Levels of six antibiotics used in China estimated by means of wastewater-based epidemiology
Su-Fen Yuan, Ze-Hua Liu, Ri-Ping Huang, Hua Yin and Zhi Dang

ABSTRACT
Due to lack of proper regulation, information about antibiotics consumption in many countries such as China is difficult to obtain. In this study, a simple method based on wastewater-based epidemiology was adopted to estimate their usage in four megacities of China. Six antibiotics (norfl Rox, ofloxacin, sulfamethoxazole, trimethoprim, erythromycin and roxithromycin), which are the most frequently consumed antibiotics in China, were selected as the targets. Based on our results, Chongqing had the largest total annual consumption of the selected six antibiotics among the four megacities, followed by Guangzhou, then Hong Kong, with Beijing having the least, with values of 4.4 g/y/P, 4.0 g/y/P, 1.6 g/y/P, and 1.3 g/y/P, respectively. Compared with the daily consumption per capita in Italy, the estimated consumption levels of the selected six antibiotics in four cities of China were 12–41 times those of Italy. Our results suggested that the consumption of antibiotics in China was excessive.

INTRODUCTION
Antibiotics have been widely used for disease treatment or growth promotion in livestock. The global annual consumption of antibiotics has been estimated to be 100,000–200,000 tons, among which over 25,000 tons was consumed in China (Xu et al. 2007a). The latest research based on market survey revealed that the total usage of 36 antibiotics in China was 92,700 tons in 2013, which is nearly a four-fold increase within the past 6 years (Zhang et al. 2015). Research has indicated that antibiotics are poorly absorbed in the body, and about 30–90% of these compounds are excreted unchanged in urine or feces, and then flow into the domestic sewage system (Sarmah et al. 2006). Antibiotics in influent and effluent of municipal wastewater have been widely detected and reported (Gobel et al. 2005; Karthikeyan & GMeyer 2006; Gao et al. 2012a).

Due to incomplete removal of antibiotics by wastewater treatment plants (WWTPs), they have been found with high concentrations in our environment including surface waters (Jiang et al. 2011, 2013; Gao et al. 2012b), sediments (Yang et al. 2010; Zhou et al. 2011) and soils (Schlusener et al. 2003; Stoob et al. 2006). With the wide distribution of antibiotics in the environment, they may pose potential risks to humans and wildlife and one of the biggest concerns is the increased antibiotic resistance in microorganisms under the long-term exposure of these antibiotics. It was reported (Cardo et al. 2004; Klevens et al. 2007) that about 98,000 patients die each year from hospital acquired infections in US, a notable increase from 13,300 deaths in 1992. For this reason, many efforts have been paid to counter the challenge (Baquero et al. 2008; Martínez 2008; Allen et al. 2010). As a basic but important parameter, the consumption levels of antibiotics in a specific region or country urgently needs to be known. However, such information in China is rare, and the annual consumption of antibiotics is difficult to obtain due to lack of proper regulation of drugs. Antibiotics administration in China differs for different antibiotics. For example, in Hong Kong, antibiotics for community acquired pneumonia is administered topically, while ofloxacin (OFL) and erythromycin (ERY) are administered systemically (Leung et al. 2012). In mainland China, most antibiotics are not prescription medicines, and they can be directly purchased by individual consumers from pharmaceutical stores.

To overcome this difficulty, there is a method called wastewater-based epidemiology which is based on the fact...
that traces of almost everything we consume are excreted unchanged or as a mixture of metabolites in our urine and/or feces, which ultimately ends up in municipal WWTPs. This approach has been successfully used to calculate the consumption of illicit, or excretion of natural, estrogens (Liu et al. 2009, 2015a, b; Bijlsma et al. 2014; Castiglioni et al. 2014; Senta et al. 2014). Therefore, the main objective of this study is to estimate the consumption levels of antibiotics in four megacities of China with the wastewater-based epidemiology, with the purpose of understanding their usage status in China.

EXPERIMENTAL

Target antibiotics

The antibiotics fluoroquinolone, macrolide and sulfonamide groups are the most frequently used antibiotics in China, contributing to approximately 15%, 20% and 12% of the total amount of antibiotics used for human and livestock purposes, respectively (Xu et al. 2007b). Macrolides have been reported not to be removed from wastewater by WWTPs (Xu et al. 2007b; Gulkowska et al. 2011; Gao et al. 2012a). Therefore, five antibiotics, norfloxacin (NOR), OFL, sulfamethoxazole (SMX), roxithromycin (ROX), and ERY, belonging to the above three groups were selected. In addition, SMX is commonly used as part of a synergistic combination with trimethoprim (TMP), thus TMP was also selected. In total, these six antibiotics were chosen as the targets. Some basic properties of these selected antibiotics are summarized in Table 1.

City information of the selected municipal WWTPs

With a thorough reference survey, there were in total 12 municipal WWTPs in China which reported the concentrations of antibiotics in wastewater, simultaneously, the information about served populations (SP) and wastewater flow (WF) rates was also available. These WWTPs belong to four cities of China as shown in Figure 1. Beijing is the capital of China, which is located in north China and has a typical monsoon climate in the North Temperate Zone. Chongqing is located in south-west China, while Guangzhou and Hong Kong are located in south China; all three cities belong to the typical subtropical monsoon climate. Due to regional differences, their average monthly temperature changes are different, take 2014, for example, as shown in Figure 2, Beijing has evident cold seasons between December and February, while Guangzhou and Hong Kong have long periods with hot weather.

Estimation method for antibiotics consumption

The daily consumption of antibiotics per capita $U$ (μg/d/P) is calculated using Equation (1):

$$U = \frac{C \times Q}{P \times E}$$

where $C$ is the influent concentration of antibiotics in municipal wastewater (μg/m³), $Q$ is the daily flow rate of wastewater (m³/d), $P$ is the SP of each sewage treatment plant, and $E$ is the excretion ratio of antibiotics after intake. All published data available were collected and are summarized in Table 2, the

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CAS no.</th>
<th>$K_{ow}$</th>
<th>$K_{oc}$</th>
<th>Treatment</th>
<th>Excreted factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOR</td>
<td>70458–96–7</td>
<td>1.744 ± 0.831</td>
<td>1.0</td>
<td>Urinary tract infections</td>
<td>0.30a</td>
</tr>
<tr>
<td>OFL</td>
<td>82419–36–1</td>
<td>1.855 ± 0.875</td>
<td>2.15</td>
<td>Bacterial exacerbations of chronic bronchitis</td>
<td>0.80a</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMX</td>
<td>723–46–6</td>
<td>0.659 ± 0.409</td>
<td>7.22</td>
<td>Urinary tract infections</td>
<td>0.12b</td>
</tr>
<tr>
<td>Macrolides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROX</td>
<td>80214–83–1</td>
<td>2.842 ± 0.869</td>
<td>67.5</td>
<td>Respiratory tract, urinary and soft tissue infections</td>
<td>0.85a</td>
</tr>
<tr>
<td>ERY</td>
<td>114–07–08</td>
<td>1.909 ± 0.841</td>
<td>21.0</td>
<td>Infections caused by Gram-positive bacteria and mycoplasma</td>
<td>0.05a</td>
</tr>
<tr>
<td>Pyrimidine antibiotics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMP</td>
<td>738–70–5</td>
<td>0.594 ± 0.385</td>
<td>23.7</td>
<td>Urinary tract infections</td>
<td>0.80a</td>
</tr>
</tbody>
</table>

*Verlicchi et al. (2014).*

*Leung et al. (2012).*

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concentrations of antibiotics in wastewaters were all analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/(MS)). The daily consumption rate as a whole is shown in Table 2, \( U_a, \mu g/d/P \) is calculated using Equation (2)

\[
U_a = \frac{\sum_i U_i \times P_i}{\sum_i P_i} \tag{2}
\]

where \( U_i \) and \( P_i \) are the daily consumption rate based on one municipal wastewater and the corresponding SP, respectively. As shown in Table 2, the information about the SP and WF provided are always the designed values rather than the actual ones when the wastewater samples were collected. Meanwhile, the sampling type (ST) varies with composite sampling (C) and grabbing sample (G). These two differences may give some uncertainty. However, based on our previous work on natural estrogens, their predicted concentrations of natural estrogens in six municipal WWTPs were close to the measured values (Liu et al. 2015b). Therefore, this factor should not affect the validity of the prediction method.

RESULTS AND DISCUSSION

Based on the summary of the published data available, the daily consumption levels of antibiotics per capita among the four megacities of China varied greatly, as shown in Table 2. For antibiotics NOR and OFL, the average consumption in Beijing was the largest with a volume of 710 and 629 \( \mu g/d/P \), followed by Hong Kong with 328 and 169 \( \mu g/d/P \), then Chongqing with 264 and 169 \( \mu g/d/P \). Guangzhou had the lowest average consumption of 234
Table 2 | Concentrations of antibiotics in raw municipal wastewater and their estimated consumption levels in four cities of China

<table>
<thead>
<tr>
<th>Cities</th>
<th>SP</th>
<th>WF m³/d</th>
<th>ST</th>
<th>LOD (ng/L)</th>
<th>Concentration in raw municipal wastewater (μg/m³)</th>
<th>Daily consumption per capita (μg/d/p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NOR</td>
<td>OFL</td>
</tr>
<tr>
<td>Beijing</td>
<td>2,400,000</td>
<td>1,000,000</td>
<td>C</td>
<td>1.6–50</td>
<td>339</td>
<td>1208</td>
</tr>
<tr>
<td></td>
<td>814,000</td>
<td>200,000</td>
<td>–</td>
<td>0.5–37</td>
<td>775</td>
<td>1287</td>
</tr>
<tr>
<td></td>
<td>810,000</td>
<td>200,000</td>
<td>C</td>
<td>0.01–0.25</td>
<td>1813</td>
<td>2794</td>
</tr>
<tr>
<td></td>
<td>4,024,000</td>
<td>1,400,000</td>
<td>–</td>
<td>–</td>
<td>612</td>
<td>1446</td>
</tr>
<tr>
<td>As a whole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hong Kong</td>
<td>369,512</td>
<td>150,000</td>
<td>G</td>
<td>4.0–93</td>
<td>460</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>376,077</td>
<td>94,300</td>
<td>G</td>
<td>4.0–94</td>
<td>110</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>300,000</td>
<td>95,000</td>
<td>C</td>
<td>1.0–10</td>
<td>54</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3,500,000</td>
<td>1,377,000</td>
<td>C</td>
<td>1.0–10</td>
<td>263</td>
<td>368</td>
</tr>
<tr>
<td>As a whole</td>
<td>4,545,589</td>
<td>1,716,300</td>
<td>–</td>
<td>–</td>
<td>260</td>
<td>349</td>
</tr>
<tr>
<td>Guang zhou</td>
<td>80,000</td>
<td>30,000</td>
<td>G</td>
<td>1.0–10</td>
<td>229</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>1,050,000</td>
<td>400,000</td>
<td>G</td>
<td>1.0–10</td>
<td>179</td>
<td>359</td>
</tr>
<tr>
<td></td>
<td>380,000</td>
<td>100,000</td>
<td>–</td>
<td>0.52–6.0</td>
<td>92</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>425,000</td>
<td>74,400</td>
<td>–</td>
<td>0.52–6.0</td>
<td>649</td>
<td>634</td>
</tr>
<tr>
<td>As a whole</td>
<td>1,935,000</td>
<td>604,400</td>
<td>–</td>
<td>–</td>
<td>225</td>
<td>337</td>
</tr>
<tr>
<td>Chong qing</td>
<td>1,540,000</td>
<td>600,000</td>
<td>G</td>
<td>0.2–17.5</td>
<td>203</td>
<td>346</td>
</tr>
<tr>
<td>As a whole in China</td>
<td>12,044,589</td>
<td>4,320,700</td>
<td>–</td>
<td>–</td>
<td>361</td>
<td>724</td>
</tr>
</tbody>
</table>

*aXiao et al. (2008).  
bJia et al. (2012).  
cLi et al. (2013).  
dGulkowska et al. (2008).  
eXu et al. (2007b).  
fZhou et al. (2013).  
gYan et al. (2014).
and 132 μg/d/P. For SMX and ROX, Chongqing had the largest average consumption of 9531 and 185 μg/d/P, but the average consumptions in the other three megacities were mixed. The average consumption of SMX in Beijing was ranked the second largest with 1021 μg/d/P, while the corresponding consumption of ROX was the lowest with 38 μg/d/P. Hong Kong had the second largest average consumption of ROX with 69 μg/d/P, while its average consumption of SMX was the lowest with 78 μg/d/P. The average consumptions of SMX and ROX in Guangzhou were all ranked third at 455 and 47 μg/d/P. The order of the average consumption of ERY from the largest to the smallest was Guangzhou, Hong Kong, Chongqing, and Beijing, and the consumptions were 9958 μg/d/P, 3651 μg/d/P, 1981 μg/d/P, and 1091 μg/d/P, respectively. The consumption of TMP in Beijing was not available as its concentration in municipal wastewater of this district was not reported. Among the other three cities, Hong Kong had the greatest average consumption of TMP with 99 μg/d/P, followed by Chongqing with 38 μg/d/P, with Guangzhou having the lowest consumption of 32 μg/d/P.

The consumption discrepancies between different districts seem to have strong correlation with climate difference. For example, ERY is mainly used for treatment of infections caused by Gram-positive bacteria, anaerobic bacteria and mycoplasma purposes, which are common diseases more readily occurring in hot locations (Figure 2). On the other hand, SMX and OFL had higher consumption in Beijing, which are mainly used for bacterial exacerbations of chronic bronchitis and urinary tract infections, and such diseases more often occur in districts with cold weather (Figure 2). This insight is common sense, but no attention was paid to the fact that consumption of different antibiotics in different districts varies with different climates. To evaluate the potential risks of antibiotics, it is suggested that regional differences with climate discrepancy is considered, given that different antibiotics may pose different antibiotic resistance. Considering the total annual consumptions per capita of the six antibiotics, Chongqing had the largest total annual consumption among the four megacities, followed by Guangzhou, then Hong Kong, with Beijing having the lowest consumption, the results being 4.4 g/y/P, 4.0 g/y/P, 1.6 g/y/P, and 1.3 g/y/P, respectively (Figure 3).

In contrast to other countries, where all antibiotics are prescription medicines, most antibiotics are purchased as over-the-counter medicines in China. The potential consequence is that antibiotics in China might be excessively used. To give a rough profile on the excessive usage of antibiotics, the estimated consumptions in this study were compared with those in Italy. As can be seen in Table 3, the average consumptions of the selected six antibiotics per capita in the four studied cities were 12–41 times (25 times the average) of those in Italy (Verlicchi et al. 2014). Although people in different regions may use different drugs, the big difference between Italy and the four cities of China may still indirectly indicate the excessive usage of antibiotics in China. Owing to the above-mentioned potential adverse effects on the environment, efforts including strict regulation and proper guidance must be paid to change the unfavorable situation. According to the 2013 statistics from NBSPC (2014), the total population in China, was 1,360,720,000. Based on the estimated consumption per capita in Table 2, the calculated annual consumptions of antibiotics in China for NOR, OFL, SMX, TMP, ERY, and ROX were 215 t/y, 162 t/y, 1025 t/y, 25 t/y, 2235 t/y and 41 t/y, respectively. There is no corresponding statistical data on the annual consumption of antibiotics available, thus the estimated data here cannot be compared. It has been reported that the total sales of OFL in Chongqing was 294 kg in 2012 based on the total prescription amounts (Yan et al. 2014), but the corresponding estimated annual consumption in this study was 2,056 kg based on the estimated consumption per capita in Table 2. Our estimated value is seven times that of the reported value by the public agency of Shanghai Food and Drug Administration (Yan et al. 2014). The great discrepancy between this study and the statistical data may be explained by the fact that some released antibiotics from other medical sources, such as numerous private practitioners, were not included.
in the statistics, resulting in great underestimation of the statistics by the public agency.

### CONCLUSIONS

Information about consumption level of antibiotics is very important; however, the lack of such data is not only limited to China, but also to most other countries. This study introduced a simple but effective method based on the concentrations of antibiotics in raw wastewater called wastewater-based epidemiology, with which the consumptions of the six selected antibiotics (NOR, OFL, SMX, TMP, ERY and ROX), in the four megacities of China, were estimated. The results showed antibiotics consumption levels varied greatly among the four cities. Compared to those in Italy, the much higher estimated consumptions of antibiotics per capita in four megacities of China suggested that antibiotics in China were excessively used. Actions must be carried out to reduce the unnecessary usage.

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### REFERENCES


