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Occurrence and removal of multiple classes of antibiotics and antimicrobial agents in biological wastewater treatment processes

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ABSTRACT

Very little information on the occurrence and fate of multiple classes of antimicrobials in the aquatic environment is reported for the Southeast Asian region. This study provides the first and comprehensive data on the occurrence of ten different classes of antimicrobials in wastewater samples for Singapore. Among the investigated antimicrobials, 19 out of 21 target compounds were detected in 100% of the collected raw influent samples. Concentrations of the detected antimicrobials in raw influent varied from 23.8 to 43,740 ng/L. Removal of antimicrobials by conventional activated sludge (CAS) and membrane bioreactor (MBR) systems at a local wastewater treatment plant was evaluated. MBR exhibited better performance over CAS for most target antimicrobials. Beta-lactam, glycopeptide, and fluoroquinolone classes were largely eliminated by biological wastewater treatment processes, whereas trimethoprim and lincosamides appeared to be persistent. Effects of physicochemical properties and chemical structures of target antimicrobials on their removal efficiencies/mechanisms during wastewater treatment process were also discussed.

1. Introduction

In recent years, the occurrence of antimicrobials in the environment has been recognized as an emerging environmental problem due to their potential in causing undesirable ecosystem and human health (Díaz-Cruz and Barceló, 2005; Diaz-Cruz et al., 2008; Kummerer, 2009; Le-Minh et al., 2010; Richardson and Ternes, 2011; Luo et al., 2014). Antimicrobials, such as antibiotics, are one of the most important drugs to prevent and treat infectious diseases. In addition, a certain fraction of antibiotics is also used as feed additives to promote the growth rate of livestock and poultry animals (Kummerer, 2009; Le-Minh et al., 2010). It is reported that approximately 50–90 percent of antibiotics administrated by humans or animals are excreted via urine and feces as a mixture of parent and metabolite forms (Kummerer, 2009; Le-Minh et al., 2010). After administration, large amounts of antibiotics or their metabolites are released into municipal wastewater due to excessive consumption and disposal of unused antibiotics (Kummerer, 2009). Human and veterinary antibiotics can enter the aquatic environment via a number of routes, including (i) direct discharge of animal wastewater from poultry and meat processing, aquaculture as well as from household pets (Kummerer, 2009); discharge of treated wastewater effluents from wastewater treatment plants (WWTPs) (Le-Minh et al., 2010; Luo et al., 2014); (iii) sewer leaking/sewer overflow (Tran et al., 2014); (iv) surface runoff; and (v) infiltration from manure-amended agricultural lands (Cha and Cupples, 2009). Till now, the major concerns of the occurrence of antimicrobials in the environment are the development of antimicrobial resistance genes (ARG) and antimicrobial resistance bacteria (ARB), which reduce the therapeutic potential against human and animal bacteria pathogens (Kim and Aga, 2007; Rizzo et al., 2013; Blair et al., 2015b). Another concern of the occurrence of antimicrobials in the aquatic environment is possible toxicity to sensitive organisms (Richardson and Ternes, 2011).

The removal of antibiotics and antimicrobial agents in WWTPs was earlier reported in previous studies (Gobel et al., 2007; Radjenovic et al., 2007, 2009; Watkinson et al., 2007). For example, Gobel et al. (2007) investigated the removal of

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sulfonamides, macrolides and trimethoprim by different treatment technologies. They reported that the membrane bioreactor (MBR) system showed better removal efficiency than conventional activated sludge (CAS) system for most of the investigated compounds. In contrast, Radjenovic et al. (2009) found that no significant difference in removal efficiency between MBR and CAS systems was observed for several antibiotics, including erythromycin, sulfamethoxazole, and trimethoprim. It was also reported that removal efficiency of antibiotics in wastewater treatment process was not only dependent on treatment technologies employed at WWTPs, but also other factors, such as seasons and nature of antibiotics (Joss et al., 2006; Gobel et al., 2007; Kimura et al., 2007; Watkinson et al., 2007; Guerra et al., 2014).

Hitherto, the occurrence and fate of several classes of antimicrobials in different environmental compartments (wastewater, surface water, groundwater and soils) have been documented in some geographical regions of the world, such as North America, Europe, and Japan (Díaz-Cruz and Barceló, 2005; Karthikeyan and Meyer, 2006; Kobayashi et al., 2006; Gobel et al., 2007; Radjenovic et al., 2007, 2009; Kummerer, 2009; Garcia-Galan et al., 2010; Le-Minh et al., 2010; Blair et al., 2015a), while very little information on the occurrence and fate of antibiotics in the Southeast Asian region has been reported.

In addition, most of the previous studies only focused on a small number of antimicrobials as well as antimicrobial classes (Gobel et al., 2005, 2007; Gros et al., 2006b; Terzic et al., 2008; Cha and Cupples, 2009; Tong et al., 2009; Garcia-Galan et al., 2010; Behera et al., 2011). In another study, Cha et al. (2006) developed an analytical method for determination of the second generations of β-lactams, such as amoxicillin, ampicillin, and oxacillin. To the best of our knowledge, no or limited information on the occurrence and fate of new generations of β-lactam antibiotics (i.e. ceftazidime and meropenem) or other antibiotic classes, e.g. glycopeptide (vancomycin) and lincosamide (clindamycin), in the environment has been reported in the earlier studies, particularly for tropical regions.

Therefore, the first objective of this study is to fill the existing gap by providing the first and comprehensive data on the occurrence of 21 commonly used antimicrobials belonging to 10 different classes in wastewater for the tropical region (Singapore), where weather conditions, land use, population size, population density, demographic pattern and usage patterns of antibiotics are different from those in North American and European countries. These differences may subsequently impact on the occurrence distribution and concentration of antimicrobials in the water environment.

The second objective was to investigate the removal of the target antimicrobials during biological wastewater treatment processes at a local WWTP. The removal efficiencies for target antimicrobials in dissolved phase by different wastewater treatment technologies, i.e. CAS and MBR, were also evaluated via an intensive sampling campaign. Meanwhile, insights into the relationship between the physicochemical properties (i.e. log K_{ow}, log D_{ow}, pK_a, and ionization state)/chemical structures of antimicrobials and their removal efficiencies/mechanisms were also taken into account.

2. Materials and methods

2.1. Target antimicrobials, chemical reagents and solvents

In this study, 21 antimicrobials belonging to ten different classes were investigated, including:

(i) β-lactam: ceftazidime [CFZ], meropenem [MER], and amoxicillin [AMX].
(ii) Fluoroquinolone: ciprofloxacin [CIPX].
(iii) Lincosamides: lincomycin [LIN] and clindamycin [CLI].
(iv) Macrolides: erythromycin [ERY], azithromycin [AZT], clarithromycin [CLAR], and tylosin [TYL].
(v) Sulfonamide antibiotics: sulfamethazine [SMZ] and sulfamethoxazole [SMX].
(vi) Reductase inhibitor: trimethoprim [TMP].
(vii) Tetracycline family: tetracycline [TET], minocycline [MIN], chlortetracycline [CTC], and oxytetracycline [OXY].
(viii) Glycopeptide: vancomycin [VCM].
(ix) Chloramphenicol [CAP].
(x) Antiseptic additives: triclosan [TCS] and triclocarban [TCC].

The physicochemical properties of the target antimicrobials are presented in Table A.1 (Supplementary Information). All the target antimicrobials as well as other chemical reagents/solvents are of high purity grade (>99%) and were purchased from Sigma–Aldrich (Sigma–Aldrich, Singapore). Fifteen ^{2}H and ^{13}C-isotope labeled internal/surrogate standards (ILISs) were purchased from Toronto Research Chemicals (Toronto, Canada), including ceftazidime-d_5 [CFZ-d_5], meropenem-d_6 [MER-d_6], ciprofloxacin-d_8 [CIPX-d_8], lincomycin-d_3 [LIN-d_3], clindamycin-d_3 [CLI-d_3], azithromycin-d_3 [AZT-d_3], clarithromycin-d_3 [CLAR-d_3], erythromycin-d_6 [ERY-d_6], sulfamethazine-d_4 [SMZ-d_4], sulfamethoxazole-d_4 [SMX-d_4], trimethoprim-d_3 [TMP-d_3], tetracycline-d_6 [TET-d_6], chloramphenicol-d_5 [CAP-d_5], triclosan-d_3 [TCS-d_3], and triclocarban-$^{13}C_6$ [TCC-$^{13}C_6$].

2.2. Wastewater treatment plant

To investigate the occurrence and removal of target antimicrobials during wastewater treatment processes, a routine sampling and monitoring campaign was conducted at a local wastewater treatment plant (WWTP). Detailed information on the investigated WWTP is provided elsewhere (Tran et al., 2015). Briefly, the investigated WWTP is constructed to treat wastewater mainly from municipal sources (approximately 90%), with a total design capacity of 361,000 m^3/d. The influent of the WWTP is treated in two concurrent liquid streams, i.e. South-works [Train-A] and North-works [Train-B], as illustrated in Fig. 1. Train-A is a conventional activated sludge system (CAS), which includes the following treatment units: primary settling tanks, Modified Ludzack–Ettinger (MLE) tanks (including anoxic tanks, followed by aerobic tanks with internal cycling) and secondary settling tanks. Train-B is a membrane bioreactor (MBR) system that consists of primary settling tanks, MLE tanks and microfiltration (MF) membrane unit.

The major difference between the two treatment trains is that Train-A uses conventional sedimentation for solid–liquid separation, whereas Train-B uses a MF membrane unit with a design flow rate of 23,000 m^3/d to retain the suspended solids (Tran et al., 2015). In addition, the operating parameters, such as mixed liquor suspended solid (MLSS), hydraulic retention time, and sludge retention time between CAS and MBR systems were also different. The main operating parameters of CAS (Train-A) and MBR (Train-B) are summarized in Table A.2 (Supplementary Information).

2.3. Sample collection

An intensive sampling campaign was carried out from April to May 2015 at five different sampling points (INFL, A1, A2, B1, and B2) as shown in Fig. 1. These sampling points were selected to evaluate the occurrence and change in antimicrobial concentrations at different treatment units on Train-A and Train-B. For example, the sampling point (INFL) was chosen to evaluate the characteristics of raw influent (raw wastewater) before entering the treatment

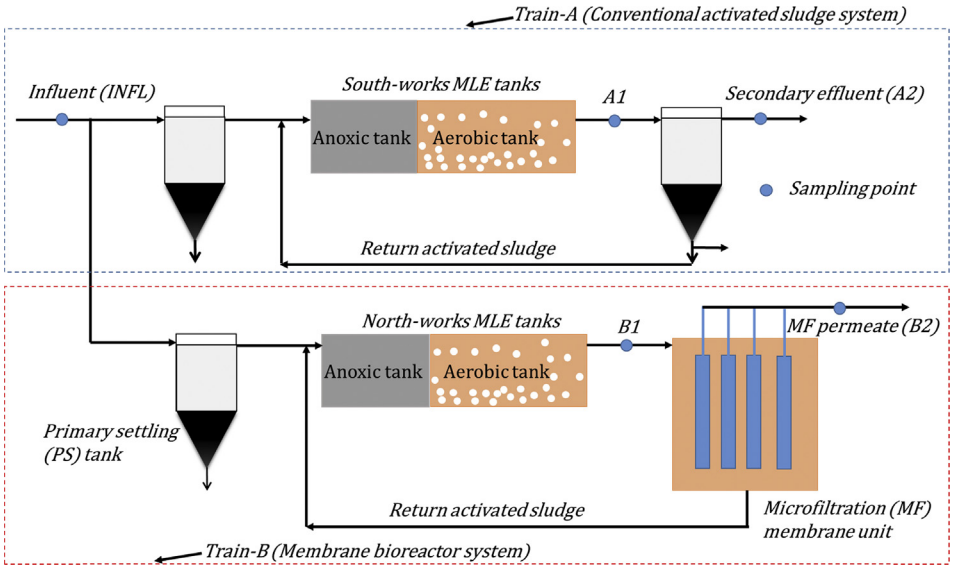

Fig. 1. Schematic diagram of the investigated wastewater treatment plant (WWTP). Train-A represents conventional activated sludge (CAS) system; Train-B is membrane bioreactor (MBR) system.

systems. The sampling sites A1 and B1 to assess the changes in antimicrobial concentrations after physical and biological treatment processes at primary settling tanks and MLE tanks of the corresponding treatment trains (Train-A and Train-B). A2 and B2 were selected to elucidate the water quality of secondary effluent from Train-A and MF permeate from Train-B, respectively.

All wastewater samples were collected as grab samples and filled in 1 L amber glass bottles and immediately transported to the laboratory in ice-packed containers. Once samples arrived at the laboratory, the samples were filtered using 1.2μ m glass fiber filters (GF/C, Whatman, UK), followed by 0.45μ m membrane filters (PALL, corporation, US). Subsequently, the filtrate samples were spiked with a constant amount of ILISs (100 ng) before doing solid phase extraction (SPE) on the same day. However, the filtrate samples spiked with a constant amount of ILISs (100 ng) could also be stored in a dark room at 4 °C until SPE was performed, but no later than 24 h after the collection to minimize the degradation/hydrolysis of target analytes, particularly in terms of beta-lactam antibiotics that have been reported to be hydrolyzed under ambient water conditions (Hirte et al., 2016). The addition of ILISs (i.e. CFZ-d_5, MER-d_6, CIPX-d_8, LIN-d_3, CLI-d_3, AZT-d_3, CLAR-d_3, ERY-d_6, SMZ-d_4, SMX-d_4, TMP-d_3, TET-d_6, CAP-d_5, TCS-d_3, and TCC-$^{13}C_6$) to the filtered water samples before storage at 4 °C allows compensation of the hydrolysis/degradation and the loss of target analytes during the storage period of water samples as well as SPE process.

2.4. Chemical analysis

Dissolved-phase concentrations of 21 target antimicrobials in aqueous phase of the collected wastewater samples were analyzed using SPE coupled with ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) and isotope dilution. Detailed information about the analytical procedures, such as UHPLC-MS/MS parameters, extraction recoveries, calibration curves, method detection methods (MDLs) and method quantification methods (MQLs) have been fully described in our recent study (Tran et al., 2016). Briefly, the relative SPE recoveries for target antimicrobials in wastewater samples varied from 90.8 to 109.6% for treated effluent samples and from 86.5 to 116.5% for raw influent samples (Table 1). MQLs for the target antimicrobials ranged from 0.2 to 150 ng/L, depending on each target compound and each environmental matrix (Table 1).

The concentration of dissolved organic carbon (DOC) and total nitrogen (TN) were measured by Total Organic Carbon Analyzer (TOC-L/CS, Shimadzu, Japan) while the concentrations of ions, such as NH_4^+, NO_2^-, and NO_3^- in the samples were determined using ion chromatography (Thermo Scientific Dionex ICS-1600, United States).

2.5. Statistical analysis

An unpaired T-test was used to examine the statistically significant difference between mean values of two independent groups. A significance level of 0.05 was used for all statistical tests in this study. Minimum, maximum and median values were calculated based on detectable values and values below MDLs were set at 50% of MDLs.

3. Results and discussion

3.1. Occurrence of antimicrobials in raw influent and treated effluent

Table 2 shows the concentrations and detection frequencies of the target antimicrobials in raw wastewater (INFL), mixed liquor suspended solid at MLE tanks (A1, B1), secondary effluent (A2), and MF permeate (B2) in the Train-A and Train-B of the WWTP. It can be seen that all the target antimicrobials, except CFZ and TYL, were detected in 100% of the collected raw influent (INFL) samples. The absence of TYL in all the collected raw wastewater samples may be well interpreted by the fact that TYL is mainly used in veterinary medicine and frequently detected in animal wastewater sources from livestock and poultry farms (Angenent et al., 2008). However, the WWTP in this study receives raw wastewater from the three main sources (i.e. residential, commercial, and hospital sources), and the contribution of animal wastewater from livestock/poultry to the raw influent of the WWTP can be deemed to be negligible. That is why TYL was absent in all the collected raw influent samples. In previous studies in North American and European countries (Gracia-Lor et al., 2012; Guerra et al., 2014), it was also reported that

Table 1
Method validation data for treated effluent and raw influent samples.

Target analyte	Corresponding ILISs	Treated effluent				Raw influent			
		Absolute SPE recovery mean (RSD) %	Relative SPE recovery mean (RSD) %	MDL ng/L	MQL ng/L	Absolute SPE recovery mean (RSD) %	Relative SPE recovery mean (RSD) %	MDL ng/L	MQL ng/L
CFZ	CFZ-d_5	67.8 (4.4)	101.3 (7.1)	25	75	62.7 (3.4)	96.7 (3.9)	40	125
MER	MER-d_6	47.1 (7.9)	95.3 (1.4)	2.5	10	46.8 (6.3)	105.2 (13.2)	5.0	15
AMX	MER-d_6	47.1 (14.9)	94.9 (8.0)	20	65	46.8 (8.9)	106.3 (13.1)	50	150
CIPX	CIPX-d_8	75.8 (12.2)	100.2 (6.8)	0.8	2.5	82.9 (14.9)	94.7 (4.4)	2.0	5.0
LIN	LIN-d_3	83.4 (8.1)	101.1 (4.7)	0.05	0.2	81.8 (10.9)	99 (5.5)	0.1	0.3
CLI	CLI-d_3	93.9 (5.8)	99.3 (4.9)	0.05	0.2	94.5 (10.1)	101 (4.8)	0.1	0.3
ERY	ERY-d_6	50.3 (14)	97.3 (11.6)	0.15	0.5	54.6 (11.9)	98.7 (10.4)	0.3	1.0
ERY*	CLAR-d_3	97 (4.7)	108 (9.7)	0.1	0.3	88.7 (3.2)	94.2 (10.5)	0.2	0.6
AZT	AZT-d_3	94.8 (2.6)	98.9 (2.3)	0.08	0.2	104.9 (6.6)	107.3 (8.9)	0.15	0.5
CLAR	CLAR-d_3	84.7 (10.4)	99.4 (5.4)	0.06	0.2	92.5 (11.4)	97.6 (5.0)	0.15	0.5
TYL	AZT-d_3	87.2 (8.8)	90.8 (6.9)	0.3	1.0	85.7 (8.0)	87.7 (3.4)	0.5	1.5
SMZ	SMZ-d_4	75.3 (6.2)	99.3 (4.9)	0.06	0.2	74.2 (8.2)	102.6 (9.5)	0.1	0.3
SMX	SMX-d_4	76.6 (10.2)	102.6 (5.3)	0.15	0.5	73.2 (12.5)	102.1 (4.2)	0.2	0.6
TMP	TMP-d_3	97.3 (8.7)	99.8 (4.4)	0.15	0.5	95.1 (2.8)	102 (4.5)	0.25	0.8
TET	TET-d_6	94.2 (4.9)	103.4 (6.4)	8.0	25	98.3 (4.8)	104.1 (1.0)	15	50
MIN	TET-d_6	84.3 (8.8)	92.3 (5.4)	16	50	81.8 (10.7)	86.5 (7.7)	40	125
CTC	TET-d_6	93.9 (5.7)	103.2 (8.7)	1.5	5.0	95.5 (2.9)	101.1 (2.5)	2.5	7.5
OXY	TET-d_6	96.2 (5.5)	105.5 (5.3)	12	40	97.8 (9.0)	103.6 (9.4)	23	75
TCS	TCS-d_3	95.1 (2.2)	100.5 (6.4)	1.0	3.5	85.9 (11.2)	98.9 (6.7)	3.0	10
TCC	TCC-$^{13}C_6$	97.7 (5.3)	101.3 (5.5)	0.6	2.0	93.1 (11)	102.9 (4.4)	1.4	4.5
VCM	CFZ-d_5	73.5 (7.2)	109.6 (4.3)	4.5	15	75.5 (5.9)	116.5 (1.9)	12	40
CAP	CAP-d_5	94.4 (3.1)	102.3 (2.9)	0.5	1.5	92.7 (2.1)	100.3 (4.4)	0.6	2.0

ERY: indicating ERY that was detected and quantified based on the precursor 734.47 $[M+H]^+$.
ERY*: indicating ERY that was detected and quantified based on the precursor 716.5 $[M\text{-}H_2O + H]^+$.
RSD: relative standard deviation (%).

TYL was not detected in raw influent samples (Table 3).

Regarding the absence of the third generation β-lactam (CFZ) in the raw influent samples, this might be due to the lower consumption of this compound compared to other human antibiotics. Another possible reason for the absence of CFZ in the raw influent samples might be due to its rapid degradation in the human body via renal route (Kemmerich et al., 1983) as well as in sewer pipe systems. To date, no information on the occurrence of CFZ in wastewater has been reported.

Among the antimicrobial classes detected in the raw influent samples, the glycopeptide VCM appeared to be the most abundant compound with its concentration up to 43.74 μg/L. In fact, VCM is often used to treat serious, life-threatening infections by Gram-positive bacteria unresponsive to other antibiotics. Till now, very little data on the occurrence and fate of VCM in the aquatic environment has been reported. Therefore, this study provided the first and quantitative data on the occurrence and fate of VCM in a WWTP for the Southeast Asian region.

The second most abundant antimicrobial class in raw wastewater belonged to the tetracycline family (CTC, OXY, TET, and MIN). In fact, tetracyclines are commonly used antibiotics to treat both Gram-positive and Gram-negative bacterial infections. The concentrations of tetracyclines in raw wastewater sampled ranged from several hundred ng/L to a few ten μg/L (Table 2), depending upon the compound. Previously (Kim et al., 2007), and (Zhou et al., 2013) reported that tetracyclines (CTC and TET) were the most frequently detected antibiotics in raw wastewater. The concentrations of TET in treated effluent samples of this study are comparable with those reported in North American countries (Miao et al., 2004; Karthikeyan and Meyer, 2006).

Beta-lactams (AMX and MER) were the third most abundant antimicrobials detected in the raw influent samples. Indeed, β-lactams are known as the most commonly prescribed and wide-spectrum antibiotics to treat bacterial infections by Gram-negative and Gram-positive genera, such as *Streptococcus*, *Gonococcus* and *Staphylococcus* (Le-Minh et al., 2010). Previous studies found that several β-lactams belonging to the second and third generations (i.e. AMX, cefaclor, cephalexin, and cloxacillin) were predominant antibiotics detected in raw wastewater (Watkinson et al., 2007). The concentrations of β-lactams significantly varied from 264.8 to 6516 ng/L, depending on the compound and sampling date. For example, the concentration of the 2nd generation β-lactam (AMX) in raw influent could go up to 6516 ng/L, while concentration of the new generation β-lactam (MER) was significantly lower than that of AMX by one order of magnitude. This might be due to the difference in usage pattern of these antibiotics since MER is an ultra-broad-spectrum injectable antibiotic used to treat a wide variety of bacterial infections. To the best of our knowledge, this study provided the first quantitative data on the occurrence and fate of new generation β-lactam antibiotic (MER) in wastewater treatment plant.

Fluoroquinolones (CIPX) and macrolides (AZT, CLAR, ERY, and ERY-H_2O) were the most frequently detected antibiotic classes in the raw influent and treated effluent samples. It can be seen from Table 2 that concentrations of CIPX in the raw influent samples appeared to be consistently higher than those of the macrolides (AZT, CLAR, ERY, and ERY-H_2O). This could be also explained by the difference in the usage pattern of these antibiotic classes. For example, fluoroquinolone (CIPX) is commonly prescribed to treat infectious diseases caused by several types of Gram-negative and Gram-positive bacteria, while macrolides (i.e. ERY) are only effective for treating infectious diseases by Gram-positive bacteria.

For the macrolides, AZT was found in the highest concentrations (1537–2951 ng/L), followed by CLAR (1201–1854 ng/L), ERY-H_2O (299.3–737 ng/L), and ERY (111.4–403.3 ng/L). The distribution characteristics of macrolides in wastewater in this study is different from other countries (Miao et al., 2004; Gobel et al., 2007). For example, in Switzerland, CLAR was more frequently detected in raw wastewater at higher concentrations compared to AZT and ERY-H_2O, which was demonstrated to be correlated to consumption data (Gobel et al., 2007). In contrast, Miao et al. (2004) and (Zhou et al., 2013) found that ERY-H_2O was more often detected at higher concentrations in raw wastewater than CLAR for the cases of Canada and China, respectively. However, the concentrations of ERY

Table 2
Concentrations and detection frequencies of the target antimicrobials in raw influent (INFL), mixed liquor suspended solids in MLE tanks, and treated effluents.

Target compound	Raw influent (INFL) (n = 4)			Train-A (CAS system)						Train-B (MBR system)					
				MLE tanks (A1), (n = 4)			Secondary effluent (A2), n = 4			MLE tanks (B1), n = 4			MF permeate (B2), n = 4		
	Range (ng/L)	Median (ng/L)	DF (%)	Range (ng/L)	Median (ng/)	DF (%)	Range (ng/L)	Median (ng/)	DF (%)	Range (ng/L)	Median (ng/)	DF (%)	Range (ng/L)	Median (ng/)	DF (%)
CFZ	<MQL	<MQL	0	<MQL	<MQL	0	<MQL	<MQL	0	<MQL	<MQL	0	< MQL	<MQL	0
MER	264.8–433.6	322.1	100	20–63.1	35.8	100	27–67.9	48	100	27–31.6	29.3	100	34.2–60.7	45.5	100
AMX	2935–6516	3746	100	<MQL–485.4	<MQL	25	<MQL–1129	<MQL	25	<MQL	<MQL	0	<MQL–833	<MQL	25
CIPX	2241–6453	3496	100	639.8–1053	807.9	100	321.3–524.1	495.5	100	326.3 –972.2	452	100	5–421	333.3	100
LIN	57.8–96	65.5	100	37.7–48.1	40.2	100	36.1–35.1	40.7	100	27.5–44.8	31.5	100	12.8–62.5	31.2	100
CLI	23.8–26.6	24.7	100	2.4–3.64	2.8	100	3.41–4.24	4.0	100	2.4–3.6	3.21	100	2.94–3.62	2.98	100
ERY	111.4–403.3	272.5	100	49.3–240.5	144.9	100	89.8–112	98.2	100	64.1–90.4	76.5	100	70–186.6	118.4	100
ERY-H_2O	299.3–737	652.1	100	289.4–671.2	425.5	100	194.5–381	272.6	100	243.4 –280.6	267.6	100	164.8 –267.5	216.4	100
AZT	1537–2951	1949	100	168–327	222.7	100	367.3–980	469.5	100	100 –661.9	483.2	100	60.1–278.5	164.1	100
CLAR	1201–1854	1497	100	214–883.1	522.1	100	387.3–637.1	531.7	100	194.6 –470.8	419.3	100	158.8 –635.3	425	100
TYL	<MQL	< MQL	0	<MQL	<MQL	0	<MQL	<MQL	0	<MQL	<MQL	0	< MQL	<MQL	0
SMZ	449.9–1814	802.8	100	32.5–238	47.3	100	73–260.8	135.9	100	21.6–51.6	29.7	100	41.1–105.2	86.2	100
SMX	893.4–1389	1172	100	268.4–477.9	352.1	100	301.5–463.4	311.3	100	302.1 –489.3	389.4	100	290.2–562	336	100
TMP	197.6–251.2	235.5	100	44.3–115.2	90.4	100	124.9–178.6	151.6	100	1.5–88.4	9.4	100	60.6–80.37	70	100
TET	1240–12,340	3604	100	330.9–589.1	426.7	100	691.2–1536	766.4	100	55.6–555	181.9	100	122.5 –645.4	245.6	100
MIN	730.9–3808	1233	100	174.7–749.9	361.7	100	<MQL	< MQL	0	177.9 –403.6	207.9	100	<MQL	<MQL	0
CTC	2333–15,911	6434	100	684.6–1433	1292	100	1472–1986	1757	100	401.6 –915.3	654.9	100	505.3 –1732	807	100
OXY	1629–30,049	4887	100	713.7–1099	1024	100	839.8–2014	1469	100	366.8 –779.7	581.9	100	335.4 –1069	387.3	100
TCS	341.1–743.9	426.1	100	50.3–63	56.1	100	28.5–45.9	43	100	27.2 –133.3	46.1	100	8.4–120.8	15	100
TCC	423.9–933.9	700.9	100	157.7–288.9	174.1	100	143.1–214.5	199	100	71.3 –294.4	149.7	100	49.1–263.9	110.5	100
VCM	962–43,740	20,730	100	<MQL	<MQL	0	<MQL	<MQL	0	< MQL	<MQL	0	<MQL	<MQL	0
CAP	62–80	72.5	100	<MQL	<MQL	0	<MQL	<MQL	0	< MQL	<MQL	0	<MQL	<MQL	0

< MQL: below method quantification limit.
DF: detection frequency (%).
ERY-H_2O is the degradation products of ERY (i.e. ERY-H_2O, with molecular weight of 715 Da), which existed in the environmental samples due to acid-catalyzed degradation, and not the result of the loss of one water molecule during HPLC-MS/MS analysis.

and its degradation products (i.e. ERY-H_2O) in the raw influent and treated effluent samples in this study are comparable with those reported in other countries (Karthikeyan and Meyer, 2006; Zhou et al., 2013). The differences in the distribution tendencies and concentrations in wastewater of an antibiotic could be attributed to the differences in its usage pattern in each country, water catchment characteristics (i.e. land use and population), weather conditions, and sewer systems (i.e. combined or separate sewer systems).

Sulfonamides (SMX and SMZ) and TMP were ubiquitously detected in both the raw influent and treated effluent samples with their concentrations ranging from a few hundred ng/L to thousand ng/L. The co-occurrence of SMX and TMP in the raw influent samples could be linked to the simultaneous consumption of these two antibiotics in effective treatment against a wide variety of potential bacterial infections. It is widely reported that SMX and TMP are often administered in combination at a ratio 1:5 (Gobel et al., 2005). It was interesting to observe that the concentration of TMP in the raw influent samples in this study was lower than that of SMX by a factor of approximately 5, which is consistent with the typical prescription ratio.

Other antibiotic classes, such as lincosamides (CLI and LIN) and chloramphenicol (CAP), were more frequently found in the raw influent at lower levels compared to other antibiotic classes. Concentrations of these antibiotics in the raw influent sample were below 100 ng/L. To date, the occurrence of lincosamides (CLI and LIN) has been less reported in domestic wastewater (Watkinson et al., 2007) since these drugs are mainly applied to animals. In this study, the presence of CLI and LIN in the raw influent might have resulted from animal waste of household pets or disposal of unused drugs. Similar to lincosamides, CAP was found in the raw influent at very low levels (62–80 ng/L). The low levels of CAP in the raw influent might be related to the banned use of CAP in livestock breeding in Singapore and many other countries (Kasprzyk-Hordern et al., 2009; Tong et al., 2009).

In addition to antibiotic classes, in this study, the occurrence and fate of the two widely used antimicrobial agents (TCC and TCS) were also investigated. It can be seen from Table 2 that TCC and TCS were ubiquitously detected in the raw influent and treated effluent samples. In fact, these compounds are commonly used in many household and personal care products, i.e. soaps, toothpastes, and shampoos (Cha and Cupples, 2009). In this study, TCC was more often detected at higher concentrations than TCS. In contrast, in most studies in North America and Europe (Table 3), TCS frequently showed higher concentration than TCC in both raw influent and treated effluent (Guerra et al., 2014; Kosma et al., 2014).

For treated wastewater samples, it can be seen from Table 2 that concentrations of most target antimicrobials in secondary effluent

Table 3
A comparison of occurrence of antibiotics and antimicrobial agents in influent and treated effluent samples of this study with those from different North American and European countries.

Target compound	This study			North America			European countries		
	Influent	Train-A effluent	Train-B effluent	Influent	Effluent	Reference	Influent	Effluent	Reference
CFZ	<MQL	<MQL	< MQL	–	–	–	–	–	–
MER	264.8–433.6	27–67.9	34.2–60.7	–	–	–	–	–	–
AMX	2935–6516	<MQL–1129	<MQL–833	n.r	<MQL	[1]	<MQL	<MQL	[4]
CIPX	2241–6453	321.3–524.1	5–421	17–2500	22–620	[2]	<MQL–591	<MQL–591	[4]
				<MQL–210	<MQL–140	[3]	<MQL–2610	11–168	[5]
LIN	57.8–96	36.1–35.1	12.8–62.5	11–110	4.9–65	[2]	<MQL–281	<MQL	[4]
CLI	23.8–26.6	3.41–4.24	2.94–3.62	–	–	–	<MQL	10–20	[6]
ERY	111.4–403.3	89.8–112	70–186.6	–	–	–	<MQL–320	<MQL	[4]
ERY-H_2O	299.3–737	194.5–381	164.8–267.5	14–600	27–270	[2]	60–190	n.r	[7]
				<MQL–1200	<MQL–300	[3]	48–420	15–59	[5]
							242–6755	292–2841	[10]
AZT	1537–2951	367.3–980	60.1–278.5	61–2500	57–1300	[2]	77–1139	38–784	[5]
							90–380	n.r	[7]
CLAR	1201–1854	387.3–637.1	158.8–635.3	48–8000	130–7000	[2]	n.r	25–133	[5]
							330–600	n.r	[7]
TYL	<MQL	<MQL	< MQL	<MQL	21–47	[2]	<MQL	<MQL	[6]
SMZ	449.9–1814	73–260.8	41.1–105.2	17–45	<MQL	[2]	<MQL	<MQL	[6]
				<MQL–210	<MQL	[3]	<MQL–2	<MQL	[8]
SMX	893.4–1389	301.5–463.4	290.2–562	n.r	<MQL–111	[1]	<MQL–507	<MQL–80	[4]
				59–3100	33-1800	[2]	293–11,555	119–544	[5]
				<MQL–1250	<MQL–370	[3]	230–570	n.r	[7]
TMP	197.6–251.2	124.9–178.6	60.6–80.37	n.r	<MQL–37,000	[1]	<MQL–200	<MQL–95.8	[4]
				79–810	18–580	[2]	35–3422	75–245	[5]
				170–1300	<MQL–550	[3]	210–440	n.r	[7]
TET	1240–12,340	691.2–1536	122.5–645.4	24–120	6.8–36	[2]	<MQL–85	<MQL–24	[8]
				<MQL–1200	<MQL–850	[3]			
					<MQL–977	[12]			
MIN	730.9–3808	<MQL	<MQL	<MQL	<MQL	[2]	–	–	–
CTC	2333–15,911	1472–1986	505.3–1732	<MQL	<MQL	[2]	–	–	–
OXY	1629–30,049	839.8–2014	335.4–1069	<MQL	<MQL	[2]	<MQL–7	<MQL–5	[8]
TCS	341.1–743.9	28.5–45.9	8.4–120.8	340–2900	64–490	[2]	<MQL-1742.5	<MQL–452.1	[9]
							33–463	13–82	[10]
TCC	423.9–933.9	143.1–214.5	49.1–263.9	14–270	3.1–33	[2]	97–140	n.r	[11]
VCM	962–43,740	<MQL	<MQL	–	–	–	–	–	–
CAP	62–80	<MQL	<MQL	–	–	–	<MQL	<MQL	[6]
							<MQL–319	<MQL	[10]

MQL: method quantification limit.
n.r: not reported.
–: data were not found in the literature.
[1] (Palmer et al., 2008); [2] (Guerra et al., 2014); [3] (Karthikeyan and Meyer, 2006); [4] (Papageorgiou et al., 2016); [5] (Senta et al., 2013); [6] (Gracia-Lor et al., 2012); [7] (Gobel et al., 2007); [8] (Pailler et al., 2009); [9] (Kosma et al., 2014); [10] (Kasprzyk-Hordern et al., 2009); [11] (Gasperi et al., 2014); [12] (Miao et al., 2004).

(A2) and MF permeate (B2) samples were significantly lower than those in the raw influent (unpaired T-test, $p < 0.05$), indicating a high elimination of these compounds in municipal WWTP.

3.2. Removal of antimicrobials by CAS and MBR systems

Table 4 shows the removal efficiencies of the target antimicrobials from the aqueous phase while passing through the whole wastewater treatment processes in Train-A and Train-B of the investigated WWTP. In this study, the calculations of all the removal efficiencies in the whole treatment processes were calculated as mean values with their standard deviations of the aqueous-phase concentrations of the raw influent (C_{INFL}) and secondary effluent (C_{A2}) for the CAS system (Train-A) or MF permeate (C_{B2}) for Train-B, as described in the following equations:

$$\text{Removal efficiency by CAS } (\%) = \frac{(C_{INFL} - C_{A2}) \times 100}{C_{INFL}} \quad (1)$$

$$\text{Removal efficiency by MBR } (\%) = \frac{(C_{INFL} - C_{B2}) \times 100}{C_{INFL}} \quad (2)$$

It can be seen from Table 4 that the removal efficiencies for the target antimicrobials varied significantly from −8.1 to 99.9%, depending on the compound and wastewater treatment system. The apparent negative removal efficiencies of a compound might be a result of the transformation of the conjugated forms into the original parent compound by microorganisms as well as the grab sampling strategies (Gobel et al., 2007; Radjenovic et al., 2007, 2009). In earlier studies, it was reported that several pharmaceuticals exhibited negative removal in biological wastewater treatment process (Gobel et al., 2007; Radjenovic et al., 2007, 2009; Blair et al., 2015a). For example, Gobel et al. (2007) reported that negative elimination was observed for many antibiotics, including AZT (−26 ± 8%), CLAR (−45 ± 7%), ERY-H_2O (−14 ± 4%), SMX (−107 ± 8%), and TMP (−1 ± 6%). Similarly, in a recent study, Blair et al. (2015a) also showed that some antibiotics exhibited negative removal during wastewater treatment, e.g. CIPX (−88.6%), CLAR (−72%), LIN (−50.4%), SMX (−35.8%), SMZ (−4.6%), and TMP (−53.1%).

In general, the MBR system (Train-B) showed higher removal efficiencies than the CAS system (Train-A) for the majority of the target antimicrobials, except ERY (Fig. 2). For example, better removal performance of lincosamides (CLI and LIN), sulfonamides (SMX and SMZ), macrolides (AZT, CLAR, and ERY-H_2O), tetracyclines (CTC, TET, OXY, and MIN), TMP, TCC, and TCS was noted by the MBR than CAS system. However, regarding other antibiotic classes, such

Table 4
Overall removal efficiencies of the detected antimicrobials in aqueous phase in Train-A (CAS system) and Train-B (MBR system).

Target compound	Removal efficiency by CAS system (n = 4)			Removal efficiency by MBR system (n = 4)		
	Removal range (%)	Median (%)	Mean ± SD (%)	Removal range (%)	Median (%)	Mean ± SD (%)
MER	80.7–92.6	84.4	85.5 ± 5.0	81–92.3	84.5	85.6 ± 4.9
AMX	99.3–99.7	99.5	99.5 ± 0.2	69.9–99.7	99.5	92.1 ± 14.8
CIPX	76.6–92.4	87.8	86.2 ± 6.8	84.9–99.9	88.6	90.5 ± 6.8
LIN	8.1–56.1	42.1	37.1 ± 21	−8.1–79.3	62.1	48.8 ± 38.8
CLI	83.6–85.7	83.9	84.3 ± 1.0	85.8–88.9	87.5	87.4 ± 1.3
ERY	31.4–77.7	63.8	59.2 ± 19.7	26.6–74.9	54.8	52.3 ± 19.8
ERY-H_2O	35–64.7	49.3	49.6 ± 13.8	49.9–67.7	64.8	60.6 ± 10.5
AZT	48.8–80.9	78.0	71.4 ± 15.3	88.6–96.8	91.4	90.1 ± 3.4
CLAR	51.3–73.8	67.0	64.8 ± 10.1	57.8–89.3	71.3	72.4 ± 13.8
SMX	62.8–77.7	66.6	68.4 ± 4.5	54–74.9	69.0	66.8 ± 8.9
SMZ	52.2–96	80.3	76.9 ± 19	78.4–96.2	88.1	87.7 ± 9.6
TMP	23.8–42.2	33.1	33.0 ± 7.8	67.7–73.3	69.1	69.8 ± 2.4
TET	44.3–87.6	67.1	66.5 ± 23.4	83.3–95.5	92.4	90.9 ± 5.6
MIN	44.8–86.9	70.2	68.1 ± 20.8	70.1–86.9	84.7	81.6 ± 7.8
CTC	31.4–88	58.8	59.2 ± 31.6	84–97.8	87.9	89.4 ± 6.1
OXY	54.6–93.9	80.3	77.3 ± 16.8	89.3–96.3	93.4	93.1 ± 3.5
TCS	87.4–94.2	91.1	90.9 ± 3.6	83.8–97.6	96.4	93.5 ± 6.6
TCC	51.1–84.7	69.9	68.9 ± 14.9	67.9–93.5	80.4	86.6 ± 12.3
VCM	96.6–99.9	99.9	99.1 ± 1.7	97.2–99.9	99.9	99.3 ± 1.4
CAP	98.4–98.8	98.6	98.6 ± 0.2	98.4–98.8	98.6	98.6 ± 0.2

CAS: conventional activated sludge.
MBR: Membrane bioreactor.
SD: Standard deviation.

as β-lactams (i.e. AMX and MER), CAP, CIPX and VCM, it was found that no significant difference (unpaired T-test, $p > 0.05$) in removal efficiencies was observed between CAS and MBR. The lower removal of ERY and AMX in MBR compared to CAS might be attributed to their difference in the degradation/hydrolysis rate under different acidic/alkaline conditions and temperature between CAS and MBR systems. It has been proved that MBR often has a faster response to variable influent concentrations and operational perturbation (i.e. pH, temperature, etc.) than CAS.

In addition to better removal performance, MBR particularly exhibited more stable performance than the CAS system in the elimination of most antimicrobials, with the standard deviation (RSD) of removal efficiencies for most target compounds below 15% (Table 4). For example, the removal efficiencies of tetracyclines (e.g. CTC, TET, OXY and MIN) fluctuated significantly by CAS (31.4–93.9%), while their removal efficiencies by MBR were relatively stable (70.1–97.8%). The robustness of MBR could be its faster response to variable influent concentrations and operational perturbations.

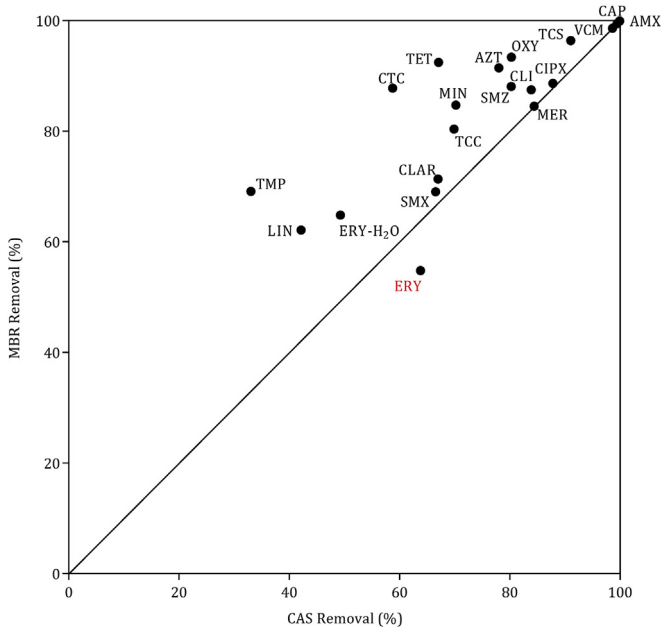

Fig. 2. Comparison of the median removal of the detected antimicrobials in CAS and MBR systems.

Better performance of MBR over CAS in the elimination of target antimicrobials could be explained by the higher biomass concentration, longer solid retention time (SRT), and the complete retention of solids and microorganisms of MBR compared to CAS (Clara et al., 2005; Joss et al., 2006; Kimura et al., 2007; Radjenovic et al., 2007, 2009). In particular, higher biomass (9648 mg MLSS/L) in MBR (Table A.2, Supplementary Information) might result in a lower food to microorganisms (F/M) ratio. Under these conditions, the shortage of biodegradable organic matter may force microbes to metabolize poorly degradable compounds (i.e. target antimicrobials) and positively affect the elimination of antimicrobials undergoing co-metabolism (Gobel et al., 2007). Longer SRT would allow the enrichment of slowly growing bacteria (e.g. autotrophic nitrifying bacteria) and establishment of a more diverse microbial population in the activated sludge system (Kimura et al., 2007; Radjenovic et al., 2007, 2009). A number of studies revealed that autotrophic nitrifying bacteria showed a high co-metabolic degradation for a broad-spectrum of emerging micropollutants (Batt et al., 2006; Tran et al., 2009, 2013). In an earlier study, Gobel et al. (2007) found that higher removal efficiencies of several antibiotics (including AZT, TMP, ERY, and CLAR) were observed at SRTs of 60–80 days.

3.3. Contribution of MF membrane unit to overall removal of MBR system

To further evaluate the performance of the MF membrane unit to the overall removal of antimicrobials in the aqueous phase, concentrations of antimicrobials in different treatment units in the MBR system (Train-B) were analyzed in order to determine their removal contributions. The removal contribution of the primary settling tank (PS) and MLE tanks to the overall removal of MBR

system (Train-B) was calculated based on the aqueous concentrations of the raw influent (INFL) and the concentration of target compounds leaving MLE tanks (B1) according to Eq. (3).

$$\text{Removal contribution}_{[\text{PS+MLE}]}\ (\%) = \frac{(C_{\text{INFL}} - C_{\text{B1}}) \times 100}{C_{\text{INFL}}} \quad (3)$$

Similarly, the removal contribution of MF membrane unit to the overall removal of MBR system (Train-B) was calculated using Eq. (4).

$$\text{Removal contribution}_{[\text{MF membrane unit}]}\ (\%) = \frac{(C_{\text{B1}} - C_{\text{B2}}) \times 100}{C_{\text{INFL}}} \quad (4)$$

As shown in Fig. 3, the treatment in PS and MLE tanks appeared to be the most important processes for removal of most target antimicrobials. More than 75% of most target compounds, except CIPX, ERY-H_2O, LIN, and SMX, were removed after the treatment processes in PS and MLE tanks. The MLE tanks (including anoxic and aerobic tanks) in the MBR system can be considered to be crucial treatment units for biodegradation of antimicrobials. It can be seen from Fig. 3 that MF membrane unit showed negative removal contribution for most compounds, including AMX, CAP, CLAR, CLI, ERY, LIN, MER, SMZ, TET, and VCM. The negative removal for several pharmaceuticals during biological wastewater treatment process was also reported in the literature (Gobel et al., 2007; Wick et al., 2009; Jelic et al., 2011). This phenomenon of negative removal contribution might be interpreted by the presence of conjugates. Most target antimicrobials, except TCC and TCS, can be excreted as unchanged parent compounds or conjugates of glucuronic acid (Gobel et al., 2007; Jelic et al., 2011), so the conjugation during contact with activated sludge might occur resulting in an increased concentration in the MF permeate (B2). In addition, most antimicrobials are excreted via urine and feces. As a result, they could be enclosed in feces particles in wastewater samples and released during wastewater treatment, leading to an increase in apparent concentration in the treated effluent (Gobel et al., 2007). Another possible interpretation is that grab sampling strategies could lead to false apparent removal efficiency by comparing the concentration before and after MF membrane unit. In fact, the contribution of MF was very low compared to the overall removal for most of the target antibiotics.

3.4. Insights into the relationships between physicochemical properties/chemical structures and removal efficiency/mechanisms

3.4.1. β-Lactams

Beta-lactams (MER and AMX) were highly removed by conventional activated sludge (CAS) or membrane bioreactor (MBR) systems of the examined WWTP. The median removal efficiencies of β-lactam antibiotics ranged from 84.4 to 99.5% (Table 4). A number of previous studies also reported that β-lactam antibiotics were highly susceptible to chemical or enzymatic hydrolysis in WWTPs (Cha et al., 2006; Watkinson et al., 2007; Le-Minh et al., 2010). The degradation of β-lactam antibiotics can take place under acidic/alkaline conditions or by reactions with weak nucleophiles, e.g. water or metal ions (Le-Minh et al., 2010; Hirte et al., 2016). Hirte et al. (2016) have recently found that the half-life of AMX in water was 128.2 h under acidic condition (pH 3), 208.3 h in neutral condition (pH 7) and only 9.7 h in alkaline condition (pH 11). Alternatively, β-lactam antibiotics can be enzymatically hydrolyzed by β-lactamases, which are widespread enzymes and produced by many species to inactivate the pharmacological effects of the β-lactam antibiotics. That is reason why β-lactam antibiotics are generally detected at a very low concentration in treated wastewater or not detected at all, although they are among the most widely used prescribed antibiotics (Cha et al., 2006; Watkinson et al., 2007).

3.4.2. Sulfonamides

Sulfonamide antibiotics (i.e. SMX and SMZ) were moderately eliminated by biological wastewater treatment processes, presenting the median removal efficiencies between 66.6 and 88.1%. Previous studies also reported that removal efficiencies of sulfonamide antibiotics (i.e. SMX and SMZ) varied significantly from 18 to 100% (Gros et al., 2006a; Gobel et al., 2007; Le-Minh et al., 2010).

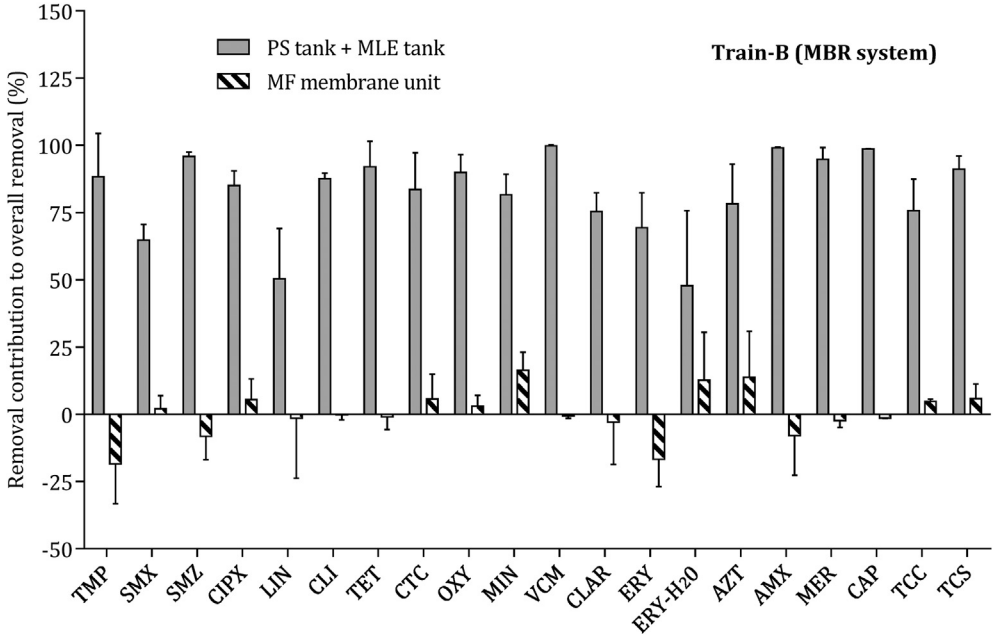

Fig. 3. Removal contribution of PS + MLE tanks and MF membrane unit to the overall removal of MBR system (Train-B) of the investigated WWTP.

The removal of sulfonamide antibiotics in conventional WWTPs is assumed to be due to moderate sorption onto activated sludge and limited biodegradation. It is widely accepted that chemicals with a low octanol-water distribution coefficient (i.e. log D_{ow}<2.5) are deemed to have a low hydrophobic sorption potential (Tadkaew et al., 2010, 2011). As a result, sulfonamide antibiotics (SMX and SMZ) are expected to have a low potential for hydrophobic partitioning since their log D_{ow} values vary from 0.02 to 0.57 at environmental pH 6–8 (Table 5).

Previous studies also reported that sulfonamides were ultimately biodegradable in activated sludge systems (Ingerslev and Halling-Sørensen, 2000), while others appeared to contradict this (Brown et al., 2006; Gros et al., 2006a). The contradiction might be due to the differences in WWTP operating conditions, such as solid retention time (SRT), hydraulic retention time (HRT), temperature, and composition of microbial community among activated sludge systems.

3.4.3. Reductase inhibitor

TMP was poorly removed in biological wastewater treatment processes, indicating the median removal efficiencies between 33.1 and 69.1% (Table 4). This result is consistent with that reported in the literature (Brown et al., 2006; Gobel et al., 2007; Lin et al., 2009), in which TMP has been reported to be persistent during conventional biological wastewater treatment processes. The removal of TMP may be mainly attributed to biodegradation; sorption of TMP onto activated sludge can be negligible due to its low octanol-water distribution coefficient (logD_{ow}<0), as shown in Table 5. Enhanced biodegradation of TMP during WWTPs can be done under nitrification (Batt et al., 2006; Khunjar et al., 2011).

3.4.4. Macrolides

Macrolide antibiotics, including AZT, CLAR, ERY, and ERY-H_2O, showed moderate removal during biological wastewater treatment processes. The median removal efficiencies of macrolides significantly varied from 54.8 to 91.4% (Table 4). Earlier studies revealed that macrolide antibiotics are often moderately removed by conventional WWTPs (Karthikeyan and Meyer, 2006; Kobayashi et al., 2006; Zhou et al., 2013). For example, removal efficiencies of ERY (including ERY-H_2O) varied from 43 to 99% by secondary wastewater treatment processes employing either activated sludge or aerated lagoons (Karthikeyan and Meyer, 2006). Similarly, Kobayashi et al. (2006) found that average removal efficiencies of macrolide antibiotics (i.e. CLAR and AZT) in conventional WWTPs in Japan were about 50%.

So far, sorption and biodegradation are considered as the main processes for removal of macrolide antibiotics during WWTPs. It has been widely accepted that sorption of macrolides (i.e. ERY and CLAR) onto activated sludge/suspended solids during WWTPs is mainly controlled by hydrophobic interactions due to their

Table 5
Relationship between physicochemical characteristics (i.e. $logK_{ow}$, $logD_{ow}$and ionization states) of the target antimicrobials and their removal controlling mechanisms in biological wastewater treatment processes.

Target compound	Removal (%)	Major regimes controlling removal efficiency	*log* K_{ow}	Hydrophobicity and ionization states at pH of 6.0					Hydrophobicity and ionization states at pH of 8.0				
				log D_{ow}	Microspecies (%)				*log* D_{ow}	Microspecies (%)			
					Anion	Neutral	Cation	Zwitterion		Anion	Neutral	Cation	Zwitterion
CFZ	n.a	n.a	−1.21	−4.87	98.2	0	0	1.8	−5.78	0	0	0	100
MER	80.7–92.6	Biodegradation/chemical hydrolysis	−1.25	−3.49	99.67	0.33	0	0	−3.49	100	0	0	0
AMX	99.3–99.7	Biodegradation/chemical hydrolysis	0.87	−2.77	0.3	0	0	99.7	−3.82	19	0	0	81
CIPX	76.6–92.4	Biodegradation/non-hydrophobic interactions	−2.82	−0.24	0	0	36.3	63.7	−0.25	17.3	0	0.5	82.2
LIN	8.1–56.1	Biodegradation/non-hydrophobic interactions	0.72/0.2	−1.4	0.3	0	0.3	99.4	−1.7	28	0	0	72
CLI	83.6–85.7	Biodegradation/non-hydrophobic interactions	2.16	−0.44	0	2.73	97.27	0	0.9	0	73.73	26.27	0
ERY	31.4–77.7	Biodegradation/non-hydrophobic interactions	3.06/3.6	0.73	0	0.4	99.6	0	2.41	0	29.4	70.6	0
AZT	48.8–80.9	Biodegradation/non-hydrophobic interactions	4.02	−2.8	0	0.1	99.9	0	0.47	0	0.3	99.7	0
CLAR	51.3–73.8	Degradation/non-hydrophobic interactions	3.16	1.43	0	0.4	99.6	0	3.09	0	29.4	70.6	0
TYL	n.a	n.a	3.27	1.36	0	6	94	0	2.7	0	86.4	13.6	0
SMZ	62.8–77.7	Biodegradation	−0.092	0.55	9.2	90.8	0	0	0.02	91	9	0	0
SMX	52.2–96	Biodegradation	0.89	0.57	40.9	59.1	0	0	0.08	98.6	1.4	0	0
TMP	23.8–42.2	Biodegradation/non-hydrophobic interactions	0.91	−0.85	0	0	18.3	81.7	−0.53	0	0	0.2	99.8
TET	44.3–87.6	Biodegradation/non-hydrophobic interactions	−1.3	−2.67	0	0	0	100	−3.11	5.2	0	0	94.8
MIN	44.8–86.9	Biodegradation/non-hydrophobic interactions	−0.42	−2.83	0	0	0	100	−3.27	0	0	0	100
CTC	31.4–88	Biodegradation/non-hydrophobic interactions	−0.62	−3.54	0	0	0	100	−4.46	0	0	0	100
OXY	54.6–93.9	Biodegradation/non-hydrophobic interactions	−0.89	−4.15	3.8	0	0.1	96.1	−4.46	52.9	0	0	47.1
TCS	87.4–94.2	Biodegradation/hydrophobic interaction	4.8	5.05	2	98	2	2	4.19	67.6	32.4	0	0
TCC	51.1–84.7	Biodegradation/hydrophobic interaction	4.9	5.27	0	0	0	100	5.27	0	0	0	100
VCM	96.6–99.9	Biodegradation	−1.44	−5.11	0	0	0.6	99.4	−3.51	0	0	7.6	92.4
CAP	98.4–98.8	Biodegradation	1.14	−1.52	0	0	0	100	−1.52	0	0	0	100

Octanol-water distribution coefficients (*log* D_{ow}) and percentages of microspecies (i.e anion, neutral, cation, and zwitterion) of the target antimicrobials under environmental conditions (pH 6–8) were estimated from ChemAxon software.

relatively high octanol-water distribution coefficient (logD_{ow} 0.73–3.09), as shown in Table 5. However, cation exchange processes may govern the sorption of macrolides onto activated sludge biomass since macrolide antibiotics (ERY, AZT, and CLAR) mainly exist as cation forms (70.6–99.6%) through the protonation of the basic dimethylamino group under typical WWTP conditions (pH 6–8) as presented in Table 5. As a consequence, these cation forms tend to be easily sorbed onto activated sludge flocs via electrostatic interactions because activated sludge biomass is widely assumed to have negatively charged surfaces.

3.4.5. Fluoroquinolones

In this study, fluoroquinolones, such as CIPX, was highly eliminated during wastewater treatment processes of the investigated WWTP. The removal efficiencies of CIPX from aqueous phase varied from 76.6 to 99.9% (Table 4). Previous studies demonstrated that sorption is the predominant mechanism for removal of fluoroquinolones during conventional WWTPs (Golet et al., 2003). Despite the negative logD_{ow} value (Table 5), CIPX has been demonstrated to have a high sorption potential because of its ionization states as zwitterion (63.7–82.2%) and cation (0.5–36.3%) under WWTP conditions (pH 6–8), as shown in Table 5. Consequently, electrostatic interactions between the positive charge of solute (CIPX) and negatively charged surface of activated sludge biomass are considered to be the main mechanism controlling the sorption of CIPX onto activated sludge during wastewater treatment processes. Due to its high sorption capability, CIPX was revealed to have the highest concentration in sewage sludge samples (Golet et al., 2003; Zhou et al., 2013).

3.4.6. Tetracyclines

The class of tetracyclines (i.e. TET, CTC, OXY, and MIN) showed a moderate removal by CAS system (Train-A), with median removal efficiencies ranging from 58.8 to 80.3%, depending on the compound (Table 4). However, their removal efficiencies were substantially enhanced in the MBR system (Train-B), exhibiting median removal efficiencies between 84.7 and 93.4%. The results in this study are comparable with those reported in the literature (Karthikeyan and Meyer, 2006; Zhou et al., 2013), in which removal efficiencies of tetracyclines more often varied from 67 to 100% in biological wastewater treatment processes. The elimination of tetracyclines in biological activated sludge processes are assumed to be attributed to biodegradation activities of microorganisms (i.e. autotrophs and heterotrophs) in activated sludge (Kim et al., 2005; Song et al., 2015). For example, Song et al. (2015) revealed that a higher biodegradation of TET was conducted under the presence of autotrophic nitrifying bacteria. In addition to biodegradation, sorption of tetracyclines onto the activated sludge biomass might play a considerable role in their overall removal. Despite the negative logD_{ow} values (Table 5), tetracyclines (CTC, TET, OXY, and MIN) may still exhibit strong sorption potential during wastewater treatment processes. The sorption mechanisms of tetracyclines onto activated sludge can be attributed to non-hydrophobic interactions, such as electrostatic interactions between the positive charges of zwitterion species of tetracyclines and the negatively charged surface of activated sludge biomass. The formation of complexes of tetracyclines with divalent metal cations (i.e. Mg^{2+}, Ca^{2+}, and Cu^{2+}) present in WWTP may also contribute to their overall removal (Carlotti et al., 2012). In addition, pH and temperature are reported to affect chemical hydrolysis rates of tetracyclines. This is also considered a possible removal mechanism contributing to the elimination of tetracyclines in wastewater treatment, particularly in terms of tropical regions, such as Singapore, where temperature is commonly above 30 °C.

3.4.7. Lincosamides

Lincosamides, including CLI and LIN, were poorly eliminated during biological wastewater treatment in the Train-A and Train-B, with removal efficiencies ranging from −8.1–88.9%. Poor removal of LIN observed in this study is consistent with that reported in the previous literature (Watkinson et al., 2007; Behera et al., 2011). The sorption of LIN onto activated sludge may be negligible because of its low octanol-water distribution coefficient (logD_{ow}<−1.4) and under typical WWTP operating conditions, as presented in Table 5. However, the sorption CLI onto activated sludge biomass may play a role in the overall removal since CLI exists as cation species (26.27–97.27%) under environmental pH 6–8 (Table 5). Till now, it is widely accepted that biodegradation is the principal regime controlling the removal of lincosamides in biological wastewater treatment processes (Watkinson et al., 2007; Zhou et al., 2013). Enhanced biodegradation of LIN could be achieved under nitrification with the presence of nitrifying bacteria (Rattier et al., 2014).

3.4.8. Other antimicrobial classes

Glycopeptide antibiotics (i.e. VCM) and chloramphenicol are other antibiotic classes of interest. In this study, VCM and CAP were ubiquitously detected in the raw influent samples, but not found in any secondary treated effluent (A2) as well as MF permeate (B2) samples. Both VCM and CAP were mostly easily eliminated (>98%) in biological wastewater treatment processes. Hitherto, there is no information on the occurrence and fate of VCM in the environment. Therefore, this study seems to be the first one to report the occurrence and fate of VCM in municipal WWTPs.

For the two commonly used antimicrobial agents (TCS and TCC), they were highly removed during biological wastewater treatment processes, with median removal efficiencies varying from 69.9 to 96.4% (Table 4). The removal efficiencies of TCS and TCC in this study are comparable with those reported in the literature (Ying and Kookana, 2007; Lozano et al., 2013). So far, it has been widely accepted that biodegradation is the predominant removal mechanism for TCC and TCS in a WWTP (Ying and Kookana, 2007; Behera et al., 2011; Lozano et al., 2013). However, sorption onto activated sludge/solids may a significant role in the elimination of these compounds in wastewater treatment processes due to their high octanol-water distribution coefficients (logD_{ow} 4.19–5.27) as shown in Table 5. That is the reason why TCS and TCC were more often detected at a high concentration in the primary sludge from WWTP. Previously (Lozano et al., 2013), also found that the average concentration of these antimicrobial agents in primary sludge varied from 13.1 to 20.3 μg/g dry wt.

4. Conclusion

This study provided the first and comprehensive quantitative data on the occurrence and removal of ten different classes of antimicrobials during biological treatment at a WWTP for the Southeast Asian region. 19 out of 21 target antimicrobials were ubiquitously detected in raw influent samples. The MBR system showed more stable performance and higher removal efficiencies than the CAS system for the majority of target antimicrobials. Beta-lactam, glycopeptide, and fluoroquinolone antibiotics were easily removed by biological wastewater treatment processes. Conversely, LIN and TMP showed poor removal efficiencies during biological wastewater treatment process. Further studies on the occurrence, fate and transport of these antibiotics in surface waters and groundwater are recommended.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.08.040.

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