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Monitoring and antibiotic detection from a pharmaceutical wastewater treatment plant: A case study from an Indonesia pharmaceutical industry

Fatkhiyatus Sa'adah[1,4], Heri Sutanto[2,4*], Hady Hadiyanto[3], Ali Khumaeni[2], Wahyu Zuli Pratiwi[4]

[1] Doctoral Program of Environmental Science, School of Postgraduate, Diponegoro University, Semarang, Indonesia
[2] Department or Physics, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia
[3] Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Semarang, Indonesia
[4] Smart Material Research Center (SMARC), Diponegoro University, Semarang, Indonesia
* Corresponding author's e-mail: herisutanto@live.undip.ac.id

ABSTRACT

Pharmaceutical wastewater treatment plants (WWTPs) are critical points for the removal of pharmaceutical residues, including antibiotics, from industrial effluents before discharge into the environment. This study evaluated the effluent quality from a pharmaceutical WWTP in Indonesia, focusing on both standard physicochemical parameters and antibiotic residue levels. The physicochemical properties, such as COD, BOD, TSS, and pH, were compared to Indonesian regulatory standards. At Company XX, the removal efficiencies were 82.15% for COD, 82.71% for BOD, and 66.27% for TSS. However, the final effluent exceeded the discharge limits for COD and BOD by 22.74 and 2.38 times, respectively. In contrast, Company YY achieved a higher COD removal efficiency of 85.41%. The analysis of antibiotic residues focused on oxytetracycline (OTC), ciprofloxacin (CIP), amoxicillin (AMX), doxycycline (DOX), and cefadroxil (CEF). The results showed significant concentrations of OTC (3.887 mg/L in Company XX and 3.1473 mg/L in Company YY), CIP (2.849 m/L in Company XX and 1.711 mg/L in Company YY), and CEF (2.3871 mg/L in Company XX). AMX was detected at 2.1207 mg/L in Company XX. In contrast, AMX were below the LOD in Company YY. DOX was not detected in either company. The findings suggest that conventional treatment methods in pharmaceutical WWTPs are inadequate for eliminating antibiotic residues, underscoring the need for improved treatment technologies to mitigate antibiotic contamination and its ecological impact.

Keywords: wastewater treatment plants, antibiotics, occurrence, pharmaceutical effluent.

INTRODUCTION

Water pollution aggravates the issue of water scarcity, a growing concern intensified by climate change and the advancing spread of desertification. This growing issue presents a significant obstacle to the successful realization of the United Nations Sustainable Development Goals (SDG's No. 3, 6, 12, and 13) (Yang et al., 2025). Pharmaceutical companies play an important part in enhancing human health, but the production processes of these companies handle considerable quantities of wastewater having an intricate composition, comprising organic and inorganic contaminants (Adetunde et al., 2025). Pharmaceutical contaminants originate from various stages of the drug production process. Pharmaceutical compounds are recognized as potentially toxic pollutants in aquatic environments. Due to their persistence, they pose long-term risks to both aquatic ecosystems and human health. These substances contribute significantly to the development of antibiotic-resistant bacterial strains and can cause endocrine disruption. Pharmaceutically

active compounds (PhACs) also threaten ecosystem functionality and can diminish the overall quality as well as appeal of the environment (Bai et al., 2018; Kumar et al., 2023).

A major environmental challenge today is the frequent detection of low-concentration pollutants in various water bodies (K. Wang et al., 2021). Among these pollutants, residual antibiotics are specific interest. The antibiotics that are not adequately removed by wastewater treatment plants (WWTPs) are eventually released into natural aquatic systems, where they pose considerable ecological risks due to their harmful ecotoxicological effects (N. Wang et al., 2024). Globally, final effluents from WWTPs have been reported to contain traces of antibiotic residues. Recent studies have also identified antibiotics and other pharmaceutical compounds in surface waters, primarily originating from wastewater samples (Fiaz et al., 2021; Liu et al., 2023; Saidulu et al., 2021; K. Wang et al., 2021). Since conventional WWTPs are often unable to completely remove these compounds, treated effluent has become a major pathway through which antibiotics enter the environment. Their persistence in treated wastewater also poses significant challenges for water reuse initiatives (Tran et al., 2018). In Indonesia, research on the occurrence of antibiotics in the effluents of pharmaceutical WWTPs remains limited. Nevertheless, a few studies have reported the presence of antibiotics in surface waters, such as the Ciliwung River (Shimizu et al., 2013), Citarum river (Astuti et al., 2023), Loji river (Alam et al., 2024), and the Cirata reservoir (Ariyani et al., 2024). Against this background, industrial pollution by antibiotics is an escalating issue in Indonesia, primarily on the part of pharmaceutical production (Alifdini et al., 2018).

Indonesia has a large and growing pharmaceutical sector that is an integral part of its public health and economy. Nevertheless, legislation and control on discharging wastewater containing antibiotics is still incomplete and ineffective in its capacity to identify trace-level contaminants (Nurlaela Arief et al., 2022). In addition, the persistent selective pressure from these residues promotes the emergence and spread of antibiotic-resistant traits, which in turn presents a serious threat to public health and global development (Guo et al., 2025). To address the growing threat of antimicrobial resistance (AMR), the Government of Indonesia has implemented the National Action Plan for Antimicrobial Resistance Control *(Peraturan Menteri Koordinator Bidan Pembangunan Manusia dan Kebudayaan Republik Indonesia Nomor 7 tahun 2021 tentang Rencana Aksi Nasional Pengendalian Resistensi Antimikroba)*. One of the central strategies of RAN-PRA is the monitoring and regulation of residual antibiotics in environmental matrices, particularly from pharmaceutical manufacturing sources. In accordance with the principles of the One Health approach and international commitments to AMR mitigation (Velazquez-Meza et al., 2022), the pharmaceutical industries in Indonesia are mandated to comply with the national regulations regarding the prevention and detection of antibiotic contamination in wastewater discharges.

The current monitoring practices in Indonesia for pharmaceutical wastewater effluent primarily focus on standard physicochemical parameters, such as pH, chemical oxygen demand (COD), biological oxygen demand (BOD, and total suspended solids (TSS) based on the national wastewater quality standard (Rukmini et al., 2019). While these indicators provide a general overview regarding the wastewater quality, they are not adequate for measuring ecological risk imposed by residual antibiotics and other trace pollutants. To address this challenge, five antibiotics: oxytetracycline (OTC), ciprofloxacin (CIP), amoxicillin (AMX), doxycycline (DOX), and cefadroxil (CEF), were selected as model target compounds for detection. The identification of antibiotic compounds in WWTPs, particularly those containing specific pharmaceuticals, can be effectively carried out using High-Performance Liquid Chromatography (HPLC) (Abera et al., 2025). HPLC has become a powerful analytical technique due to its sensitivity and accuracy in quantifying antibiotic residues within complex environmental matrices. Additionally, the use of high-resolution mass spectrometry (LC/MS) enables the identification and characterization of over 100 antibiotics and their metabolites in water sources at extremely low concentrations, reaching parts per trillion (ppt) (B.Q. Wang et al., 2024). This technique is highly effective for detecting trace levels of antibiotics and is widely applied in environmental monitoring and risk assessment studies.

Therefore, this study aimed to evaluate the effluent quality of the wastewater treatment plant (WWTP) of a pharmaceutical company in Indonesia by combining conventional physicochemical analyses with advanced HPLC and LC/MS-based detection of antibiotic residues. The findings aim

to enhance the understanding of pharmaceutical pollution dynamics as well as support the development of improved monitoring strategies and regulatory frameworks to protect environmental and public health.

MATERIALS AND METHOD

Study area and sampling locations

The real industrial pharmaceutical wastewater (PW) was provided by two pharmaceutical manufacturing companies located in Semarang City, Central Java, Indonesia, and herein are known as Company XX and Company YY. WWTPs are operated by both companies within their environmental management system. The treated effluents from the WWTPs are released to the municipal drain system. The geographical coordinates of both companies were noted by GPS at the time of sampling. The site map indicating the area where Company XX and Company YY are situated is given in Figure 1.

Pharmaceutical companies both use multi-stage WWTP facilities that are capable of handling high-strength industrial wastewater. The treatment processes of the WWTPs at company XX and YY illustrated in Figure 2. The treatment method used for the WWTP of company XX involves chemical, biological, and physical processes to treat its pharmaceutical influent, as illustrated in Figure 2a. Influent (IN) is first collected in an equalization tank (ET) where pH adjustment and initial chemical coagulation occurred. Following this, the wastewater is transferred to an anaerobic reactor (AAT), and continue to a sedimentation tank (ST). After sedimentation, the wastewater enters an aeration tank (AT) and chlorination tank (CT). Before final discharge, the treated wastewater passes through a fish pond. İn parallel, solid waste or the sludge generated during the treatment process is dewatered using drying beds. Company YY also employs a multi stage WWTPs system, as seen in Figure 2b. The treatment begins in an equalization tank (ET), followed by a secondary equalization tank (SET), and then directed to a sedimentation tank

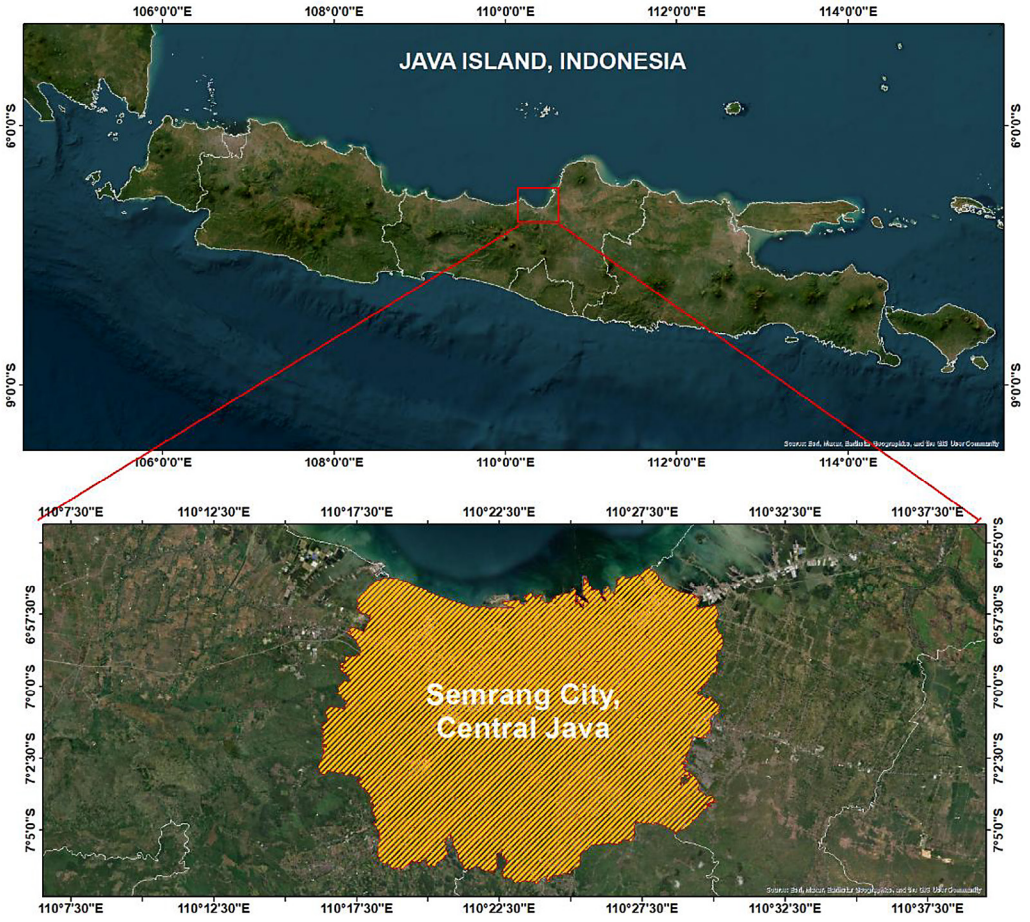


Figure 1. Map location of company XX and YY in Semarang, Central Java

(ST). After sedimentation, the wastewater moves to an aeration tank (AT). The resulting sludge is transferred to a drying bed, and the treated water undergoes a sand filter. Wastewater samples at each treatment stage were analyzed to assess the physicochemical quality parameters and monitoring for antibiotic residues. The wastewater samples were stored at 4°C until chemical analysis was carried out within 48 h.

Physicochemical parameter analysis

Physicochemical parameters including pH, chemical oxygen demand (COD), biological oxygen demand (BOD), and total suspended solids (TSS) were analyzed. The pH was measured using a calibrated digital pH meter (Laqua PD2000, Horiba). The pH measurement provides essential information about the acidity or basicity of wastewater, which can significantly impact chemical reactions and biological treatment processes. The COD was measured via the dichromate reflux method, colorimetric technique, following the procedures outlined in the Standard Methods for the Examination of Water and Wastewater. The wastewater samples were digested with potassium dichromate under acidic conditions and then measured spectrophotometrically at a wavelength of 600 nm. The COD value indicates the amount of oxygen required to chemically oxidize organic and inorganic substances in the sample. BOD was determined by the 5-day incubation method. The samples were incubated in darkness at 20°C for a period of five days. The dissolved oxygen (DO) levels were recorded both before and after incubation with a DO meter. The reduction in DO reflects the quantity of biodegradable organic matter present in the water. TSS concentration was measured by using the gravimetric method. A predetermined volume of wastewater was filtered through a pre-weighed glass fiber filter. The collected solids on the filter were dried at 103–105°C until a constant mass was achieved. TSS was calculated based on the weight gain of the filter, representing the concentration of suspended particles in the sample. All analyses were conducted in triplicate to ensure reliability, and standard quality control procedures were followed based on standard methods for the examination of water and wastewater 23rd Edition (APHA-AWWA-WEF) (Hong Dao et al., 2023).

Antibiotic detection using LC/MS and HPLC

Chemicals and reagents

Analytical-grade standards of oxytetracycline (OTC), ciprofloxacin (CIP), amoxicillin (AMX), doxycycline (DOX), and cefadroxil (CEF) were obtained from Sigma-Aldrich. Syringe filters (0.45 µm, PTFE) were sourced from Thermo Fisher Scientific (USA). Solid-phase extraction (SPE) was performed using Oasis hydrophilic-lipophilic balanced (HLB) cartridges (6 mL, 200 mg), purchased from Waters Co. (Milford, MA, USA). HPLC-grade solvents, including methanol, acetonitrile, and formic acid, were procured from Merck. Ultrapure water was generated using a Milli-Q® water purification system (Millipore, USA).

Stock solutions of each antibiotic (1000 mg/L) were individually prepared in methanol and stored at –18°C until further use. Working solutions for calibration and quality control were freshly prepared on the day of analysis by serial dilution in ultrapure water or the appropriate mobile phase. The molecular formula and weight, antibiotics class, structure and wavelength of maximum absorption of studied antibiotics, presented in Table 1.

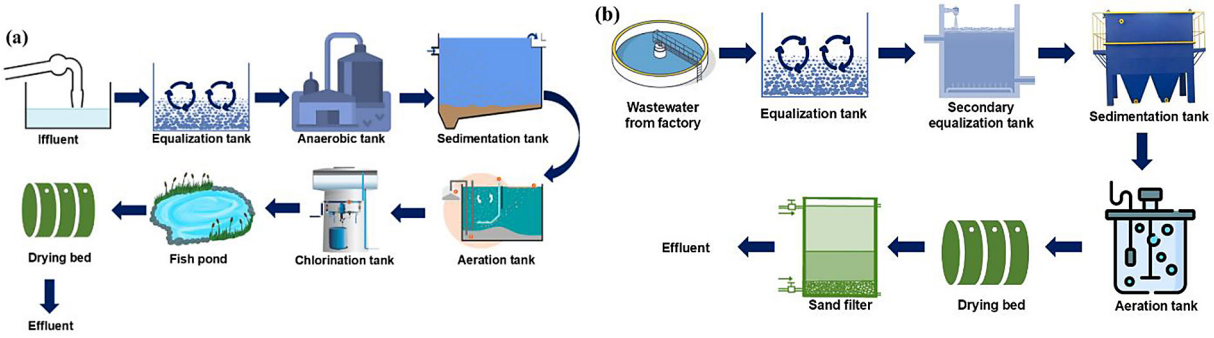
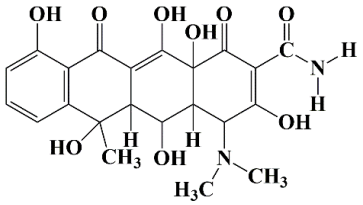
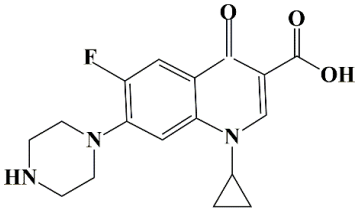
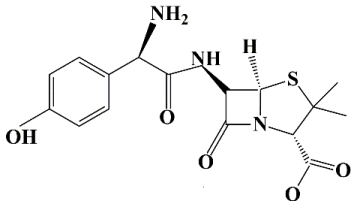
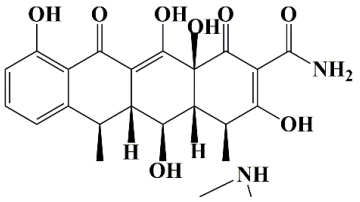
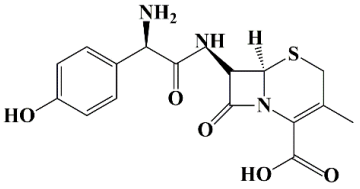


Figure 2. Flow diagram of WWTPs from pharmaceutical company (a) company XX, and (b) company YY

Table 1. The chemical characteristics of antibiotics

Antibiotics	Molecular formula and weight (g/mol)	Class	Chemical structure	λ_{max} (nm)	Ref
Oxytetracycline (OTC)	$C_{22}H_{24}N_2O_9$ (460.4)	Tetracycline		254	(Haq et al., 2025)
Ciprofloxacin (CIP)	$C_{17}H_{18}FN_3O_3$ (331.4)	Quinolones		278	(Cheng et al., 2025)
Amoxicillin (AMX)	$C_{16}H_{19}N_3O_5S$ (365.4)	Penicillin		220	(Khrushchev et al., 2022)
Doxycycline (DOX)	$C_{22}H_{24}N_2O_8$ (444.4)	Tetracycline		280	(Melissaropoulou et al., 2024)
Cefadroxil (CEF)	$C_{16}H_{17}N_3O_5S$ (363.39)	Cephalosporin		262	(Elbalkiny et al., 2020)

Sample preparation

Water samples from the WWTP effluents were collected in pre-cleaned amber glass bottles with the total volume of 1 L, and then keep stored in a freezer at -18°C. Prior to analysis, the wastewater samples were filtered through 0.45 µm PTFE membrane filters (Millipore, USA) to remove particulate matter. Solid-phase extraction (SPE) was used for sample clean-up and concentration using Oasis HLB cartridges. The SPE cartridges were conditioned with 5 mL of methanol followed by 5 mL of ultrapure water. Subsequently, 500 mL of filtered effluent was loaded onto each cartridge at a flow rate of approximately 5 mL/min. After sample loading, the cartridges were washed with 5 mL of ultrapure water and then dried under vacuum for 30 minutes. The analytes were eluted with 5 mL of methanol. The eluates were evaporated to near dryness under a gentle nitrogen stream at 40°C and reconstituted in 1 mL of 10% methanol in water. Finally, the reconstituted samples were filtered again through 0.22 µm PTFE filters and transferred into LC vials for HPLC and LC-MS analysis.

HPLC conditions

Initial screening of antibiotics was performed using Shimadzu LC 40-D machine equipped with a quaternary pump, auto sampler, temperature control module for column, and a PDA detector. The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) with a flow rate of 1.0 mL/min. A gradient elution was applied, starting with 95% A, decreasing

linearly to 70% A from 5 to 20 minutes, then to 50% A at 25 minutes, followed by re-equilibration at 95% A until 30 minutes. The injection volume was 10 µL for all analyses. The PDA monitored the eluent at detection wavelengths based on the maximum absorbance of each antibiotic: 254 nm for oxytetracycline, 280 nm for doxycycline, 278 nm for ciprofloxacin, 220 nm for amoxicillin, and 262 nm for cefadroxil. Calibration curves for each analyte were prepared by plotting peak areas against concentrations in the range of 0.1 0.25, 1.0, 2.5, and 5 mg/L. The residual concentration of the antibiotics in the samples was calculated according to the calibration curves.

LC-MS analysis

Further confirmation and sensitive quantification of the antibiotics were conducted using an Agilent 6130 single quadrupole liquid chromatography–mass spectrometry (LC-MS) system equipped with an electrospray ionization (ESI) source. Chromatographic separation was performed using the same column and mobile phase program, as described for HPLC analysis. The mass spectrometer was operated in positive ESI mode with the following parameters: a drying gas temperature of 350 °C, a drying gas flow rate of 10 L/min, a nebulizer pressure of 35 psi, and a capillary voltage of 4000 V. Targeted detection was performed in selected ion monitoring (SIM) mode, monitoring the [M+H]+ ions of each antibiotic.

Method validation

The analytical method was validated based on several key parameters, including linearity, sensitivity, recovery, precision, and matrix effects. Calibration curves were prepared using matrix-matched standards at five concentration levels (0.1, 0.25, 1.0, 2.5, and 5 mg/L), with each level analyzed in triplicate. The linearity of the method was assessed by calculating the coefficient of determination (R^2). The limits of detection (LOD) and limits of quantification (LOQ) were determined according to the guidelines established by the International Conference on Harmonisation (ICH), using the equations provided in Equations 1 and 2 (Kolla, 2018).

$$LOD = 3.3\ x\ \frac{\sigma}{m} \quad (1)$$

$$LOQ = 10\ x\ \frac{\sigma}{m} \quad (2)$$

where: σ represents the slope of the calibration curve, and m represents the residual standard deviation.

The qualitative evaluation of antibiotic residues in pharmaceutical effluent samples was performed based on four analytical criteria: response factor, linearity (correlation coefficient), ion ratio deviation, and retention time (RT) deviation. A sample was considered positive for an antibiotic only when all four criteria were satisfied. This approach aligns with the Commission Implementing Regulation (EU) 2021/808 of 22 March 2021, which outlines the performance requirements for analytical methods used to detect residues of pharmacologically-active substances in food-producing animals, as well as the interpretation of results and methods for sampling, repealing Decisions 2002/657/EC and 98/179/EC (EU, 2021).

The response factor, determined as the ratio of the area of the first product ion to that of the internal standard, offers a semi-quantitative indication of the analyte presence. In this study, the response factor values for confirmed antibiotics were within an acceptable range of ≤ 20% relative to the standard, indicating a consistent signal response and effective performance of the internal standard across all samples. The linearity of each antibiotic was assessed using matrix-matched calibration curves made from fortified blank samples. The calibration curves demonstrated strong correlation coefficients ($R^2 \geq 0.980$), confirming that the analytical system reliably produced a linear response across the tested concentration range. Ion ratio consistency is crucial for accurate compound identification in mass spectrometry. The ion ratio, calculated as the ratio of the second product ion area to the first product ion area, was compared between samples and standards. For confirmed positives, the maximum relative deviation in ion ratio did not exceed 40%, in accordance with the established guidelines, ensuring that the fragmentation pattern in samples matched that of the reference standard.

Retention time stability is also vital in LC–MS/MS analysis. The RT deviation between the analyte and its internal standard was found to be within 1% for all positive detections, confirming the chromatographic identity of the compound. Only samples that met all four criteria – response factor ≤ 20%, linearity (R^2) ≥ 0.980, ion ratio deviation ≤ 40%, and RT deviation ≤ 1% – were classified as positive for specific antibiotics. The samples that

did not meet any one of the criteria were not considered positive, even if the target compound was initially detected. This multi-criteria approach enhances the reliability of qualitative results, minimizing the risk of false positives caused by matrix interference or analytical variability.

RESULTS AND DISCUSSION

Physicochemical characteristics of the treatment pharmaceutical WWTPs

The physicochemical characteristics of wastewater from pharmaceutical companies XX and YY, collected at various stages of treatment, are illustrated in Figures 3 and 4. The permissible limits for discharge are based on the Indonesian regulatory standard outlined in the Ministry of Environment and Forestry Regulation No. 5 of 2014. In company XX, the removal efficiencies for COD, BOD, and TSS were 82.15%, 82.71%, and 66.27%, respectively, across all treatment technologies employed. Most wastewater samples from different WWTP stages exhibited elevated concentrations of COD and BOD. As depicted in Figure 3a, the influent pH was distinctly acidic, suggesting the presence of acidic substances possibly originating from industrial processes. While the pH and TSS concentrations in the treated effluent complied with regulatory limits, COD and BOD levels exceeded the discharge standards by approximately 22.74 and 2.38 times, respectively. The presence of COD and BOD concentrations significantly above the regulatory thresholds highlights the necessity for further improvements in the wastewater treatment processes at company XX.

The analysis of the different tested treatment stages revealed that the highest efficiencies for the removal of COD, BOD, and TSS were 37.28%, 39.35%, and 28.74%, and these were detected in the AT, AAT, and CT. AT removed the 37.28% of the COD, 15.38% of BOD, and 11.25% of TSS, indicating an effective reduction of these components due to the number of bacteria in AT. Aeration in industrial wastewater treatment refers to the introduction of oxygen into the wastewater to enhance the biological degradation of organic pollutants. This process plays a critical role in secondary treatment, where aerobic microorganisms utilize the supplied oxygen to decompose complex organic compounds into simpler, more stable substances (Bakhtiyari-Ramezani et al., 2025). The removal efficiencies of COD, BOD, and TSS in the AAT process were 22.42%, 39.35%, and 19.31%, respectively. AAT is the second stage of the WWTP at company XX and demonstrates the highest removal efficiencies among all treatment stages. Anaerobic treatment is a promising method for converting organic matter in wastewater into methane while minimizing waste production (Tian et al., 2025). This process typically relies on anaerobic bacteria, and in this company, the bacteria used are *Hitreat bactery* sp. 585. For the

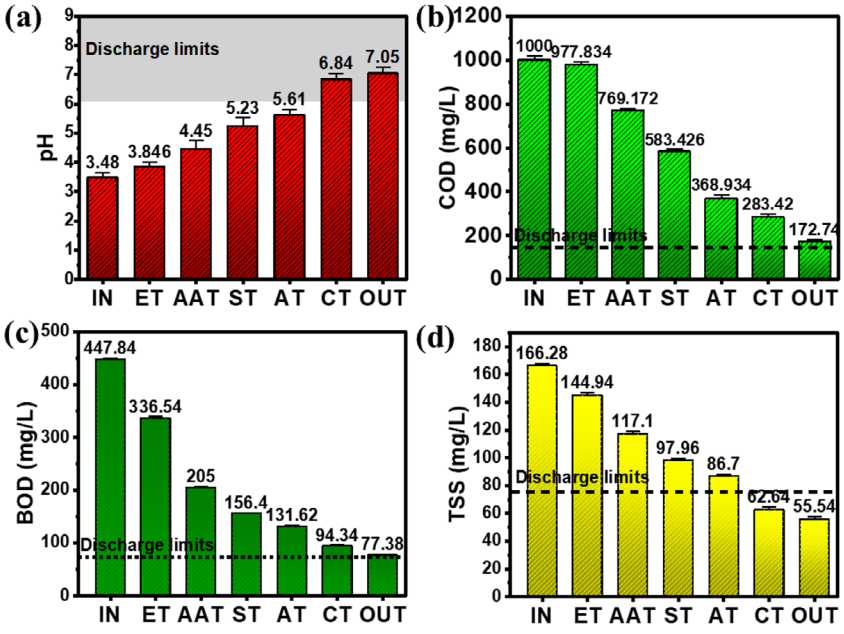


Figure 3. The physicochemical properties of wastewater from company XX

chlorination treatment, the removal efficiencies of COD, BOD, and TSS were 21.97%, 28.79%, and 28.74%, respectively. Chlorination treatments in WWTPs typically involve disinfection processes aimed at inactivating pathogens, such as bacteria and viruses. Additionally, this process helps oxidize and break down dissolved organic and inorganic contaminants (Zhang et al., 2022).

The conventional characteristics of wastewater from Company YY, collected at various treatment stages, are presented in Figure 4. As illustrated in Figure 4a, the initial pH of the influent was 3.634, indicating acidic conditions, though slightly higher than those observed at Company XX. Acidic pH in industrial wastewater is commonly attributed to the presence of acidic compounds originating from industrial processes. Throughout the treatment stages, specifically ET and SET, the pH gradually increased to 5.57, reflecting ongoing neutralization and dilution. Subsequent treatment stages led to continued improvement, with the final effluent (OUT) reaching a pH of 7.15, which falls within acceptable discharge standards. These findings highlight the effectiveness of the treatment system in neutralizing acidic wastewater. Figure 4(b) displays the reduction in chemical oxygen demand (COD) throughout the treatment process, achieving a total removal efficiency of 85.41%. The influent showed a high COD concentration of 998.18 mg/L, indicating a significant organic and chemical load. Compared to Company XX, the initial COD level at Company YY was slightly lower. However, the wastewater treatment plant at Company YY demonstrated superior COD removal efficiency. After progressing through each treatment stage, COD levels decreased markedly to 790.12 mg/L (ET), 427.62 mg/L (SET), and 284.02 mg/L (ST), with further reductions observed in the AT (198.22 mg/L) and final effluent (145.84 mg/L). Despite this substantial reduction, the final COD concentration remained slightly above regulatory discharge limits, suggesting a need for additional polishing steps to meet more stringent environmental standards.

The BOD profile exhibited a similar trend to COD, as shown in Figure 4c. Initial BOD concentrations were notably high, recorded at 405.36 mg/L for the influent and 398.66 mg/L after ET. Significant reductions were observed throughout the treatment process, with BOD levels dropping to 310.48 mg/L (SET), 120.38 mg/L (ST), 89.02 mg/L (AT), and 78.34 mg/L in the final effluent. Although the final BOD removal efficiency reached 80.64%, the value still slightly exceeded discharge standards, indicating that while biological treatment was effective, further optimization or the incorporation of advanced treatment technologies may be required. Figure 4d presents the TSS profile, which followed a decreasing trend similar to that of the organic load parameters. The TSS removal efficiency was calculated at 46.36%. Initial TSS concentrations of 109.98 mg/L gradually

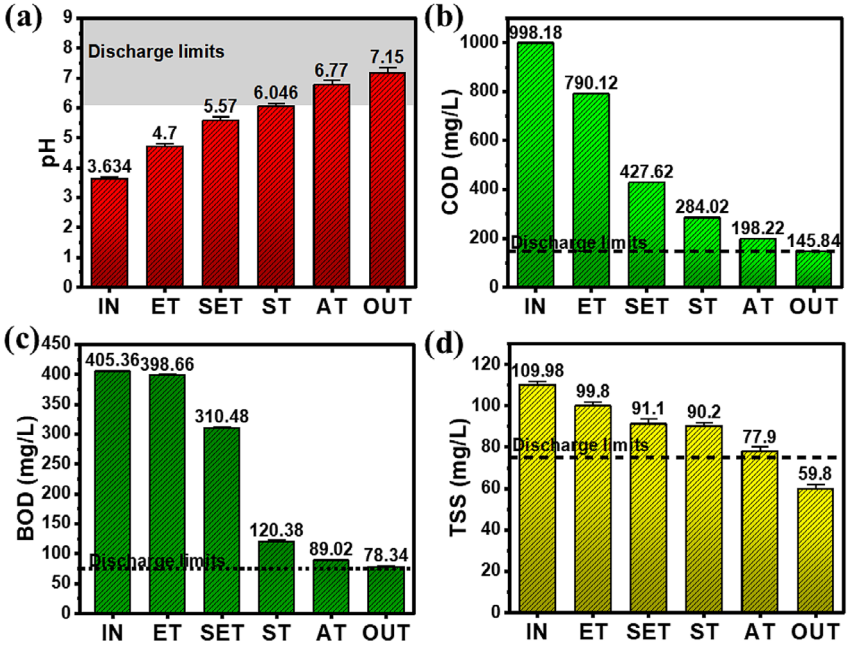


Figure 4. The physicochemical properties of wastewater from company YY

decreased to 99.8 mg/L (ET), 91.1 mg/L (SET), and 90.2 mg/L (ST), ultimately reaching 59.8 mg/L in the final effluent successfully meeting discharge requirements. This highlights the effectiveness of sedimentation and filtration units in removing suspended solids from the wastewater.

Both Company XX and Company YY demonstrated efficient wastewater treatment performance, particularly in terms of pH neutralization and TSS removal, achieving compliance with applicable discharge standards. However, while COD and BOD removals were considerable, they still require further optimization to meet increasingly strict environmental regulations. Minor performance differences were observed between the two companies, likely attributable to variations in both physicochemical and biological treatment. Company YY achieved slightly better COD reduction, which may be due to more effective biological treatment stages, like aeration and sand filtration, that promote microbial degradation of organic pollutants. In contrast, Company XX, although utilizing both anaerobic and aerobic treatment units, may have experienced lower efficiency due to suboptimal operating conditions or differences in microbial community composition. In contrast, Company XX demonstrated marginally higher efficiency in TSS removal. Overall, these findings indicate that organic-based pollutants are the primary contributors to water pollution from pharmaceutical wastewater in the industrial. Consistent with prior studies, the complex and poorly biodegradable nature of the organic compounds present in pharmaceutical wastewater poses significant challenges for treatment. The current study reaffirms that removing organic and nitrogenous pollutants remains the most difficult aspect of pharmaceutical wastewater management (Ma et al., 2016).

While the current study provides valuable insights into the composition and treatment efficiency of pharmaceutical wastewater in Companies XX and YY, it is important to acknowledge that sampling was conducted at a single time point. Seasonal or cyclical variations in production schedules, rainfall intensity, and operational efficiency may influence the physicochemical characteristics and antibiotic concentrations in effluent samples. As such, the findings represent a temporal snapshot rather than a comprehensive longitudinal profile. Future studies are recommended to adopt a long-term monitoring framework to better capture the dynamics of antibiotic discharge throughout different seasons.

Quality assurance of analytical procedure

The linearity data, along with the detection and quantification limits for the HPLC-PDA method applied to various antibiotics, are presented in Table 2. A linear calibration curve was successfully developed for five antibiotics, as illustrated in Figure 5. Each antibiotic demonstrated a strong linear correlation between peak area and concentration across the tested range (0.1, 0.25, 1, 2.5, and 5 mg/L), with correlation coefficients (R^2) exceeding 0.99. These high R^2 values confirm the strong linearity and reliability of the method for quantitative analysis. The corresponding linear regression equations and R^2 values are provided in Table 2. Among the antibiotics analyzed, ciprofloxacin (CIP) showed the highest correlation coefficient (R^2 = 0.9990), indicating excellent detector response consistency and low variability (Zambre et al., 2025). On the other hand, oxytetracycline (OTC) had the lowest R^2 (0.9903), though it still demonstrated a sufficiently linear relationship for accurate quantification. DOX exhibited the steepest slope among the compounds, suggesting greater detector sensitivity toward this antibiotic.

Table 2. Method validation for determination of OTC, CIP, AMX, DOX, and CEF

Antibiotics	Acronym	Retention time (RT)	Linearity (R^2)	Linear regression (Equation)	LOQ (mg/L)	LOD (mg/L)
Oxyetracycline	OTC	3.94	0.9903	1.476-E7 + 8.451-E5	2.626	0.866
Ciprofloxacin	CIP	3.54	0.9990	9.080-E6 + 1.630-E5	3.242	1.071
Amoxicillin	AMX	2.60	0.9977	8.986-E6 + 2.469-E5	2.013	0.664
Doxycycline	DOX	6.48	0.9935	3.055-E8 + 1.431E7	6.599	2.178
Cefadroxil	CEF	7.79	0.9979	1.012-E7 + 2.703-E5	1.431	0.473

Method sensitivity was evaluated based on the calculated LOD and LOQ. The LOQ values ranged from 1.43 to 6.59 mg/L, with CEF showed the lowest LOQ, indicating superior sensitivity. Additionally, CEF also exhibited the lowest LOD, further confirming the heightened sensitivity of the HPLC method for this compound. The low LOD and LOQ values support the suitability of the method for detecting and quantifying antibiotics at trace levels, which is critical for monitoring environmental contamination and pharmaceutical effluent discharges. The retention times for OTC, CIP, AMX, DOX, and CEF were 3.9, 4.38, 2.60, 6.48, and 7.79 minutes, respectively. These distinct retention times demonstrate the selectivity of the method and its capability to accurately detect and confirm antibiotic residues.

The occurrence of antibiotics compound in wastewater

The presence of antibiotic compounds in wastewater discharged by Company XX was evaluated using both HPLC and LC-MS analyses. The HPLC analysis verified the occurrence of all five targeted antibiotics in the effluent samples. Example chromatograms from the wastewater of Company XX are illustrated in Figure 6. The retention times for each compound closely aligned with those of their respective standard references, supporting the precision and dependability of the method used. Moreover, LC-MS analysis corroborated the findings from HPLC and additionally revealed the presence of trace-level antibiotic compounds that were not detected in the HPLC profiles.

Quantitative analysis showed that the concentrations of OTC, CIP, AMX, and CEF in the wastewater samples from Company XX were 3.887 mg/L, 2.849 mg/L, 2.1207 mg/L, and 2.3871 mg/L, respectively (refer to Table 3). Among these, OTC was detected at the highest level, suggesting it is the dominant antibiotic in the effluent, with CIP following closely behind. The high concentration of OTC could be attributed to its extensive application in both human and veterinary healthcare, along with its low biodegradability and strong resistance to conventional wastewater treatment methods (Jan et al., 2025). OTC is recognized for its strong chemical stability and low removal efficiency in biological wastewater treatment processes, leading to its continued presence in treated effluents (Idrees et al., 2025; Santajit et al., 2023). These findings indicate a potential ecological threat, especially in terms of promoting antibiotic resistance and posing toxic effects to aquatic organisms. While AMX and CEF were present at comparatively low levels, their simultaneous presence with other antibiotics may lead to synergistic or additive interactions, potentially enhancing ecological harm even at concentrations below inhibitory thresholds (Rzymski et al., 2024).

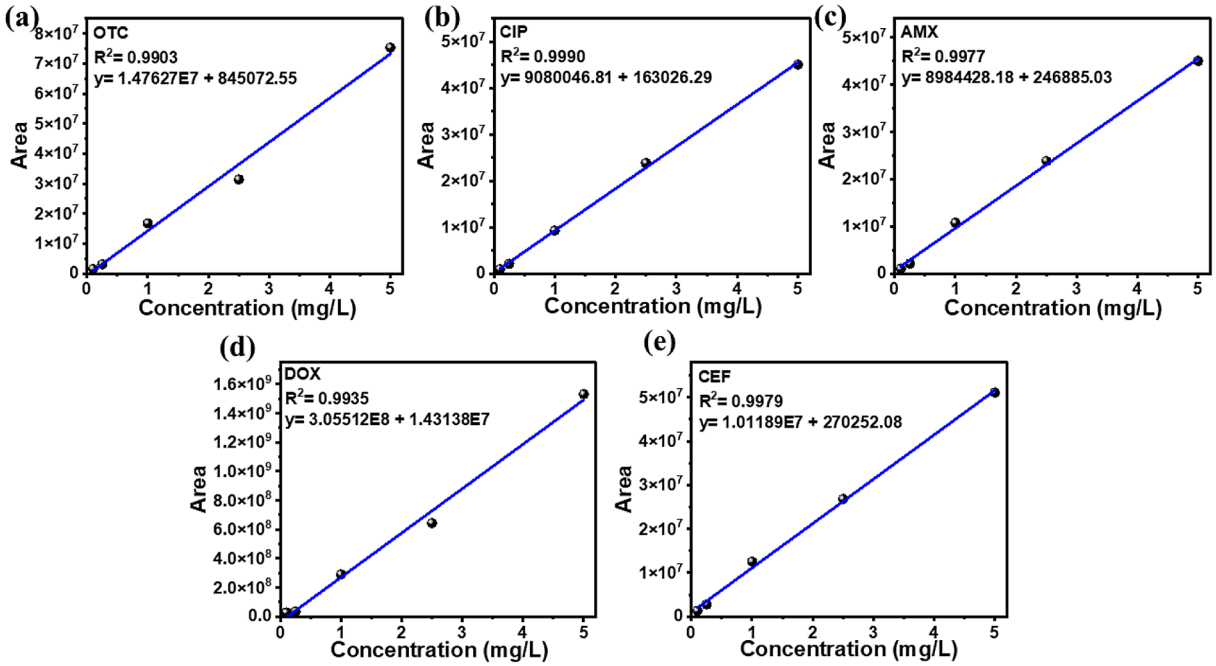


Figure 5. The calibration curves of HPLC test for antibiotic (a) OTC, (b) CIP, (c) AMX, (d) DOX, and (e) CEF

When comparing the obtained results to previous studies, the concentrations of OTC and CIP detected in this research are similar to those found in pharmaceutical wastewater from other Southeast Asian countries (Anh et al., 2021; Thai et al., 2018). This suggests that the discharge of antibiotics from pharmaceutical manufacturing is a widespread global issue. In contrast, the lower levels of DOX observed in the conducted study may be attributed to several factors, such as reduced usage at the source, more efficient removal during treatment processes, or potentially faster degradation under local environmental conditions (Yan et al., 2013).

The analysis of final effluents from pharmaceutical WWTPs of company YY identified several antibiotic residues at varying concentrations, as shown in Table 3. The HPLC chromatograms from the YY wastewater are illustrated in Figure 7. Specifically, the effluent from Company YY contained OTC, CIP, and CEF at concentrations of 3.1473 mg/L, 1.711 mg/L, and 0.578 mg/L, respectively. In contrast, AMX and DOX were not detected in the effluent from Company YY, as their concentrations were below the LOD. The absence of beta-lactam antibiotics such as AMX in the effluent suggests that they were effectively removed during the treatment process.

The detection of OTC, belonging to the tetracycline class of antibiotics, indicates that these compounds are likely used or produced during the manufacturing processes at Company YY. While

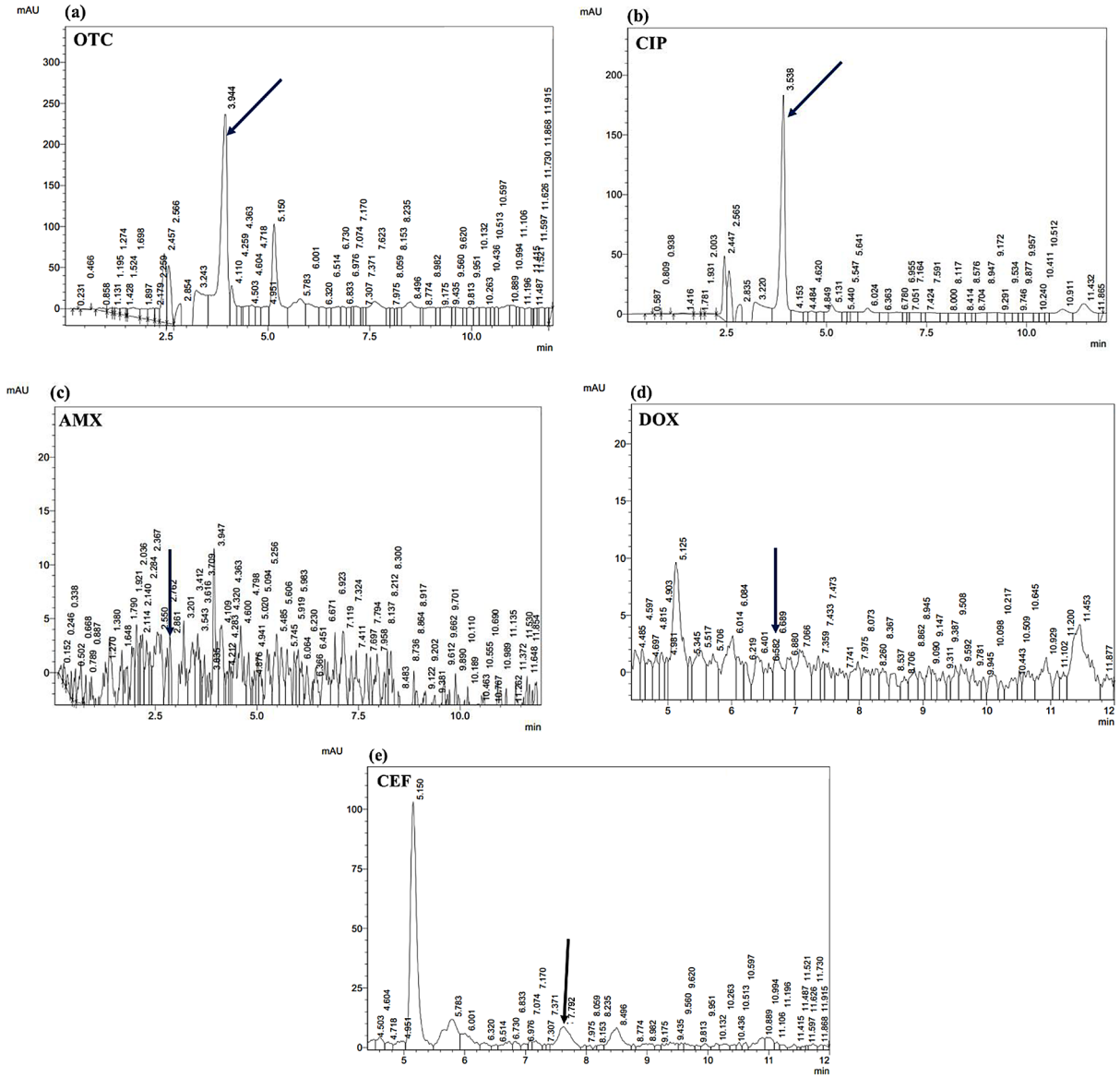


Figure 6. HPLC chromatograms of antibiotic residues detected in pharmaceutical effluent from company XX: (a) OTC, (b) CIP, (c) AMX, (d) DOX, and (e) CEF

OTC was found at higher concentrations in the effluent from Company XX, its presence in Company YY remained notable as well. These findings suggest that conventional treatment systems are not fully effective in removing tetracyclines, likely due to their stable chemical structure and low biodegradability (Sharma et al., 2024). CIP, a fluoroquinolone antibiotic, was detected in the effluent from Company YY at a concentration of 1.711 mg/L, indicating its widespread use or persistent presence due to its resistance to conventional treatment methods. Given its high environmental persistence and toxicity to aquatic organisms, ciprofloxacin poses a significant concern in pharmaceutical wastewater (Idrees et al., 2025).

The persistent exceedance of COD and BOD standards in the effluents of both companies raises the possibility of synergistic effects between antibiotics and other organic pollutants present in the wastewater. Although a direct correlation analysis between antibiotic concentrations and COD/BOD was not performed, the presence of multiple bioactive compounds, including antibiotics, may contribute to the total organic load and potentially interfere with microbial degradation processes (Guo et al., 2025; Rzymski et al., 2024). Company XX exhibited a broader range of antibiotic residues, including AMX, which were not detected in Company YY. These β-lactam antibiotics are known to

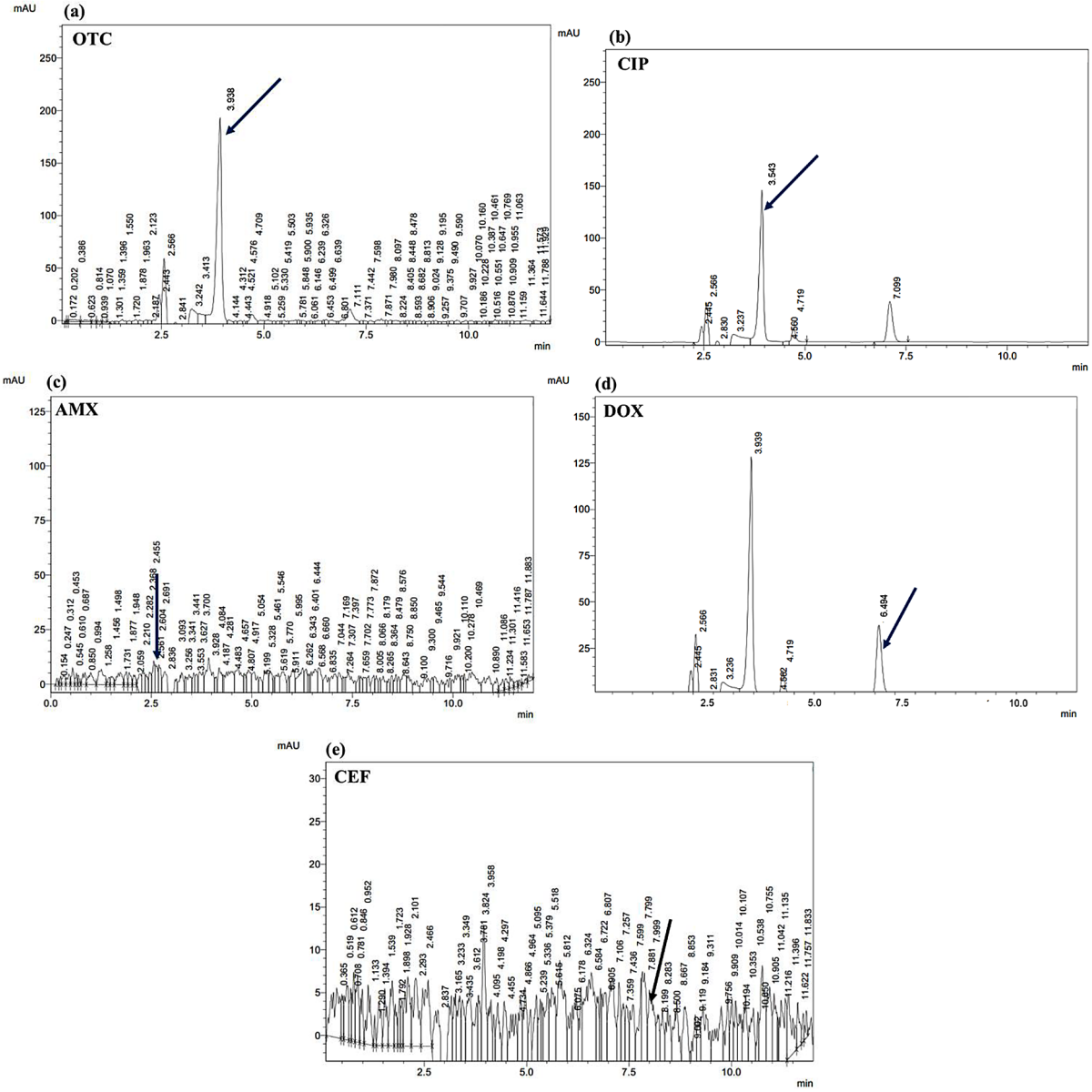


Figure 7. HPLC chromatograms of antibiotic residues detected in pharmaceutical effluent from company YY: (a) OTC, (b) CIP, (c) AMX, (d) DOX, and (e) CEF

Table 3. Concentrations of antibiotic residues in the effluent samples from company XX and YY

Antibiotic	Company XX (mg/L)	Company YY (mg/L)
Oxytetracycline (OTC)	3.887	3.147
Ciprofloxacin (CIP)	2.849	1.711
Amoxicillin (AMX)	2.1207	<LOD
Doxycycline (DOX)	< LOD	<LOD
Cefadroxil (CEF)	2.3871	0.578

degrade rapidly under specific conditions but may persist in effluents depending on the efficiency of treatment processes and the intensity of production. Although the concentrations detected in this study are in the nanogram-per-liter range, they remain environmentally significant. The continued presence of these antibiotics in treated effluents highlights the need to reassess and enhance the current wastewater treatment approaches (Öztürk et al., 2025; Y. Wang et al., 2025). Conventional methods are often insufficient, particularly for compounds like CIP and OTC, which are resistant to biodegradation. Advanced treatment technologies, such as ozonation, photocatalysis, and membrane bioreactors, have shown promising potential in addressing these challenging contaminants (Jin et al., 2025; Rab et al., 2025; Yang et al., 2024). Furthermore, the co-occurrence of multiple antibiotics in the effluent adds another layer of concern. Even at low concentrations, their combined presence can promote the development of antibiotic-resistant genes (ARGs), which poses an escalating public health risk that requires urgent attention from regulators and technology innovators.

CONCLUSIONS

The treatment performance for COD, BOD, TSS, and pH revealing significant removal efficiencies across company XX and YY. Company XX achieved removal efficiencies of 82.15% for COD, 82.71% for BOD, and 66.27% for TSS, but the final effluent still exceeded discharge standards for COD and BOD by 22.74 and 2.38 times, respectively. In contrast, Company YY showed superior treatment performance, particularly in COD removal, with a total removal efficiency of 85.41%. The final effluent at Company YY achieved near-compliance with regulatory limits, though COD and BOD concentrations slightly exceeded discharge standards, necessitating further polishing steps. A total of five antibiotics were analyzed, including OTC, CIP, AMX, DOX, and CEF using HPLC and LC/MS. The results showed significant concentrations of OTC (3.887 mg/L in Company XX and 3.1473 mg/L in Company YY), CIP (2.849 m/L in Company XX and 1.711 mg/L in Company YY), and CEF (2.3871 mg/L in Company XX). AMX was detected at 2.1207 mg/L in Company XX. In contrast, AMX was below the LOD in Company YY. DOX was not detected in either of the companies. These results highlight the persistence of antibiotics in treated wastewater and emphasize the potential risks they pose in terms of antibiotic resistance development and ecological disruption. One limitation of this study is that it used sampling at only one point in time. This approach may not reflect changes in wastewater quality due to production cycles or seasonal variation. Future studies should use repeated sampling over time to provide more reliable and representative data. While the results may not apply to all pharmaceutical facilities in Indonesia, due to differences in production scale, treatment methods, and local conditions, this study offers a useful reference. It also provides a practical method that can support future monitoring and wastewater management efforts across the country.

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