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Wastewater-based epidemiology in Beijing, China: Prevalence of antibiotic use in flu season and association of pharmaceuticals and personal care products with socioeconomic characteristics

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ABSTRACT

Wastewater-based epidemiology is an emerging field that has mostly been applied to investigate consumption of illicit drugs. In this study, the wastewater-based epidemiology approach was employed to study consumption of pharmaceuticals and personal care products (PPCPs) and measure their prevalence of use in eight densely populated, urban areas of Beijing, China. Ammonium loads were used to estimate the population equivalents of each sewershed. These estimates were applied to calculate population-normalized antibiotic consumption and prevalence of use during flu season, when antibiotics are frequently misused as a medical treatment. Results indicated that 21.9 g d^{-1} (10^4 people)$^{-1}$ of ten popular antibiotics were consumed across the eight sewersheds, indicating that 1.98‰ of the 12.5 million population equivalents used these antibiotics during the sampling period. A comparison of these results to calculations made using previously reported data from 2013 suggest that recent Chinese antibiotic control policies have been effective. Uncertainty analyses were conducted to identify the 95% confidence range for antibiotic prevalence of use as 1.44–3.61‰. Human excretion factors were identified as the most sensitive variable. The wastewater-based epidemiology methods were also applied to a wider range of PPCPs, and the results indicated positive relationships between consumption and socioeconomic factors, such as housing price and population density. Overall, this work provides important public health information on antibiotic use and elucidates relationships between PPCP consumption and socioeconomic characteristics.

1. Introduction

The occurrence of pharmaceuticals and personal care products (PPCPs) in wastewater has drawn increased attention and represents a grand environmental challenge (Boxall et al., 2012). Among the hundreds of PPCPs that have been identified in wastewater, antibiotics comprise a specific threat to public health. The mass production and consumption of antibiotics, along with their recalcitrance in conventional wastewater treatment processes and long-range transport in the environment, contribute to the development and spread of antibiotic resistance (Crofts et al., 2017; Zhao et al., 2016). The prevalence of antibiotic resistant bacteria and antimicrobial resistance genes has continued to increase for various antibiotic classes (Baker et al., 2018). These phenomena compromise the ability of medical health professionals to treat a wide variety of potentially lethal infections, raising the possibility of a post-antibiotic era in this century (Baker, 2015; Baker et al., 2018). To prevent such a catastrophe, countries around the world have begun to control and restrict antibiotic use in both humans and animals (Van Epps and Blaney, 2016).

Self-medication and misprescription practices lead to antibiotic overuse/misuse and, ultimately, increase the mass loading of antibiotics into wastewater (Adriaenssens et al., 2011; Al Rasheed et al., 2016; Ocan et al., 2015). The average Chinese citizen now consumes more antibiotics each year than their American counterparts (Li, 2014).

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Despite the ineffectiveness of antibiotics to cure viral infections, such as influenza, many people in China regard antibiotics as a panacea, causing widespread misuse of antibiotics (Wang et al., 2017; Ye et al., 2017). In an interesting medical audit, Currie et al. (2011) asked 229 healthy participants from two large urban areas of China to visit their physician and simulate identical flu-like conditions. After seeing their physicians, 65% of the patients received antibiotic prescriptions. Similarly, Wang et al. (2017) conducted a questionnaire-based survey on antibiotic use with 11,192 university students in China. The results indicated that 41% of students believed that antibiotics could speed up recovery from the flu, 63% of students kept a stock of antibiotics, and 56% of students had purchased antibiotics without a prescription. Among the 913 students that had visited a physician complaining of flu-like symptoms, 66% were prescribed antibiotics (Wang et al., 2017). To regulate antibiotic use/misuse and minimize the development and spread of antibiotic resistance, the Chinese government implemented strict controls on antibiotic prescription and use in 2012 (NHFPC, 2012). However, the impacts of these policies, especially on the misuse of antibiotics, are difficult to quantify and evaluate with traditional approaches.

To address this problem, we propose application of the wastewater-based epidemiology concept (Daughton, 2001) to relate wastewater concentrations of antibiotics with upstream antibiotic consumption. Wastewater-based epidemiology was originally developed to investigate illicit drug consumption in cities around the world (Castiglioni et al., 2013; van Nuijs et al., 2011; Zuccato et al., 2008). Recent applications of wastewater-based epidemiology have involved estimation of dynamic populations and measurement of exposure to hazardous chemicals (Been et al., 2014; O'Brien et al., 2013; Rousis et al., 2017; Thomas et al., 2017). The major advantage of wastewater-based epidemiology is that it enables estimation of antibiotic consumption as a function of time, providing critical information on the connection between antibiotic loads in wastewater, public health, and socioeconomic status for distinct sewersheds (Lopardo et al., 2017).

In the present study, wastewater samples were collected from the eight largest wastewater treatment plants (WWTPs) in Beijing, China during flu season. These facilities were specifically chosen to collect wastewater from the most prosperous urban area in China, one of the most intensely populated regions in the world, and one of the most strictly controlled areas of China with regard to antibiotic prescriptions. Like many major cities, Beijing is a metropolis with a highly mobile population that is difficult to accurately count. The Beijing subway system transports > 10 million passengers each day (BJG, 2016), complicating efforts to estimate *per capita* consumption of antibiotics (or other chemicals). Under such circumstances, an accurate measure of the population in a particular sewershed is necessary to confidently back-calculate antibiotic consumption from wastewater concentrations. Previous studies have recommended the use of human biomarkers for this purpose (Gracialor et al., 2017; Lai et al., 2015; O'Brien et al., 2013). Been et al. (2014) normalized illicit drug concentrations to ammonium (NH_4-N) levels to estimate population in dry weather conditions. The same approach was employed in this work.

The specific objectives of this study were as follows: (1) estimate sewershed population using the NH_4-N load for eight wastewater facilities in Beijing, China during the flu season; (2) employ wastewater-based epidemiology to calculate average, *per capita* antibiotic consumption and prevalence of use (*i.e.*, the portion of the population consuming antibiotics) in Beijing during flu season; and, (3) assess the impact of antibiotic control regulations. After validation of the wastewater-based epidemiology approach for antibiotics, this strategy was applied to other PPCPs to explore relationships between PPCP loads and socioeconomic characteristics of particular sewersheds. This study is the first to consider wastewater-based socioeconomic analyses, which may serve as a powerful tool for developing data-derived regulations of other PPCPs.

2. Materials and methods

2.1. Chemicals

Based on reported wastewater concentrations, national consumption data, and local environmental occurrence (Ma et al., 2016; Zhang et al., 2015; Zhang et al., 2018), 21 antibiotics, namely trimethoprim (TP), nalidixic acid (NA), chloramphenicol (CP), erythromycin (EM), clarithromycin (CAM), roxithromycin (RXM), tylosin (TS), clindamycin (CDM), lincomycin (LCM), chlortetracycline (CTC), sulfadiazine (SD), sulfathiazole (ST), sulfamerazine (SMR), sulfisoxazole (SIX), sulfisomidine (SIM), sulfamethoxypyridazine (SMP), sulfaquinoxaline (SQX), sulfamethazine (SMT), sulfamethoxazole (SMX), sulfamethizole (SMZ), and sulfamonomethoxine (SM), and 14 other PPCPs, including propranolol (PPN), carbamazepine (CBZ), *N*,*N*-diethyl-meta-toluamide (DEET), sulpiride (SP), metoprolol (MTP), caffeine (CF), diclofenac (DF), indomethacin (IM), ketoprofen (KP), mefenamic acid (MA), bezafibrate (BF), clofibric acid (CA), gemfibrozil (GF), and acetaminophen (ATP), were selected for inclusion in this study. Details on chemical purity and analytical methods are available in Zhang et al. (2018).

2.2. Wastewater treatment plants and sewersheds

The WWTPs examined in this study represent the largest wastewater facilities in Beijing, which is one of the largest cities in the world; furthermore, three of these plants are among the largest in China. Together, the eight WWTPs treat approximately 70% of the wastewater generated in Beijing, a total that amounts to > 2.7 billion L per day (BWA, 2017). The sewersheds of WWTPs A, B, D, and G are commercially developed areas with higher housing prices, while WWTPs C, E, F, and H mostly collect wastewater from residential sectors of the city (see population density map in Fig. S1 of the Supporting information (SI)). The WWTPs receive and treat domestic wastewater from a densely populated and urbanized area that accounts for approximately 60% of the urban population of > 8 million registered residents (BMBS, 2017). Housing prices were acquired from a local real estate agency and used to create the heat map shown in Fig. 1, from which weighted average values were calculated for each sewershed (see Table S1 in the SI), serve as an indirect, but convenient and available, index of social and economic development in the sewersheds (Chen et al., 2011).

2.3. Flu season sampling and analytical procedures

In the winter of 2016–17, the flu season lasted from the 46th week of 2016 to the 15th week of 2017 (*i.e.*, 11 November 2016 to 9 April 2017) (see Fig. S2 in the SI). According to the Beijing Municipal Commission of Health and Family Planning (BJCHFP, 2018), > 96,000 cases of flu treatment were reported from the beginning of November to the middle of December. The magnitude of influenza cases during this period was over a hundred times more than the average number of cases in other seasons. WWTP H was not included in the antibiotic analysis, because samples were collected before flu season.

The 24-h volumetric flow rates for the eight WWTPs and sample collection protocols were reported by Zhang et al. (2018), who also described the occurrence, mass load, and fate of multiple PPCPs in the studied WWTPs. A one-day, high-resolution (10-min interval) composite sample collection strategy was applied on normal work days during flu season (see Table S2 in the SI). The sampling campaigns were all conducted in dry weather to eliminate possible contamination from run-off (see Table S2 in the SI). Samples were concentrated using solid-phase extraction and analyzed by HPLC-MS/MS. The analytical methods for measurement of antibiotic concentrations were reported in Zhang et al. (2018).

Ammonia concentrations (as NH_4-N) were measured on-site in the 24-hour composite samples according to the Chinese national standard method HJ 535-2009 to minimize effects of redox reactions. The NH_4-N

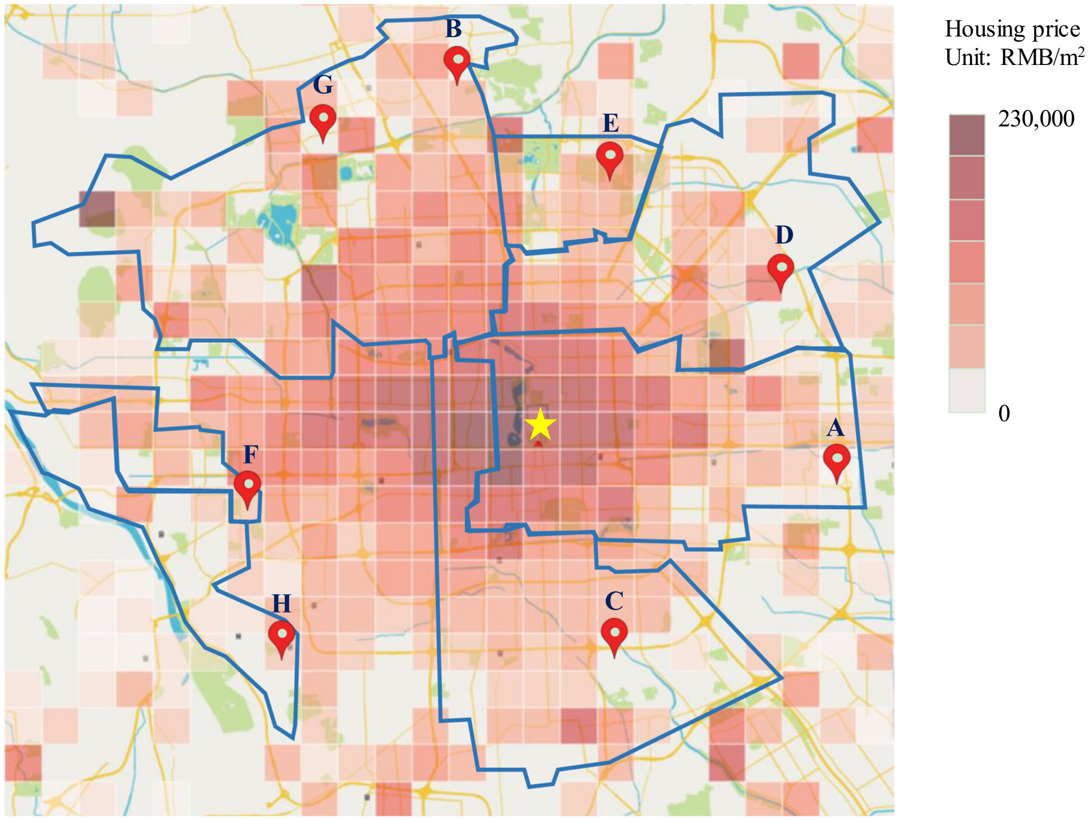

Fig. 1. Housing price map with WWTP sewershed overlays. The blue outlined regions are the boundaries of the WWTP sewersheds, the red position marks represent the location of WWTPs, and the yellow star is the center of Beijing City. The color gradient in the heat map indicates the housing price per square meter from the lightest color (¥ 0) to the darkest color (¥ 230,000). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

calibration curves always demonstrated coefficients of determination > 0.999 and relative standard deviations < 7%.

2.4. Average PPCP consumption and prevalence of use

2.4.1. Real-time population estimation

Accurate population estimates are necessary to obtain reliable statistics on average pharmaceutical consumption and prevalence of use (Castiglioni et al., 2013; Gao et al., 2016). Generally, population is estimated by one of four approaches: (1) the WWTP design capacity (Zuccato et al., 2008); (2) a simultaneous census (O'Brien et al., 2013); (3) the use of human-related biomarkers, *e.g.*, artificial sweeteners (Gao et al., 2016); or, (4) correlation with routinely tested water parameters, such as chemical oxygen demand or ammonia (van Nuijs et al., 2011). Nevertheless, population estimation is still a challenging task. In major cities with mobile populations that travel across sewersheds between work and home, the WWTP capacity and census techniques are not able to capture population dynamics; furthermore, the logistical challenges associated with accurate census counts during a simultaneous sampling campaign may be insurmountable for large cities, such as Beijing. Biomarkers, while promising, involve high costs and analytical uncertainties at the low concentrations present in wastewater. In comparison, routine, economical, and available measurements of wastewater parameters have a high potential for use as population markers. Sewershed characteristics are critical when considering the use of typical wastewater parameters for population estimates. For example, some parameters are biased by contributions from industrial wastewater sources (Rico et al., 2017; van Nuijs et al., 2011). In this study, the selected WWTPs only receive and treat wastewater from highly urbanized, residential areas of Beijing City, preventing influence from industrial effluents. NH_4-N was used as a calculation-based population index (see Eq. (1)) since this parameter is less affected by non-human sources compared to other wastewater parameters (Been et al., 2014; Castiglioni et al., 2013).

$$P_j = \frac{C_{N,j}\, Q_j}{m_{N,j}} \tag{1}$$

In Eq. (1), P_j (capita) is the real-time population of the sewershed for WWTP j, $C_{N,\,j}$ (g m^{-3}) is the measured concentration of NH_4-N in raw wastewater from WWTP j, Q_j (m^3 d^{-1}) is 24-hour cumulative flow rate measured during the sampling campaign for WWTP j, and $m_{N,\,j}$ (g d^{-1} capita^{-1}) is the average, daily NH_4-N emission *per capita* in the sewershed of WWTP j.

2.4.2. Population-normalized PPCP consumption

Drug consumption can be back-calculated from the mass load in raw wastewater (Castiglioni et al., 2013). The population-normalized, one-day mass loads for the selected PPCPs were calculated using Eq. (2).

$$\mathrm{M}_{i,j} = \frac{C_{i,j}\, Q_j}{10^6\, P_j} \tag{2}$$

In Eq. (2), $\mathrm{M}_{i,\,j}$ is the population-normalized, one-day mass load of PPCP i (g d^{-1} capita^{-1}) in raw wastewater from WWTP j, $C_{i,\,j}$ is the concentration of PPCP i (ng L^{-1}) in raw wastewater from WWTP j, and 10^6 is a conversion factor. PPCP mass loads and NH_4-N equivalent populations are reported in Tables S2–S3 in the SI and Table 1, respectively.

The average PPCP consumption can be calculated from the population-normalized, one-day mass load; however, human metabolism and sewer losses must be considered. These losses were incorporated into the correction factor (CF) described by Rousis et al. (2017) and

Table 1
Wastewater flow rate, NH_4-N concentration in raw wastewater, calculated NH_4-N equivalent population, and reported population of investigated WWTPs.

WWTP	Flow	NH_4-N	NH_4-N equivalent population	Reported population
	($10^4\ m^3\ d^{-1}$)	($mg\ L^{-1}$)	(10^4 capita)	(10^4 capita)
A	111.5	39.9	468	240
B	59.0	39.4	245	190
C	57.5	49.5	300	210
D	20.4	60.2	129	50
E	8.5	45.6	41	40
F	9.0	40.5	38	20
G	7.4	33.8	26	15

shown in Eq. (3).

$$CF_i = \frac{1}{r_{ex,i}\,(1 - r_{a,i})} \tag{3}$$

In Eq. (3), CF_i is the correction factor for PPCP i, $r_{ex.\ i}$ is the human excretion factor for PPCP i, and $r_{a,\ i}$ is the fraction of PPCP i lost in the sewer system based on results from the laboratory stability tests discussed in Section 2.5. The corrected, population-normalized PPCP consumption was calculated using Eq. (4).

$$\text{Consumption}_{i,j} = CF_i\,M_{i,j} \tag{4}$$

2.4.3. PPCP prevalence of use

The prevalence of use, which is defined as the fraction of a population that consumes a PPCP, was calculated by dividing the corrected, population-normalized consumption by the daily intake, as shown in Eq. (5).

$$P_{ac,i}(\%) = \frac{\sum\left(\frac{Consumption_{i,j}}{DDD_i}\right)}{\sum Pop_j}100\% \tag{5}$$

In Eq. (5), DDD_i ($g\ d^{-1}$ capita^{-1}) is the defined daily dose of antibiotic i, which is the recommended PPCP dose per person per day (WHOCC, 2018).

2.5. Antibiotic stability and potential use as biomarkers

Many antibiotics are stable in wastewater (Gracialor et al., 2017; Lindberg et al., 2014; Rossmann et al., 2014). In this work, the stability of the target compounds was investigated by spiking $100\ ng\ L^{-1}$ antibiotics into raw wastewater. These solutions were maintained at 4 °C in the dark to mimic the sewer environment during winter. Samples were collected at 0 and 24 h, considering that the average wastewater residence time in the Beijing sewer system is 1–6 h. The data from these experiments indicated minimal changes in antibiotic concentration for the 24-hour period (see Table S3 in the SI), suggesting that antibiotics were relatively stable in the Beijing wastewater collection system during the sampling period; however, further studies are recommended to assess biofilm-based degradation of antibiotics in the wastewater collection system (McCall et al., 2016). For this reason, the $r_{a,\ i}$ term in Eq. (3) was assumed to be negligible with respect to calculation of the correction factor; however, this term was considered in the uncertainty analysis.

2.6. Uncertainty and sensitivity analyses

The Monte-Carlo method was used to evaluate the uncertainty in the calculation of antibiotic prevalence of use (Moschandreas and Karuchit, 2002; Plummer et al., 2016). In the prevalence of use calculations, all elements were accounted for and assumed to fit normal distributions (see Table S4 in the SI). For measured factors, μ and σ were assigned to the mean and standard deviation of measured results, respectively; for correction factors, μ was assigned to values acquired from literature reports and σ was assigned to 10% of μ as a conservative estimate of standard deviation. One million simulations were run to obtain significant results for the uncertainty on calculated values. Sensitivity analyses were also carried out to evaluate the contribution of each input variable to the overall uncertainty. All statistical analyses and data visualizations were conducted in R (the R foundation), SPSS (IBM), and Excel (Microsoft).

3. Results and discussion

3.1. Wastewater-based epidemiology of antibiotic use during flu season in Beijing

The influenza virus is not treatable with antibiotics. Nevertheless, this practice represents one of the major causes of antibiotic overuse/misuse in China (Currie et al., 2014; Li, 2014; Wang et al., 2017). In this study, wastewater-based epidemiology was used to determine the prevalence of antibiotic use during flu season, evaluate the efficacy of antibiotic control policies in Beijing, and assess an alternative approach to appraise antibiotic use.

3.1.1. Population-normalized mass load of antibiotics in wastewater

Due to the high mobility of people in Beijing, an accurate estimate of population is critical for the proposed wastewater-based epidemiology approach. Previous studies reported a wide range of daily NH_4-N emission *per capita* (*i.e.*, m_N = 4–8 $g\ d^{-1}$ capita^{-1}) (Been et al., 2014; Zheng et al., 2017). However, due to influences from sewershed-specific characteristics, the NH_4-N production coefficient was set to 9.5 $g\ d^{-1}$ capita^{-1} in accordance with available data from the national waste production and discharge coefficient report for Beijing (MEP, 2011). The population equivalents for the eight WWTPs were calculated using Eq. (1) and compared to the reported populations served by these facilities (see Table 1).

For most plants, the NH_4-N equivalent population was larger than the reported population. This result likely stems from unregistered migrants, commuters, tourists, and other people that were not included as registered inhabitants in the WWTP census. During workdays, a large population flows into business centers located in sewersheds A and D (net increase in population) from the residential sewersheds C and E (net decrease in population). The NH_4-N equivalent population estimates were compared to those reported by the plant managers in Fig. 2(A) (O'Brien et al., 2013; Zheng et al., 2017). A significant linear relationship was found between the NH_4-N equivalent population and the reported population (*i.e.*, R = 0.95, $p < 0.01$), suggesting that the relative populations are similar between sewersheds but the magnitude of populations for each sewershed may be underreported by the census. Importantly, NH_4-N is less affected by non-human sources compared to other wastewater parameters and has been successfully applied to estimate dynamic populations (Been et al., 2014; Castiglioni et al., 2013). To verify the reasonableness of this approach, the uncertainty of the NH_4-N population estimate was determined to be 12% by Monte-Carlo simulation. This result is consistent with the comprehensive review of Castiglioni et al. (2013), in which the range of uncertainty associated with population estimates was reported to be 7–55%.

Fig. 2(B) reveals a significant linear correlation between the antibiotic load in raw wastewater and NH_4-N equivalent population (*i.e.*, R = 0.94, $p < 0.01$), suggesting that the population estimation model can be effectively applied to antibiotic monitoring. The slope of this relationship provides the average mass load for the 21 detected antibiotics in Beijing wastewater, namely 5.9 $g\ d^{-1}$ (10^4 capita)$^{-1}$, but does not account for human metabolism and sewer losses.

3.1.2. Antibiotic consumption patterns and effects of control policies

The ten most abundant antibiotics in Beijing wastewater were

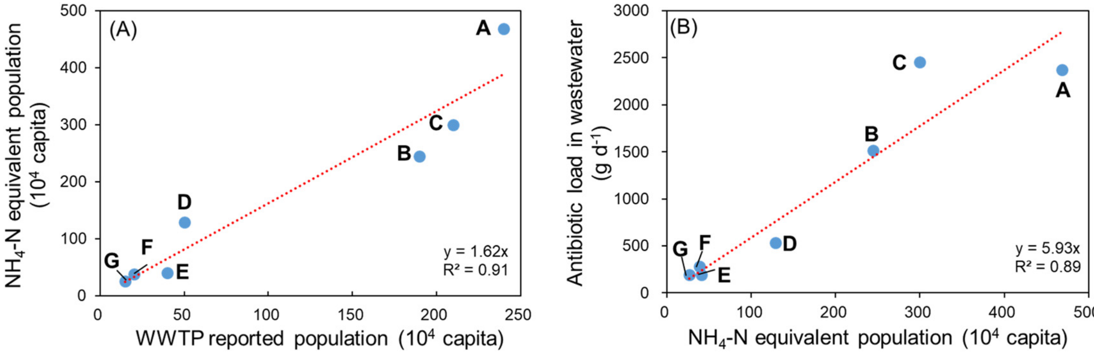

Fig. 2. Linear correlation of (A) NH_4-N equivalent population with reported WWTP population and (B) antibiotic load with NH_4-N equivalent population.

Table 2
Per capita antibiotic consumption and prevalence of use.

Antibiotics	ΣInput	Excretion	DDD	*Consumption*$_{2013}$	*Consumption*$_{2016}$	P_{ac} in 2016
	$g\,d^{-1}$	%	$g\,d^{-1}\,capita^{-1}$	$g\,d^{-1}\,(10^4\,capita)^{-1}$	$g\,d^{-1}\,(10^4\,capita)^{-1}$	‰
Trimethoprim	479	80–90 (85)[a]	0.4	9.93	0.45	0.11
Nalidixic acid	475	25[b]	4.0	–	1.52	0.04
Chloramphenicol	105	30[c]	3.0	4.27	0.28	0.01
Erythromycin	951	2–15 (8.5)[d]	2.0	24.7	8.98	0.45
Clarithromycin	1442	45[e]	0.5	1.31	2.57	0.51
Roxithromycin	1012	63[f]	0.3	3.66	1.29	0.43
Clindamycin	153	13.6[a]	1.2	–	0.90	0.08
Lincomycin	943	4.9–30.3 (17.6)[a]	1.8	19.8	4.30	0.24
Sulfadiazine	188	43–60 (51.5)[c]	0.6	4.73	0.29	0.05
Sulfamethoxazole	1477	80–100 (90)[a]	2.0	0.04	1.32	0.07
Sum				68.4	21.9	1.98

Acronyms: DDD is defined daily dosage; *Consumption*$_{2016}$ is the back-calculated *per capita* antibiotic consumption from this study; *Consumption*$_{2013}$ is the *per capita* antibiotic consumption calculated with data from 2013 (Zhang et al., 2015); and, "–" indicates that data are not reported.
The values in parentheses are the means used for calculation purposes.
Excretion factors were acquired from
[a] RxList database.
[b] McChesney et al. (1964).
[c] Drugbank database.
[d] Pubchem database.
[e] Lebel (2016).
[f] Puri and Lassman (1987).

selected as representative compounds, based on prescription rates in China and input from doctors (personal communications), to study consumption patterns during flu season. The daily, per-capita consumption of antibiotics was calculated using Eq. (4) and is reported as *Consumption*$_{2016}$ in Table 2. The total consumption of the ten antibiotics was 21.9 $g\,d^{-1}\,(10^4\,capita)^{-1}$. Erythromycin and lincomycin were the most consumed antibiotics in Beijing and represent the most prevalent human-use antibiotics in China (Zhang et al., 2015) due to their widespread availability and low cost. In Table 2, the *per capita* antibiotic consumption is also reported for 2013 (see the *Consumption*$_{2013}$ column), which was the year that the official antibiotic control policies were adopted in China, using data reported by Zhang et al. (2015). Antibiotic consumption during the 2016 flu season was only 32% of the 2013 level; however, we acknowledge that different methodologies were employed in the 2015 report and the present study, potentially affecting conclusions stemming from this comparison. The 2016 chloramphenicol concentration decreased by 30% since 2013 (Sui et al., 2011), and the 2016 sulfadiazine consumption was 90% lower than 2013 levels (Gao et al., 2012; Li et al., 2013). These findings suggest that the antibiotic controls measures implemented in China have been effective (NHFPC, 2012).

3.1.3. Estimation of the prevalence of antibiotic use

The prevalence of antibiotic use was calculated with Eq. (5) and reported in Table 2. According to this analysis, 1.98‰ of the population from the eight sewersheds, or 25,000 people, took antibiotics during the sampling period. Given the reported number of flu cases (*i.e.*, 96,000), approximately 26% of people suffering from flu may have misused antibiotics as a medical strategy. This study only accounted for ten antibiotics, but 36 human-use antibiotics have been frequently detected in Chinese wastewater (Zhang et al., 2015). The prevalence of use could, therefore, reach 8‰ if other antibiotics were used in equal proportion. This situation raises public health concerns since it corresponds to almost 100,000 people potentially misusing antibiotics. The calculated prevalence of use is influenced by three major pathways of human consumption: (1) legal and valid prescriptions; (2) antibiotic misuse; and, (3) exposure from water and food products (Li et al., 2017). The contribution of each of these pathways is difficult to deconvolute, although the total mass of antibiotic consumption from legal/valid prescriptions for infections and ingestion of antibiotics from food and drink is not expected to vary greatly throughout the year. However, the misuse of antibiotics is much more unpredictable, especially in flu season (Al Rasheed et al., 2016; Li, 2014). For these reasons, a more robust evaluation of the prevalence of antibiotic use and misuse is needed for different periods of the year.

3.1.4. Uncertainty and sensitivity analysis

The prevalence of antibiotic use (P_{ac}) from Eq. (5) can also be expressed as Eq. (6).

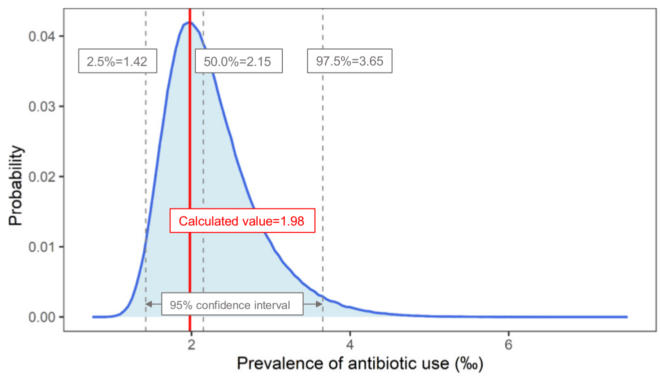

Fig. 3. Distribution diagram for the prevalence of antibiotic use from Monte Carlo analysis.

$$P_{ac}\,(‰) = \left(\frac{m_N}{1 - r_a}\right)\sum_{i=1,j=1}^{m=10,n=7}\left[\left(\frac{C_{i,j}\,Q_j}{DDD_i\,r_{ex,i}}\right)\left(\frac{1}{\sum_{j=1}^{n=7} C_{N,j}\,Q_j}\right)\right]1000‰ \tag{6}$$

From this expression, the uncertainty associated with the prevalence of use calculation stems from the following parameters: antibiotic and NH_4-N concentrations in raw wastewater; raw wastewater volumetric flow rate; NH_4-N discharge coefficient; antibiotic excretion factors; compliance with DDD recommendations; and, antibiotic losses in the wastewater collection system. The uncertainty in each of these parameters affects the calculated prevalence of antibiotic use; therefore, Monte Carlo simulations were introduced to quantify and compare the uncertainties for each variable. The input parameters and a detailed explanation of the protocol are detailed in Table S4 of the SI. After one million simulations, the possibility distribution shown in Fig. 3 was obtained for antibiotic prevalence of use during the 2016–17 flu season in Beijing. The median prevalence of use for the ten highly consumed antibiotics in Table 2 was 2.15‰, and the 95% confidence interval ranged from 1.42‰ to 3.65‰. The 1.98‰ prevalence of antibiotic use calculated by wastewater-based epidemiology overlapped with this range, supporting the validity of that approach.

A sensitivity analysis was conducted to evaluate the influence of each variable (see Eq. (6)) on the prevalence of antibiotic use. The results presented in Fig. S4 of the SI showed that the antibiotic excretion rate (r_{ex}), NH_4-N emission coefficient (m_N), and rate of antibiotic loss in the wastewater collection system (r_a) were the most sensitive factors, contributing 52.3%, 20.4%, and 20.2% of the total uncertainty, respectively. These three variables are region-specific and, therefore, vary between sewersheds. More precise predictions of the prevalence of antibiotic use could, therefore, be achieved by improving the accuracy of these regional factors.

3.2. Wastewater-based socioeconomic analysis

The methodologies used to estimate antibiotic consumption and prevalence of use were also applied to a wider suite of PPCPs to investigate the application of wastewater-based epidemiology to socioeconomic descriptors. In particular, the same approach was used to estimate population-normalized mass loads of 17 PPCPs, including three antibiotics, from seven pharmaceutical classes. The results were correlated with socioeconomic parameters, including housing prices and population density.

3.2.1. PPCP composition profiles for the eight WWTPs

PPCP concentrations in the investigated WWTPs are summarized as rose diagrams in Fig. S3 of the SI. The results revealed high concentrations of acetaminophen and caffeine in all eight WWTPs, indicating that these two compounds are commonly used in all sewersheds. Further analysis showed that WWTPs E, F, and G exhibited high concentrations of ketoprofen and carbamazepine. Several PPCPs were reported at similar concentrations in WWTP D and H. These findings suggest that PPCP compositional profiles may be similar in some facilities and different in others. For that reason, an unsupervised cluster analysis was conducted for PPCP composition across the eight WWTPs (SPSS 21.0, IBM). This analysis confirmed the clustering of three main groups, as shown in Fig. S4 of the SI. Group 1 included the three largest plants (*i.e.*, WWTPs A, B, and C); Group 2 comprised the three smallest facilities (*i.e.*, WWTPs E, F, and G); and, Group 3 involved the medium-capacity WWTPs D and H. Although the population-normalized PPCP mass loads vary between clusters, the overlapping rose diagrams in Fig. 4 confirm that similar consumption profiles exist within individual sewersheds.

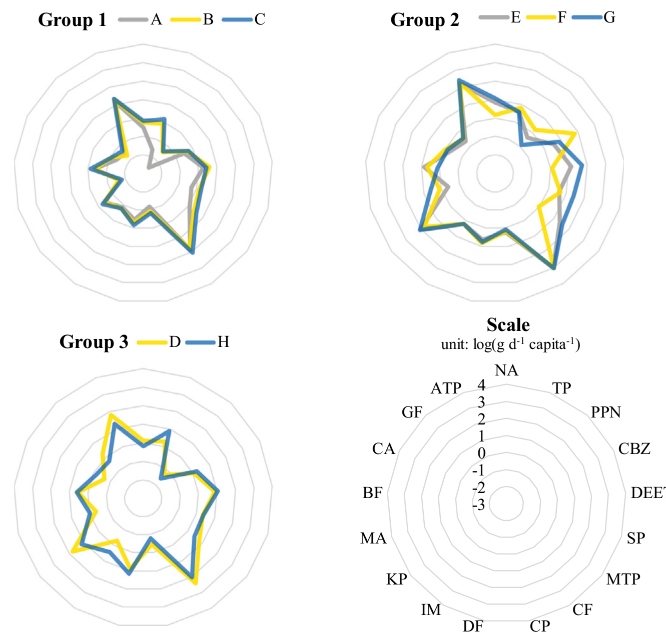

Fig. 4. PPCP composition rose diagrams for raw wastewater from eight WWTPs grouped as three main clusters (*i.e.*, Groups 1, 2, and 3). Distance from the center is measured by the log of the population-normalized, daily mass load.

3.2.2. Correlation of estimated PPCP mass load with housing price and population density

Wastewater-based population estimates have been successfully correlated to pharmaceutical consumption patterns (Castiglioni et al., 2013; Gao et al., 2016). That concept was extended here by relating socioeconomic factors to wastewater-based PPCP consumption estimates. Housing price is a major indicator of the social development level (Dietz and Haurin, 2003); therefore, a heat map of housing prices was overlaid on the sewershed boundaries in Fig. 1. The combined map reveals that the larger WWTPs and sewersheds (*i.e.*, WWTPs A, B, and C) tend to have higher housing prices. A subsequent analysis of the weighted average housing prices in the sewersheds of WWTPs A through H verified this trend (see Table S1 in the SI).

Socioeconomic factors have been correlated to different diseases (Jones et al., 2008; Ahn et al., 1998; Van Boeckel et al., 2014), a situation which leads to different pharmaceutical consumption and prescription preferences in different areas (Beuscart et al., 2017; Ramay et al., 2015; Van Boeckel et al., 2014). For this reason, correlations were explored between socioeconomic conditions and PPCP composition and load in raw wastewater for each sewershed. Specifically, the equivalent (ammonia-based) population density and housing price were employed as primary socioeconomic factors (Chen et al., 2011; Glaeser and Gyourko, 2018; Knoll et al., 2017). Fig. 5 demonstrates positive linear relationships for population density (*i.e.*, R = 0.93, $p < 0.01$) and

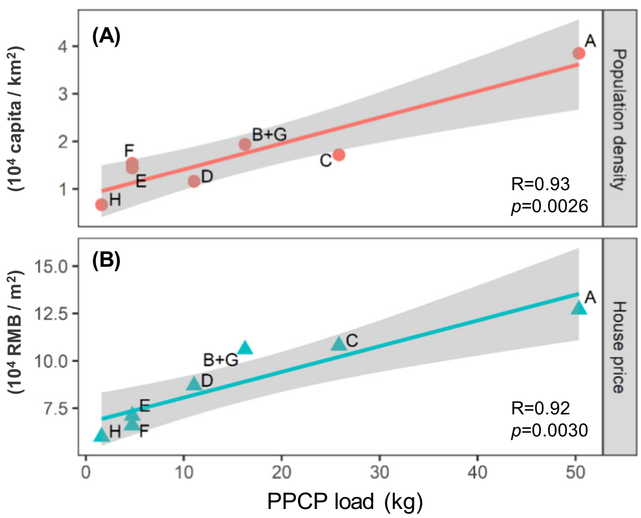

Fig. 5. Linear relationships between (a) population density and (b) housing prices with the PPCP load in raw wastewater from the eight WWTPs. The grey shading represents the 95% confidence band. The PPCP loads for WWTPs B and G were combined due to challenges associated with deconvoluting the sewersheds.

housing cost (*i.e.*, R = 0.92, $p < 0.01$) with the overall PPCP load in raw wastewater from the corresponding sewershed. Notably, the difference in housing prices is reflected by the distance between points in Fig. 5. For example, the housing price in the sewershed of WWTP A is ~18,000 RMB/m^2 higher than in the sewershed of WWTP C and, accordingly, the PPCP load in WWTP A is almost two times that of WWTP C. On the other hand, the sewersheds for WWTPs E and F, which exhibit similar real estate values, are effectively co-located in Fig. 5. The geographical proximity of WWTP B and G makes it difficult to deconvolute the individual sewersheds. For this reason, the B + G data point includes two sewersheds and, according to the cluster analysis in Fig. 4, the corresponding WWTPs exhibit different PPCP concentration profiles; therefore, combination of the associated socioeconomic data may compromise the cluster analysis.

3.2.3. Implications for wastewater-based socioeconomics

The differences in PPCP composition for raw wastewater in the eight WWTPs suggest similar pharmaceutical consumption patterns in the Group 1, 2, and 3 sewersheds (see Fig. 4). The reason that sewersheds clustered in a particular group may stem from a number of PPCP consumption factors: doctor/patient prescription preferences; patient age and sex distribution; pharmaceutical availability; local policy; and/or, mobility of population into and out of the sewershed (Regitz Zagrosek, 2012; Zhong et al., 2013). Socioeconomic factors affect many of these parameters (Beuscart et al., 2017; Ramay et al., 2015; Van Boeckel et al., 2014). However, pharmaceutical loads measured in wastewater are impacted by complex sources (*e.g.*, mobile populations) and fate mechanisms. For these reasons, the influence of individual factors cannot be identified without exhaustive study. Nevertheless, the overall relationships between PPCP loads and socioeconomic factors identified in this work highlight the possibility that PPCP levels are not only determined by physicochemical characteristics, but also demographic factors. This work, therefore, establishes opportunities to further extend wastewater-based epidemiology strategies in mega cities.

The correlations of housing prices and population density with PPCP mass loads provide additional evidence of the approach employed in this work. Such phenomena indicate that socioeconomic development indices (*i.e.*, housing price and population density) not only agree with each other, but also correspond to pharmaceutical composition patterns observed in Beijing wastewater. More importantly, the wastewater-based epidemiology results were consistent with socioeconomic characteristics, highlighting potential application of social/economic approaches in mega cities. Given the major impacts of mega cities on the adjacent environment, further efforts are needed to refine this approach given the specific challenges of working in these large, complex cities.

3.3. Limitations of the approach

This study is the first attempt to investigate antibiotic consumption and prevalence of use patterns with wastewater-based epidemiology. The innovative wastewater-based epidemiology strategy was also applied to explore potential relationships between PPCP consumption and socioeconomics. While this work advances the application of wastewater-based epidemiology into new and exciting fields for a mega city, certain limitations are noted with respect to interpretation of results and generalization of the reported approach. Wastewater sampling occurred over representative 24 h periods for each WWTP in the flu season; however, extended sampling campaigns are recommended to better understand antibiotic consumption and prevalence of use throughout different seasons. Long-term field measurements of PPCP concentrations in the sewer system are also needed to validate chemical stability and suitability as epidemiological biomarkers. Nevertheless, these large-scale, long-term efforts are temporally- and economically-expensive, and so optimization of sampling plans is required to pragmatically incorporate wastewater-based socioeconomic analysis. Hospitals were not considered major sources of pharmaceuticals in municipal wastewater (Le Corre et al., 2012; Ort et al., 2010); however, the well-constrained boundaries of hospitals (*e.g.*, pharmaceutical orders, drug consumption, number of patients, *etc.*) may provide important insight for future wastewater-based epidemiology studies. For similar reasons, application of wastewater-based epidemiology in small/isolated regions has achieved significant results due to the readily available data on population and pharmaceutical sales (Devault et al., 2018). In urbanized regions with mobile populations, such data contain more uncertainty, but we believe that wastewater-based epidemiological approaches still have merit and provide critical information on contaminant sources. Future investigations must also consider critical ethical issues, such as confidentiality, non-maleficence, and beneficence (Prichard et al., 2014), within the scope of wastewater-based epidemiology and socioeconomic studies. Although other sources of PPCPs in raw wastewater were not an issue in Beijing, landfill leachate and industrial wastewater could influence the results of wastewater-based epidemiological and socioeconomic analysis in other regions (Sui et al., 2017).

4. Conclusions

In this study, wastewater-based epidemiology was used to investigate antibiotic consumption in Beijing, China. Sewershed population was estimated using the NH_4-N biomarker, and the population-normalized mass load of 21 antibiotics was estimated to be 5.9 g d^{-1} (10^4 capita)$^{-1}$. After correcting for human metabolism and sewer losses, the average consumption of ten commonly used antibiotics was calculated to be 21.9 g d^{-1} (10^4 capita)$^{-1}$. These results are approximately 70% lower than estimates from previous studies, which were carried out shortly after implementation of strict antibiotic control policies in China. The wastewater-based epidemiological approach was also used to estimate the prevalence of antibiotic use in the flu season and, thereby, inform potential antibiotic misuse. Monte Carlo simulations indicated a 95% confidence interval of 1.42‰ to 3.65‰ for antibiotic prevalence of use. PPCP excretion factors, NH_4-N emission coefficients, and sewer loss rates were the most sensitive factors for calculation of the prevalence of antibiotic use, suggesting that further work is needed to improve these regionally-influenced variables. Correlations were established between socioeconomic factors and wastewater-based epidemiology estimates of PPCP consumption for sewersheds with comparable characteristics, housing price, and population density. Overall, this study suggested the potential application of wastewater-based epidemiology as an effective tool to inform and improve sampling plans for PPCP analysis and to elucidate PPCP consumption patterns between sewersheds of mega cities due to socio-economic drivers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.01.061.

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