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Adaption of the microbial community to continuous exposures of multiple residual antibiotics in sediments from a salt-water aquacultural farm

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HIGHLIGHTS

- Sediments from ponds and channels exposed to 11 residual antibiotics at low levels.
- No significant shifts in microbial community structure were found in sediment.
- Roxithromycin residuals weakly related to microbial community structure in sediments.
- Microbial community structure adapts to long-term exposure to residual antibiotics.

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ABSTRACT

Residual antibiotics from aquacultural farming may alter microbial community structure in aquatic environments in ways that may adversely or positively impact microbially-mediated ecological functions. This study investigated 26 ponds (26 composited samples) used to produce fish, razor clam and shrimp (farming and drying) and 2 channels (10 samples) in a saltwater aquacultural farm in southern China to characterize microbial community structure (represented by phospholipid fatty acids) in surface sediments (0–10 cm) with long-term exposure to residual antibiotics. 11 out of 14 widely-used antibiotics were quantifiable at $\mu g\,kg^{-1}$ levels in sediments but their concentrations did not statistically differ among ponds and channels, except norfloxacin in drying shrimp ponds and thiamphenicol in razor clam ponds. Concentrations of protozoan PLFAs were significantly increased in sediments from razor clam ponds while other microbial groups were similar among ponds and channels. Both canonical-correlation and stepwise-multiple-regression analyses on microbial community and residual antibiotics suggested that roxithromycin residuals were significantly related to shifts in microbial community structure in sediments. This study provided field evidence that multiple residual antibiotics at low environmental levels from aquacultural farming do not produce fundamental shifts in microbial community structure.

1. Introduction

Antibiotics are vital for human healthcare and animal production activities. The animal industry uses antibiotics as feed additives to treat diseases and to promote animal growth as feeding additives [1]. Various antibiotics have been detected in aquatic environments around the world, including coastal wetlands [2], freshwater streams, estuarine sediments [3–8], and sewage sludge [9].

There is increasing concern about the biological effects of antibiotic contamination on aquatic ecosystems [10–12]. Antibiotic-resistant genes (ARGs) have been found in many aquatic environments [13] and ecological functions such as nitrate reduction have been shown to affected by residual antibiotics [14]. However, these laboratory studies, with individual antibiotics at high concentrations indicated that the adverse effects were

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not target-specific to aquatic organisms and ecological processes [15–17]. There have been few of field studies where environments have been to multiple antibiotics, for long time periods, at low concentrations.

Saltwater aquaculture provides over half of the seafood production in China (http://data.stats.gov.cn-2013). As medicines for disease treatment and disease prevention and as additives for growth promotion, antibiotics are increasingly used in aquaculture [18,19]. As in many nations, over half the consumption of antibiotics in China occurs in livestock farming, including aquaculture [20]. The unprecedented usage of antibiotics in aquaculture has resulted in substantial concentrations of antibiotic residuals in surface water and sediment within farming sites and in surrounding environments receiving aquacultural wastewater [2,7,21,22]. The persistence of antibiotic compounds in aquatic environmental depends on their bio-stability and dosage, as well as on site characteristics. In many saltwater aquaculture sites, rotation practices have been applied as a strategy to reduce disease outbreaks and antibiotic resistance [18,23]. However, the rotation practice also increases the range of antibiotic compounds that aquatic organisms are exposed to.

Responses of microbial community (structure and function) to multiple residual antibiotics in actual environments are complex and poorly studied. Laboratory studies have been done with a few compounds, for instance, ciprofloxacin in salt marsh sediments [24], sulfadiazine in two agricultural soils with manure amendments [25], sulfamethoxazole in an agricultural soil with other substrate additions [26], and oxytetracycline exposure in a wheat rhizosphere soil [27]. However, aquacultural sediments are exposed to multiple antibiotics at concentrations that are much lower than what is used in laboratory experiments. Bernier and Surette [28] pointed out that in natural environments antibiotic activity is concentration-dependent, i.e., antimicrobial activity at high concentrations and diverse biological responses (including resistance/tolerance) in microbes at subinhibitory concentrations. There is a great need to understand changes in microbial community structure and activity to multiple antibiotic residuals, at the levels that are actually found in the environment.

In this study, we characterized microbial community (structure and biomass) in surface sediments from a saltwater aquacultural site in Zini Town, Fujian Province, China where antibiotics have been used over multiple years. Fourteen antibiotic compounds, frequently detected in aquatic ecosystems worldwide [5,6,9], were determined in the surface sediments as well. Our objective was to characterize in situ changes caused by long-term exposure to multiple antibiotic compounds at actual residual concentrations. We hypothesized that microbial community structure would vary in different farming systems (fish, razor clam, shrimp) due to differences in antibiotic application (type and dosage) in different systems.

2. Materials and methods

2.1. Chemicals and standards

All the standard compounds, listed in Table 1, used for analyses were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), except norfloxacin (NFC), ofloxacin (OFC), C_{13}-caffeine, d_5-chloramphenicol (d_5-CAP), and d_4-sulfadiazine (d4-SMZ) which was obtained from Sigma–Aldrich Inc. HPLC grade solvents, including methanol, formic acid, chloroform, acetone, methyl-butyl ether, hexane, and acetonitrile, were provided by the Tedia (Ohio, USA). Guaranteed reagents, including phosphoric acid, hydrochloric acid, sodium hydroxide, dipotassium hydrogen phosphate, and disodium EDTA, were purchased from the Sinopharm Chemical

Table 1
The protocol and performance for quantification and qualification of the selected antibiotic compounds.

Antibiotic compound	Abbreviation	Precursor ion (m/z)	Product ion (m/z)[a]	Ion mode	DP (V)	CE (V)	Regression equation[b]	R^2[c]	Recovery rate (%)[d]	Detection limit ($\mu g\,kg^{-1}$)
Oxytetracycline	OTC	461.2	**426.3/444.0**	Positive	45/45	25/25	$y = 1.72e3x + 22.1$	0.9966	80.1 ± 7.6	0.25
Tetracycline	TCC	445.2	**410.3/427.0**	Positive	45/45	25/25	$y = 1.85e3x + 2.1$	0.9988	96.4 ± 17.1	0.35
Chlortetracycline	CTC	479.2	**444.0/462.0**	Positive	51/51	26/26	$y = 575x - 0.00108$	0.9935	86.4 ± 10.0	0.64
Roxithromycin	RTM	837.6	**158.2/679.6**	Positive	65/60	50/35	$y = 969x + 22.1$	0.9997	81.3 ± 9.0	0.02
Erythromycin–H_2O	ETM	716.4	**158.2/558.2**	Positive	68/66	35/27	$y = 480x + 22.1$	0.9915	70.9 ± 11.5	0.07
Norfloxacin	NFC	320.0	**302.3/276.4**	Positive	60/55	25/21	$y = 28x + 0.000599$	0.9928	89.2 ± 2.6	0.68
Ofloxacin	OFC	362.3	**261.2/318.0**	Positive	50/53	37/17	$y = 2.69e3x - 0.0104$	0.9959	81.8 ± 7.1	0.28
Sulfadiazine	SDZ	251.0	**91.9/155.9**	Positive	51/51	35/35	$y = 2.52e3x + 66.2$	0.9976	98.5 ± 3.4	0.05
Sulfamethazine	SMZ	279.1	**124.2/186.2**	Positive	55/55	35/22	$y = 5.17e3x + 0.0204$	0.9988	48.1 ± 7.4	0.03
Sulfamethoxazole	SMX	256.0	**156.0/108.1**	Positive	45/45	18/30	$y = 4.65e3x + 88.3$	0.9997	37.0 ± 4.3	0.06
Trimethoprim	TMP	291.2	**260.9/132.2**	Positive	45/45	35/50	$y = 8.54e3x + 22.1$	0.9936	33.2 ± 1.9	0.07
Thiamphenical	TAP	356.1	**184.9/291.8**	Negative	−35/−35	−22/−22	$y = 986x + 0.9983$	0.9983	30.0 ± 4.5	0.10
Florfenicol	FF	357.9	**184.7/338.0**	Negative	−35/−35	−22/−22	$y = 2.23e3x + 26.4$	0.9965	102.6 ± 7.1	0.07
Chloramphenicol	CAP	320.8	**152.1/257**	Negative	−35/−35	−22/−22	$y = 1.12e3x + 8.81$	0.9978	95.1 ± 5.8	0.11

[a] The bold figures of product ions were used for quantification and paired-ions were used for confirmation.
[b] Regression equation was for calibration curves of 10.0, 20.0, 50.0, 100.0, and 200.0 μg L for each antibiotic compound.
[c] R^2 was the correlation coefficient of the regression equation.
[d] Recovery rates were presented as mean ± RSD (relative standard deviation).