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Assessment of community-wide antimicrobials usage in Eastern China using wastewater-based epidemiology

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ABSTRACT

Wastewater-based epidemiology (WBE) has potential to identify the epidemiological links between people, animals, and the environment, as part of antimicrobial resistance (AMR) surveillance. In this study, we investigated six wastewater treatment plants (WWTPs) serving six communities located in two regions in Eastern China: Site A in Zhejiang and site B in Jiangsu province to assess the public use of antimicrobial agents (AA). Fifty antimicrobials and 24 of their metabolites were quantified using ultraperformance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UPLC-MS/MS). Spatiotemporal trends were established for measured concentrations, daily loads, and population-normalised daily loads. Daily AA mass loads varied between 1.6 g/day and 324.6 g/day reflecting the WWTP scales, with macrolides and β-lactams showing the highest overall environmental burden at 223.7 g/day and 173.7 g/day, respectively. Emissions of antibiotic residues from manufacturing have been observed, with the peak daily load 12-fold higher than the overall load from a community serving a population of over 600,000. Community exposure levels of 225.2 ± 156.2 mg/day/1000 inhabitant and 351.9 ± 133.5 mg/day/1000 inhabitant were recorded in site A and B, respectively. Paired parent-metabolites analysis identified a large proportion (64–78%) of un-metabolised metronidazole and clindamycin at site B, indicating improper disposal of unused drugs either in the community or in livestock production. Consumption levels, calculated via WBE, suggested relatively low antimicrobial usage in Eastern China compared to other areas in China. This first application of WBE in Eastern China to assess the community-wide exposure to AAs has potential to inform regional antimicrobial stewardship.

1. Introduction

Antimicrobial resistance (AMR) has been identified by the World Health Organisation (WHO) as one of the top 10 global public health threats facing humanity. A recent comprehensive study of the global burden of AMR reported an estimated 4.95 million deaths associated with bacterial AMR in 2019 (Murray et al., 2022). In addition, the COVID-19 pandemic has also caused an unforeseen impact on AMR, particularly in developing countries (Ghosh et al., 2021). This is partially due to the widespread use of antibiotics to treat COVID-19 patients regardless of severity of illness in the first phase of the pandemic (Cong et al., 2021). While global and coordinated action plans are required in order to address the spread of AMR, the lack of surveillance data in many locations is one of the major challenges in need of resolution (Murray et al., 2022).

Compared to the traditional clinical surveillance systems, a wastewater-based approach could be a promising tool especially for low- and middle-income countries because of lower resource-demand (Larsson and Flach, 2021). Wastewater-based epidemiology (WBE) is a new epidemiology approach that can be applied for environmental

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surveillance of AMR to identify epidemiological links between humans, animals and the environment (Sims and Kasprzyk-Hordern, 2020). Wastewater represents a complex matrix containing a wide range of chemical and biological markers including city stressors (e.g., toxicants and infectious agents) and physiological processes (e.g., specific disease-linked proteins, antibiotic resistance genes, and metabolites) (Choi et al., 2018; Kasprzyk-Hordern et al., 2021a). Some recent studies have successfully applied WBE to investigate AMR status both regionally and nationally. For example, quinolones and quinolone resistance gene loads have been monitored in wastewaters across seven European countries (Castrignanò et al., 2020b); antibiotic use has been estimated during a flu season in eight densely populated areas in Beijing, China (Zhang et al., 2019); WBE combined with local prescription data has been applied in Southwest England to quantify antimicrobials usage (Holton et al., 2022b). Parent compounds (active antimicrobial agents, AAs) are usually selected as biomarkers; however, the inclusion of AA metabolites in WBE analyses adds a further dimension to AMR exposure assessment. The ratios between parent drugs and their corresponding metabolites in wastewater can also be used to verify AA consumption levels and direct disposal of unused hence unmetabolised drugs (Kasprzyk-Hordern et al., 2021b). In the context of an ecological risk assessment, metabolites can be found at high concentrations with high toxicity, mobility and persistence in the environment (Singh et al., 2021). It is, therefore, advisable to include key metabolites (where possible) to fully understand the overall environmental burden of antibiotics. For example, the human metabolite sulfoxide clindamycin has been detected in influent samples to obtain accurate estimates of clindamycin input loads (Ávila et al., 2021; Marx et al., 2015; Oertel et al., 2014). Recent studies in China have shown the potential to use sulfonamide, quinolone and macrolide parent drugs and metabolites to estimate the nationwide consumption level (Gao et al., 2022; Han et al., 2022).

There are several considerations for the application of WBE to assess community-wide exposure to antimicrobials. Firstly, in order to capture the dynamics of wastewater, a flow- or time-proportional sampling strategy should be adopted. Secondly, daily flow rates of influent are essential to convert the measured concentration into daily mass loads. Thirdly, the population size in the WWTP catchment is key to allowing direct comparison between different communities. Finally, the in-sewer stability and in-sample stability of the selected biomarkers should be considered, for instance longer sewer residence times could lead to degradation/transformation of oxidative stress biomarkers (Gracia-Lor et al., 2017; O'Brien et al., 2019).

To date, a few studies have reported mass loads of AAs per capita in WWTPs in China (Gao et al., 2022; Han et al., 2022; Wang et al., 2018; Yuan et al., 2015; Zhang et al., 2019), two of which have included metabolite biomarkers in the consumption estimation. Due to dense populations, an intensive livestock production industry and extensive antibiotic use, Eastern China has been identified as a hotspot for AMR (Cai et al., 2022b). Two economically and epidemiologically distinct residential areas, Zhejiang Province (Site A) and Jiangsu Province (Site B) located in Eastern China were selected in the present study. The study sites were selected to facilitate the use of existing epidemiology study cohorts to explore the potential long-term effects of antibiotic use and exposure to environmental antibiotic residues. Seventy-four AAs including both parent AAs and their metabolites were monitored in the surveyed communities in two sampling campaigns accounting for different seasons. The aim of this study is to assess the spatiotemporal community-wide public exposure to AAs (public intake vs total environmental burden) using a WBE approach. This has potential to inform antimicrobial stewardship interventions in the local area.

2. Materials and methods

2.1. Chemicals and materials

A total of 50 AAs and 24 major metabolites were targeted in the present study. The selected AAs covered a wide range of antibiotic classes including β-lactams, sulfonamides and trimethoprim, macrolides and lincomycin, quinolones, azoles, phenicols, cyclines, nitrofurans, and tuberculosis (TB) drugs. Two antiretrovirals, emtricitabine and lamivudine, were included due to their significant association with TB and HIV coinfection. A total of 21 stable isotope–labelled internal standards (ISTD) were used for the quantification of the target compounds. A full list is provided in Table 1. More details on the chemical information can be found elsewhere (Holton and Kasprzyk-Hordern, 2021). Analytical standards and deuterated standards were purchased from Sigma-Aldrich (Gillingham, UK), TRC (Toronto, Canada), LGC (Middlesex, UK), or MCE (Cambridge, UK). HPLC grade methanol (MeOH), acetonitrile and water were purchased from VWR (UK) and formic acid (> 95%) was purchased from Sigma-Aldrich, respectively. Stock solutions were prepared in MeOH or acetonitrile at 1.0 mg/mL and mixed working solutions containing all analytes were diluted from the stock solutions to a final concentration of 1.0 mg/L in methanol. Stock and working solutions were stored at -20 ℃ freezer.

2.2. The study areas and sampling sites

Sampling regions A and B were located in Zhejiang and Jiangsu provinces, respectively, in Eastern China. A map showing the sampling locations can be found in Fig. S1 (Supplemental material). Six WWTPs were selected, serving population sizes ranging from 12,000 to 150,000 in site A (WWTP-1, -2 and -3) and from 8730 to 634,000 in site B (WWTP-4, -5, and -6). Wastewater types included domestic, industrial, and combined domestic and industrial input. Detailed information on WWTPs coverage and population size are provided in Table S1. Population size (population equivalent, PE) served by selected WWTPs was estimated using local census data. WWTP-5 (region B) is an exception, where ammonia parameters were used to estimate PE (Table S2) (Zhang et al., 2019). Two sampling campaigns were carried out over 7 consecutive days in August 2020 and January 2021, respectively, which are considered as wet and dry seasons in the study areas. WWTP 1-3 are grouped as AS (summer) and AW (winter) in site A; while WWTP 4-6 are grouped as BS (summer) and BW (winter) in site B. In order to obtain a representative influent sample, a flow-proportional composite sampling strategy was adopted by using an auto-sampler (JH-8000D, Qingdao) to collect 10 mL of the influents at regular 15-min time intervals for 24 h. On the day of collection, 50 mL of the composite wastewater samples were spiked in duplicate with 50 ng of an internal standard mixture (50 μL of 1 mg/L mix). Samples were transported to the laboratory on ice and frozen (-20 ℃) for shipment to the UK and processing.

2.3. Sample preparation and analysis

Solid phase extraction (SPE) was conducted at Fudan University using sample preparation protocol developed by Holton and Kasprzyk-Hordern (2021). Briefly, 50 mL of samples were filtered through a GF/F glass fibre filter (0.7 μm, Whatman) and the filtrates were loaded under vacuum onto pre-conditioned Oasis HLB cartridges (60 mg, Waters, UK) at 5.0 mL/min. The cartridges were conditioned and equilibrated with 2 mL of MeOH, followed by 2 mL of HPLC water under gravity. After loading, cartridges were dried under vacuum, sealed with parafilm, and stored at -20 ℃ freezer, until being shipped on dry ice to the University of Bath. Once received, analytes were eluted using 4 mL MeOH under gravity and the eluate was collected in silanised vials (Thermo Scientific, UK) to prevent the analytes and internal standards from adsorbing onto the glass surface. After elution, extracted samples were evaporated to dryness at 40 ℃ under a gentle nitrogen flow

Table 1
Target antimicrobials and abbreviations, ordered by drug classes.

Drug class	Compounds	Parent/ Metabolite	Abbrev.	Drug class	Compounds	Parent/ Metabolite	Abbrev.
Sulfonamide & Trimethoprim	Sulfadiazine	Parent	SDZ	Quinolone	Besifloxacin	Parent	BSF
	Sulfapyridine	Parent & active metabolite	SPY		Ciprofloxacin	Parent & active metabolite	CIP
	Sulfamethoxazole	Parent	SMX		Danofloxacin	Parent	DFX
	Sulfasalazine	Parent (prodrug)	SLZ		Enrofloxacin	Parent	ENR
	Trimethoprim	Parent	TMP		Flumequine	Parent	FLU
	N-acetyl sulfadiazine	Metabolite	aSDZ		Gatifloxacin	Parent	GAT
	N-acetyl sulfapyridine	Metabolite	aSPY		Lomefloxacin	Parent	LOM
	N-acetyl sulfamethoxazole	Metabolite	aSMX		Moxifloxacin	Parent	MXF
	4-hydroxy-trimethoprim	Metabolite	hTMP		Nadifloxacin	Parent	NAD
	*Sulfamethoxazole-d4	-	SMX-d4		Nalidixic acid	Parent	NAL
	*Sulfasalazine-d4	-	SLZ-d4		Norfloxacin	Parent	NOR
	*Trimethoprim-d9	-	TMP-d9		Ofloxacin	Parent	OFX
Macrolide & lincosamide	Clarithromycin	Parent	CLR		Prulifloxacin	Parent (prodrug)	PFLX
	Clindamycin	Parent	CLI		Sarafloxacin	Parent	SRF
	Erythromycin	Parent	ERY		Desethylene ciprofloxacin	Metabolite	deCIP
	Roxithromycin	Parent	ROX		Hydroxy-norfloxacin	Metabolite	hNOR
	N-desmethyl erythromycin	Metabolite	dmERY		Ofloxacin N-oxide	Metabolite	OFXo
	N-desmethyl clarithromycin	Metabolite	dmCLR		Desmethyl-ofloxacin	Metabolite	dmOFX
	N-desmethyl clindamycin	Metabolite	dmCLI		Ulifloxacin	Metabolite (active)	UFX
	*Clarithromycin-d3	-	CLR-d3		*Flumequine-13C3	-	FLU-13C
	*Erythromycin-13C,D3	-	ERY-13C		*Ofloxacin-d3	-	OFX-d3
	*Roxithromycin-d7	-	ROX-d7		*Ofloxacin desmethyl-d8	-	dmOFX-d8
Nitrofuran	Nitrofurantoin	Parent	NIT	Antiretrovirals	Emtricitabine	Parent	FTC
	1-(2-nitrobenzylidenamino)-2,4-imidazolidinedione	Metabolite	NPAHD		Lamivudine	Parent	3TC
	*Nitrofurantoin-13C3	-	NIT-13C3	Oxazolidinone	Linezolid	Parent	LZD
Azole	Metronidazole	Parent	MTZ	Amphenicol	Chloramphenicol	Parent	CHL
	Ketoconazole	Parent	KTC		Florfenicol	Parent	FLO
	Hydroxy-metronidazole	Metabolite	hMTZ		2-Amino-1-(4-nitrophenyl)-1,3-propanediol	Metabolite	S,S-ANP
	Deacetyl-ketoconazole	Metabolite	daKTC		*Chloramphenicol-d5	-	CHL-d5
	*Metronidazole-d4	-	MTZ-d4	Cycline	Chlortetracycline	Parent	CTET
	*Ketoconazole-d3	-	KTC-d3		Doxycycline	Parent	DOX
β-lactam	Amoxicillin	Parent	AMOX		Oxytetracycline	Parent	OTC
	Ampicillin	Parent	AMP		Tetracycline	Parent	TET
	Flucloxacillin	Parent	FLX		*Doxycycline-d3	-	DOX-d3
	Penicillin G	Parent	PenG		*Tetracycline-d6	-	TET-d6
	Penicillin V	Parent	PenV	TB drug	Isoniazid	Parent	INH
	Amoxicilloic acid	Metabolite	AMXa		Pyrazinamide	Parent (prodrug)	PZA
	Ampicilloic acid	Metabolite	AMPa		Ethambutol	Parent	EMB
	Penicilloic G acid	Metabolite	PenGa		Rifampicin	Parent	RMP
	Cefalexin	Parent	LEX		Rifabutin	Parent	RFB
	Cefixime	Parent	CFX		Isonicotinic acid	Metabolite	Ina
	Ceftiofur	Parent	CTF		Acetyl-isoniazid	Metabolite	aINH
	Ceftriaxone	Parent	CRO		5-Hydroxy-pyrazinoic acid	Metabolite	hPZA
	Aztreonam	Parent	ATM		25-desacetyl rifampicin	Metabolite	daRMP
	*Amoxicillin-d4	-	AMOX-d4		25-O-desacetyl rifabutin	Metabolite (active)	daRFB
	*Ampicillin-d5	-	AMP-d5		*Isoniazid-d4	-	INH-d4
	*Cefalexin-d5	-	LEX-d5		*Rifabutin-d7	-	RFB-d7
	*Penicillin G-d7	-	PenG-d7				

* Internal standards.

(TurboVap, UK) and reconstituted in 500 μL of 80:20 H_2O:MeOH before transferring to injection vials (Waters, UK). Mobile phase and matrix quality controls were prepared in parallel with wastewater samples by spiking analyte mix at 10, 100, and 1000 ng/L and ISTDs at 50 ng/L for each campaign to monitor analyte calibration and physical-chemical behaviour between the varying sample compositions. Method detection and quantification limits (MDL and MQL) are as described in previous study (Holton and Kasprzyk-Hordern, 2021).

Liquid chromatography-mass spectrometry (LC-MS/MS) was performed using a Waters ACQUITY UPLC™ system coupled to a Xevo TQD-ESI Mass Spectrometer. A reverse-phase BEH C18 column (50 × 2.1 mm, 1.7 μm) with Acquity column in-line 0.2 μm pre-filters (Waters, Manchester, UK) were used to separate analytes under a 19 min mobile phase gradient using 95:5 H_2O:MeOH with 0.1% formic acid (mobile phase A) and 100% MeOH (mobile phase B). Mass spectrometry was performed via fully targeted multiple reaction monitoring (MRM), involving two MRM transitions per analyte and one per ISTD. Detailed information on chromatographic separation as well as mass

spectrometry parameters (MRM masses, cone voltage and collision energies) can be found elsewhere (Holton and Kasprzyk-Hordern, 2021).

2.4. Quality control and statistics

Calculations for the daily load (DL, mg/day), population-normalised daily load (PNDL, mg/day/1000 inhabitant) and population-normalised daily intake (PNDI, mg/day/1000 inhabitant) are calculated using the following Eqs. (1)–(3) according to previous WBE publications (Holton et al., 2022b; Kasprzyk-Hordern et al., 2009; Van Nuijs et al., 2009).

$$DL_{Analyte}(\mathrm{mg/day}) = C_{Analyte} \times \mathrm{F} \quad (1)$$

$$\mathrm{PNDL\ (mg/day/1000\ inhabitant)} = \frac{DL_{Analyte}}{PE \times 10^3} \quad (2)$$

$$\mathrm{PNDI\ (mg/day/1000\ inhabitant)} = \mathrm{PNDL} \times \mathrm{CF} \quad (3)$$

In Eq. (1), C_{Analyte} is the concentration of the analyte (ng/L) in influent wastewater, and F is the flow rate of wastewater (L/day); in Eq. (2), PE is the population size of the surveyed community; and in Eq. (3), CF is the correction factors used to justify metabolism rate for each analyte.

Raw instrumental data was produced and integrated using MassLynx software packages (Waters Lab Informatics, UK). A custom Excel template was used for data processing and validation assessment (Holton and Kasprzyk-Hordern, 2021). OriginPro 2019 was used to draw histogram and line graphs. Mean and standard deviation calculations were performed with Microsoft Excel 2016. One-way analysis of variance (ANOVA), Pearson correlation analysis and principal component analysis (PCA) were performed using OriginPro 2019.

3. Results and discussion

3.1. Detection frequency, classification, and distribution of antimicrobials

A total of 39 out of 74 AAs were detected in all samples. Both mixed type wastewater and domestic wastewater showed similar detection frequencies (73%) and industrial wastewater showed a lower detection frequency (14%). Overall detection frequency and concentration range for each AA in the sampling sites (excluding industrial wastewater) are shown in Fig. 1. In general, macrolides and sulfonamides showed the highest detection frequencies, followed by quinolones and other AAs. β-lactams were found at the lowest detection frequencies (20–40%) in influent samples from all WWTPs. The majority of analytes recorded a concentration range between 10 and 100 ng/L in the influents, with site A showed a slightly lower mean value at 44.0 ± 55.3 ng/L than site B (63.9 ± 116.0 ng/L). Highest concentration range > 500 ng/L was seen for ceftriaxone and roxithromycin, and the latter varied the most in the two sites. Numbers of AAs detected daily are provided in Fig. S2.

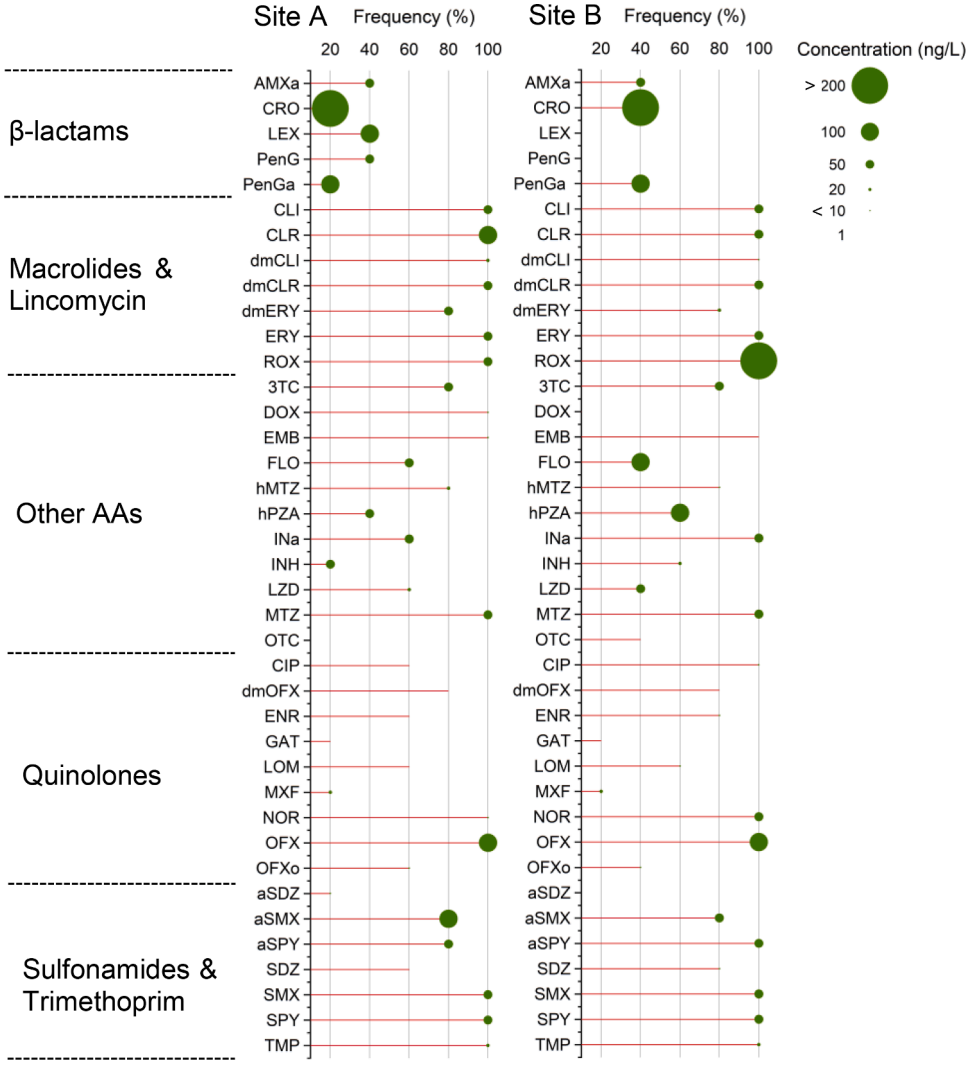

Fig. 1. Detection frequencies and concentration range of the antimicrobial agents presented. Others include amphenicol, tetracycline, azole, linezolid and TB drugs.

According to the WHO AWaRe (Access, Watch and Reserve) metrics, Access group antibiotics (9 in total) accounted for 11.5%–52.5% of all antibiotics detected, while Watch group antibiotics (11 in total) accounted for 46.9%–88.5% (Fig. S3). A target indicator proposed by WHO specifies a country-level target of at least 60% of overall antibiotic consumption from the Access group (WHO, 2021). Higher percentage of the Watch group observed is mainly due to the contribution of quinolones and macrolides, indicating that these drugs need to be prioritised for antibiotic stewardship. Table S3 shows the differences in AB classification in the study areas. The levels calculated in wastewaters are commensurate with hospital antibiotic use in China (Hsia et al., 2019). For instance, WHO Watch group antibiotics accounted for up to 82.2% of all antibiotic therapies in children (Wang et al., 2020). Proportional abundances for each drug class across all sites/seasons are provided in Fig. S4.

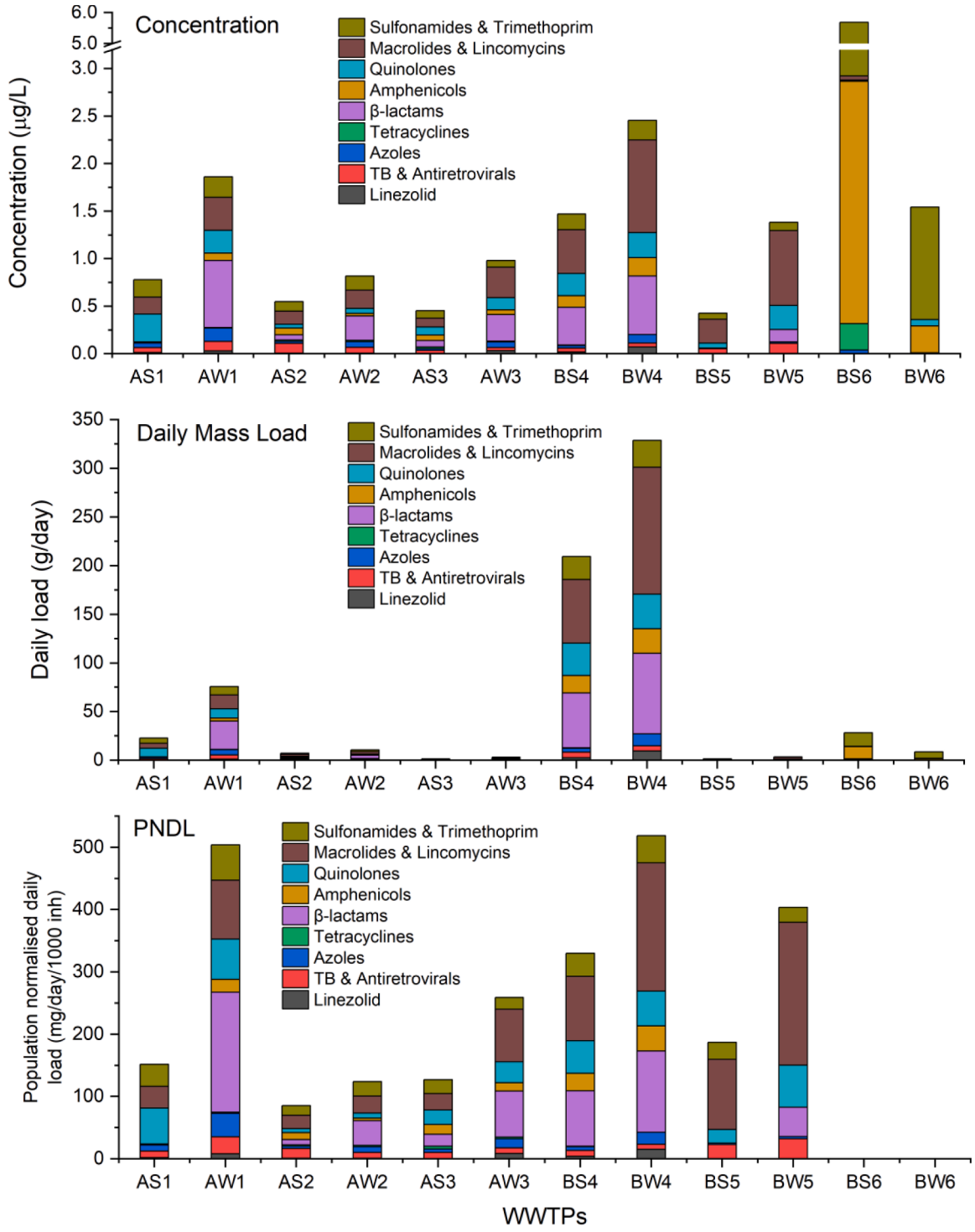

Fig. 2. Concentration, daily mass load and population-normalised daily load of antimicrobials in all WWTPs. A and B: sampling sites; S and W: summer and winter season; 1-6 refer to WWTPs.

3.2. Overall spatial-temporal trends for concentrations, daily mass loads, and PNDLs

Spatiotemporal week-averaged antibiotic (by drug class) concentrations, daily mass loads and population-normalised daily loads are presented in Fig. 2. Industrial wastewater (WWTP-6) showed the highest overall concentration as a result of drug manufacturing, mainly due to the contribution of sulfonamide and amphenicol. Seasonal increases in winter were seen for all domestic-associated wastewater, among which WWTP-5 in site B increased the most (220%). Flow rate varied considerably according to the capacity of each WWTP, ranging from 2.4×10^6 L/day in BW5 to 1.4×10^8 L/day in BS4 (Fig. S5). Overall daily mass loads varied between 1.6 g/day and 324.6 g/day as a result of changing flows (Fig. 2). Spatiotemporal trends of PNDLs are presented in Fig. 2. Site B showed a higher community exposure level (351.9 ± 133.5 mg/day/1000 inh) than site A (225.2 ± 156.2 mg/day/1000 inh). Higher mass loads at 672 ± 182 mg/day/1000 inh have been reported in Guangdong, South China (Zhou et al., 2013) and at 630 ± 42 mg/day/1000 inh in Wuxi City, East China (Yuan et al., 2015). WWTP-4 in site B, which served the largest population in the area and represented the most complex influent compositions, recorded the highest PNDLs at 422.6 ± 126.5 mg/day/1000 inh compared to other WWTPs.

Correlation analysis on the population size with overall concentration and daily load is shown in Fig. 3A. Antibiotic residue levels and

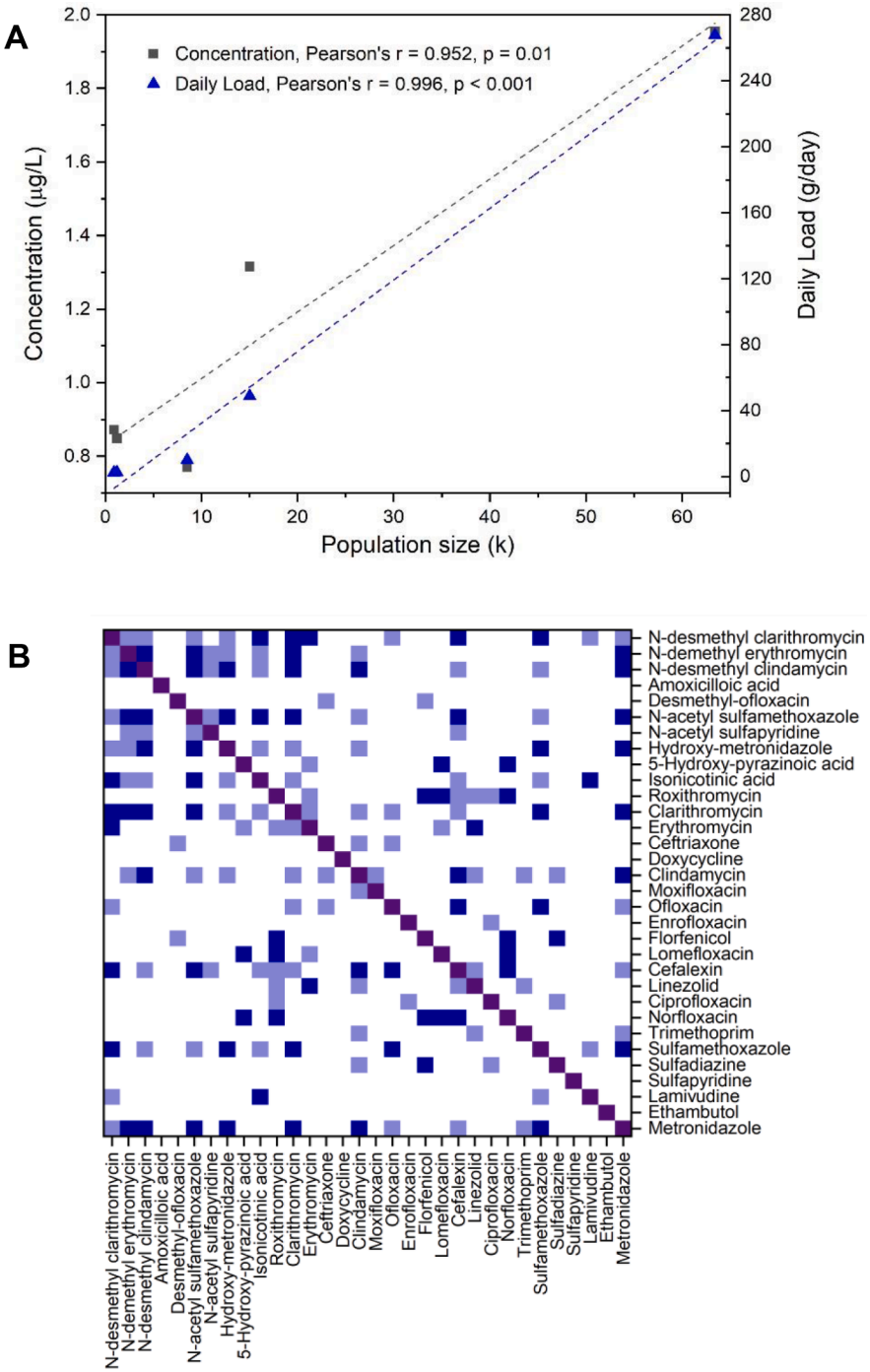

Fig. 3. A: Correlation of population size with antibiotic residual concentration and daily load; B: Correlations among all antibiotics (white: no statistical correlation; light blue: significance at 0.05; dark blue: significance at 0.01. Purple: correlation analysis is not applicable for the same compound).

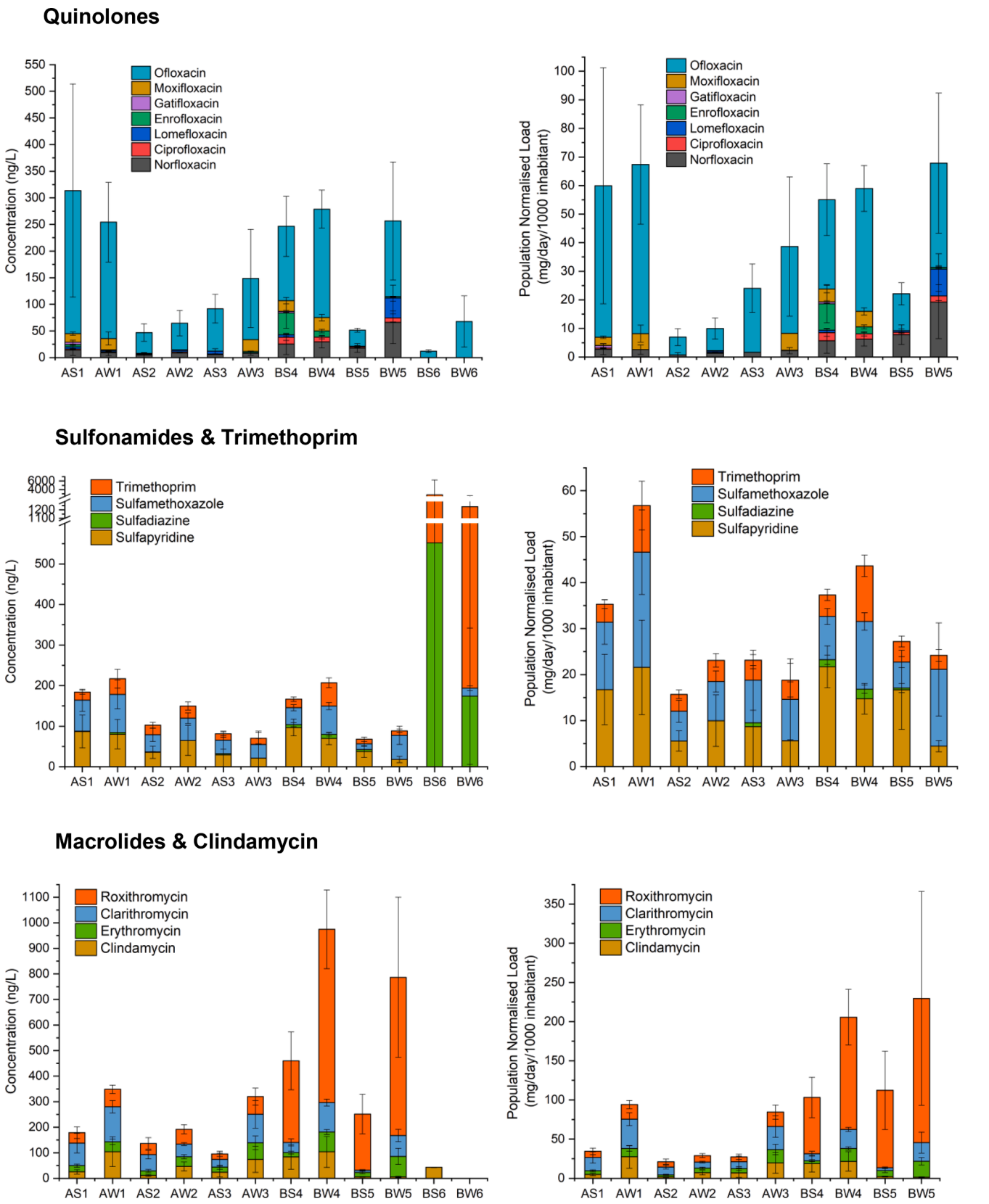

Fig. 4. Concentrations (left) and PNDL (population-normalised daily loads, right) for quinolones, sulfonamides, macrolides, beta-lactams, and others in WWTPs. A and B: sampling sites; S and W: summer and winter season; 1-6 refer to WWTPs.

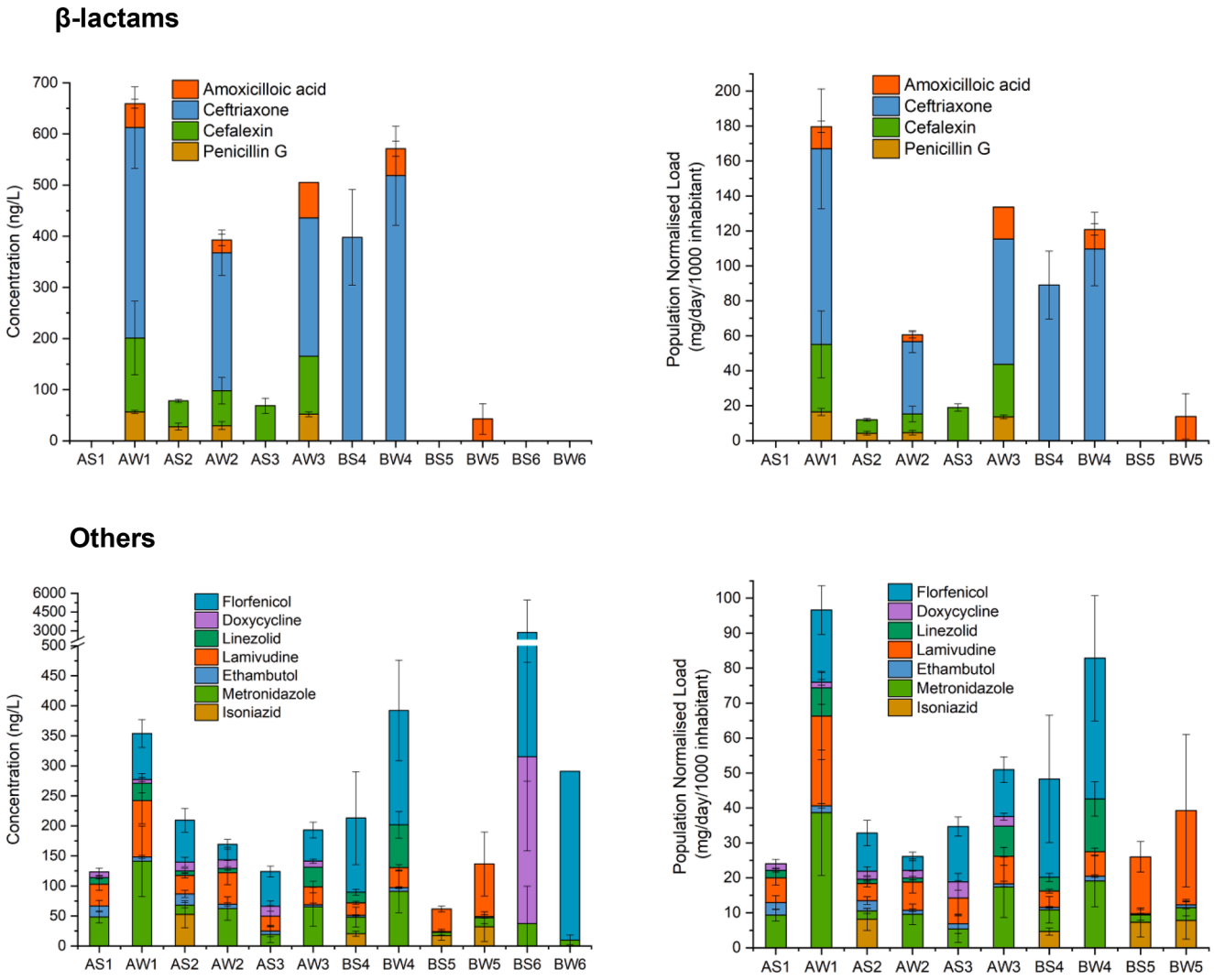

Fig. 4. (*continued*).

daily mass loads were driven by community size, as evidenced by the strong and significant positive correlations (Pearson's r > 0.95, $p < 0.05$) between these parameters. This observation is consistent with previous studies (Elder et al., 2021; Kasprzyk-Hordern et al., 2021a). Correlation analysis on the weekly average was performed to explore the potential of co-prescribing patterns. Metronidazole, cefalexin, clindamycin, and clarithromycin, as well as their metabolites, showed significant correlations ($p < 0.05$) with more than 35% of all analytes, indicating their popularities as broad-spectrum antibiotics for co-prescribing with other drug classes (Fig. 3B, Table S4).

3.3. Drug class observations

Spatiotemporal trends have been subdivided by drug classes for the following data interpretation (Fig. 4). Results classified by WWTPs are provided in supplementary material Fig. S6.

3.3.1. Quinolones and their metabolic consumption markers

Nine out of 19 quinolones, including metabolites, were detected in influents across all sampling sites, among which ofloxacin showed the highest abundance in both sites. Influent ofloxacin concentration exceeding Predicted No Effect Concentration (PNEC, derived from Minimal Inhibitory Concentration (MIC) data) at 500 ng/L (Bengtsson-Palme and Larsson, 2016) was observed in AS1 (Fig. S6), with daily loads increasing accordingly from 5.4 g/day to 15.7 and 17.9 g/day. This indicates that quinolone resistance could potentially be selected for in-sewer and across the wastewater treatment process, especially when activated sludge or sequencing batch reactor treatment is adopted (Castrignanò et al., 2020a). Norfloxacin is the second prevalent quinolone in wastewater influent, detected in all domestic-associated WWs. Average daily loads of norfloxacin ranged from 20.1 mg/day in AW3 to 3.9 ± 1.5 g/day in BW4. No noticeable seasonality was observed for either ofloxacin (*P*-value 0.46) or norfloxacin (*P*-value 0.78). Lomefloxacin showed a lower overall detection frequency at 48%, among which 30% was quantifiable. Regardless of the relatively low detection frequency, lomefloxacin presented consistently in all BW5 samples with an average influent concentration at 37.3 ± 24.4 ng/L (Fig. S6). This level is 19-fold higher than the average value (2.0 ± 1.2 ng/L) from other influent samples. The use of lomefloxacin, norfloxacin, and ofloxacin has been banned in food-producing animals in China since 2016 (MAPRC, 2015). As a 'Reserve' group antibiotic that should be treated as a last-line option, the high level of lomefloxacin in BW5 may indicate an overuse or misuse of this drug in the community.

Gatifloxacin and moxifloxacin were only sporadically detected across the sampling sites and not present in pure domestic WWs. Gatifloxacin was found only in summer samples at 3.7 ± 1.1 ng/L, while moxifloxacin showed a higher detection frequency at 15% and was present in both seasons. Moxifloxacin drug production in site B may have also contributed to its presence in the influent. In contrast, a lower concentration range between 0.6 and 6.7 ng/L has been reported in

European WWs (Castrignanò et al., 2020b).

Enrofloxacin, a veterinary antibiotic, was found consistently in 57% (30% quantifiable) of all samples. Daily influent concentration ranged between < 0.25 ng/L and 93.7 ± 9.7 ng/L in BS4. As expected, WWTP-4 in site B represented the highest environmental enrofloxacin residues presumably due to the contribution from veterinary settings such as pig, chicken, and sheep farming. Notably, enrofloxacin was the most frequently detected veterinary antibiotic in manure-based fertilizers in the sample region (Qian et al., 2016). Ciprofloxacin is an 'Access' group human antibiotic but is also preferred as veterinary medicine in China. This drug was present in 64% (29% quantifiable) of all samples. Enrofloxacin is metabolised to a large extent into active ciprofloxacin in most species (Trouchon and Lefebvre, 2016). A ratio between ciprofloxacin and enrofloxacin at 0.34 was reported in animals (Rao et al., 2002), which is comparable with the ratio observed at 0.38 ± 0.19 in BS4. Higher ratios at 0.81 ± 0.25 and 8.6 ± 5.3 were recorded in BW4 and BW5, suggesting that high prevalence of ciprofloxacin in winter samples originated from human consumption; direct disposal as well as its formation as a metabolite of enrofloxacin.

Ofloxacin N-oxide was detected largely below the quantification limit, therefore has been excluded for parent/metabolite analysis. OFX/dmOFX ratio (Ofloxacin/Desmethyl-Ofloxacin) varied considerably across sampling sites and seasons (Fig. S7), ranging from 8.8 ± 5.3 in BS5 to 57.3 ± 29.4 in AS1. This is not uncommon for a high OFX/dmOFX ratio, as the median human excretion rate (urine and faeces) of dmOFX is 3.56% (Holton et al., 2022b). A case study of wastewater from a hospital inpatient department reported at least one magnitude lower level of metabolite compared to ofloxacin (Cai et al., 2022a). WWTP-1 in site A recorded the highest OFX/dmOFX ratios in both seasons (Fig. S7), likely due to the contribution from the production of ofloxacin eye drops in this site.

3.3.2. Sulfonamides, trimethoprim, and their metabolic consumption markers

Sulfonamide antibiotics and their metabolites were detected consistently across all sampling sites. As sulfapyridine is not licensed in China as human or veterinary medicine, the presence of this drug is only considered as an active metabolite of human anti-inflammatory sulfasalazine (Liu et al., 2017; Yang et al., 2011). For industrial wastewater (WWTP-6 in site B, Fig. S6), trimethoprim and sulfadiazine are the two predominant drugs present in both seasons. Trimethoprim was detected as high as up to 8.6 ± 0.4 μg/L in BS6, nearly 350-fold higher than the average concentration of trimethoprim (24.5 ± 14.3 ng/L) in domestic-associated WWs. It should be noted that the concentration level reached the estimated upper boundary for the minimal selective concentration for trimethoprim-resistant bacteria (Bengtsson-Palme and Larsson, 2016). Sulfadiazine was also found at 2.62 ± 0.27 μg/L on the same day, 830-fold higher than the average concentration found (<MQL to 9.8 ng/L) in other types of WWs. This highlights the importance of acknowledging pharmaceutical factories as potential major contributors of antibiotics entering the environment.

Interestingly, a strong positive correlation (Pearson's r = 0.94, $p < 1.0 \times 10^{-5}$) was found between trimethoprim and sulfadiazine in industrial wastewater, indicating the potential concurrent mass production of these two drugs in local manufacturing plants. Both sulfamethoxazole and trimethoprim showed seasonality, and the daily load of the latter increased significantly ($P < 0.05$) in winter. As sulfonamides are generally thermally stable (Holton et al., 2022a), usage patterns may correspond to disease seasonality. Co-prescribing patterns were observed for sulfamethoxazole and trimethoprim (as co-trimoxazole), as evidenced by the significant positive correlations (Pearson's r = 0.70 and 0.53 in site A and B, respectively, $p < 0.004$) between the two drugs. Results showed a ratio of 2.4 ± 1.2 for SMX/TMP in wastewater containing domestic sewage. The value is within the range between 1.1 and 3.3 for the theoretical ratio of SMX/TMP in raw wastewater, indicating that human consumption/excretion of co-trimoxazole is the main origin for the presence of these two drugs in the study areas (Thiebault, 2020). Sulfadiazine showed the lowest detection frequency (64%) compared to other sulfonamides. Average daily influent concentrations of sulfapyridine ranged from 18.0 ± 7.9 ng/L (BW5) to 96.8 ± 21.0 ng/L (BS4). Much lower levels of sulfapyridine have been reported in previous case studies, where this drug was either not detectable, or present at very low concentrations ranging between 0.4 and 2.2 ng/L in wastewater influents (Chen et al., 2015; Hanna et al., 2018). These variations reflect the regional difference in the usage patterns of sulfasalazine.

SMX/aSMX (N-acetyl SMX) and SPY/aSPY (N-acetyl SPY) are discussed here to verify consumption levels vs direct disposal of unused drugs. Sulfadiazine and its metabolite were only present sporadically and have been excluded for paired parent/metabolite analysis. In general, both SMX/aSMX and SPY/aSPY showed consistent ratios across the sampling sites (Fig. S7), ranging from 0.5 to 1.2 and from 1.1 to 2.4, respectively. This is somewhat expected, as the acetyl metabolites of sulfonamides are excreted as a large proportion of the drug (22–40%), where parent compounds excreted at a relatively lower portion (15–37%) (Holton et al., 2022b). No seasonal patterns were observed for the ratios regardless of a higher antibiotic consumption in winter, indicating rational uses of these drugs in the area.

3.3.3. Macrolides, lincomycin, and their metabolic consumption markers

Distinctive clindamycin usage patterns were found in the two sites. Significant seasonal increases in winter were seen in site A (P-value < 0.001, < 0.01, = 0.05 for WWTP-1, 2, and 3, respectively), while no such trends were observed in site B. Concentration of clindamycin in domestic wastewater in site B were recorded at 5.8 ± 3.0 ng/L. In Nanjing (Jiangsu Province), clindamycin was found in influent at similar concentrations (< 10 ng/L) in December 2019 (Chen et al., 2020), suggesting a relatively low clinical use of clindamycin in Jiangsu. Nevertheless, this drug has been frequently detected in animal faeces in Jiangsu (Guo et al., 2016), indicating that the high level of clindamycin in WWTP-4 (93.6 ± 14.1 ng/L) in site B could be attributable to its veterinary use. Paired parent-metabolite analysis showed that CLI/dmCLI (clindamycin/N-desmethyl clindamycin) varied significantly (Fig. S7) in summer and winter ($P < 0.01$). Potential explanations are (1) temperature-dependent degradation of both clindamycin and its metabolite (Holton et al., 2022a); or (2) in-sewer transformation of metabolite to clindamycin in summer (Ávila et al., 2021). Notably, clindamycin was found largely unmetabolised in BS4, evidenced by the consistent higher level of parent/metabolite ratio (16.9 ± 9.6) compared to other sites (6.1 ± 0.9). This could be attributed to the imprudent use of clindamycin in animal husbandry in site B.

Erythromycin, clarithromycin, and roxithromycin were detected in 98.6% of domestic-associated WW. Erythromycin and roxithromycin are mainly used as human antibiotics but also have limited usage in animals, while clarithromycin is human-use only (Wang et al., 2016). Seasonal increases in winter were seen for all macrolides, albeit at various extents. Comparable PNDLs were observed for both erythromycin and clarithromycin in the two sites; roxithromycin, by contrast, was 11-times higher in site B ($PNDL_B$ = 124.5 mg/day/1000 inh) than in site A ($PNDL_A$ = 11.1 mg/day/inh). This clearly indicated the distinctive usage patterns for macrolides. Nationwide surveillance data (CARSS, China Antimicrobial Resistance Surveillance System) has revealed that bacterial resistance rate to erythromycin is the highest in Jiangsu compared to all other provinces in China, suggesting a history for the overuse of erythromycin and lower effectiveness in killing bacteria in clinical settings in site B (CARSS, 2022). This could explain why roxithromycin, which is pharmacologically similar to erythromycin, was much more preferred in site B than site A. Worryingly, potential overuse of roxithromycin was also observed in site B. For instance, a concentration level lower than 50 ng/L has been reported in influent samples in Nanjing (capital city in Jiangsu) in winter 2019 (Chen et al., 2020), while much higher levels ranging between 402.6 ng/L and 1304.9 ng/L

were recorded in site B (Fig. S6). The highest level detected exceeded $PNEC^{MIC}$ for roxithromycin at 1 μg/L (Bengtsson-Palme and Larsson, 2016), meaning that resistance selection could occur during wastewater transport and across the treatment process. Furthermore, a previous study investigating 23 veterinary antibiotics in Jiangsu province has reported roxithromycin, albeit at low concentration levels, as the most frequently detected macrolide (up to 90%) in manure and manure-amended soils (Guo et al., 2016). The extensive use of roxithromycin in site B may have accelerated resistance development and induced multi-drug resistance to macrolides.

Paired parent-metabolite analysis showed that CLR/dmCLR (clarithromycin/N-desmethyl clarithromycin) ratios were consistent across sampling sites and seasons (Fig. S7), 2.2 ± 0.6 in site A and 2.0 ± 0.5 in site B. The ratios are comparable with previous study, where influent CLR:dmCLR ratio was reported at 1.85 ± 0.55 in South Africa samples (Holton et al., 2022a). In contrast, ERY/dmERY (erythromycin/N-desmethyl erythromycin) showed spatial patterns, recorded at 1.4 ± 0.7 in site A, and 2.3 ± 0.4 and 5.9 ± 3.4 in WWTP-4 and -5 in site B, respectively. A ratio of approx. 1:1 in wastewater influent has been reported previously (Holton et al., 2022a). Erythromycin is well known for its variable bioavailability (18–45%) after oral administration, which may lead to differing extents of human metabolism.

3.3.4. Beta-lactams and their metabolic consumption markers

First- and third-generation cephalosporins, cefalexin and ceftriaxone, were detected in influents. Cefalexin showed an overall detection frequency of 42.8% in site A, among which 33.3% presented in summer and 52.4% in winter. Cefalexin has been reported in surface waters in Zhejiang at concentrations up to 283 ng/L (Yuyang et al., 2021). Due to the lack of oral absorption for ceftriaxone, this drug can only be given as an injection, either intramuscularly or intravenously. Therefore, the use of this drug in healthcare facilities was considered as the sole source. Detection frequency for ceftriaxone increased from 10.0% in summer to 22.9% in winter. Highest concentration recorded in the present study was at 518.4 ± 96.9 ng/L in BW4. In site B, ceftriaxone was only observed in WWTP-4, with daily loads increasing from 56.4 ± 12.3 g/day in summer to 69.5 ± 13.3 g/day in winter. The temporal trends observed can be attributed to both seasonal change in temperature, where thermal-degradation of ceftriaxone was expected (Holton et al., 2022a); and potential increased prescription in treating seasonal infectious diseases such as respiratory tract infections. Ceftriaxone is one of the most frequently prescribed antibiotics for hospitalised COVID-19 patients, accounting for 17.8% of total usage (Cong et al., 2021).

Because of its poor oral bioavailability, penicillin G is generally given intramuscularly or intravenously as a salt solution. Penicillin G was detected frequently in domestic wastewater in site A with no seasonal pattern observed. Although penicillin G parent compound was not seen in site B, its primary metabolite penicilloic G acid was measurable in winter samples (weekly average 193.8 ± 71.3 ng/L in BW4 to 206.1 ± 157.6 ng/L in BW5). Similarly, amoxicilloic acid presented in 80% of all winter samples while its parent compound amoxicillin was <MDL. Given that they are reported to be the most commonly misused antibiotic groups in China (Li, 2014), the overall low detection frequencies of cephalosporins and penicillins were likely to be mainly due to their instability in natural aquatic environments, where chemical hydrolysis or cleavage of the unstable β-lactam ring by β-lactamases could be expected (Kulkarni et al., 2017). For those quantifiable samples, a ratio at 0.52 ± 0.06 was observed for Penicillin G/Penicilloic G acid. Comparisons of the β-lactams usage patterns suggested that site B tends to prescribe more third-generation cephalosporins (ceftriaxone) instead of the first-generation cephalosporins cefalexin and penicillin G, while these drugs were still in frequent use in site A.

3.3.5. Others - Azoles, cyclines, phenicols, linezolid, TB drugs, and antivirus

Phenicols, cyclines, and linezolid. Florfenicol, a veterinary antibiotic, showed an overall detection frequency of 50%. Industrial wastewater recorded the highest daily concentration up to 5.4 μg/L in BS6 (Fig. S6), exceeding the PNEC at 2 μg/L for florfenicol (Bengtsson-Palme and Larsson, 2016). For domestic-associated wastewater, highest concentrations and daily loads were both recorded in BW4 at 190.6 ± 83.6 ng/L and 25.5 ± 11.3 g/day, respectively, suggesting contributions from industrial livestock and poultry farming in site B. Site A showed a higher detection frequency for florfenicol in winter (80.9%) than in summer (47.6%), albeit at lower levels. In fact, illicit uses of florfenicol in livestock and fishery production are not uncommon in China due to its effectiveness and low cost (Yang et al., 2021). As a result, this drug has been widely detected in food and drinking water (Hanna et al., 2018; Li et al., 2020), constituting major exposure pathways of animal antibiotic in humans. Recent study has revealed that the presence of florfenicol in urine was associated with obesity risk in children (Wang et al., 2016). Notably, linezolid, which belongs to the class of oxazolidinones and is used as a last-line human antibiotic in China, was detected frequently in four of the WWTPs in both seasons, with concentration ranging from <MDL to 96.2 ± 12.1 ng/L in BW4. Daily loads increased significantly ($P < 0.01$) in winter in WWTP-1 in site A (0.3 to 1.2 g/day) and WWTP-4 in site B (2.5 to 9.6 g/day). Clinical consumption of linezolid has increased significantly in recent years in China (Jian et al., 2020). As a critically important human medicine, WHO has warned that resistance will increase if linezolid use continues at current levels or increases. In particular, linezolid was among one of the frequently prescribed antibiotics for hospitalised COVID-19 patients in the first phase of the pandemic (Cong et al., 2021), which might have accelerated resistance development. For cyclines, overall detection frequencies for doxycycline and oxytetracycline were 56% and 17%, respectively. Doxycycline presented in industrial wastewater in summer (daily loads 1.4 ± 0.7 g/day) as a result of drug production. In domestic-associated wastewater, highest doxycycline concentration was recorded at 16.5 ± 8.6 ng/L (< MQL) in AS3.

Azoles, TB drugs, and antiretrovirals. Due to its wide application, metronidazole is among one of the most prevalent antibiotics detected in the present study and showed significant correlations with almost all drug families (Xie et al., 2015). Seasonal trends were observed for both sites (P-value = 0.01 and 0.05 for site A and B, respectively), among which WWTP-1 in site A increased the most from 1.4 ± 0.3 g/day in summer to 5.8 ± 2.7 g/day in winter. Temperature-dependent degradation of metronidazole (approx. 20–40%) has been reported (Holton et al., 2022a), which may have contributed to decreased loads detected in summer. Metronidazole also presented in industrial wastewater at concentrations ranging between 1.7 and 130.3 ng/L. The metronidazole/hydroxy-metronidazole ratio showed site- and season-specific patterns (Fig. S7). In site A, average ratio was 6.6 ± 0.8 and 3.6 ± 0.6 in summer and winter, respectively. In site B, the ratios varied considerably in WWTP-4, recorded at 18.1 ± 10.2 in summer and 11.6 ± 5.6 in winter, while domestic WWTP-5 showed comparable levels in both seasons, 4.0 ± 1.5 in summer and 3.3 ± 2.3 in winter. The ratios calculated in this study were constantly higher than that reported by Holton et al. (2022a) at 1.56 and 0.64 in South Africa wastewater samples. Notably, the highest parent-metabolite ratio observed in WWTP-4 in site B suggested a largely unmetabolised portion of metronidazole, which is indicative of poor animal farming practice and/or direct disposal of unused drug.

First-line TB drugs, ethambutol, isoniazid and its acid metabolite, and a metabolite of pyrazinamide have been detected in 100%, 32.9%, 78.6% and 42.9% of all domestic-associated wastewater, respectively. In general, site A showed an overall higher TB-drug PNDLs (3.5 mg/day/

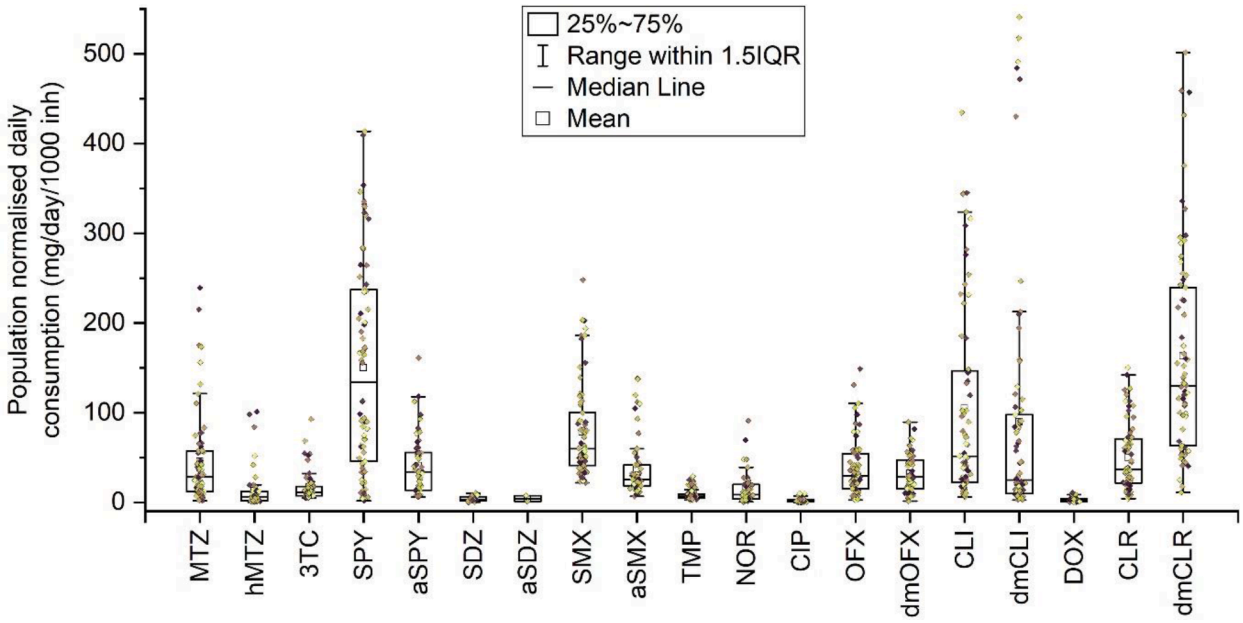

Fig. 5. Predicated daily consumption levels in the surveyed community.

1000 inh) compared to site B (2.1 mg/day/1000 inh). This is consistent with the nationwide surveillance data, where reported incidence of tuberculosis (per 100,000 people) was 31.25 in Jiangsu and 45.79 in Zhejiang in 2019 (WANG Qian, 2021). An antiretroviral, lamivudine, has been found present in 87% of domestic-associated wastewater. Similarly to TB drug patterns, PNDLs of lamivudine in site A were higher than in site B.

3.4. *Community-wide antibiotic consumption estimation*

Back-calculations estimating population-normalised daily intake were performed for 17 compounds using correction factors and PNDLs. Where applicable, consumption estimates were calculated for both parent AA and metabolite (Fig. 5). Comparable parent-metabolite estimates (no statistical differences, $P > 0.05$) were observed for SDZ-aSDZ, SMX-aSMX, OFX-dmOFX and CLI-dmCLI; while MTZ-hMTZ, SPY-aSPY, and CLR-dmCLR showed inconsistent estimates (statistical differences, $P < 0.05$). In general, the parent estimations gave higher consumption levels compared to their metabolites. This is consistent with the observations by Holton et al. (2022b), where antimicrobials excreted in an unchanged form were often observed to over-estimate daily intake due to biotransformation, via glucuronide cleavage or direct disposal of unused drug practices or topical applications not involving metabolic pathways of AA degradation. As the prescription or consumption of antibiotics in the study area is not precisely monitored, it should be noted that the following assumptions may be speculative. Table 2 lists the predicted mass of antibiotics consumed in the communities and comparisons between different areas in China.

A previous catchment study has validated that the acetyl metabolites of sulfonamides and the unchanged form of TMP were typically better at estimating community antibiotic consumption (Holton et al., 2022b). After correction, a ratio at 4.9 ± 2.9 was observed for SMX/TMP, which is closer to the expected co-prescription pattern for SMX/TMP at 5:1. Both SPY and aSPY were considered as sulfasalazine metabolites and the higher SPY estimate could be due to the glucuronide-cleavage, and biotransformation from sulfasalazine. The averaged estimated sulfasalazine daily intake (using SPY) was 150.3 mg/day/1000 inh, similar to the consumption level at 131.6 mg/day/1000 inh reported in a case study in south China (Zhou et al., 2013). The unchanged MTZ showed a 4-times higher consumption estimate than hMTZ. As suggested in the previous study, hMTZ should be considered as a better predictor when comparing to prescription data using a correction factor of 3.66. After applying the corresponding correction factors, OFX remained the most popular quinolone consumed, especially in site A. CIP and NOR were more preferred in site B than site A. It is interesting to note that OFX (CF = 1.25) and dmOFX (CF = 29.23) showed good concordance (< 20% variation) after correction. As quinolones are largely excreted in the unchanged form, parent OFX estimation is more favoured due to its high detection frequency in wastewater sample. Overall, quinolone consumption levels in the study sites were generally lower than that reported in other areas in China (Table 2). CLI and dmCLI estimates were also consistent at 14% variation. However, CLI was observed largely unmetabolised in WWTP-4 in site B, which could overestimate the daily intake. As validated in previous study, dmCLI should be used for predicting CLI intake (Holton et al., 2022b). Mean consumption value at 89.6 mg/day/1000 inh was similar to the CLI level reported in a case study in north China at 90 mg/day/1000 inh (Zhang et al., 2019). Statistically significant difference ($P = 0.002$) was observed for CLR and dmCLR estimates using CFs of 2.92 and 18.2, respectively. An overestimation of dmCLR was verified in the previous study (Holton et al., 2022b), therefore, the unchanged parent was preferred for predicating CLR consumption and the calculated mean value was 49.7 mg/day/1000 inh. This level was comparable to CLR usage in south China and 5-times lower than its usage in north China (Table 2).

3.5. *Limitations*

Two limitations should be considered for the back-calculated daily intake. Although this study has discussed stability of the analytes with regards to temporal variations using Holton et al. (2022b) observations, potential degradation/transformation during in-sewer transport introduces uncertainties in consumption estimation (Gracia-Lor et al., 2017). Mean residence time of wastewater in sewers varied according to the population size, while the current calculation assumed that the losses from toilets to WWTPs were the same across all sites. This may cause an underestimation in consumption for certain compounds which underwent longer sewer residence times. Another limitation is the potential impact of COVID-19 pandemic on seasonal antibiotic usage patterns. It was clear that during both sampling campaigns, COVID-19 measures were strictly in place in the study regions, especially for the

Table 2
Population-normalised daily intake of antimicrobials (mg/day/1000 inhabitant) in this study and in different areas in China.

Compound	Correction factors (Holton et al., 2022b)	Site A (east China) August 2020			January 2021			Site B (east China) August 2020		January 2021			Consumption level calculated from literatures South (Zhou et al., 2013)	South-west (Yan et al., 2014)	North (Zhang et al., 2019)	South (Chen et al., 2015)	South-east (Wang et al., 2018)	East (Yuan et al., 2015)
		1	2	3	1	2	3	4	5	4	5							
SMX	6.57	96.4	42.5	60.7	164.6	55.9	58.9	61.8	37.0	96.9	109.7		453.3	7513.8	132	0.74		362.7
aSMX*	1.96	34.3	17.0	55.1	115.9	21.9	66.2	20.8	15.1	28.3	29.5							
SDZ	2.68	0.2	0.2	2.3	0.5	0.0	0.1	4.2	1.1	5.6			13.7	240.1	29			2.8
aSDZ*	3.88		0.9		7.6													
SPY*	11.96	200.2	66.2	103.8	257.8	119.3	67.3	259.2	199.5	176.3	53.0		131.6					
sSPY	3.62	45.3	15.9	40.0	86.3	22.2	112.4	38.9	66.4	46.4	8.4			184.3		3.7	332.4	84.2
TMP*	1.58	6.2	5.8	6.9	16.1	7.3	6.6	7.4	7.1	19.1	4.8		39.0	47.6	45	4.2	15.1	37.8
MTZ	3.98	37.2	9.4	21.4	153.7	38.1	69.1	24.6	8.6	76.0	14.7						109.8	206.9
hMTZ*	3.66	5.2	1.5	6.7	57.4	11.0	15.4	1.4	3.3	6.9	6.9							16.4
NOR*	2.33	6.3	1.8	3.9	6.0	3.2	5.2	13.3	18.2	14.5	44.8		227.9	184.3		3.7	332.4	84.2
CIP*	1.99	0.9	0.3	0.4	0.7	0.6		5.7	1.7	3.9	4.2		31.0				15.1	37.8
OFX*	1.25	66.2**	7.8	28.0	73.9**	9.7	37.9	39.1	16.2	53.8	45.7		113.5	168.5		11.7	109.8	206.9
dmOFX	29.23	31.9	16.3	43.4	36.2	8.3	28.0	34.8	40.6	43.3	32.1				90			
CLI	9.07	45.0	16.2	63.7	249.3	65.6	178.3	170.0	20.9	196.6	12.1							
dmCLI*	19.62	17.4	5.1	22.4	390.0	79.5	214.0	21.7	10.2	111.0	25.2							
CLR*	2.92	49.4	29.1	25.0	109.3	22.3	85.7	26.0	10.5	70.7	69.3		45.3		257	26.8		123.0
dmCLR	18.2	134.6	62.1	125.5	302.9	92.5	238.3	113.2	54.5	204.3	293.7							
DOX*	1.22	2.3	2.8	5.7	2.0	2.7	3.3											1.0
3TC*	1.45	10.3	7.1	10.7	37.3	11.8	11.5	6.8	23.6	10.2	39.1							
Sum		398.7**	145.2	268.1	1073.3**	289.2	513.1	401.3	305.4	494.7	322.4						261-900	

* Compounds selected for total consumption calculation.
** Potential overestimation due to contribution from drug production. 1-5 refer to WWTPs.

management of antibiotics commonly used for respiratory tract infections (data not shown). Further data triangulation will be conducted using local medicine diary/record data collected during the sampling period to explore the impact of COVID-19 measures on AMR at the community level.

4. Conclusions

Wastewater-based epidemiology (WBE) was applied for the first time in Eastern China for the estimation of community-wide exposure to antibiotics including the analysis of both parent and metabolic consumption biomarkers in wastewater. The main conclusions are as follows:

1. Environmental sample determined AA concentrations and daily loads allowed direct comparison between catchments of different population sizes. Results indicated that the population sizes showed a strong correlation with both concentrations and daily loads; and site B represented higher community-wide exposure level to antibiotics, which is consistent with trends reported from the national antimicrobial surveillance network at the provincial level.
2. WHO AWaRe (Access, Watch and Reserve) metric analysis suggested that there is more conservative use of antibiotics in site A. The Access group accounted for 42% and 20% of total antibiotics detected in site A and B, respectively, which do not reach the target indicator proposed by WHO of 60%. This indicates that antibiotic stewardship policies need to be optimised in the study regions. The high level of the reported Watch group (up to 88.5%) was mainly due to the contribution of quinolones and macrolides.
3. Paired parent-metabolites analysis from one WWTP revealed the potential inappropriate use of antibiotics in one community at site B, evidenced by the higher parent:metabolite ratios for both clindamycin and metronidazole compared to other communities. High levels of veterinary antibiotic residues in site B may potentially reflect poor animal farming practice in the area.
4. Emissions of antibiotic residues from manufacturing processes have been observed in this study. When entering the local WWTP after discharge from the production site, the highest concentrations for specific antibiotics were well above the Predicted No Effect Concentrations ($PNEC^{MIC}$) and the highest daily mass loads exceeded the overall load from the community serving over 600,000 population.
5. Correction factors accounting for AA metabolism were applied to the environmental data to estimate community consumption levels (Holton et al., 2022b). Comparable parent-metabolite estimates were observed for sulfadiazine and sulfamethoxazole and their acetyl metabolites; ofloxacin and clindamycin and their desmethyl metabolites, indicating the potential for using either parent or metabolite biomarkers for consumption estimation. In contrast, inconsistent estimates were observed for metronidazole and its hydroxy metabolite; sulfapyridine and its acetyl metabolite; and clarithromycin and its desmethyl metabolite. Additional local clinical data on consumption (e.g., prescription records; medicine diaries, etc.) are needed to further validate the suitability of these biomarkers.

CRediT authorship contribution statement

Like Xu: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Jinxin Zang:** Conceptualization, Investigation, Methodology, Data curation, Writing – original draft. **Wenjuan Cong:** Conceptualization, Methodology, Writing – review & editing. **Elizabeth Holton:** Methodology, Writing – review & editing. **Lufang Jiang:** Investigation, Writing – review & editing. **Samuel K. Sheppard:** Conceptualization, Methodology, Writing – review & editing. **Yingying Wang:** Investigation. **Na Wang:** Conceptualization, Investigation, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition,

Resources. **Jason Weeks:** Conceptualization, Methodology, Writing – review & editing. **Chaowei Fu:** Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition, Resources. **Qingwu Jiang:** Conceptualization, Methodology, Writing – review & editing, Project administration, Funding acquisition. **Helen Lambert:** Conceptualization, Methodology, Writing – review & editing, Project administration, Funding acquisition. **Barbara Kasprzyk-Hordern:** Conceptualization, Methodology, Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118942.

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