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# Occurrence and Removal of Antibiotics in Hospital Wastewater Treatment Plants

Panadda Kanmalai[1], Yanasinee Suma[2], Chongrak Polprasert[3], and Numfon Eaktasang[1*]

[1] *Faculty of Public Health, Thammasat University, Pathumthani, Thailand*
[2] *Faculty of Public Health, Thammasat University (Lampang Campus), Lampang, Thailand*
[3] *Faculty of Engineering, Thammasat University, Pathumthani, Thailand*

*Corresponding author: numfon.e@fph.tu.ac.th
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## Abstract

In hospitals, antibiotics are utilized to treat human infectious illnesses, where extra antibiotics are frequently discharged and left in hospital wastewater. However, investigating antibiotic residues using conventional activated sludge wastewater treatment plants in hospitals has still been very limited, especially in Thailand. This research studied the contamination of antibiotics in the conventional activated sludge wastewater treatment plants from three hospitals: a first-level hospital (H1), a middle-level hospital (H2), and an advanced-level hospital (H3). The antibiotics were focused on 3 groups: bata-lactams, quinolones, and tetracyclines, which included 7 types of antibiotics (amoxicillin, ampicillin, ciprofloxacin, norfloxacin, levofloxacin, ofloxacin, and doxycycline). Antibiotics were extracted by solid-phase extraction and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS). The results showed that amoxicillin had the highest concentrations of H2, H1, and H3 in the influent (18,434 ± 99, 11,541 ± 42 and 10,994 ± 56 ng/L, respectively). Furthermore, with 7,990 ± 53, 5,879 ± 52 and 4,892 ± 63 ng/L of H2, H3, and H1, respectively, amoxicillin appeared to have the highest concentrations in the effluent. The removal efficiency was the highest for norfloxacin (94.78%) in H1, followed by doxycycline (93.99%) in H2. However, ampicillin had the lowest removal efficiency (19.02%) in H2. This finding from the research on antibiotic contamination in hospital wastewater reveal that the conventional activated sludge treatment system could not completely remove all antibiotics in the wastewater. Therefore, the advanced treatment technology should be investigated to increase the antibiotics removal efficiency.

***Keywords:*** Antibiotic; Conventional activated sludge; Hospital wastewater; Wastewater treatment system

## 1. Introduction

Pharmaceutical product demand is increasing globally, and it has been found frequently in aquatic environments, raising concerns about potential ecological consequences (Tewari *et al.,* 2013). Pharmaceutical residues may contain endocrine disrupting chemicals that disrupt natural hormones (Ji *et al.,* 2010). In developing countries, the antibiotic drugs can be easily bought without a prescription from drugstores or pharmacies (Sommanustweechai *et al.,* 2018). In 2009, the value of antibiotics imported into Thailand or manufactured in the country was about 315 million USD, and this value represented about 10% of the total value of the medicines consumed (Jitraknatee, 2011). There appears to be widespread and often unregulated use of antibiotics, not only for human and pet health but also for the treatment of livestock both on farms and in households (Sommanustweechai *et al.,* 2018). Therefore, inappropriate use can cause the discharge of many antibiotic compounds into the environment. Hospitals have been identified as significant sources of antibiotics released into the water environment (Verlicchi *et al.,* 2010). It has been reported that the wastewater generated from hospitals contained various antibiotics such as ofloxacin, levofloxacin, norfloxacin, and ciprofloxacin (Hamjinda *et al.,* 2018). The antibiotics sulfamethoxazole and ciprofloxacin were found in hospital wastewater treatment plants in the USA (Brown *et al.,* 2010). Ciprofloxacin, metronidazole, and sulfamethoxazole have the highest residue concentration of hospital effluent in Benin (Deguenon *et al.,* 2022). Also, the antibiotics (amoxicillin, ofloxacin, and sulfamethoxazole) have been detected in the effluent of a municipal wastewater treatment plant in Thailand (Eaktasang *et al.,* 2021). In Greece, sulfamethoxazole was discovered in the influent of hospital wastewater treatment plants as well as the influent of urban wastewater treatment plants (Kosma *et al.,* 2014). The contaminated antibiotics were also found in the Chaophraya River, Thailand (Honda *et al.,* 2016).

Conventional treatment systems do not completely remove all antibiotics (Watkinson *et al.,* 2007). Unfortunately, most hospitals in Thailand used conventional activated sludge (CAS), and some antibiotics still exist in the effluent. Recently, the antibiotics in wastewater discharged were not standard for controlling wastewater quality, although many previous studies reported the acute and chronic toxicity of antibiotics in low concentration. However, because different levels of hospitals have different patient sizes and numbers, the amount and contaminants of hospital wastewater vary. Therefore, this study was interested in comparing antibiotics found in wastewater treatment systems from various hospitals. In Thailand, there was a scarcity of data on antibiotic contamination from various hospital levels of wastewater.

Therefore, this study aimed to investigate the antibiotic concentration in the influent and effluent of wastewater from three hospitals in Saraburi province. This study focused on the seven antibiotics (amoxicillin, ampicillin, ciprofloxacin, levofloxacin, norfloxacin, ofloxacin, and doxycycline). The general information and antibiotic usage in hospitals were collected and compared in relation to antibiotic detection in hospital wastewater. The wastewater characteristics and antibiotic removal efficiency of CAS were analyzed. The results could be applied to the improvement of hospital wastewater treatment systems to reduce antibiotic residues prior to discharge into the environment and the development of antibiotic standards for hospital effluents.

## 2. Materials and Methods

### *2.1 Wastewater sampling*

Wastewater samples were obtained from the CAS of three hospitals located in Saraburi province, Thailand. The hospital was divided into 3 levels: the first-level hospital (H1), the middle-level hospital (H2), and the advanced-level hospital (H3). Influent and effluent were sampled at points of wastewater discharge (Figure 1). The samples were replicated by sampling with the composite method. A mixture of individual samples (grab samples) was collected over a period of 2 hours for a total of 24 hours (n = 3). Each sample was fully collected in a 1.5 L PET (polyethylene terephthalate) bottle. All samples were preserved by adding $Na_2$-EDTA (1 g/L) and kept at 4 °C before being analyzed in a laboratory (Faculty of Public Health, Thammasat University).

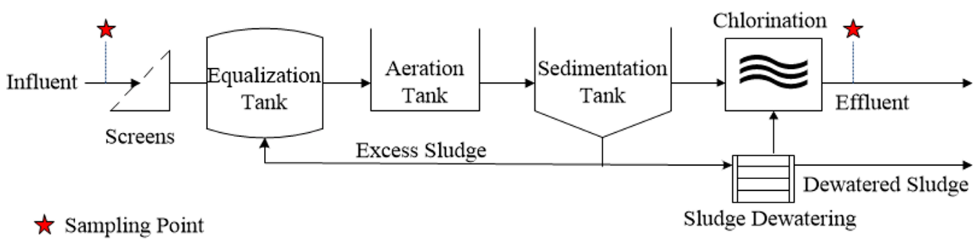


**Figure 1.** Schematic diagram for selected hospital wastewater treatment plants and wastewater sampling points in Saraburi province, Thailand

*2.2 Wastewater analysis*

The pH, Biochemical Oxygen Demand (BOD), Suspended Solid (SS), Sulfide, Total Dissolved Solids (TDS), Settleable Solid, Oil and Grease, Total Kjeldahl Nitrogen (TKN), Chemical Oxygen Demand (COD), Coliform Bacteria, and Faecal Coliform Bacteria were analyzed in the influent and effluent. The Standard Methods for the Examination of Water and Wastewater were followed for wastewater analysis (APHA, 2012).

*2.3 Antibiotic analysis*

Wastewater samples were extracted as described in EPA Method 1694 (EPA, 2007). The samples were extracted using the OASIS HLB extraction cartridge for solid-phase extraction (SPE) and eluted with pure methanol. The target antibiotic compounds in the extracts were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) (Agilent 1100 series, Palo Alto, USA). Analysis focused on the 7 antibiotics listed as presented in Table 1. The antibiotics in this study were generally found in hospital wastewaters (Schröder *et al.,* 2012; Hamjinda *et al.,* 2018).

*2.4 Antibiotic removal efficiency analysis*

The antibiotic removal efficiency (%) was evaluated using Equation 1.

$$\text{Removal efficiency (\%)} = \left(\frac{C_i - C_e}{C_i}\right) \times 100 \qquad (1)$$

where $C_i$ and $C_e$ are the antibiotic concentrations of influent and effluent (ng/L), respectively.

## 3. Results and Discussion

*3.1 General information on hospitals under study*

Based on the number of beds, the three hospitals in this study were divided into 3 levels: first-level hospital (H1), middle-level hospital (H2), and advanced-level hospital (H3). The CAS treatment system served three hospitals' wastewater treatment needs. The general information of the studied hospitals is shown in Table 2. The presented data was collected in 2018.

**Table 1.** Antibiotics list in this study

| Class | Antibiotics |
|---|---|
| Beta-lactams | Amoxicillin<br>Ampicillin |
| Quinolones | Ciprofloxacin<br>Levofloxacin<br>Norfloxacin<br>Ofloxacin |
| Tetracyclines | Doxycycline |

**Table 2.** General information about the three hospitals studied

| Information | H1 | H2 | H3 |
|---|---|---|---|
| Type | Governmental hospital | Governmental hospital | Governmental hospital |
| Sizing (beds) | 69 | 154 | 700 |
| Inpatient department (person/year) | 3,357 | 10,019 | 48,242 |
| Outpatient department (person/year) | 118,334 | 209,241 | 924,266 |
| Wastewater generation ($m^3$/day) | 60 | 150 | 680 |
| Wastewater treatment process | CAS | CAS | CAS |
| Disinfection process | Chlorination | Chlorination | Chlorination |

*3.2 Antibiotic usage information*

The information on antibiotics used in inpatient departments in 2018 for three hospitals showed the highest use of amoxicillin, ampicillin, and ciprofloxacin, respectively (Table 3).

*3.3 Wastewater characteristics*

The pH ranged from 7.4 – 8.2 as a result of the wastewater characteristics obtained from the CAS of three hospitals, with a BOD of 14 – 79 mg/L in the influent. In addition, pH, BOD, SS, sulfide, TDS, settleable solids, oil and grease, and TKN met the effluent standard, according to the Ministry of Natural Resources and Environment (Table 4).

*3.4 Antibiotic concentrations*

Wastewater samples that were collected from CAS treatment plants were extracted by SPE and then analyzed by LC-MS/MS. Three groups of antibiotics were beta-lactams, quinolones, and tetracyclines, which included seven antibiotics: amoxicillin, ampicillin, ciprofloxacin, levofloxacin, norfloxacin, ofloxacin, and doxycycline. Table 5 shows the outcome. In this study,

**Table 3.** Antibiotics used for inpatient

| Class | Antibiotics | Antibiotic usage of inpatient department (g/year) | | |
|---|---|---|---|---|
| | | H1 | H2 | H3 |
| Beta-lactams | Amoxicillin | 553.8 | 3,575.8 | 36,148.5 |
| | Ampicillin | N/A | 3,241.0 | 1,225.0 |
| Quinolones | Ciprofloxacin | N/A | 1,984.6 | 1,350.2 |
| | Levofloxacin | N/A | N/A | N/A |
| | Norfloxacin | 10.8 | 715.8 | 633.2 |
| | Ofloxacin | 26.6 | 67.0 | 1,120.4 |
| Tetracyclines | Doxycycline | 54.3 | 108.4 | 1,215.0 |

N/A = Not available

**Table 4.** Wastewater characteristics of studied hospitals

| Parameters | H1 | | H2 | | H3 | | Standard[1] |
|---|---|---|---|---|---|---|---|
| | Influent | Effluent | Influent | Effluent | Influent | Effluent | |
| pH | 7.6 ± 0.3 | 7.7 ± 0.2 | 8.2 ± 0.1 | 7.7 ± 0.2 | 7.4 ± 0.2 | 7.6 ± 0.1 | 5 - 9 |
| BOD (mg/L) | 14 ± 8 | 2 ± 1 | 79 ± 6 | 2 ± 1 | 64 ± 10 | 3 ± 1 | < 20 |
| SS (mg/L) | 8 ± 4 | 4 ± 1 | 94 ± 12 | 4 ± 2 | 66 ± 8 | 6 ± 2 | < 30 |
| Sulfide (mg/L) | 0.21 | 0.04 | 0.23 | 0.04 | 0.35 | N/D | < 1.0 |
| TDS (mg/L) | 364 ± 15 | 303 ± 12 | 762 ± 14 | 491 ± 10 | 397 ± 8 | 369 ± 15 | < 500* |
| Settleable solids (mg/L) | 0.1 ± 0.05 | < 0.1 | 0.3 ± 0.1 | < 0.1 | 1.5 ± 0.5 | < 0.1 | < 0.5 |
| Oil and grease (mg/L) | 3.4 ± 1 | 0.7 ± 0.05 | 13.2 ± 4 | 3.2 ± 1 | 29.1 ± 5 | 0.8 ± 0.2 | < 20 |
| TKN (mg/L) | 13.8 ± 2 | 0.9 ± 0.4 | 46.9 ± 5 | 1.8 ± 0.5 | 30.5 ± 4 | N/D | < 35 |
| COD (mg/L) | 77 ± 6 | 26 ± 5 | 273 ± 12 | 51 ± 7 | 276 ± 15 | 32 ± 6 | < 120[2] |
| Coliform bacteria (MPN/100 mL) | > 16,000 | > 1.8 | > 16,000 | > 1.8 | > 16,000 | 4.5 | < 5,000[3] |
| Faecal coliform bacteria (MPN/100 mL) | > 16,000 | > 1.8 | > 16,000 | > 1.8 | > 16,000 | 4.5 | < 1,000[3] |

N/D = Not detected
Number of samples = 3
Average ± Standard deviation
[1] Building effluent standards; type A, Ministry of Natural Resources and Environment
[2] Industrial effluent standard, Ministry of Natural Resources and Environment
[3] Hospital accreditation standard, Institute of Hospital Quality Improvement and Accreditation, Ministry of Health
* Additional of TDS from water supply

seven antibiotics were discovered in the influent. Amoxicillin had the highest concentrations of 18,434 ± 99, 11,541 ± 42, and 10,994 ± 56 ng/L in H2, H1, and H3, respectively. Ofloxacin was 13,470 ± 75, 10,343 ± 38, and 8,247 ± 44 ng/L in H2, H1, and H3, respectively. In addition, the antibiotic contamination was found in effluent, with the highest concentrations of amoxicillin at 7,990 ± 53, 5,879 ± 52, and 4,892 ± 63 ng/L in H2, H3, and H1, respectively. Ofloxacin was found to be at 3,687 ± 35, 1,874 ± 22, and 1,629 ± 25 ng/L in H3, H2, and H1, respectively.

*3.5 Antibiotic treatment efficiency of CAS*

The result of antibiotic removal with the CAS treatment plants of three hospitals was the highest removal efficiency of 94.78% for norfloxacin, followed by 93.99% for doxycycline in H1 and H2, respectively. In addition, the norfloxacin had an 87.30% removal efficiency of H3. Otherwise, ampicillin had the lowest removal efficiency of 19.02% in H2. The antibiotic removal efficiency is presented in Figure 2.

As a result, the three hospitals have different levels and numbers of beds. The average wastewater generation in hospitals was approximately 1,070 L/bed/day, which was higher when compared to the average estimation of the Pollution Control Department of about 800 L/bed/day (PCD, 2017). The total wastewater was generated from other services besides inpatient care, whereas the wastewater generation was calculated based on the number of beds or beds occupied. The hospitals are operated under the Department of Health in Thailand and

**Table 5.** Antibiotic contamination in studied hospitals

| Antibiotics | Average ± Standard deviation (ng/L)[1] | | | | | |
|---|---|---|---|---|---|---|
| | H1 | | H2 | | H3 | |
| | Influent | Effluent | Influent | Effluent | Influent | Effluent |
| Amoxicillin | 11,541 ± 42 | 4,892 ± 63 | 18,434 ± 99 | 7,990 ± 53 | 10,994 ± 56 | 5,879 ± 52 |
| Ampicillin | 1,078 ± 21 | 238 ± 9 | 163 ± 6 | 132 ± 10 | 771 ± 18 | 399 ± 21 |
| Ciprofloxacin | 225 ± 12 | 38 ± 5 | 4,294 ± 41 | 754 ± 17 | 1,460 ± 22 | 392 ± 13 |
| Levofloxacin | 1,988 ± 24 | 315 ± 12 | 2,504 ± 28 | 384 ± 15 | 1,510 ± 25 | 751 ± 10 |
| Norfloxacin | 690 ± 15 | 36 ± 4 | 1,003 ± 19 | 754 ± 16 | 559 ± 12 | 71 ± 5 |
| Ofloxacin | 10,343 ± 38 | 1,629 ± 25 | 13,470 ± 75 | 1,874 ± 22 | 8,247 ± 44 | 3,687 ± 35 |
| Doxycycline | 2,081 ± 23 | 710 ± 14 | 1,297 ± 11 | 78 ± 8 | 376 ± 24 | 85 ± 12 |

[1] Number of samples = 3

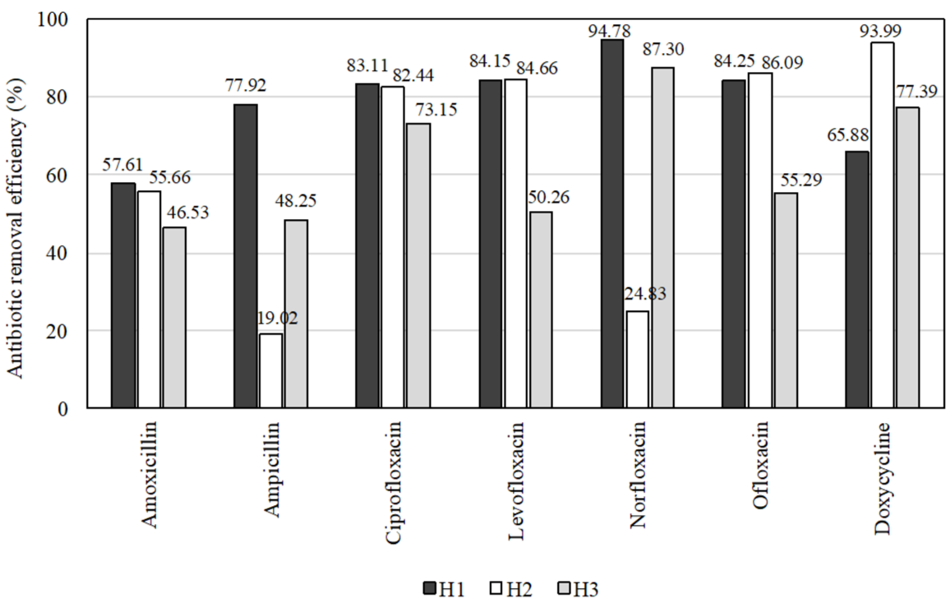


**Figure 2.** Antibiotic removal efficiency in studied hospitals

have the same design of wastewater treatment plant as the CAS system. The chlorination was used for the disinfection method of effluent before discharge to the environment. It has been reported that disinfection units such as chlorination and UV could increase the antibiotic removal efficiency, with chlorination being more efficiency than UV (Sinthuchai *et al.,* 2016). The chlorination could not completely remove all antibiotic but was most effective for ciprofloxacin, which was similar to this study. The removal efficiency of ciprofloxacin was 73.15 – 83.11% in three hospitals.

Based on the information about antibiotic usage for inpatients, the beta-lactams group (amoxicillin and ampicillin) was the most commonly used when compared to other antibiotics in a similar previous report (Jitraknatee *et al.,* 2011). Beta-lactams are the most widely used group of antibiotics. Elander (2003), reported that more than half of all commercially available antibiotics in use were beta-lactam compounds. Therefore, it is possible to detect high concentrations of the beta-lactams group in the influent.

The wastewater characteristics of H1, H2, and H3 did not exceed the standard for all parameters, but the TDS of effluent quite low removal efficiency. The advanced treatment process, such as adsorption using activated coal, is also recommend by a previous study to remove the TDS (Ani *et al.,* 2019). Therefore, the TDS treatment process in CAS should be investigated for hospital wastewater treatment. In addition, the pH value is an important parameter in chemical reaction, which may affect antibiotic reactions. The $pK_a$ was related to protonated and deprotonated water solution. For example, the sulfonamides are characterized by two $pK_a$ values at pH 2 – 3 due to protonation of the amino group and at pH 5 – 11 due to the deprotonation of the $R_1SO_2NHR_2$ moiety (Ngigi *et al.,* 2020). As a result, sulfonamides were prevalent in the water at pH levels greater than 5.0. In contrast, ampicillin was highly soluble in water at a pH greater than 7.3 (Ngigi *et al.,* 2020). As a result, a different pH value in the wastewater treatment unit could affect the antibiotics in the water. At pH 7.3, ampicillin was highly soluble in water (Ngigi *et al.,* 2020), and the pH of the influent in this study ranged from 7.6 to 8.2. As a result, ampicillin was abundant in the influent. Moreover, others form of antibiotics was not only detected in water but also in sediment (Ngigi *et al.,* 2020). Hence, the antibiotics residue should be studied further in sludge and sediment in wastewater treatment plants (Sinthuchai *et al.,* 2016).

The antibiotics were detected in the influent and effluent of three hospitals for seven antibiotics, and there was a high concentration of amoxicillin in the influent of the three hospitals, which is similar to the previous report that detected amoxicillin and tetracycline in the CAS of the municipal treatment plant (Sawaittayothin *et al.,* 2016). The presence of amoxicillin in the influent could be due to inpatients usage. Therefore, it was not surprising that there were high residuals of amoxicillin in the influent hospital wastewater. The human body's metabolism of beta-lactams includes both absorption and excretion. The antibiotic usage was 50 – 70% beta-lactams when compared to other antibiotics, followed by sulfonamides, macrolides, and fluoroquinolones (Kummerer *et al.,* 2009). Otherwise, the result of this study varied from a previous study that found high concentrations of norfloxacin, ciprofloxacin, ofloxacin, and levofloxacin in hospital wastewater (Hamjinda *et al.,* 2018), and cefazolin (Sinthuchai *et al.,* 2016).

The influent amoxicillin concentration ranged from 10,994 – 18,434 ng/L, which is higher than the previous study value of 240 – 2,400 ng/L (Hamjinda *et al.,* 2018). The ofloxacin concentration ranged from 8,247 – 13,470 ng/L, which is higher when compared to the previous report (1,600 – 4,800 ng/L) (Hamjinda *et al.,* 2018). Moreover, the high concentration of amoxicillin in effluent ranged from 4,892 – 7,990 ng/L, which is higher than the previous study's 90 ng/L in CAS and 60 ng/L in rotating biological contactor (RBC) systems (Hamjinda *et al.,* 2018). In addition, the concentration of amoxicillin in this study was higher than the study of Sawaittayothin, as reported amoxicillin in effluent was 640 ng/L treated by activated sludge system (Sawaittayothin *et al.,* 2016).

The antibiotic treatment efficiency of CAS was high, with high removal of norfloxacin (94.78%) and doxycycline (93.99%) in H1 and H2, respectively. The removal efficiency of norfloxacin in this study was higher when compared to a prior report that found it to be 69% and 64% of amoxicillin, respectively (Hamjinda *et al.,* 2018). In this study, the amoxicillin was the most contaminated. Furthermore, the CAS treatment system is only capable of removing 53.3% of the amoxicillin, resulting in a high concentration in the effluent. The amoxicillin removal efficiency of 25.03% was reported by Al-Gheethi and Ismail (2014), whereas amoxicillin had a high removal efficiency by CAS (99%) (Hamjinda *et al.* (2018). The results show the beta-lactams group had low removal from wastewater when compared to quinolones and tetracyclines groups in CAS. The ciprofloxacin removal efficiency by CAS was over 73% in three hospitals, which is similar to a prior study that found ciprofloxacin removal at over 80% by CAS (Sinthuchai *et al.,* 2016). Although the CAS served as a hospital wastewater treatment plant, the removal efficiency varied, which could be attributed to operational conditions and system maintenance.

The wastewater treatment system was not completely remove all antibiotics, therefore the water discharge from hospitals might accumulate in the environment and induce antibiotic resistance in microorganisms (Polprasert *et al.,* 2015; Zieliński *et al.,* 2019). Based on these results, the suggestion for the control of antibiotics contamination in the environment include, firstly, controlling the amount of antibiotic usage by patients, such as using a specific antibiotic for treatment and avoiding broad antibiotic application. In addition, the government has to control the drug store so it does not dispense the antibiotics without a prescription from a hospital or medical care center. Secondly, control the antibiotic residue in wastewater treatment process by using advanced treatment and disinfection processes to increase the antibiotic removal efficiency, such as the adsorption method using activated carbon. Thirdly, the government or related agencies should provide the guidelines or regulations to control the antibiotics in the wastewater treatment process and establish the antibiotic discharge standard.

## 4. Conclusion

According to the findings of this study, the CAS system of three hospitals could effectively treat organic and inorganic matters but could not complexly remove all antibiotics. Although the concentration of antibiotics in water discharged from wastewater treatment is low, they can accumulate in the environment. Most wastewater treatment plants were not designed to treat the antibiotic. Therefore, to increase the efficiency of wastewater treatment systems for the removal of antibiotic contamination, this study was intended to suggest that the integration of wastewater treatment technologies should be investigated and operated in hospital wastewater treatment plants.

## Acknowledgement

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