in antibiotic resistance. Among the tested antibiotics, tetracycline exhibited the highest resistance rate (66.7%), followed by sulfamethoxazole/trimethoprim

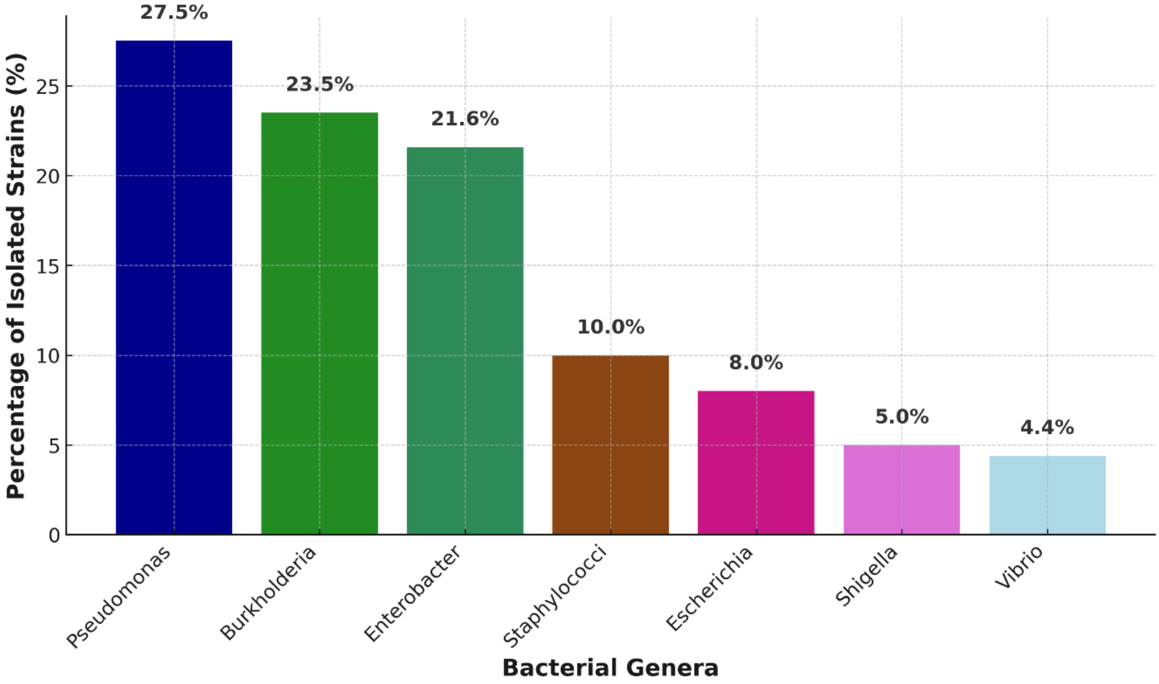

Fig. 2. Detailed distribution of bacterial genera in wastewater samples

Table 2. Antibiotic resistance rates

Antibiotic	Resistance rate (%)
Tetracycline (Te)	66.7
Sulfamethoxazole/Trimethoprime (Bt)	33.3
Ciprofloxacin (Ci)	28.2
Azithromycin (Az)	17.4
Ceftazidime (Cz)	17.6
Gentamicin (Ge)	13.2
Piperacillin/Tazobactam (Pt)	16.1
Meropenem (Me)	10.4

Multidrug resistance across sample origins

(33.3%) and ciprofloxacin (28.2%). The resistance rates to other antibiotics, including azithromycin, ceftazidime, gentamicin, piperacillin/tazobactam, and meropenem, were below 20%, with meropenem exhibiting the lowest resistance rate (10.4%). Overall, resistance rates for all antibiotics ranged from 10% to 67%, reflecting a diverse profile of bacterial resistance in wastewater samples. Notably, meropenem retained its high effectiveness, with 89.6% of the tested strains remaining sensitive (data not shown). The antibiotic resistance patterns are summarized in Table 2.

The analysis of multidrug resistance revealed that industrial sources contributed the largest number of resistant strains across all categories (Figure 3). A total of 22 strains exhibited resistance to 0 antibiotics, with consistent contributions across higher antibiotic resistance levels. Pharmaceutical sources also displayed significant resistance to strains resistant to 3 and 8 antibiotics. Farming and food sources made smaller contributions, with 2 and 4 resistant strains, respectively, for lower resistance levels (1 or 0 antibiotics). Residential sources showed limited resistance, and a few resistant strains to specific categories, such as 3 antibiotic

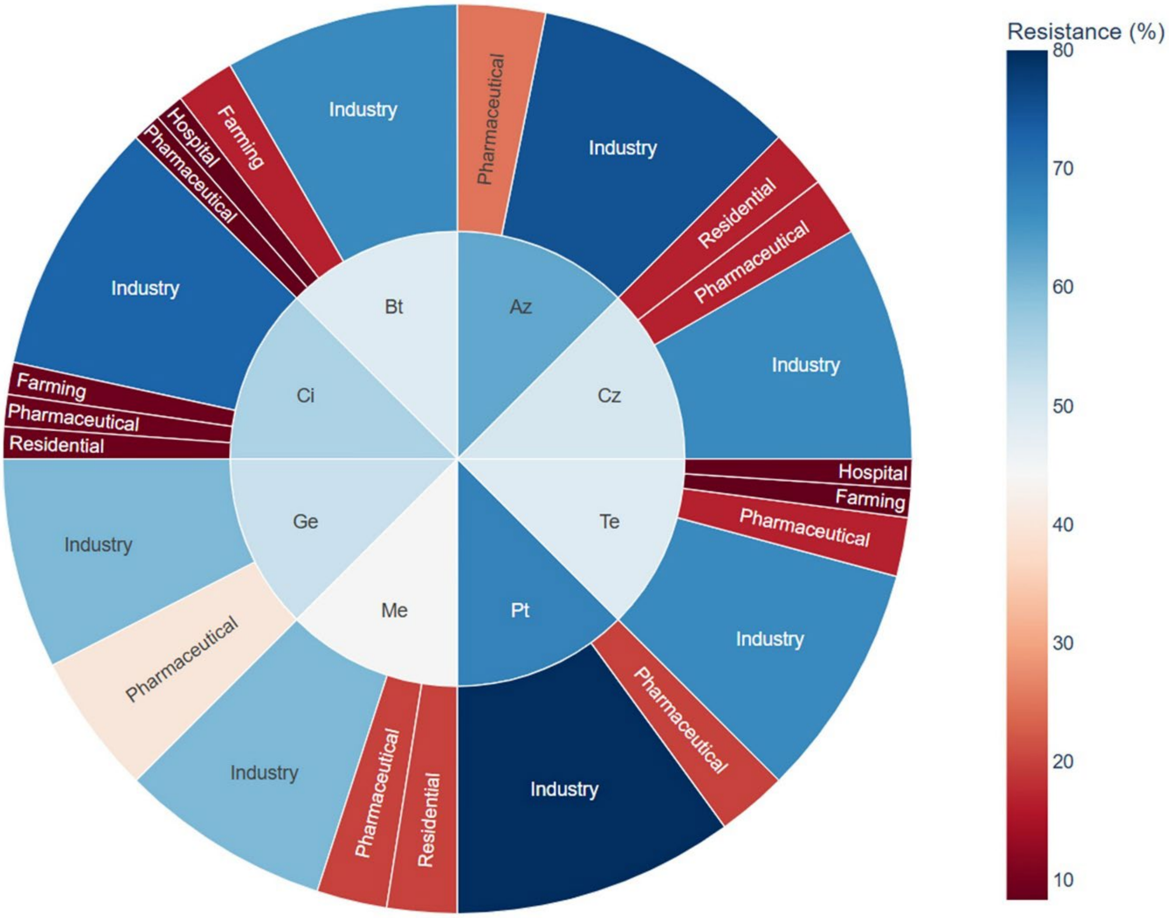

Fig. 3. Multidrug resistance across antibiotic categories and samples

resistance. Hospital sources contributed minimally, with only one resistant strain recorded for two antibiotic resistance cases. The distribution of resistance progressively decreased as the number of antibiotics increased, with resistance predominantly concentrated in industrial and pharmaceutical sources.

Impact of wastewater treatment on resistance

The comparative analysis of resistance levels in untreated and treated wastewater samples revealed a significant effect of wastewater treatment (Figure 4). The untreated samples exhibited a considerably higher resistance rate compared with the treated samples, highlighting the effectiveness of treatment processes in mitigating antibiotic resistance. In untreated samples, resistance was highest for tetracycline (66.7%), sulfamethoxazole/trimethoprim (58.3%), and ciprofloxacin (63.6%). Although treated samples exhibited reduced resistance to these antibiotics, substantial residual resistance persisted, particularly for tetracycline (33.3%) and sulfamethoxazole/trimethoprim (41.7%). Notably, azithromycin and meropenem showed near-complete elimination of resistance in treated samples, with no detectable resistance to azithromycin.

Discussion

This study provides critical insights into the prevalence, distribution, and antibiotic resistance patterns of bacteria isolated from wastewater in Southern Vietnam, specifically from the regions

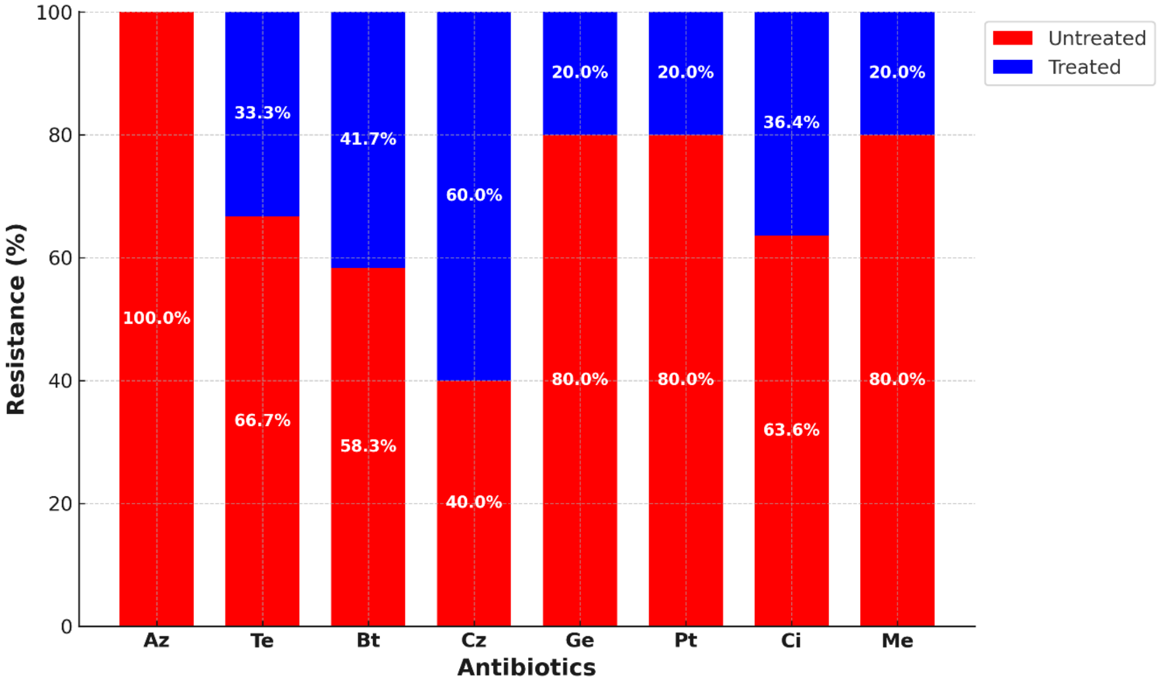

Fig. 4. Resistance rates of treated and untreated wastewater to antibiotics

of Dong Nai, Binh Duong, Tay Ninh, and Ho Chi Minh City. These locations are of particular significance due to their rapid industrialization, urbanization, and expansion of the healthcare sector (Viet et al., 2013). As the largest economic and industrial hubs in Southern Vietnam, Ho Chi Minh City and the surrounding provinces experience substantial wastewater discharge from various sources, including the healthcare, pharmaceutical, industrial, and agricultural sectors (Theuretzbacher et al., 2023). The unique dynamics of these regions contribute to the growing global challenge of AMR, adding novelty to the study by providing region-specific data that can inform targeted public health policies and environmental management strategies.

The bacterial contamination rate observed in this study was 33.3%, with MDRB present in 25.5% of the isolates, highlighting wastewater's significant role as a reservoir for antibiotic-resistant bacteria and resistance genes. These findings align with global reports identifying wastewater as a critical source of AMR dissemination and underscoring the necessity for improved surveillance and mitigation strategies (Fouz et al., 2020; Hendriksen et al., 2019; Liguori et al., 2022). The results further revealed that untreated wastewater exhibited significantly higher contamination and resistance rates than treated wastewater, emphasizing the crucial role of efficient wastewater management systems in controlling the spread of resistant pathogens. However, despite the obvious reduction in contamination after treatment, resistance rates remained substantial in treated wastewater, particularly for antibiotics such as tetracycline and sulfamethoxazole/trimethoprim. This indicates that although wastewater treatment reduces bacterial loads, it may not be entirely effective in eliminating resistant strains and their genetic material (Kalli et al., 2023).

The persistence of antibiotic resistance in treated wastewater can be attributed to several factors. First, conventional wastewater treatment methods, including activated sludge processes, can reduce bacterial populations but often fail to remove ARBs and resistance genes (Alam et al., 2021). However, these methods may struggle to eliminate resistant strains because of the limitations of current treatment technologies (Cheng et al., 2020). Second, residual antibiotic concentrations in treated effluent continue to exert selective pressure on bacterial populations, favoring the survival and propagation of resistant bacteria (Tello et al., 2012). Additionally, biofilm formation and other mechanisms of bacterial persistence may contribute to the survival of ARBs in treated wastewater (Flores-Vargas et al., 2021). The detection of resistance in treated effluent emphasizes the need for the implementation of advanced treatment technologies, such as advanced oxidation processes (AOPs) or membrane bioreactors (MBRs) (Azuma et al., 2022). These methods are more effective at breaking down contaminants and eliminating resistant organisms, offering potential solutions for improving wastewater treatment efficacy (Mosaka et al., 2023).

Distinct contamination patterns were observed in the different wastewater sources, with pharmaceutical and industrial effluents exhibiting the highest bacterial prevalence and MDR rates. This is in contrast with findings from other regions, where agricultural runoff and hospital effluents are often identified as primary contributors to AMR (Manyi-Loh et al., 2018; Kotwani et al., 2021). The predominance of *Pseudomonas*, *Burkholderia*, and *Enterobacter* among the isolated bacteria further highlights their ecological adaptability to nutrient-rich environments, which are characteristic of wastewater from the industrial, pharmaceutical, and healthcare sectors. These genera are known for their ability to thrive in environments containing high levels of organic matter and contaminants (Holden et al., 2009; Wu et al., 2011). Specifically, *Pseudomonas* species are well-known for their metabolic versatility and resistance to various antibiotics, making them particularly prominent in wastewater environments (Ambreetha et al., 2024). *Burkholderia* is another resilient genus that is frequently associated with soil and water environments, further explaining its prevalence in wastewater (Tavares et al., 2020). *Enterobacter* species, known for their persistence in both hospital and industrial wastewater environments, can survive under conditions in which nutrient availability and selective pressure from antibiotics foster their persistence (Davin-Regli et al., 2019).

In the context of Vietnam, the high prevalence of these genera can be attributed to several factors, including rapid industrialization and the expansion of pharmaceutical and healthcare sectors. These industries are significant contributors to wastewater contamination, and inadequate treatment infrastructure intensifies the problem. The absence of stringent wastewater treatment regulations in certain regions may also allow for the continued proliferation of resistant bacteria. Moreover, the widespread use of antibiotics in both medical and agricultural practices further promotes the selection of resistant strains, contributing to the growing AMR burden in wastewater (Samrot et al., 2023). Specifically, the high resistance rates observed for tetracycline (66.7%) and sulfamethoxazole/trimethoprim (33.3%) reflect the extensive use of these antibiotics in medical and agricultural settings in Vietnam. In particular, resistance to tetracycline exceeds the rates reported in many developed countries, which may be attributed to differing antibiotic usage patterns, regulatory frameworks, or environmental factors (Ayukekbong et al., 2017). These findings highlight the urgent need for stricter controls on antibiotic use, particularly in agriculture and aquaculture, to mitigate the selective pressures driving resistance.

From a public health and environmental management perspective, these results underscore the critical need for enhanced wastewater treatment technologies and stricter regulations on effluent discharge, particularly from pharmaceutical and industrial sources. The detection of resistant bacteria in treated wastewater highlights significant gaps in current treatment technologies, necessitating the development and implementation of advanced methods capable of eliminating resistant pathogens (Kalli et al., 2023). Furthermore, waste management practices in the pharmaceutical and healthcare sectors require closer scrutiny to prevent the improper disposal of pharmaceutical waste and to reduce the discharge of untreated or inadequately treated effluent into municipal systems (Azam et al., 2020). Improvements in these areas, such as enhancing pharmaceutical waste disposal and upgrading treatment systems, are essential for reducing the spread of AMR (Kotwani et al., 2021).

This study contributes valuable region-specific data to the growing body of research on AMR in wastewater, offering a foundation for targeted interventions and policies aimed at mitigating the spread of resistant bacteria in Vietnam and similar regions. The findings provide important insights into the unique dynamics of wastewater contamination in rapidly industrializing and urbanizing regions, emphasizing the need for a multifaceted approach to combating AMR, including improved wastewater treatment technologies, stronger regulatory frameworks, and antimicrobial stewardship in healthcare and agriculture.

CONCLUSION

This study underscores the significant role of wastewater as a reservoir for antibiotic-resistant bacteria, with bacterial contamination rates of 33.3% and 25.5% for multidrug-resistant isolates. The analysis revealed higher contamination and resistance rates in untreated wastewater compared to treated samples, emphasizing the importance of effective treatment processes in mitigating the spread of AMR. The predominance of *Pseudomonas*, *Burkholderia*, and *Enterobacter* among the isolated bacteria, combined with high resistance rates to tetracycline (66.7%) and sulfamethoxazole/trimethoprim (33.3%), reflects the impact of antibiotic usage in healthcare and agricultural settings in Vietnam.

These findings highlight the urgent need for stricter regulations on wastewater management, particularly in the industrial and pharmaceutical sectors, which exhibit the highest contamination and MDR rates. Enhanced treatment technologies, such as AOPs like ozonation, ultraviolet (UV) light irradiation, and photocatalysis, should be prioritized. These technologies are effective at breaking down organic pollutants, including antibiotic residues, and could play a crucial role in removing resistant bacteria from wastewater. Additionally, MBRs and biological nutrient removal (BNR) systems offer promising options for improving treatment efficiency, particularly in regions with limited access to large-scale infrastructure.

Given the specific needs of Vietnam's infrastructure, it is essential to consider cost-effective, scalable solutions that can be integrated into existing wastewater treatment plants. Pilot projects and case studies should be conducted to evaluate the feasibility of these advanced technologies in local contexts, especially for smaller or decentralized wastewater treatment systems. In addition, ongoing monitoring and surveillance programmes must be established to track antibiotic residues and resistance patterns in treated effluents. These data are critical for informing public health policies and guiding future interventions.

On a broader scale, efforts to address AMR in wastewater systems should be integrated with antimicrobial stewardship programs aimed at reducing the misuse of antibiotics in both human and veterinary medicine. Public awareness campaigns, combined with collaborations among policymakers, healthcare providers, and industry stakeholders, are crucial for addressing the environmental dimensions of AMR. The findings of this study provide valuable insights to support the development of comprehensive strategies to combat AMR, safeguard public health, and protect the environment.

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This research did not receive any financial support.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this manuscript. In addition, ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy were completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threats were employed in this research.

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