

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

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Research

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Abstract

Background

Aquatic ecosystems are considered to be among the most important reservoirs of antibiotic resistance genes (ARGs). Drinking water sources were usually parts of lakes and rivers in Yangtze River Delta, among which Qingcaosha Reservoir is the largest river impoundment and benefit the population of more than 13 million for Shanghai city. In this study, we aimed at investigating the distribution of antibiotics and ARGs to characterize the pollution across various sites in Qingcaosha Reservoir in three seasons.

Results

Sulfamethoxazole, sulfamonomethoxine and penicillin G potassium salt were the dominant antibiotics and of high detection frequencies in this reservoir. Sulfonamide resistance genes (sul1 and sul2) were the most prevalent and predominant genes. Higher total relative abundance of the ARGs were detected in the site closest to the inflow than those in other sites. Overall, the concentrations of antibiotics in May (spring) were relatively lower than November (autumn) and February (winter). Correlation analysis indicated sul1 , ermB and mphA had positive correlation with corresponding antibiotics in February and int11 was also greatly positively correlated to sul1 , sul2 , ermB and mphA .

Conclusion

In conclusion, the antibiotics and ARGs were widespread in Qingcaosha Reservoir. Our result indicated that the drinking water reservoir might serve as gene reservoir for antibiotic resistance and mobile gene element int11 can serve as a medium to contribute to the widespread of various ARGs. What is more, we considered that Reservoir could be served as a functional area contributing to the elimination of ARGs.

1 Background

Nowadays, we are in an era that kinds of antibiotics were widely used in clinical, livestock farms, aquaculture and other fields for disease treatment, prophylaxis or growth promoters [1, 2]. Most antibiotics cannot be completely absorbed by humans and animals after intake, of which nearly 25–75% was discharged via urine and feces [3]. The antibiotic residues could then enter into the environment and pose potential risk to the ecological microbes and even human health [1]. A growing amount of studies suggested that irrational usage and residues of antibiotics possessed long-term selection pressure on microbes and could induce antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) [7–10]. Especially for antibiotics such amoxicillin and erythromycin which could be used both in agriculture and clinic, the abuse of them in animals would cause more serious situation. Once bacteria developed resistance to them, it might lead to the failure of infection treatment [4–6].

ARG was firstly proposed as an emerging environmental contaminant in 2006 and attracted growing global concern of environments and public health [11]. The emergency of antibiotic resistance or the

multidrug-resistant bacteria can result in the failure of infectious diseases treatment [12]. For recent years, due to the potential risk of ARGs in environment, previous studies have been performed to assess the prevalence of various ARGs in livestock farms and aquaculture [13], clinical environment [14], wastewater treatment plants [15], surface water all over the world including Germany and Australia [16], Europe [8] and China [17–20], reservoirs [21] and even the outflow of drinking water treatment plants [20]. Therefore, it is obvious that aquatic environment plays a crucial role in the prevalence of antibiotic resistance and gradually become a reservoir of ARGs. 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]. The reservoirs, often acting as main and important drinking water sources relied on exogenous source, most of them are located in the areas with little human activities [23]. Due to the riverine inputs 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 reservoir to guarantee safety of drinking water supply.

According to the reported data, 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 Yangtze estuary were published [20, 28–30]. However, information about the spatial and seasonal variation, and a comprehensive understanding about a specific area is still lacking. Qingcaosha Reservoir is the largest impoundment reservoir of river in China. It 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 our present study, we collected 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 int β 1 in this reservoir; (2) characterize and discuss the spatial and seasonal 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.

2 Material And Methods

2.1 Study area and sampling campaign

Qingcaosha Reservoir was 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 sample site and stored in two sets of different containers. Specifically, 1.5 L water were stored in sterile polyethylene bottles and 1 L water were stored in brown glass bottles for antibiotics analysis. Appropriate amount of hydrochloric acid was added into the glass bottles 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 31th, 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.

2.2 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 - oxytetracycline (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). Antibiotic standards were purchased from Dr.Ehrenstorfer (Germany). Add 1 ml of the 100 $\mu\text{g/L}$ mixed standards to water samples (1 L) and adjust pH to 3. The mixed standards contain SMX- $^{13}\text{C}_6$, trimethoprim (TMP)- D_3 and tetracycline (TET)- D_6 (Dr.Ehrenstorfer, Germany), FLX- D_5 and furazolidone (FZD)- D_4 (Witega, Germany), olaquinox (OQX)- D_4 and AMC- $^{13}\text{C}_6$ (Cambridge Isotope Laboratories, UK). Active the column before loading and treat the samples through extraction column at a speed of 5 min/ml, then wash the column with 6 ml ultra-pure water and 6 ml 5% methanol-aqueous solution and dry by gas for 5 min after loading. Finally, eluent with 10 ml elution. 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 filtered the solution through a 0.22 μm PTFE needle filter to a brown injection bottle for testing. All the above treatments were conducted in parallel for 3 times, 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 acetonitrile and Milli-Q water with 0.1% (v/v) formic acid; gradient elution program as follows: 0-2.2 min, 16% acetonitrile; 2.2–2.5 min, 16–95% acetonitrile; 2.5–5.5 min, 95% acetonitrile; 5.5-6.0 min, 95% acetonitrile – 16% acetonitrile; 6.0–10.0 min; 16% acetonitrile.

Chromatographic separation of the analyses was conducted with ACQUITY BEH C18 column (100 mm \times 2.1 mm, 1.7 μm , Waters, USA) for antibiotics. The temperature of the columns was 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.

2.3 DNA extraction

The water samples (500 mL each) were filtered through 0.22 µm polytetrafluoroethylene membrane filters (Millipore, USA) to capture microorganisms and 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 Qubit™ 4 Fluorometer with Qubit™ dsDNA HS Assay Kit (Invitrogen, USA). All extracted DNA samples were stored at -40 °C prior to analysis.

2.4 Quantification of ARGs

The presence of 13 ARGs conferring resistance to the six antibiotic classes we monitored (as described in 2.2) as well as class I integrase gene (*intl 1*) 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 × SensiMix™ 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 (Supporting information: 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. The primer sets used for the PCR amplification of ARGs were showed in 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].

2.5 Statistical analysis

The data was 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.

3 Results

3.1 antibiotic concentrations

Fourteen of nineteen antibiotics were detected in all samples (Fig. 2), with SIZ, SDD, CIP, OFX and TILM not detected in any samples (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 a low detection, but interestingly, CTC was found to be of high concentration in February. 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%). Among quinolones, almost only ENR could be detected in November and May. The AZM and CTC were barely detected in November but were frequently present in February. Quite the opposite, the SPM, ENR and OTC were detected in February more frequently in November.

The mean concentrations of SMX, SDZ and SMM (25.07 ng/L, 1.30 ng/L, 3.82 ng/L) in November, (3.07 ng/L, 2.16 ng/L, 7.97 ng/L) in February and (5.79 ng/L, 2.31 ng/L, 3.37 ng/L) in May. The mean concentration of CTC in February (34.3 ng/L) was the highest among all antibiotics. The 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 almost 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. The SMX and PenG were much higher in November than the other two sampling months. For the detection frequencies and concentrations, the SMX, SMM and PenG were dominant antibiotic residues.

3.2 Occurrence and distribution of ARGs

The studied ARGs and *intI1* were all detected in Qingcaosha Reservoir. Summary of detection frequencies of 12 ARGs and *intI1* in water samples was 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 -lactamase 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 summarized 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. Of the two tetracycline resistance genes, *tetC* was more prevalent than *tetW*, which 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. There was no much difference in concentration of two macrolides resistance gene *mphA* in different water season, basically ranged from 2.69×10^4 to

8.78×10^5 copies/L water. Conversely, *ermB* was with low concentration near the limitation of detection. Between gene *strA* and *aacC4*, the former was detected ranged 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* was ranging 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 in February higher than other water seasons, which ranged from 2.88×10^6 to 2.95×10^7 copies/L water. The mobile genetic gene *int1* was the highest gene in this study, 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.

3.3 Seasonal and spatial 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 performed to analyze the distribution characteristics of various ARGs among the sampling sites at different time (Fig. 4). Seasonal (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 *int1* in November, February and May from 8 sampling sites in Qingcaosha Reservoir. In this study, most of the target genes were detected in this reservoir, from 3.97×10^{-8} to 3.05×10^{-1} , expected *aacC4* not detected in any of samples of February. For ARGs and *int1*, the total relative abundance of *int1* was the highest of 4.21×10^{-1} , and the sulfonamide resistance genes *sul1* and *sul2* were predominant among 12 ARGs 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} to 10^{-2} . The *mphA* and *ermB* were both highest in May, followed by February and November. The *qnrS* was special caused by the great gaps in February and others.

Obviously, Fig. 4c showed that the total relative abundance of 12ARGs with site S8 was the relatively high detected (1.88×10^{-2} , 9.63×10^{-3} and 8.70×10^{-2}) among the 8 sample 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 water seasons. For ARGs in May, it was apparently 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.

3.4 Correlation analysis between abundance of ARGs

We carried out a correlation analysis among the absolute concentrations of ARGs, gene *int1* and the antibiotics to which they confer resistance to identify potential links between all variables. Significant

positive correlation between the *int* β 1 and ARGs, or several antibiotics and their corresponding ARGs in February were observed (Fig. 5 and Fig. S1). For instance, there were correlations between the SMX and the *sul*1 ($r = 0.59, p < 0.001$), SMM and the *sul*1 ($r = 0.56, p < 0.001$), AZM and the *mphA* ($r = 0.58, p < 0.005$), AZM and the *ermB* ($r = 0.85, p < 0.05$). However, the correlations between antibiotic and ARGs in the other two seasons or for all seasons together were not observed.

4 Discussion

Antibiotics and ARGs were commonly found in Qingcaosha Reservoir across different seasons and sample sites. As a rather important source of drinking water in 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 to human as the modern drinking water treatment plants were not designed to remove these pollutants. Our result indicated that the reservoir might serve as gene reservoir for antibiotic resistance, and the presence of ARGs conferring all kinds of antibiotics in the environment were likely related with human activities, such as fecal pollution and antibiotic residues [31, 32].

The concentration 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 concentrations was comparable to the Guanting Reservoir in north China with the mean concentration $6.7 \text{ ng}\cdot\text{L}^{-1}$ [33] and was much lower than Three Gorges Reservoir Area, in which the mean concentration was $13.65 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ [34]. And it was much higher than the Taihu Lake with the mean concentration of $0.355 \text{ ng}\cdot\text{L}^{-1}$ [35].

In previous studies, the sulfonamide, tetracycline, β -lactam, macrolide, aminoglycoside, quinolone resistance genes and class β integrase gene *int* β 1 were also observed in various environments, including lakes [17], rivers [36–38], wetlands [39], small-scale poultry production [40], and air [41] in China. In our present study, sulfonamide resistance genes *sul*1 and *sul*2 were the predominant genes among the detected ARGs. The findings in this study were similar with previous studies in which the *sul*1 and *sul*2 were also the most abundant ARGs in wetland in Beijing [39] and the Pearl River [42]. The *sul*1 gene encodes dihydropteroate synthase that confers resistance to sulfonamides and is generally harbored in class β integrase carrying other resistance genes [43]. The high abundance and detected frequency of *sul*1 found in this study might result from the association with *int* β 1 and the widely use of sulfonamides in China [25]. And also, the widespread of *sul*2 was due to the fact that it usually exists on small non-conjugative or large transmissible multi-resistant plasmids [44, 45]. Compared to the survey on ARGs in Qingcaosha Reservoir in 2016 [21], there is no remarkable abundance differences which might suggest dynamic balance of ARGs in this area. The abundance of *sul*1 and *sul*2 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 [46, 47], revealed that the water quality in this reservoir keep stable and 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 is 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) [49] and Taihu Lake (10^{-4} - 10^{-3} genes copies/16S rRNA gene copies) [17], but comparable to Ba River [50]. Different from the other two tet genes, ampC was located on chromosomes and could not be transported by mobile genetic elements, which might be the reason for its lower abundance and detection frequency. The qnrS gene is related with plasmid-borne fluoroquinolone resistance that has become increasingly prevalent in anthropogenically-influenced environments [51]. Apparently, the qnrS in February much higher than other seasons with average relative abundance of 8×10^{-4} . Our results indicated that some changes of microbial taking qnrS community composition during rainfall process.

As the previous studies revealed, the int β 1 is ubiquitous and with great abundance in a large-scale of clinical and environmental isolates [52–54]. In this study, the absolute abundance of int β 1 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 int β 1 is likely to acquire and disseminate these related ARG subtypes as gene cassettes, which are unbalance in the aquatic environment and maybe ultimately derived from human waste or their domestic livestock. Likewise, in northern yellow sea and pearl river, the results were consistent with previous studies suggesting that int β 1 can serve as a medium to contribute to the widespread of various ARGs [37, 55].

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 [56]. In the present study, sul1, ermB and mphA had 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 mainly imported from external aquatic environment and low antibiotics residue did not exert strong selective pressures.

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 of ARGs. To be specific, regarding the antibiotics and ARGs results in a temporal and spatial context, some significant tendency 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 were detected in site S8 than those found in other sites. Several antibiotics (including SMX, SMM, AZM, FLX, ENR and CTC) could be found relative 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 and 3c). From the map of Qingcaosha reservoir as Fig. 1 showed, 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 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 [57, 58]. Compared to other antibiotics, macrolide consumption is more widespread in households to apply to treat specific disease such as pneumonia and bronchitis [59] 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 [60]. This phenomenon may be attributed to that the differences in the characteristics of river, temperature and location between the system and this reservoir.

5 Conclusion

In the present study, we investigated the occurrence and distribution of 19 antibiotics, 12 ARGs and *int1* in 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. Higher total relative abundance of the ARGs were detected in site S8 than those found in other sites and the overall trend of antibiotics in May were relatively lower than November and February. Correlation analysis indicated *sul1*, *ermB* and *mphA* had positive correlation with their antibiotics and *int1* was also greatly positively correlated to *sul1*, *sul2*, *ermB* and *mphA*. Due to 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.

Additional Files

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 int₁ and ARGs.

Additional File 2:

Table S2. Antibiotic residues in Qingcaosha Reservoir.

List Of Abbreviations

antibiotics resistance genes (ARGs); Quantitative Real-time PCR (qPCR); sulfamethoxazole (SMX); sulfadiazine (SDZ); sulfachlorpyridazine (SCP); sulfamonomethoxine (SMM); sulfisoxazole (SIZ); sulfamethoxypyrimidine (SMP); sulfadimidine (SIZ); oxycycline (OTC); chlortetracycline (CTC); amoxicillin trihydrate (AMC); penicillin PenG potassium salt (PenG); azithromycin (AZM); clarithromycin (CLA); spiramycin (SPM); tilmicosin (TILM) ; ciprofloxacin hydrochloride (CIP); fleroxacin (FLX); ofloxacin (OFX); enrofloxacin (ENR); trimethoprim(TMP); tetracycline(TCY); furazolidone(FZD) ; olaquinox(OQX).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

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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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Figures

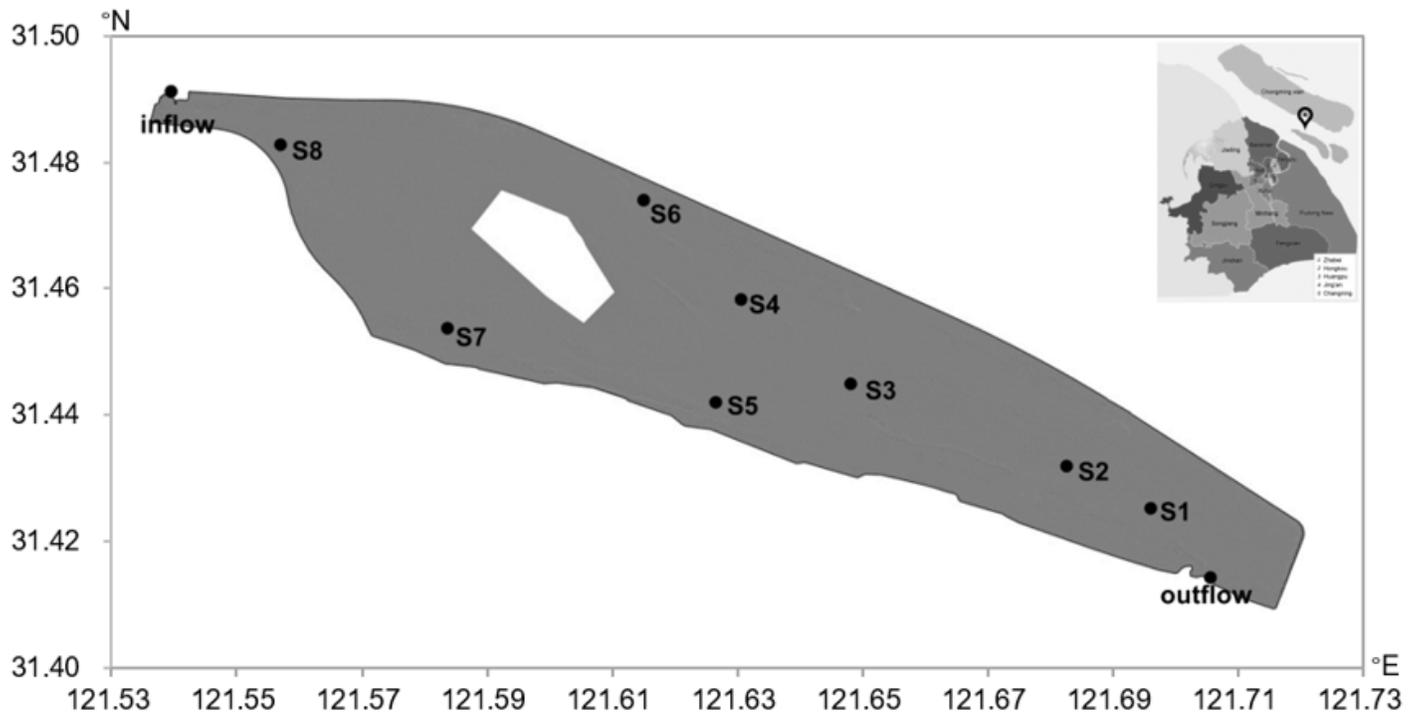


Figure 1

Map of the Qingcaosha Reservoir and the location of sampling sites. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

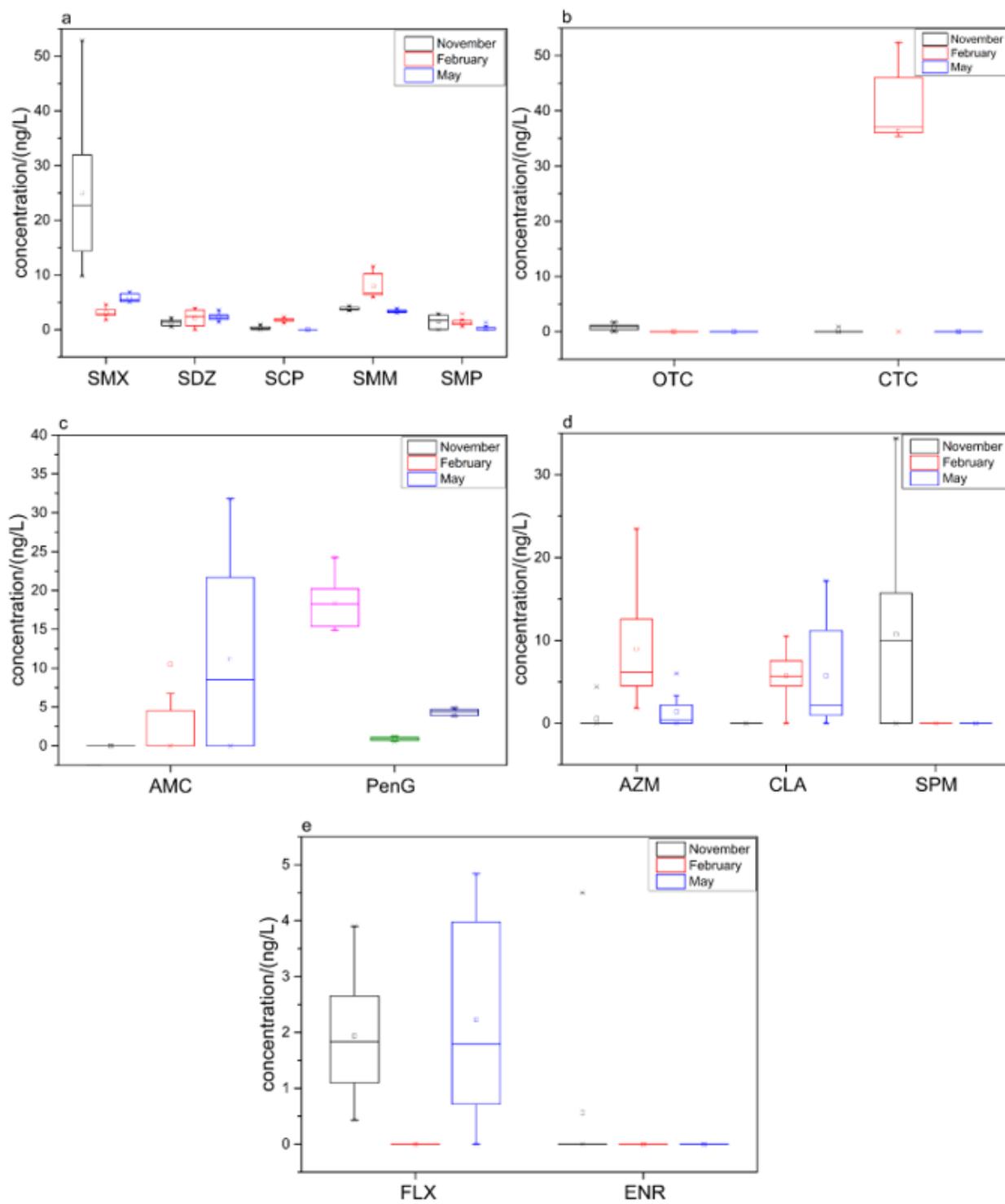


Figure 2

The residues of target antibiotics in the different water samples, (a) sulfonamides; (b) tetracycline; (c) β -lactams; (d) macrolides; (e) quinolones.

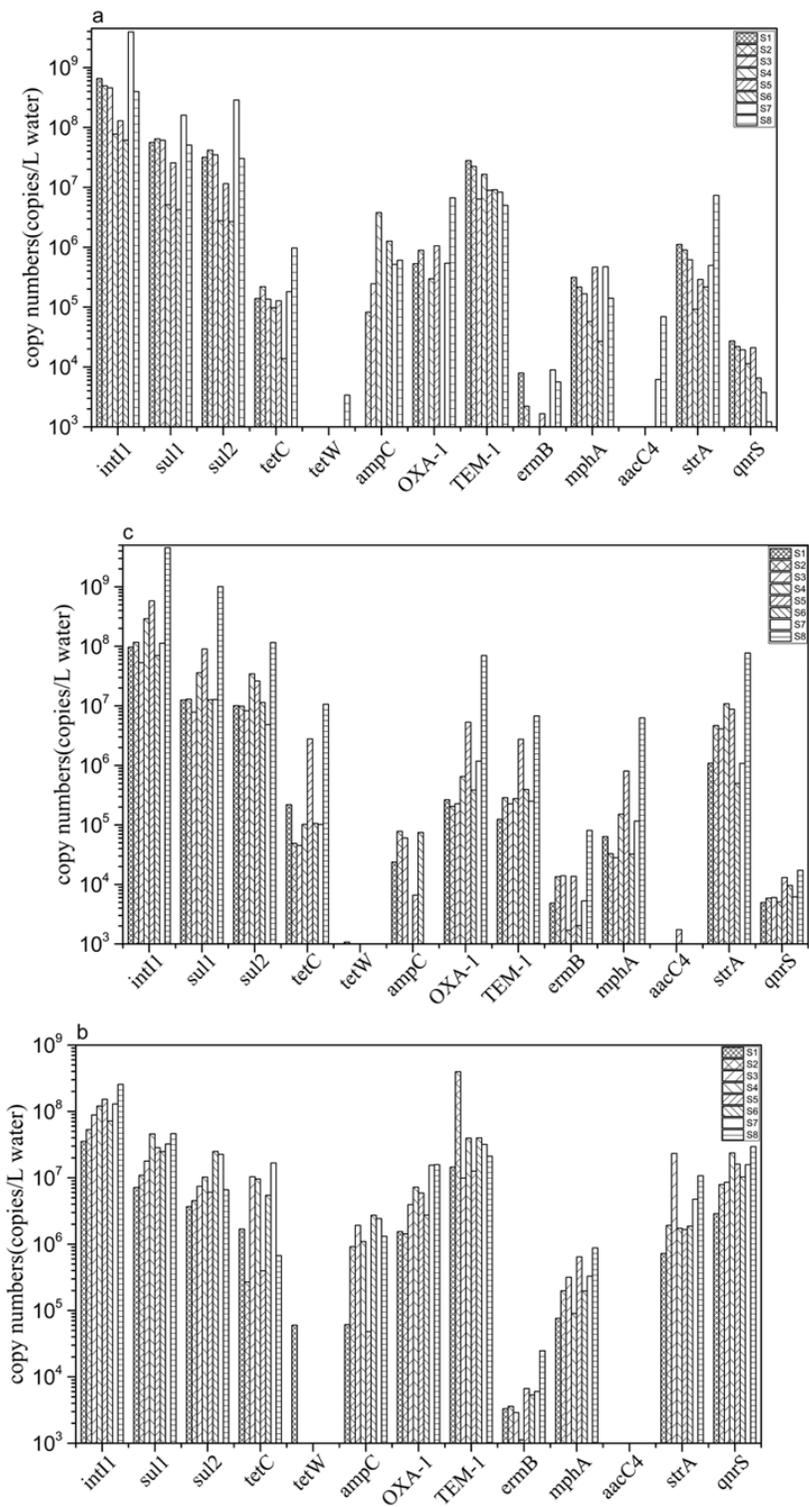


Figure 3

The absolute abundance of ARGs and *int1* in Qingcaosha Reservoir, (a) samples collected in November; (b) samples collected in February; (c) samples collected in May.

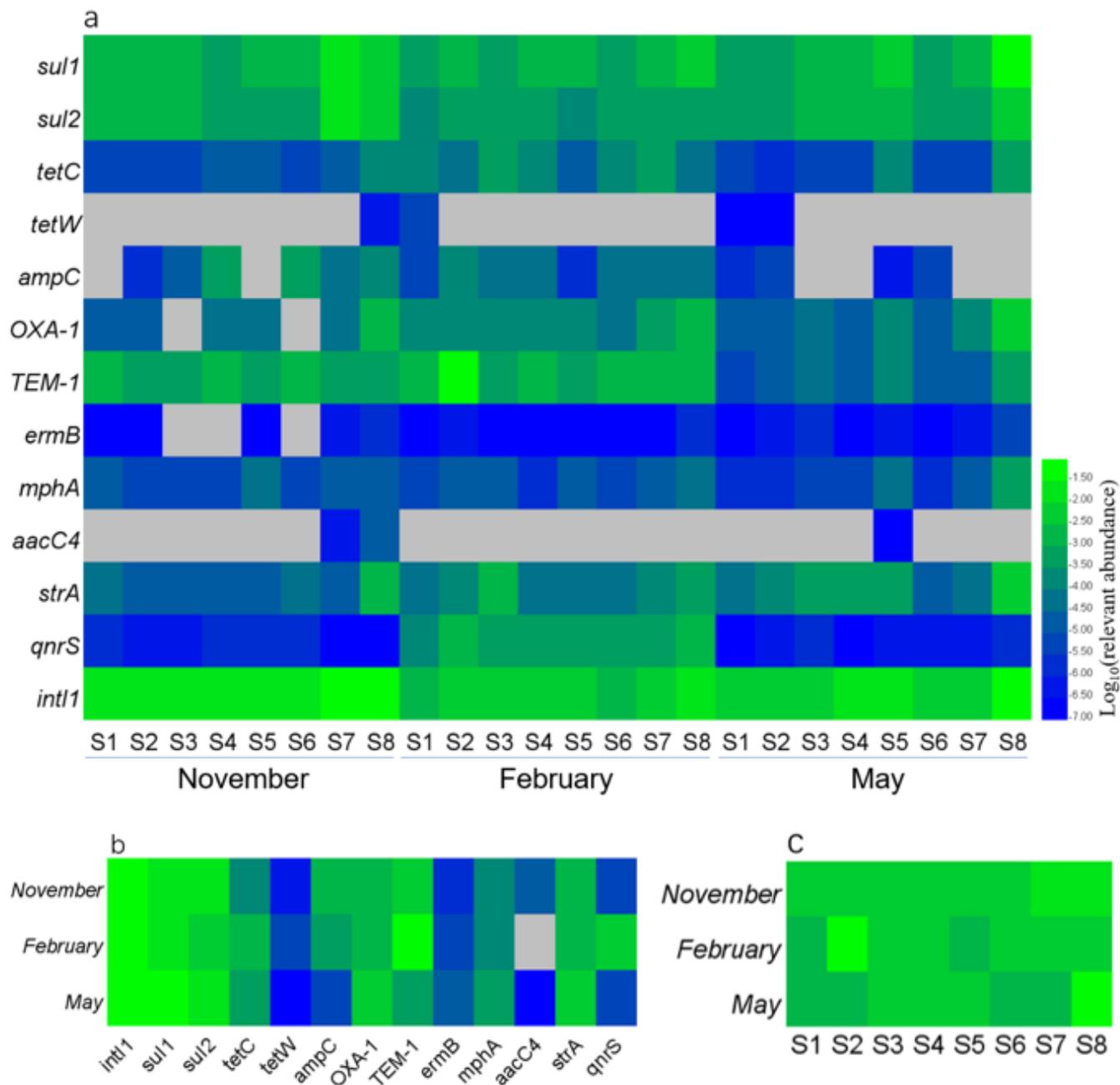


Figure 4

Heatmap of the relative abundance of target genes, (a) relative abundance of all target genes; (b) seasonal variation of ARGs in the samples.; (c) distribution of target genes in the 8 sampling sites.

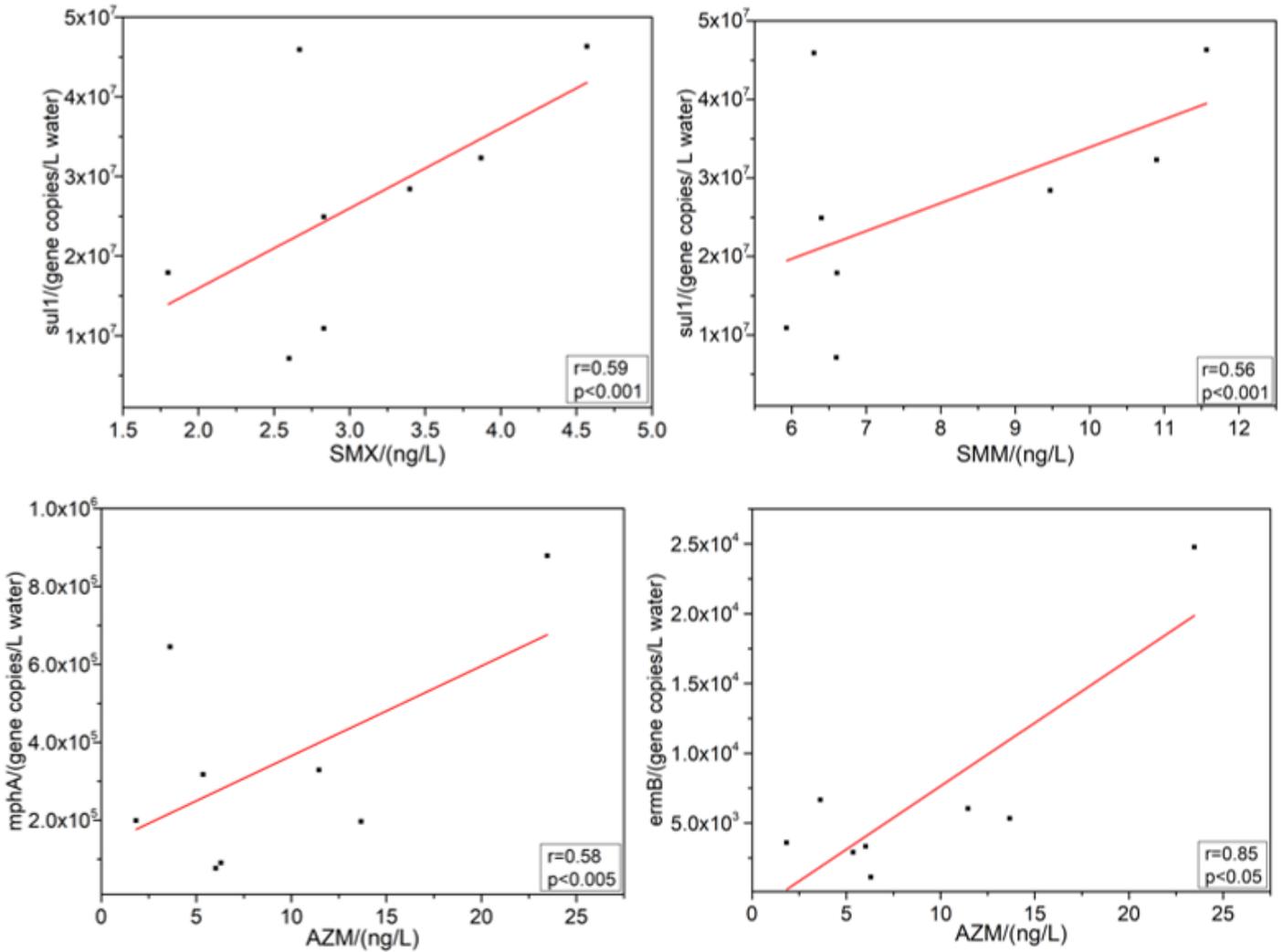


Figure 5

Correlations between several antibiotics and ARGs in February

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