Research Paper

Fate and mass loading of antibiotics in hospital and domestic wastewater treatment plants in Bangkok, Thailand

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ABSTRACT

Excessive and inappropriate use of antibiotics contributes to the spread of antibiotic resistance in the environment, especially in low- to middle-income countries. This study investigated the occurrence, relative abundance, and fate of eight antibiotics at each treatment stage in four domestic and four hospital wastewater treatment plants (dWWTPs and hWWTPs, respectively), as well as mass loadings into the receiving water environments in Bangkok, Thailand. Samples were prepared by solid-phase extraction and analyzed by high-performance liquid chromatography–tandem mass spectrometry. Antibiotic concentrations were higher in hWWTPs than dWWTPs; approximately 60 times for influents and 10 times for effluents. Ciprofloxacin concentration increased in most dWWTPs, especially in the aeration unit and return sludge, suggesting that it predominantly occurred in the solid phase. Sulfamethoxazole predominantly occurred in the dissolved form, which is more difficult to degrade, and exhibited high concentrations in effluent. Moreover, antibiotic pollutant loadings were approximately 30–3,530 times higher from dWWTPs than from hWWTPs due to higher daily discharges from the domestic sector. These plants are a major point source of antibiotic residue release to aquatic environments; thus, their efficiency should be improved by incorporating advanced treatment processes to ensure effective removal of antibiotics.

Key words: antibiotics, domestic wastewater, hospital wastewater, mass loading, wastewater treatment plant

HIGHLIGHTS

• Antibiotic concentrations in influent and effluent were 60 times and 10 times higher in hWWTPs than dWWTPs.
• Mass loading of individual antibiotics was 30–3,530 times higher from dWWTPs than hWWTPs.
• Ciprofloxacin increased substantially in aeration unit and return sludge, indicating its predominance in sludge.
• Sulfamethoxazole exhibited the highest loading as difficult to remove in domestic and hospital treatment plants.

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INTRODUCTION

Antibiotics can originate from natural organisms or be developed from semi-synthetic or synthetic materials, and are widely used to treat infections in humans and animals. Antibiotics can inhibit the growth of or eliminate microbes through different mechanisms at low concentrations (Davies & Davies 2010; Mohr 2016). They are also used for disease prevention as well as animal growth promoters in livestock and aquaculture (AlSheikh et al. 2020). During the last decade, antibiotic residues have been ubiquitously detected in various environments including surface water, drinking water, groundwater, coastal water, treated wastewater, sediments, soils, and wastewater sludge (Miege et al. 2009; Watkinson et al. 2009; Monteiro & Boxall 2010; Li et al. 2017; Chen et al. 2020; Lyu et al. 2020). A major concern related to residual antibiotics is their continued detection in the aquatic ecosystems from wastewater discharged to the environment, which can have substantial impacts on aquatic life and microbial community leading to the emergence of resistant bacteria (Batt et al. 2006; Kołodziejska et al. 2013). Antibiotic resistance refers to the loss of effectiveness of antibacterial drugs used in medical treatments and may have significant long-term implications for human health (Friedman et al. 2016). Aquatic environments receiving antibiotic residuals become
reservoirs for emerging antibiotic resistant bacteria and antibiotic resistant genes. These resistant microorganisms reduce the efficiency of the bacterial community in degradation processes such as nitrogen transformation and sulfate reduction (Keen & Patrick 2013; Qiao et al. 2017). The antibiotics and antibiotic resistant genes in the aquatic organisms are sourced from the antibiotic residuals in the water and sediments (Ben et al. 2019). Moreover, antibiotic residues in the environment can also have adverse effects on human health. The consumption of food and water contaminated with antibiotic residuals may alter the resistance of intestinal microflora (Jayalakshmi et al. 2017). Long-term exposure to even low concentrations of antibiotics has resulted in increased antibiotic or multirud occuence, which can induce treatment failure and increase the mortality rate (Baquero et al. 2008; Hidron et al. 2008; Li et al. 2017).

Antibiotics are typically dispensed in hospitals and pharmacies; however, antibiotic dispensing is not controlled and often occurs without a prescription. In Thailand, people can easily access antibiotics not only from public and private health facilities but also retail pharmacies and grocery stores. Moreover, high levels of self-medication but a poor understanding of the harmful impacts of antibiotics can lead to the misuse and overuse of these drugs (Chalker et al. 2005; Apisarnthanarak & Mundy 2008; Sommanu et al. 2018; Chanvamit et al. 2019). As a result, residual antibiotics are found in domestic wastewater treatment plants (dWWTPs), hospital wastewater treatment plants (hWWTPs), and receiving surface waters (Fuentefria et al. 2011; Dutta et al. 2014). As point sources of antibiotics released to the aquatic environment (Kim & Aga 2007), WWTPs play an important role in the life cycle of antibiotics. Several studies have identified sewage system discharge as a source of parent pharmaceutical compounds and metabolites (Daughton 2003; Tewari et al. 2013; Lu et al. 2020). Another study in 2007 showed that surface waters near WWTPs contain increased amounts of antibiotic-resistant Escherichia coli (Watkinson et al. 2007). Moreover, the behavior of antibiotic-resistant genes in the various treatment processes of WWTPs is significantly correlated with the concentrations of antibiotic residue in effluents (Mao et al. 2015). The irrigation of agricultural lands with treated wastewater and biosolids from WWTPs can cause the accumulation of antibiotics and antibiotic resistant microorganisms in food crops and livestock, eventually leading to resistant bacteria in the human gut (Sorinolu et al. 2021).

Antibiotics excreted by humans are mainly transported via sewage to the WWTPs and the residuals are even found in treated water. However, public concern regarding antibiotics is almost negligible, especially in developing countries that demonstrate poor performance in controlling the over use and dispensing of antibiotics into the aquatic environments. Studies on antibiotics mass loadings from hWWTP and dWWTP along with the estimation of the impact of these contaminants on the environment are still limited and unclear. As a source of antibiotic residues in receiving water environments, WWTPs in Thailand should be urgently monitored to determine the fate and occurrence of antibiotics. Particular attention should be paid to the fate of antibiotics at different treatment stages in hWWTPs and dWWTPs as the treatment system employed in a WWTP affects the efficiency of antibiotic removal (Gulkowska et al. 2008). Therefore, this study investigates the occurrence, relative abundance, and fate of selected antibiotics at each treatment stage of four hWWTPs and four dWWTPs in Bangkok, Thailand. Moreover, antibiotic pollutant loadings discharged to the receiving water environments are compared between hWWTPs and dWWTPs.

MATERIALS AND METHODS

Sample collection and pre-treatment conditions

Four hWWTPs (designated as H1, H2, H3, and H4) were selected according to their different types of secondary treatment and disinfection methods. Four dWWTPs (designated as D1, D2, D3, and D4) were selected based on the types of secondary treatment and due to their capacity to receive over 65% of total wastewater carried in the sewerage system in Bangkok (Department of Environment 2019). Details of the WWTPs and sampling points in Bangkok, Thailand, are shown in Table 1.

Grab sampling was employed for all sample collection. After collecting the samples in 1.5 L polyethylene terephthalate bottles, ascorbic acid was added until a pH of 3 was reached to inhibit microorganism activities and neutralize chlorine residues, which are strong oxidizing agents. All samples were carefully placed in an ice-cooler container with light protection and transported to the laboratory.

Chemicals and standard solutions

Eight target antibiotics were chosen in this study based on the statistics of the most frequently dispensed antibiotics in the study area (Ministry of Public Health 2012). Standards for the selected target antibiotics included dicloxacillin sodium (99.4%), piperacillin (≥77%), cefalexin (95.0%), cefazolin sodium (89.1%), clarithromycin (95.0%), ciprofloxacin (98.0%),...
metronidazole (99.8%), and sulfamethoxazole (99.6%). The solvents used in this study included acetonitrile (99.8%), formic acid (98.0%), and methanol. Other chemicals used were ascorbic acid (99.8%), Na₂EDTA (98.0%) and NaCl (99.5%). List of chemicals and consumable materials used in this study is given in Table S1.

Stock antibiotic standard solutions of dicloxacillin (DX), piperacillin (PC), cefalexin (CF), cefazolin (CZ), sulfamethoxazole (ST), clarithromycin (CM), and metronidazole (MD) were prepared individually by dissolving 2 mg of powdered target antibiotics in methanol in 10-mL volumetric flasks. The final concentrations of individual antibiotic standards were between 200 and 350 mg/L. Finally, these stock solutions were kept in closed amber glass bottles at 4 °C in a refrigerator, except for ciprofloxacin (CP), which was freshly prepared daily in 0.1% (v/v) formic acid/ultrapure water (UPW) and kept in the same condition as the other antibiotic stock solutions. Working standard mixtures were prepared daily by diluting each antibiotic standard from the stock solution at the initial condition of the binary gradient of the mobile phase prior to each analysis.

Solid-phase extraction

All samples were vacuum filtered through a glass fiber filter with a pore size of 1 μm. Subsequently, 1 g/L of Na₂EDTA and NaCl were added as chelating agents to prevent the formation of divalent ions, which can influence the measurement (Kolpin et al. 2002). Prior to solid-phase extraction (SPE), all samples were adjusted to pH 4 by adding NaOH. SPE was performed by OASIS HLB Plus cartridges. Each cartridge was preconditioned by adding 6 mL methanol (MeOH) followed by 4 mL ultrapure water (UPW) at a control pH of 4 by mixing it with H₂SO₄. Samples were percolated through the cartridges at a controlled flow rate of 5 mL/min. After complete percolation, 2 mL of pH 4 UPW was used to wash the cartridges to remove impurities. The eluent was then discarded. Vacuum drying was applied for 10–15 min in the vacuum manifold to remove excess moisture in the cartridges. After vacuum drying, all samples were eluted with 6 mL of MeOH. The eluent samples were completely dried using high-purity nitrogen gas at 40 °C. Then, each dried sample was brought to the final volume with 1 mL of 9:1 MeOH and 0.1% (v/v) formic acid in UPW. The final extracted samples were transferred to 2-mL vials for subsequent high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis.

High-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis

HPLC-MS/MS analysis was conducted using an Agilent 1,200 SL high-performance liquid chromatograph coupled with the Agilent 6,410 Triple Quadrupole MS equipped with an electrospray ionization source (Agilent Technologies, USA). The HPLC instrument consisted of an analytical column, Agilent Eclipse Plus C18 (100 × 2.1 mm, 1.8 μm particle size), and a protective guard column, Agilent Eclipse Plus C18, (12.5 × 4.6 mm, 5 μm particle size). The HPLC-MS/MS conditions applied in this study were derived from Sinthuchai and product ions (m/z) of the target antibiotics were dicloxacillin (470 → 160), piperacillin (518 → 142.8), cefalexin (348 → 158), cefazolin (455 → 323), ciprofloxacin (332 → 318.4), sulfamethoxazole (254 → 156), clarithromycin (748 → 590), and metronidazole (172.1 → 128.1), respectively. The detection limit (LOD) and quantification limit (LOQ) of the target antibiotics were in the range of 0.25–6.82 ng/L and 0.84–22.75 ng/L, respectively. The percentages of recovery ranged between 66 and 127% with precision (RSD) of 2.74–20.82%.

Table 1 | Details of sampling locations in different WWTPs in Bangkok, Thailand

<table>
<thead>
<tr>
<th>WWTPs</th>
<th>Treatment systems (secondary + disinfection)</th>
<th>Sampling points at each treatment stage</th>
<th>Daily flow (m³/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hWWTPs</td>
<td>Activated sludge + chlorination</td>
<td>Inf → Eq → Ae → Re → Pre-Cl₂ → Post-Cl₂</td>
<td>3,500</td>
</tr>
<tr>
<td>H2</td>
<td>Extended aeration + UV</td>
<td>Inf → Eq → Ae → Re → Pre-UV → Post-UV</td>
<td>1,500</td>
</tr>
<tr>
<td>H3</td>
<td>Sequencing batch reactor (SBR) + UV</td>
<td>Inf → Ae² → Ae² → Pre-UV → Post-UV</td>
<td>1,400</td>
</tr>
<tr>
<td>H4</td>
<td>Rotating biological contactor (RBC) + chlorination</td>
<td>Inf → Ae → Pre-Cl₂ → Post-Cl₂</td>
<td>200</td>
</tr>
<tr>
<td>dWWTPs</td>
<td>Activated sludge (nutritional removal)</td>
<td>Inf → Eq → Ae → Re → Eff</td>
<td>350,000</td>
</tr>
<tr>
<td>D1</td>
<td>Activated sludge (contact stabilization)</td>
<td>Inf → Ae → Re → Eff</td>
<td>30,000</td>
</tr>
<tr>
<td>D2</td>
<td>Activated sludge (sequencing batch reactor)</td>
<td>Inf → Ae → Eff</td>
<td>200,000</td>
</tr>
<tr>
<td>D3</td>
<td>Activated sludge (vertical loop reactor)</td>
<td>Inf → Eq → Ae³ → Ae⁴ → Re → Eff</td>
<td>157,000</td>
</tr>
</tbody>
</table>

Note: Inf, influent; Eq, Equalization Tank; Cl₂, Chlorination; Ae, Aeration; Re, Return sludge; Eff, Effluent.

The daily flow of hWWTPs was obtained during the survey while that of dWWTPs was gathered from the report by the Department of Environment in 2019.

Plant H3 consists of two aeration tanks that alternate operation.

Plant D4 consists of four aeration tanks (Ae1, Ae2, Ae3, Ae4) connected in series; the only available sampling points were at Ae1 and Ae4.
RESULTS AND DISCUSSION

Influent and effluent concentrations of individual antibiotics in hWWTPs and dWWTPs

Antibiotics were detected in all influent samples from all treatment plants, with some samples exhibiting very high antibiotic concentrations (≥1,000 ng/L), especially in the influent of hWWTPs. CZ, CP, ST, and CM were detected in all effluent samples. The results indicated that various antibiotics in the effluent of all investigated WWTPs were subsequently released to receiving water environments. Concentrations of target antibiotics in the influent and effluent of hWWTPs and dWWTPs are shown in Figure 1 and Table 2.

As shown in Figure 1, the average concentrations of target antibiotics in influent and effluent were approximately 60 times and 10 times higher in hWWTPs than dWWTPs, respectively. All effluent concentrations were higher in hWWTPs except for DX and CZ. Many factors affect the removal efficiency of antibiotics, e.g., the type of treatment process, wastewater components, and antibiotic concentration. The difference in concentration levels between hWWTPs and dWWTPs could have been due to the different types of secondary treatment systems, as well as the dilution effect from the combined sewerage system. As shown in Table 2, the concentrations of antibiotics in the influent and effluent of hWWTPs ranged from 15.4 to 13,166.16 ng/L and from < LOD to 1,499.16 ng/L, respectively. For dWWTPs, antibiotic concentrations in the influent and effluent ranged from < LOD to 215.40 ng/L and from < LOD to 152.40 ng/L, respectively.

The concentrations of DX in the effluent ranged from 44.32 to 152.40 ng/L for dWWTPs and < LOD to 13.53 ng/L for hWWTPs. Therefore, it appears that the activated sludge treatment system in the dWWTPs did not effectively remove DX from the wastewater, especially in plant D2, where DX concentrations were higher in the effluent than the influent. The highest concentration of DX was 152.40 ng/L in the effluent of D3. PC concentrations in the effluent of dWWTPs ranged between 2.10 and 8.92 ng/L. In a similar study in Germany, PC concentrations in dWWTP effluent were much higher, ranging from 274 to 355 ng/L (Rossmann et al. 2014). CF was not detected in the effluent of any dWWTPs, nor in three of the four hWWTPs selected in this study; the CF concentration in the effluent of H4 was 5.99 ng/L. According to previous studies, CF concentrations in dWWTPs were 240–2,900 ng/L in Hong Kong (Gulkowska et al. 2008), 283 ng/L on average in Taiwan (Lin et al. 2008), < LOD to 38.4 ng/L in Portugal, 65.2 ng/L in Spain, 65–66.3 ng/L in Cyprus, 66.4–87.6 ng/L in Ireland, 203.5–308.0 ng/L in Finland, and 60.7 ng/L in Norway (Rodriguez-Mozaz et al. 2020). CF concentrations in hWWTPs in Australia (up to 2,800 ng/L) were much higher than those in the study (Watkinson et al. 2007). CZ concentrations detected in the influent and effluent ranged from 654.91–13,166.16 ng/L and 1.32–23.57 ng/L, respectively, for hWWTPs, and 104.01–178.07 ng/L and 16.71–47.38 ng/L, respectively, for dWWTPs. Low concentrations of CF and CZ detected in the effluent of hWWTPs and dWWTPs indicated the effective removal of these antibiotics by secondary treatment. As reported in a previous study (Sinthuchai et al. 2016), ST is not easily removed by secondary treatment and disinfection of hWWTPs. This also agrees

Figure 1 | Average concentrations of eight antibiotics in the influent and effluent of four hWWTPs and four dWWTPs in Bangkok, Thailand. DX, dicloxacillin; PC, piperacillin; CF, cefalexin; CZ, cefazolin; CP, ciprofloxacin; ST, sulfamethoxazole; CM, clarithromycin; and MD, metronidazole.
Relative abundance of antibiotics in the different WWTP treatment processes

The relative abundances of antibiotics in the various treatment processes of the selected WWTPs in Bangkok, Thailand, are shown in Figure 2. According to variations in the relative abundances of different antibiotics in hWWTPs and dWWTPs, all dWWTPs exhibited similar patterns, whereas different patterns were observed among the different hWWTPs plants.

In hWWTPs, the highest relative abundances were all attributed to ST, which occurred in the post-chlorination section of H1, the post-UV section of H2, and the post-UV section of H3 (57.16%, 43.42%, and 90.59%, respectively). For plant H4, the highest relative abundance was CP in the aeration tank and return sludge in H1, H2, and H3. The proportion of CP in the aeration unit and return sludge of hWWTPs ranged from 26.96–86.60 to 83.68–93.09%, respectively. PC was highest in the influent of hWWTPs; 59.77% in H1 and 55.67% in H2.

Table 2 | Concentrations of target antibiotics in influent and effluent of hWWTPs and dWWTPs in Bangkok, Thailand

<table>
<thead>
<tr>
<th>WWTPs</th>
<th>Concentration (ng/L)</th>
<th>Treatment systems (secondary + disinfection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DX</td>
<td>PC</td>
</tr>
<tr>
<td>Hospital</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>Influent 1,300.82</td>
<td>9,913.24</td>
</tr>
<tr>
<td></td>
<td>Effluent &lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>H2</td>
<td>Influent 543.63</td>
<td>2,581.90</td>
</tr>
<tr>
<td></td>
<td>Effluent &lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>H3</td>
<td>Influent 372.58</td>
<td>50.61</td>
</tr>
<tr>
<td></td>
<td>Effluent 13.53</td>
<td>21.04</td>
</tr>
<tr>
<td>H4</td>
<td>Influent 15.40</td>
<td>725.58</td>
</tr>
<tr>
<td></td>
<td>Effluent 2.89</td>
<td>42.24</td>
</tr>
<tr>
<td>Domestic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>Influent 83.39</td>
<td>8.25</td>
</tr>
<tr>
<td></td>
<td>Effluent 67.50</td>
<td>3.85</td>
</tr>
<tr>
<td>D2</td>
<td>Influent 24.69</td>
<td>11.84</td>
</tr>
<tr>
<td></td>
<td>Effluent 44.31</td>
<td>2.10</td>
</tr>
<tr>
<td>D3</td>
<td>Influent 182.17</td>
<td>36.26</td>
</tr>
<tr>
<td></td>
<td>Effluent 152.40</td>
<td>8.92</td>
</tr>
<tr>
<td>D4</td>
<td>Influent 96.23</td>
<td>15.35</td>
</tr>
<tr>
<td></td>
<td>Effluent 85.71</td>
<td>5.84</td>
</tr>
</tbody>
</table>

Note: DX, dicloxacillin; PC, piperacillin; CF, cefalexin; CZ, cefazolin; CP, ciprofloxacin; ST, sulfamethoxazole; CM, clarithromycin; MD, metronidazole; LOD, limit of detection.

with the results of Faleyé et al. (2019) and Wang & Wang (2018). ST concentrations detected in the influent and effluent of dWWTPs in other countries are 330.7–417.40 ng/L and 56.20–73.0 ng/L, respectively, in Spain (Rodriguez-Mozaz et al. 2015), and 5.41–152 ng/L and 2.96–145 ng/L, respectively (in both summer and winter) in China (Zhang et al. 2015).

CP concentrations in the influent and effluent of dWWTPs in this study ranged from 14.83–30.95 ng/L to 4.58–11.84 ng/L, respectively. These are lower than those observed by Tewari et al. (2013) for dWWTPs in Thailand, which ranged from 66–382 ng/L to 12–251 ng/L in the influent and effluent. However, Tewari et al. (2013) reported ST concentrations in the influent and effluent of 3–30.5 ng/L and 2.5–89 ng/L, respectively, which are lower than the results of this study (94.8–215.4 ng/L in influent and 30.58–130.98 ng/L in effluent). This may be the amounts of antibiotics dispensed and the volumes transported to and from dWWTPs might differ between this study and the sampling period of Tewari et al. (2013). The concentrations of MD in the effluents of dWWTPs in this study ranged between < LOD and 15.70 ng/L. MD was not removed at all from plant D4, which might indicate an unsuitable treatment system in D4 for the removal of MD from wastewater. Similar trends were observed in a study in Taiwan that showed very poor removal efficiency of MD with influent and effluent concentrations of 1–294 ng/L and 10–126 ng/L, respectively (Lin et al. 2008).

It is worth noting that the hydraulic retention time (HRT) ranged from 3.2 to 37.2 h in hWWTPs and 3.42–13.72 h in dWWTPs, indicating lower average HRTs in dWWTPs. In addition, treatment in the dWWTPs did not include disinfection, which might explain the lower removal of antibiotics in dWWTPs than hWWTPs. The observed difference in antibiotic concentrations in the effluent of WWTPs might be due to various differences, e.g., influent wastewater characteristics, treatment systems, and removal efficiency of each wastewater treatment plant (Yan et al. 2014). Moreover, the season can affect the concentrations of antibiotics in the influent of dWWTPs derived from the combined sewage system and in the receiving surface water (Zhang et al. 2015).
Figure 2 | Relative abundance of target antibiotics in different treatment processes in the investigated WWTPs in Bangkok, Thailand. DX, dicloxacillin; PC, piperacillin; CF, cefalexin; CZ, cefazolin; CP, ciprofloxacin; ST, sulfamethoxazole; CM, clarithromycin; and MD, metronidazole.
CZ was highest in the influent of H3 at 70.83%. The highest proportion of CP in the influent of H4 was 35.25%. The different proportion patterns of antibiotics in the influent of hWWTPs are related to the proportions of antibiotic usage in each hospital.

Furthermore, the different proportion patterns of antibiotics in the influent of dWWTPs and hWWTPs likely reflect the different usage of antibiotics between hospitals and the domestic sector. The similar patterns observed in dWWTP influent might be due to similar patterns of antibiotics dispensing and usage in communities served by the investigated dWWTPs or well mixing of wastewater during transportation in the combined sewage system. In the dWWTPs, the highest proportion in the influent was ST at 36.06% in plant D1 and CZ in plant D4 at 35.75%. CZ was highest in the return sludge of D4 (68.84%). The highest effluent proportions were ST in D1 (45.48%), CZ in D2 (29.99%), and DX in D3 and D4 (41.19% and 37.48%, respectively). In the aeration units of dWWTPs, CP abundance was higher in the aeration units and return sludge of dWWTPs, which is similar to the results of Sabri et al. (2020). ST exhibited the highest abundance in D1 (35.13%) and D3 (55.48%). The ST increase in the effluent is due to the difficulty of removing ST by conventional activated sludge treatment processes. Therefore, ST is easily transferred into the receiving water bodies where it negatively affects aquatic environments (Xu et al. 2007; Cui et al. 2018). According to one study, increasing the sludge retention time can improve the removal rate of ST (Hatum et al. 2019). On the other hand, a higher removal rate of ST can generate by-products from the transformation of ST (Polesel et al. 2016). Therefore, the by-products of antibiotics and their effects on aquatic environments should be analyzed in future research.

**Fate of CP and ST in various treatment processes in WWTPs**

With the exception of CP, the concentrations of most of the selected antibiotics decreased at each treatment stage of WWTPs. Moreover, ST was very difficult to remove by secondary and disinfection treatment. Therefore, the fate of these antibiotics during each treatment process was analyzed in greater detail. CP and ST concentrations at various treatment process stages of investigated dWWTPs are shown in Figure 3. CP concentrations at the aeration unit were higher than those in the influent in all four dWWTPs due to adsorption/desorption of antibiotics to the liquid phase in the aeration unit. CP concentrations in the return sludge were also higher than those in the influent in all dWWTPs. The concentration of CP increased in the return sludge and decreased in the effluent. The ST concentration was low in the return sludge and higher in the effluent. It appears that ST preferentially remains in the liquid form, whereas CP tends to remain in the solid form. The results of previous studies on the behavior of CP and ST in WWTPs showed that CP has a high rate of sorption in the solid phase, which includes sludge in treatment plants, whereas ST exhibits weak sorption and a low rate of removal by the sorption mechanism (Min et al. 2018; Carneiro et al. 2020). Moreover, suspended solids and dissolved organic carbon in wastewater might be related to the degradation and sorption mechanisms of CP and ST during the wastewater treatment process. The CP concentration increased after untreated wastewater from the influent was mixed with return sludge. CP is a fluoroquinolones antibiotic used to treat infection in the respiratory and urinary tracts. According to the results, CP may preferentially dissolve in water, especially with high dissolved organic contents in the wastewater sludge, which is similar to the results of Sabri et al. (2020). Moreover, as CP is one of fluoroquinolones classified in the second rank of superbugs (World Health Organization 2018), further study is recommended on CP contamination in solid samples such wastewater sludge, soil, and sediment related to antibiotic resistance. However, the sampling results of this study were obtained over a short period of time; thus, the concentration of each antibiotic should be analyzed over a longer period of composite sampling.

**Mass loadings of antibiotics into receiving waters**

Previous studies on the occurrence and fate of antibiotics in hWWTPs and dWWTPs with different treatment systems have revealed that WWTPs are an important source of antibiotics released to water environments (Min et al. 2018). Antibiotic pollutant loadings from selected hWWTPs and dWWTPs in Bangkok, Thailand, into receiving waters are shown in Table 3. CF loading could not be calculated because the concentrations in dWWTP effluent were below the detection limit. However, it cannot be concluded that dWWTPs were not a source of CF contamination to aquatic environments. Despite the concentrations of target antibiotics in the effluent of dWWTPs being lower than those in the effluent of hWWTPs, antibiotic pollutant loadings were higher from dWWTPs because of the much higher wastewater flow rates from dWWTP discharge.

In this study, antibiotic mass loadings were calculated for four dWWTPs in Bangkok, Thailand, covering more than 65% of the total capacity in Bangkok (Department of Environment 2019). Antibiotic pollutant loadings from all selected dWWTPs were 70,602 mg/d for ST, 68,891 mg/d for DX, 21,082 mg/d for CZ, 15,500 mg/d for CM, 5,334 mg/d for CP, 4,113 mg/d for
Figure 3 | Fate of ciprofloxacin and sulfamethoxazole antibiotics at each treatment stage of dWWTPs in Bangkok, Thailand.

Table 3 | Antibiotic pollutant loadings from hWWTPs and dWWTPs into receiving waters

<table>
<thead>
<tr>
<th>WWTPs</th>
<th>Treatment capacity (m$^3$/d)</th>
<th>DX (mg/d)</th>
<th>PC (mg/d)</th>
<th>CZ (mg/d)</th>
<th>CP (mg/d)</th>
<th>ST (mg/d)</th>
<th>CM (mg/d)</th>
<th>MD (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hWWTPs</td>
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Note: DX, dicloxacillin; PC, piperacillin; CZ, cefazolin; CP, ciprofloxacin; ST, sulfamethoxazole; CM, clarithromycin; MD, metronidazole.
PC, and 4,014 mg/d for MD. In comparison, the antibiotic loadings from 15 municipal WWTPs in Croatia ranged from 118,900 to 134,800 mg/d for CP, 30,500–85,000 mg/d for CM, and 98,700–162,100 mg/d for ST (Senta et al. 2013). In a similar study, antibiotic pollution loadings from six sewage treatment plants in Italy were 97 mg/d for CP and 55 mg/d for CM (Castiglioni et al. 2006). Another study reported that the discharge loadings of antibiotic pollution from nine dWWTPs within the Lake Victoria Basin in Kenya ranged from 25.5 to 423.5 mg/d for CT and ND to 120 mg/d for ST (Kimosop et al. 2016). The results in this study differ from those of other countries due to differences in antibiotic dispensing in each country, the amount of wastewater discharged from WWTPs, WWTP treatment capacity, and WWTP treatment systems.

The highest antibiotic pollutant loadings in hWWTPs and dWWTPs were calculated for ST in H3 (2,098.82 mg/d) and D1 (34,058.40 mg/d), respectively. The highest total ST loadings in hWWTPs and dWWTPs were 2,492.09 mg/d and 70,602.49 mg/d, respectively. The total antibiotic pollutant loading into receiving waters were 3,107.65 mg/d from hWWTPs and 189,536.56 mg/d from dWWTPs. According to a survey, some hWWTPs discharge their treated wastewater into sewerage that is then transported to dWWTPs and treated again (Verlicchi et al. 2010). Moreover, 52.59% of total wastewater and septage in Bangkok is treated in dWWTPs (Buathong et al. 2013). Further study should evaluate the amount of antibiotic pollutant loadings in collected and treated sewage and uncollected household wastewater that directly reaches the water environment, as well as their impact on the environment.

The antibiotic pollution loadings observed in this study were higher than those reported in other studies. However, it should be noted that samples were collected from only four dWWTPs in Bangkok and that sample collection was only once a day. Therefore, some consideration should be given to the number of sampling sites, the length of the sampling period, antibiotic dispensing patterns, seasonal variations, WWTP treatment capacity, and the type of treatment system when making such comparisons.

**CONCLUSIONS**

The occurrence and fate of antibiotics in hWWTPs and dWWTPs were investigated in this study, as well as the mass loadings discharged into receiving waters in Bangkok, Thailand. All eight target antibiotics were found in the influent and various subsequent treatment stages of all selected hWWTPs and dWWTPs. Cefalexin alone was not detected in the effluent. The concentrations and pollutant loadings of antibiotics for hWWTPs and dWWTPs varied substantially. The overall antibiotic concentrations in hWWTPs ranged from < LOD to 13,166.16 ng/L, and were much higher than those in dWWTPs (< LOD – 215.4 ng/L). The highest antibiotic concentration in hWWTP influent and effluent was 13,166.16 ng/L of cefazolin and 1,499.16 ng/L of sulfamethoxazole, respectively. The highest antibiotic concentration in dWWTP influent and effluent was 215.40 ng/L of sulfamethoxazole and 152.40 ng/L of dicloxacillin, respectively. Although antibiotic concentrations in hWWTP effluent were much higher than those in dWWTP effluent, the discharge loading rates were much higher from dWWTPs. Individual antibiotic mass loadings from dWWTPs were approximately 30–3,530 times higher than those from hWWTPs. The study of mass loads revealed a substantial release of selected antibiotics from wastewater, which possibly accumulated in the environment over a period of time.

Antibiotic concentrations typically decreased during the treatment process; however, ciprofloxacin concentrations fluctuated and indicated substantial accumulation in wastewater sludge. Ciprofloxacin and sulfamethoxazole exhibited opposite trends, indicating that ciprofloxacin may be sorbed in the wastewater sludge whereas sulfamethoxazole is dominant in the clear treated wastewater due to the difficulty of ST removal in wastewater treatment plants. Therefore, further analysis of antibiotic concentrations in the liquid and solid phases of wastewater samples is important to verify their fate and behavior in wastewater treatment systems. The study of antimicrobial-resistant bacteria and genes in wastewater treatment plants and their receiving waters is also crucial for avoiding detrimental impacts on human health and the environment.

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**AUTHORS DISCLOSURE STATEMENTS**

No competing financial interests exist.
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DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

REFERENCES


