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Characteristics of antibiotics and antibiotic resistance genes in Qingcaosha Reservoir in Yangtze River Delta, China

Ting Xu^{1,2}, Wanting Zhao¹, Xueping Guo^{1,3*}, Hongchang Zhang⁴, Shuangqing Hu⁴, Zhifeng Huang¹ and Daqiang Yin^{1,2*} 

Abstract

Background: Aquatic ecosystems are considered to be among the most important reservoirs of antibiotic resistance genes (ARGs). Drinking water sources could serve as critical points of ARGs transfer from environment to human. In this study, the distribution of antibiotics and ARGs across Qingcaosha Reservoir located in the Yangtze River Delta during three seasons was investigated to characterize the pollution patterns.

Results: Sulfamethoxazole, sulfamonomethoxine and penicillin G potassium salt were the dominant antibiotics in this reservoir. Sulfonamide resistance genes (*sul1* and *sul2*) were the most prevalent and predominant genes. Higher total relative abundance of the ARGs was detected in the site closest to the inflow than those in other sites. Overall, the concentrations of antibiotics in May were relatively lower than those in November and February. Correlation analysis indicated *sul1*, *ermB* and *mphA* had positive correlation with corresponding antibiotics in February and *sul1*, *sul2*, *ermB* and *mphA* was also positively correlated to *int1*.

Conclusion: In conclusion, antibiotics and ARGs were widespread in Qingcaosha Reservoir. Our results indicated that the drinking water reservoir might serve as gene reservoir for antibiotic resistance and mobile gene element *int1* can serve as a medium to contribute to the widespread of various ARGs. As the ARGs abundance was found to be higher in the inflow of the reservoir compared to the outflow, it is considered that reservoir could serve as a functional area contributing to the elimination or dilution of ARGs.

Keywords: Antibiotic resistance genes, Drinking water reservoir, Antibiotic residues

Background

Nowadays, we are in an era where antibiotics were widely used in clinic, livestock farms, aquaculture and other fields for disease treatment, prophylaxis and growth promoters [1, 2]. Most antibiotics cannot be completely absorbed by humans and animals after intake, of which nearly 25–75% were discharged via urine and feces [3]. The antibiotic residues could then enter into

the environment and pose a potential risk to ecological microbes and even human health [1]. A growing amount of studies suggested that irrational use and residues of antibiotics possessed long-term selective pressure on microbes and could induce the spread of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs)[4–7]. Especially for antibiotics such amoxicillin and erythromycin which could be used both in agriculture and clinic, their abuse in animals would cause more a serious situation. Once bacteria have developed resistance to them, it might lead to the failure of infection treatment[8–10].

*Correspondence: xuepingg@tongji.edu.cn; yindq@tongji.edu.cn

¹ Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

Full list of author information is available at the end of the article

ARG was firstly proposed as an emerging environmental contaminant in 2006 and attracted growing global concern of environments and public health [11]. In recent years, due to the potential risk of ARGs in environment, numerous studies have been performed to assess the prevalence of various ARGs in livestock farms and aquaculture [12], clinical environment [13, 14], wastewater treatment plants [15], reservoirs [16] and even the outflow of drinking water treatment plants [17], surface water all over the world including Germany and Australia [18], Europe [8] and China [19–21]. Therefore, it is obvious that the aquatic environments played critical roles in the regional and global transmission of ARGs and could serve as an important reservoir of them.

Noteworthy, studies related to the survey of ARGs in drinking water source were not frequently reported. Drinking water usually originated from the surface water like rivers and lakes reservoirs which could be easily impacted by anthropogenic activities such as livestock and poultry breeding, agricultural and wastewater discharge [22]. In contrast, most of the reservoirs, often acting as main and important drinking water sources relied on an exogenous source, are located in areas with little human activities [23]. Due to the riverine input in source water reservoir, the spread and accumulation of ARGs to increase more serious ecological risk could be caused by some elements, such as some microorganism with antibiotic resistance in raw water [24]. Taken together, it is imperative to investigate the prevalence and distribution of ARGs and assess risk in the headwaters area of the reservoir to guarantee the safety of drinking water supply.

China is one of the largest producers and consumers of antibiotics worldwide, and the usage was estimated to exceed 6 times than the consumption in the UK and most of northern Europe [25, 26]. In recent years, some studies

revealed that the reservoir system could influence the residues and composition of antibiotics exported from the river [27]. Until now, several general studies on antibiotics and ARGs in surface water from the Yangtze estuary were published [20, 28–30]. However, information about the spatial and time variation, and a comprehensive understanding about a specific area is still lacking. Qingcaosha Reservoir is the largest impoundment reservoir of river in China, which covers nearly 66.15 km² with the effective capacity of 435 million m³. The reservoir freshwater stored and salt avoided officially opened in 2011 with a daily water supply of 7.19 million m³ and the beneficial population of more than 13 million. In this present study, we collected 24 water samples from eight sites of Qingcaosha Reservoir in 3 different months to conduct a comprehensive study. For these aspects showed above, the study aimed to (1) evaluate the frequency and occurrence of 19 antibiotics, 12 ARGs and *intI1* in this reservoir; (2) characterize and discuss the spatial and change over time distribution of antibiotics and ARGs; (3) explore the potential linkage among antibiotics, the corresponding ARGs and mobile genetic elements (MGEs). This study will help us better understand the prevalence and fate of ARGs in reservoir systems and provide data support to evaluate the ecological risks of antibiotic resistance in aquatic environment.

Materials and methods

Study area and sampling campaign

Qingcaosha Reservoir is located at the mouth of the Yangtze River south branch and north of Changxing Island. Eight sites across the Qingcaosha Reservoir were studied as shown in Fig. 1 for an overall monitor of the reservoir. Water samples were collected about 0.5 m below the water surface from each sampling site and

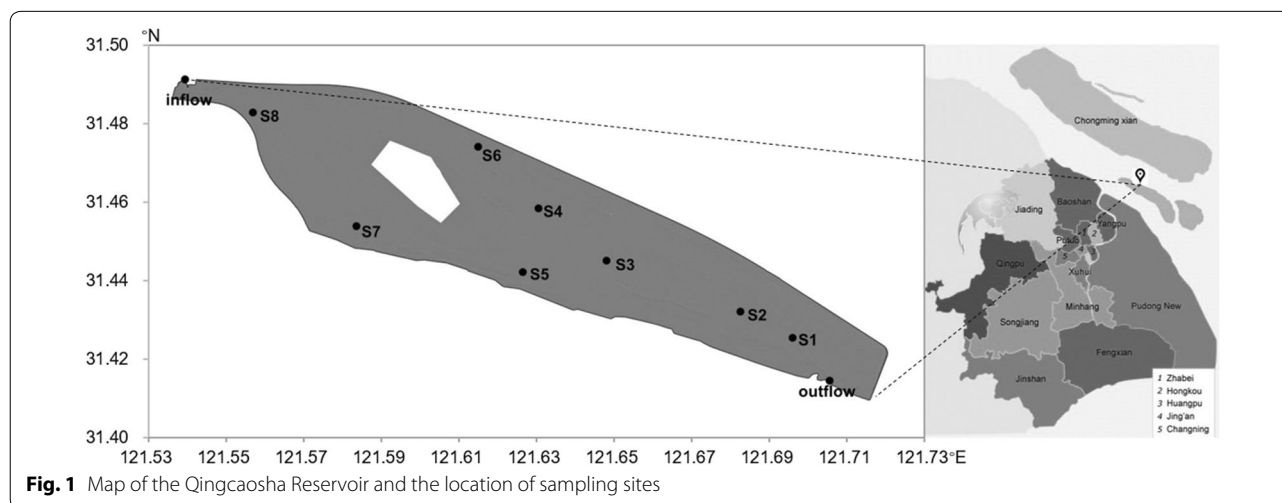


Fig. 1 Map of the Qingcaosha Reservoir and the location of sampling sites

stored in two sets of different containers. Specifically, 1.5 L water was stored in sterile polyethylene bottles for DNA extraction and 1 L water was stored in brown glass bottles for antibiotics analysis. Appropriate amount of hydrochloric acid was added into the glass bottles to a final pH less than 2 to inhibit microbial activity. Then the water samples were transported to the laboratory on ice and stored at 4 °C before treatment within 24 h. Sampling campaigns were carried out in November 26th, 2018 with air temperature (AT) ranging from 12 to 17 °C, February 24th, 2019 (AT: 6–12 °C) and May 31st, 2019 (AT: 18–25 °C), respectively. The precipitation in November 2018 and February 2019 was relatively low, the sampling campaign in May 2019 was characterized by heavy rainfalls.

Determination of antibiotic residues

Nineteen antibiotics belonging to 6 classes were studied, including seven sulfonamides—sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfachlorpyridazine (SCP), sulfamonomethoxine (SMM), sulfisoxazole (SIZ), sulfamethoxypyrimidine (SMP), and sulfadimidine (SIZ), two tetracyclines—oxycycline (OTC), chlortetracycline (CTC), two β -lactams—amoxicillin trihydrate (AMC) and penicillin G potassium salt (PenG), three macrolides—azithromycin (AZM), clarithromycin (CLA), and spiramycin (SPM), one aminoglycoside—tilmicosin (TILM) and four quinolones—ciprofloxacin hydrochloride (CIP), fleroxacin (FLX), ofloxacin (OFX), and enrofloxacin (ENR). The above-mentioned antibiotic standards were purchased from Dr. Ehrenstorfer (Germany). A mixture of SMX- $^{13}\text{C}_6$, trimethoprim (TMP)-D₃, tetracycline (TET)-D₆ (the first three standards were purchased from Dr. Ehrenstorfer, Germany), FLX-D₅, furazolidone (FZD)-D₄ (these two were from Witega, Germany), olaquinox (OQX)-D₄ and AMC- $^{13}\text{C}_6$ (the last two were from Cambridge Isotope Laboratories, UK) served as internal standards for quantification. After activating Oasis HLB column (Waters, USA), loaded the water samples onto the column at a speed of 5 mL/min. After loading, the column was washed with 6 mL ultra-pure water and 6 mL 5% methanol–aqueous solution dried by air for 5 min. Finally, the column was eluted with 10 mL elution buffer. The eluted solution was blow-dried with nitrogen at 35 °C, and the volume was fixed to 1 mL with methanol–aqueous solution with a volume concentration of 70% and the solution was filtered through a 0.22- μm PTFE needle filter to a brown injection bottle for testing. All the above treatments were conducted in triplicate, and water samples without adding antibiotics were set as blank.

Antibiotics were quantified by liquid chromatography–triple quadrupole mass spectrometer (Waters, USA).

The mobile phase consisted of (A) acetonitrile and (B) ultra-pure water with 0.1% (v/v) formic acid; gradient elution program operated as follows: 0–2.2 min, 16% A; 2.2–2.5 min, 16–95% A; 2.5–5.5 min, 95% A; 5.5–6.0 min, 95–16% A; 6.0–10.0 min; 16% A. Chromatographic separation of the analyses was conducted with BEH C18 column (2.1 \times 100 mm, 1.7 μm , Waters, USA) maintained at 35 °C. Mass spectrometric analyses were performed in a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source that operated in the positive ionization mode.

Mixed standard working fluids with antibiotic concentrations ranging from 1.37 to 500.00 $\mu\text{g/L}$ were configured for detection and analysis. The standard curves were obtained with a correlation coefficient greater than 0.993, and the detection limit was within 0.003–1.230 ng/L .

In the standard recovery experiment, the standard addition level of samples was 100 ng/L and 200 ng/L and the recovery rate ranges from 69.4 to 118.5%. The relative standard deviation is less than 10%.

DNA extraction

The water samples (500 mL each) were filtered through 0.22 μm polytetrafluoroethylene membrane filters (Millipore, USA) to capture bacteria. The collected membrane filters were stored at –20 °C before subsequent DNA extraction. Total DNA was extracted directly from the membranes by using DNeasy PowerSoil Kit (Qiagen, Germany) according to the manufactures' instructions with a final elution volume of 100 μL . DNA concentrations were determined using QubitTM 4 Fluorometer with QubitTM dsDNA HS Assay Kit (Invitrogen, USA). All extracted DNA samples were stored at –40 °C prior to analysis.

Quantification of ARGs

The presence of 12 ARGs conferring resistance to the six antibiotic classes and class I integrase gene (*intI1*) we monitored as well as 16S rRNA gene were investigated. The ARGs included sulfonamide resistance genes (*sul1* and *sul2*), tetracycline resistance genes (*tetC* and *tetW*), β -lactam resistance genes (*ampC*, *OXA-1*, and *TEM-1*), macrolide resistance genes (*ermB* and *mphA*), aminoglycoside resistance genes (*aacC4* and *strA*), and quinolone resistance genes (*qnrS*).

The target genes were quantitatively detected with a 7500 real-time PCR System (Applied Biosystems 7500, Thermo Scientific, USA). The real-time qPCR reaction system (20 μL) included 10 μL of 1 \times SensiMixTM SYBR[®] No-Rox Kit (Bioline, USA), 0.5 μL each of the forward and reverse primers (Sangon Biotech, China), 1 μL of DNA templates, 8 μL of the ddH₂O. The PCR program was as follows: initial denaturing for 5 min at 95 °C,

followed by 40 cycles including denaturing at 95 °C for 30 s, annealing at different annealing temperatures (Additional file 1: Table S1) for 1 min, extension for 30 s at 72 °C. Melting curve analysis (55–95 °C, heating rate: 0.5 °C/min, hold 30 s) was conducted for the validation of qPCR product specificity. To avoid the possible inhibition of PCR reaction, the diluted DNA template was analyzed for each sample. The primer sets used for the PCR amplification of ARGs are shown in Additional file 1: Table S1. Double distilled H₂O was used as negative control for each qPCR array, and all samples were run in triplication. The standard curves were established followed as our previous study [21].

Statistical analysis

The data were organized in Microsoft Excel 2019, and diagramming was performed with Originpro 9.1 software and Heml: Heatmap Illustrator. The correlation and statistical differences were analyzed using SPSS 22.0 (IBM, USA), one-way ANOVA following a post hoc Dunnett’s test at a *p* < 0.05 level of significance.

Results

Antibiotic concentrations

Fourteen of the 19 target antibiotics were detected in all samples (Fig. 2), with SIZ, SDD, CIP, OFX and TILM not detected in any samples (Additional file 2: Table S2). Among different classes of antibiotics, sulfonamides showed the highest detection frequencies (61.3%) and aminoglycosides (not detected) the lowest in all samples. The detection frequencies of tetracyclines, β-lactams, macrolides and quinolones were 29.2%, 64.6%, 45.1% and 16.7%, respectively. For sulfonamides, SMX and SMM could be found in all samples, and SDZ with a high detection rate of 95.8%. SMP and SCP could be detected more frequently in November and February than in May. Both OTC and CTC had low detection frequencies in February, but very differently, the concentration of CTC (36.32 ng/L) was over 44 times higher than that of OTC (0.81 ng/L). For β-lactams, PenG could be detected in all samples while the detection frequency of AMC was only 29.2%. The detection frequencies of macrolides were CLA (58.3%) > AZM (54.2%) > SPM (20.8%). For quinolones, ENR could be detected in November (1.94 ng/L) and May (2.22 ng/L) and FLX can be only detected in site S8 in November (4.5 ng/L). AZM and CTC were barely detected in November, but were frequently present in February. Quite the opposite, SPM, ENR and OTC were detected in November and more frequently in February.

The mean concentration of SMX was much higher in November (25.07 ng/L) than in February (3.07 ng/L) and May (5.79 ng/L), while the average contents of SDZ and SMM were close in all three sampling time. The

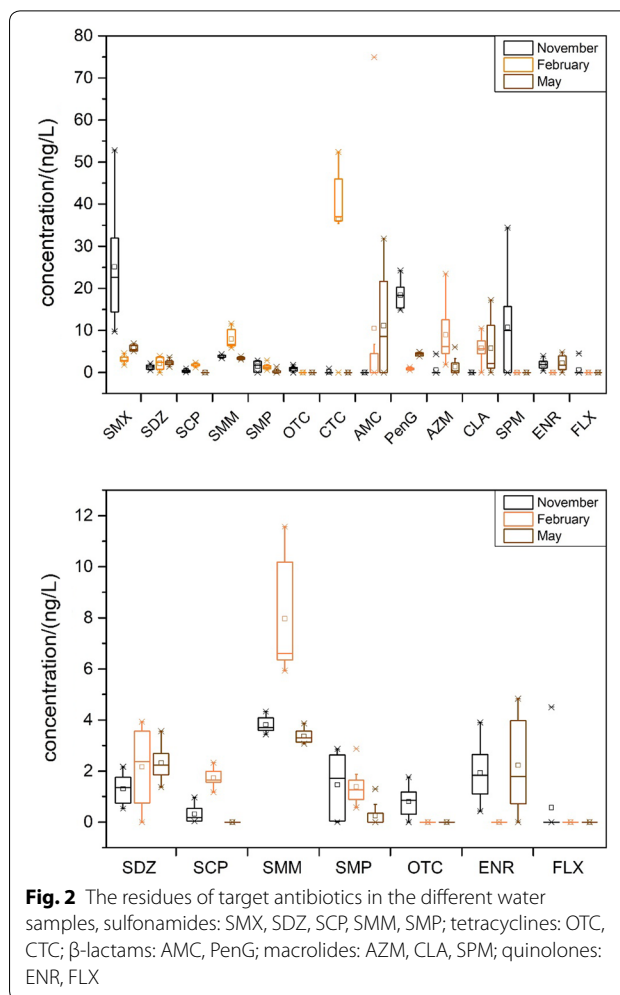


Fig. 2 The residues of target antibiotics in the different water samples, sulfonamides: SMX, SDZ, SCP, SMM, SMP; tetracyclines: OTC, CTC; β-lactams: AMC, PenG; macrolides: AZM, CLA, SPM; quinolones: ENR, FLX

concentration of PenG in November (18.37 ng/L) was significantly higher than those in February (1.14 ng/L) and May (4.33 ng/L). AZM and CLA were only detected in February (9.39 ng/L, 5.98 ng/L) and May (1.49 ng/L, 6.51 ng/L). The ENR was always in a low residue ranging from ND to 4.83 ng/L. SMX and PenG were much higher in November than the other two sampling activities. For the detection frequencies and concentrations, SMX, SMM and PenG were dominant antibiotic residues.

Occurrence and distribution of ARGs

The studied ARGs and *intI1* were all detected in Qingcaosha Reservoir. A summary of detection frequencies of 12 ARGs and *intI1* in water samples is shown in Table S3. The genes *sul1*, *sul2*, *tetC*, *TEM-1*, *mphA*, *strA*, *qnrS* and *intI1* were detected in all water samples collected in different sampling campaigns. There was little difference among different sampling activities both for *sul1* and *sul2*. The *tetW* was detected less frequently in November (12.5%) and February (12.5%) than in May (75%).

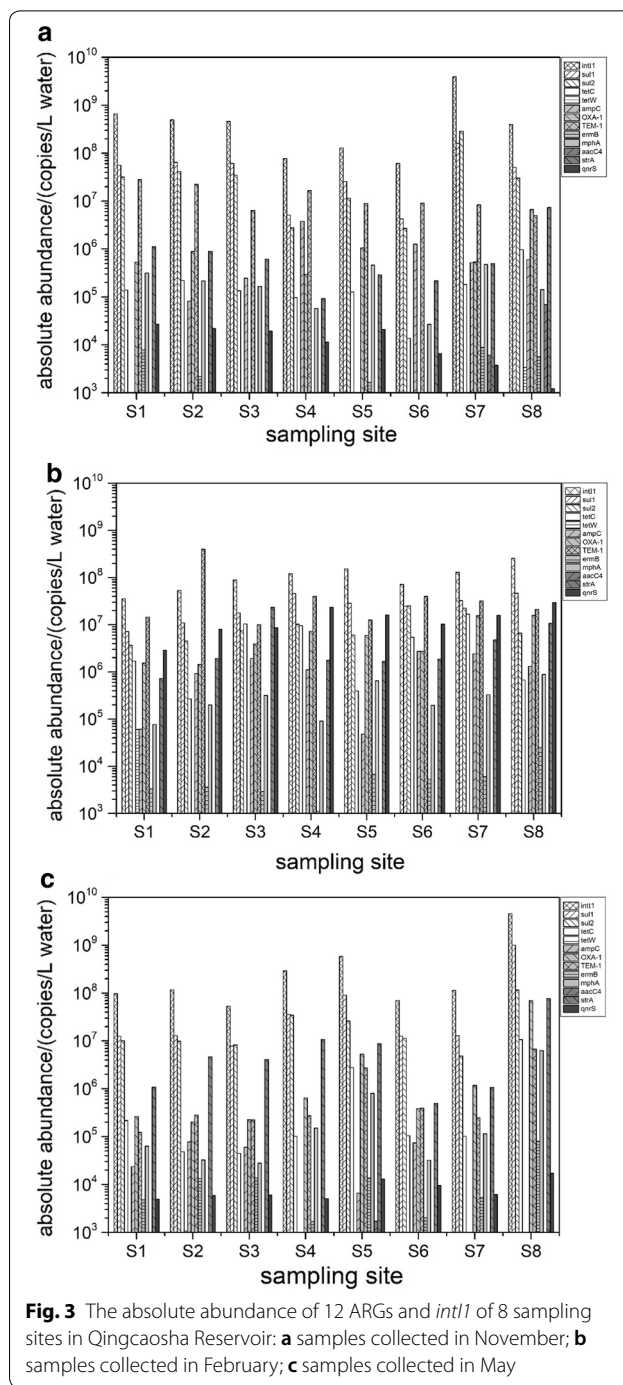
The β-lactam resistance genes were all detected in February, gene *ampC* was less detected in November (75%) and May (50%). The gene *OXA-1* was more frequently detected in February and May (100%) than November (75%). The gene *ermB* was detected in all of the samples in February and May, while 62.5% detected in November. In this study, the *aacC4* was less detected.

Figure 3 summarizes the abundance of the different subtypes of target genes at all sampling sites in different months of Qingcaosha Reservoir. Generally, the abundance of detected ARGs ranged from 1.21×10^3 to 2.87×10^8 copies/L water in November, from 1.12×10^3 to 3.97×10^8 copies/L water in February and from 2.03×10^3 to 1.01×10^9 copies/L water in May, respectively.

The *sul1* and *sul2* were predominant in terms of the 12 ARGs, up to 1.01×10^9 copies/L water and 2.87×10^8 copies/L water, respectively. For the two tetracycline resistance genes, *tetC* was prevalent of the average concentration was 2.36×10^5 copies/L water in November, 5.65×10^6 copies/L water in February and 1.76×10^6 copies/L water in May. In contrast, gene *tetW* was rarely detected. There was no much difference in the concentration of two macrolide resistance gene *mphA* in different water levels caused by rainfall, basically ranged from 2.69×10^4 to 8.78×10^5 copies/L water, while *ermB* was at a low concentration near the limit of detection. For aminoglycoside resistance genes *strA* and *aacC4*, the former was detected ranging from 9.25×10^4 to 7.36×10^6 copies/L water in November, from 7.22×10^5 to 2.33×10^7 copies/L water in February and from 4.99×10^5 to 1.09×10^7 copies/L water in May. In contrast, gene *aacC4* ranged from 1.73×10^3 to 6.92×10^4 copies/L water in November and February and not detected in February. The *qnrS* was 2–3 orders of magnitude higher in February than the other 2 months, which ranged from 2.88×10^6 to 2.95×10^7 copies/L water. As the result showed in this study, the mobile genetic gene *intI1* possessed the highest abundance ranging from 6.11×10^7 to 6.56×10^8 copies/L water in November, from 3.53×10^7 to 2.56×10^8 copies/L water in February and from 5.31×10^7 to 4.55×10^9 copies/L water in May.

Variations in the ARGs pattern

To eliminate the influence of the efficiency of DNA extraction and background interference caused by the microorganisms in aquatic environment, the relative abundance (defined as the absolute number of genes normalized to the absolute number of 16S rRNA) was applied to analyze the distribution characteristics of various ARGs among the sampling sites at different times (Fig. 4): sampling temporal (Fig. 4b) and spatial (Fig. 4c)



differences in water samples from the Qingcaosha Reservoir.

Figure 4b summarizes the total relative abundance of the 12 ARGs and *intI1* in November, February, and May from 8 sampling sites in Qingcaosha Reservoir. For ARGs and *intI1*, the total relative abundance of *intI1* was the highest of 4.21×10^{-1} , and the sulfonamide resistance genes *sul1* and *sul2* were predominant among 12 ARGs

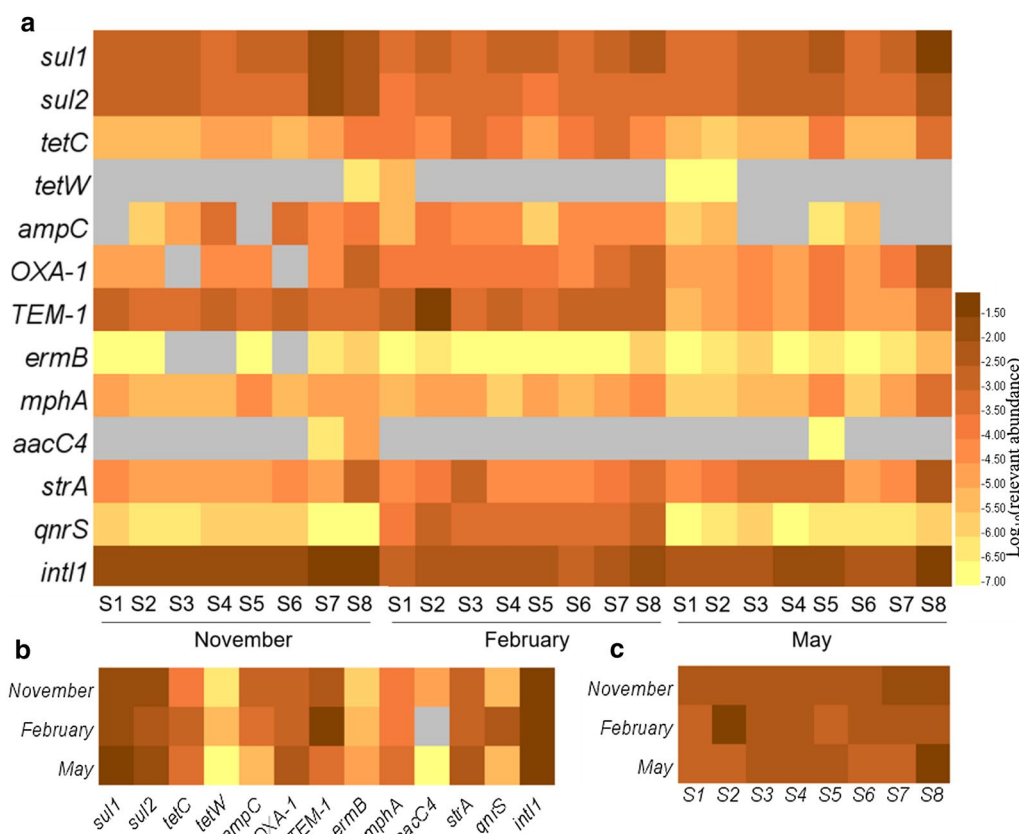


Fig. 4 Heatmap of the relative abundance of target genes: **a** relative abundance of all target genes; **b** variation of summing total target genes in the 8 sampling sites over three sampling campaigns; **c** distribution of summing total 12 ARGs in the 8 sampling sites. The grey area represented the gene was not detected

range from 3.08×10^{-2} to 1.12×10^{-1} and 4.12×10^{-3} to 2.99×10^{-2} . Two of the differently encoded genes *tetC* and *tetW*, the concentration of *tetC* was much higher than *tetW*. And the same for the aminoglycoside resistance genes, the detection of *strA* was 5 orders of magnitude higher than the *aacC4*. Among the *TEM-1*, *ampC* and *OXA-1*, the total concentration was usually between 10^{-3} and 10^{-2} . Obviously, the overall tendency of abundance of *mphA* and *ermB* was consistent, which was May > February > November. The *qnrS* was specially caused by the great gaps in February and others.

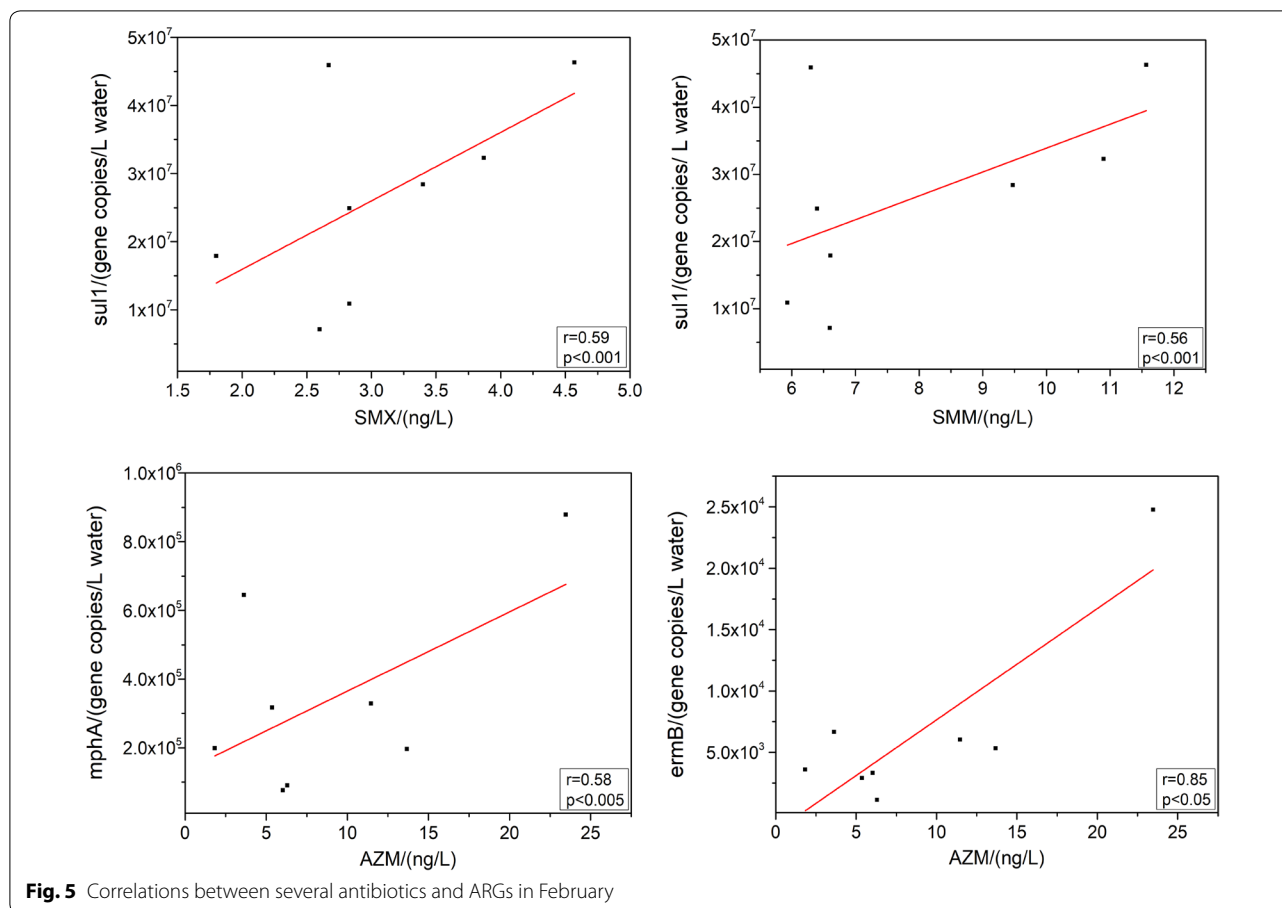
Obviously, Fig. 4c shows that the total relative abundance of 12 ARGs with site S8 was the relatively high detected (1.88×10^{-2} , 9.63×10^{-3} and 8.70×10^{-2}) among the 8 sampling locations. In particular, there were some high values in the S2 of February (6.6×10^{-2}) and S7 of November (2.84×10^{-2}) that were ten times as much as in other sampling campaigns. For ARGs in May, it was apparent that the highest relative abundance of all genes in site S8 and even the *sul1*, *OXA-1* and *mphA* in S8 were higher 2–3 magnitude orders than other sites.

Correlation analysis between abundance of ARGs

We carried out a correlation analysis among the absolute concentrations of ARGs, gene *intI1* and the antibiotics to which they confer resistance to identify potential links between all variables. A significant positive correlation between the *intI1* and ARGs, or several antibiotics and their corresponding ARGs in February was observed (Fig. 5 and Additional file 1: Fig. S1). For instance, there were correlations between the SMX and the *sul1* ($r=0.59$, $p<0.001$), SMM and the *sul1* ($r=0.56$, $p<0.001$), AZM and the *mphA* ($r=0.58$, $p<0.005$), AZM and the *ermB* ($r=0.85$, $p<0.05$) in February. However, the correlations between antibiotics and ARGs in the other two sampling times or for all sampling activities together were not observed.

Discussion

Antibiotics and ARGs were commonly found in Qingcaosha Reservoir across different sampling times and sampling sites. As a rather important source of drinking



water in the Yangtze River Delta, the investigation of Qingcaosha Reservoir could provide us general information of ARGs pollution in this area. The prevalence of ARGs and antibiotics imposed potential risks on humans as the modern drinking water treatment plants were not designed to remove these pollutants. Our result indicated that the reservoir might serve as a gene reservoir for antibiotic resistance, and the presence of ARGs conferring all kinds of antibiotics in the environment was likely related to human activities, such as fecal pollution and antibiotic residues [31, 32].

The concentrations of antibiotics in this study were at a medium level compared with previous studies as follows. Not surprisingly, sulfonamides again showed the highest detection frequencies as our previous studies [17, 20]. Despite the usage of sulfonamides, good compound stability and hydrophilia could support its transportation for a long distance in the aquatic environment [18]. The SMX concentration was comparable to the Guanting Reservoir in north China with the mean concentration of 6.7 ng L⁻¹ [33] and much lower than the Three Gorges Reservoir Area, in which the mean concentration was 13.65 μg L⁻¹ [34]. And it was

much higher than the Taihu Lake with a mean concentration of 0.355 ng L⁻¹ [35]. The detection frequency and concentration of OTC (25%, 0.81 ng L⁻¹) in this study were generally lower than previous reported in Guanting Reservoir (92.9%, 15.2 ng L⁻¹) and the lower Yangtze River located in southern Jiangsu Province in dry season (27.2%, 18.98 ng L⁻¹) [33, 36]. For CTC of low detection frequency, but with the mean determined concentration (36.32 ng L⁻¹) was much higher than in Guanting Reservoir (lower than 10 ng L⁻¹) and the lower Yangtze River located in southern Jiangsu Province in dry season (lower than 5.86 ng L⁻¹) [33, 36]. The relatively high CTC determined in this study was similar to the reported in the lower Yangtze River located in southern Jiangsu Province in January with less rainfall maybe due to slow degradation in low temperature and high consumption of CTC [36]. In contrast, the concentration of CLA was slightly different from the Yellow River and the Huai River watersheds (lower than 5 ng L⁻¹) [37]. Among quinolones group, concentration of ENR ranged from ND to 4.83 ng L⁻¹, which was much lower than in the Three Gorges Reservoir (19.32 ng L⁻¹) with 100% detection frequency [38].

In previous studies, the sulfonamide, tetracycline, β -lactam, macrolide, aminoglycoside, quinolone resistance genes, and class I integrase gene *intI1* were also observed in various environments, including lakes [17], rivers [39–41], wetlands [42], small-scale poultry production [43], and air [44] in China. In our present study, sulfonamide resistance genes *sul1* and *sul2* were the predominant genes among the detected ARGs. The findings in this study were similar to previous studies in which the *sul1* and *sul2* were also the most abundant ARGs in wetland in Beijing [39] and the Pearl River [45]. The *sul1* gene encodes dihydropteroate synthase that confers resistance to sulfonamides and is generally harbored in class I integrase carrying other resistance genes [3]. The high abundance and detected frequency of *sul1* found in this study might result from the association with *intI1* and the widespread use of sulfonamides in China [25]. And also, the widespread of *sul2* was due to the fact that it usually exists on small non-conjugative or large transmissible multi-resistant plasmids [46, 47]. The abundance of *sul1* and *sul2* were usually related to input from WWTPs effluent discharge into freshwater and inputs from urban activities such as agricultural runoff, urban discharges and other human activities in previous studies [48, 49], revealed that the water quality in this reservoir is stable and showed no tendency of deterioration in recent years.

The β -lactam resistance genes were also commonly found and of relatively high abundance, with the concentrations decreased from *TEM-1*, *OXA-1* and *ampC*. The *TEM-1* gene was the most frequently detected among the β -lactams resistance genes, the levels of *TEM-1* were higher than those in three man-made reservoirs in Spain (10^{-4} – 10^{-3} genes/16S rRNA gene abundance) [50] and Taihu Lake (10^{-4} – 10^{-3} genes copies/16S rRNA gene copies) [17], but comparable to Ba River [51]. The *qnrS* gene is related with plasmid-borne fluoroquinolone resistance that has become increasingly prevalent in anthropogenically influenced environments [52]. The abundance of *qnrS* in February was much higher than other seasons with average relative abundance of 8×10^{-4} . A possible explanation was the lowest temperature and very little precipitation in this period which affect the microbial community.

As the previous studies revealed, the *intI1* is ubiquitous and with great abundance in a large-scale clinical and environmental isolates [53–55]. In this study, the absolute abundance of *intI1* was greatly positively correlated to several target genes as showed (Fig. S1), including *sul1* ($r=0.92$, $p<0.05$), *sul2* ($r=0.87$, $p<0.05$), *ermB* ($r=0.50$, $p<0.05$), *mphA* ($r=0.69$, $p<0.05$), indicating that the *intI1* is likely to acquire

and disseminate these related ARG subtypes as gene cassettes, which are unbalanced in the aquatic environment and maybe ultimately derived from human waste or their domestic livestock. Likewise, in the northern yellow sea and pearl river, the results were consistent with previous studies suggesting that *intI1* can serve as a medium to contribute to the widespread of various ARGs, implying that the ARGs maybe transferred by mobile genetic elements from aquatic system to human is possible [37, 56, 57].

Antibiotics residue and its positive correlation with their corresponding ARG subtypes suggested sublethal concentrations of antibiotics might have a high probability to select for resistance to generate genotypic and phenotypic variability between environment and ecosystems [58]. In the present study, *sul1*, *ermB* and *mphA* had a positive correlation with their antibiotics to suggest that some ARGs and antibiotics in the reservoir had identical sources, which was similar to the study result in the urban river in Beijing [18]. However, there was no significant correlation between other antibiotics and ARGs, suggesting that the antibiotics in this reservoir were mainly imported from the external aquatic environment and low antibiotic residue did not exert strong selective pressures.

Compared to the survey on ARGs in Qingcaosha Reservoir in 2016 [16], there is no remarkable abundance of differences that might suggest a dynamic balance of ARGs in this area. But with more sampling sites in Qingcaosha Reservoir in this study, the results can better reflect about more its spatial distribution of antibiotics and ARGs. For antibiotics and ARGs in this reservoir, the studied antibiotics and ARGs tended to be of higher abundance in the sites closer to the inflow of the Qingcaosha Reservoir than those close to the outflow in most cases. Thus, we considered that reservoir could be served as a functional area contributing to the elimination or dilution of ARGs. To be specific, regarding the antibiotics and ARGs results in a temporal and spatial context, some significant tendencies between the different sites in this reservoir and the different sampling times could be observed. In terms of the different sampling sites, higher total relative abundance of the ARGs was detected in site S8 than those found in other sites. Several antibiotics (including SMX, SMM, AZM, FLX, ENR and CTC) could be found relative to high concentrations in site S8, while the slight differences among all samples for the rest of detected antibiotics, including SDZ, SCP, SMP and OTC. In contrast, the CLA, SPM, and PenG were of high residues in S5 and S6. For ARGs, it was apparent that the relative abundance was higher in S8 (Fig. 3a, c). From the

map of Qingcaosha Reservoir as Fig. 1 shows, S8 was the nearest site to the raw water from the Yangtze River, suggesting that the raw water maybe the source of the antibiotic residues. Therefore, the occurrence and distribution characteristics of ARGs may be affected by water velocity, geographical conditions and material exchange.

For the different sampling times, the CTC of tetracyclines and macrolides showed the highest concentration of 36.32 ng L⁻¹ in February maybe attribute to the tetracyclines can be degraded when exposure to sunlight combine with the weather condition (the water temperature in November and May was higher than in February) also can influence the antibiotics [59, 60]. For the other hand, CTC was the most widely used for humans among TC antibiotics, indicating that the CTC maybe used in quantity in February [33]. Compared to other antibiotics, macrolide consumption is more widespread in households to apply to treat specific diseases such as pneumonia and bronchitis [61] and the AZM can be used to treat respiratory infections that are more likely to outbreak in winter. Overall, the antibiotics detected in May were relatively low than November and February maybe caused by the rainfall. However, this concept is inconsistent with a previous finding that more rainfall could increase the absolute abundance of ARGs and MGEs in the river-reservoir system [62]. This phenomenon may be attributed to the differences in the characteristics of river, temperature and location between the system and this reservoir.

Conclusion

In the present study, we investigated the occurrence and distribution of 19 antibiotics, 12 ARGs and *intI1* in the Qingcaosha Reservoir of Shanghai, China. The SMX, SMM and PenG were dominant antibiotics residues for their detection frequencies and concentration in this reservoir. The sulfonamide resistance genes *sul1* and *sul2* were the most prevalent and predominant genes in the reservoir. The higher total relative abundance of the ARGs was detected in site S8 than those found in other sites and the overall trend of antibiotics in May was relatively lower than November and February. Correlation analysis indicated *sul1*, *ermB* and *mphA* had a positive correlation with their antibiotics and *intI1* was also greatly positively correlated to *sul1*, *sul2*, *ermB* and *mphA*. Since the reservoirs are the main source of drinking water whether directly or indirectly input, the ARGs pose a serious ecological and human health risk through the horizontal gene transfer in the environment. Overall, our results showed an important view to better understand the antibiotics and ARGs in this reservoir and to provide some information for the safety of aquatic environment management.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12302-020-00357-y>.

Additional file 1: Table S1. Primers, product lengths and annealing temperatures of quantitative PCR for target genes. **Table S3.** Detection frequencies of 13 target ARGs in Qingcaosha Reservoir. **Figure S1.** Correlations between *intI1* and ARGs.

Additional file 2: Table S2. Antibiotic residues in Qingcaosha Reservoir.

Abbreviations

ARGs: Antibiotics resistance genes; qPCR: Quantitative real-time PCR; SMX: Sulfamethoxazole; SDZ: Sulfadiazine; SCP: Sulfachlorpyridazine; SMM: Sulfamonomethoxine; SIZ: Sulfisoxazole; SMP: Sulfamethoxy pyrimidine; SIZ: Sulfadimidine; OTC: Oxycycline; CTC: Chlortetracycline; AMC: Amoxicillin trihydrate; PenG: Penicillin G potassium salt; AZM: Azithromycin; CLA: Clarithromycin; SPM: Spiramycin; TILM: Tilmicosin; CIP: Ciprofloxacin hydrochloride; FLX: Fleroxacin; OFX: Ofloxacin; ENR: Enrofloxacin; TMP: Trimethoprim; TCY: Tetracycline; FZD: Furazolidone; OQX: Olaquinox.

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Authors' contributions

TX, WZ, and XG were involved in the experiments and manuscript writing. ZH, WZ, and TX were responsible for the data analysis. ZH, TX, and HZ designed the study. TX, SH and DY contributed to correction of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets obtained and analyzed in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China. ² Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China. ³ Post-doctoral Research Station of Civil Engineering, Tongji University, Shanghai 200092, China. ⁴ Shanghai Academy of Environmental Sciences, Shanghai 200233, China.

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