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Occurrence and temporal variation of antibiotic resistance genes (ARGs) in shrimp aquaculture: ARGs dissemination from farming source to reared organisms

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HIGHLIGHTS

- Water source disseminated ARGs to aquaculture environments and reared organisms.
- The abundances of ARGs in aquaculture varied temporally during the rearing period.
- *Sul*1 is a potential indicator for ARGs in water and sediment in aquaculture.
- ARG prevalence was higher in adult than in juvenile shrimp intestinal tracts.
- Bacterial community in the intestinal tract changed greatly from juvenile to adult.

GRAPHICAL ABSTRACT

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ABSTRACT

Considerable attention has been paid to the occurrence and abundance of antibiotic resistance genes (ARGs) in aquatic environments. However, the temporal variation and dissemination of ARGs in aquaculture environments and reared organisms need further study. This study investigated the abundance and diversity of ARGs and bacterial community in water source, shrimp pond water, sediment, and shrimps during the rearing period in Pearl River Delta region, South China. The results showed that *sul*1, *qnr*D, *cml*A, and *flo*R were the predominant ARGs in the aquaculture samples. A trend of decreasing abundance of ARGs was observed for pond water samples during the rearing period, whereas an increasing trend was observed in the sediment and shrimp samples. The total concentration of ARGs in water source was significantly higher than that in shrimp pond water ($p < 0.05$). A significant negative correlation was found between the total concentrations of ARGs in pond waters and sediments ($p < 0.01$). The total abundances of ARGs in intestinal tract of adult shrimps were 4.48–19.0 times higher than those in juvenile shrimps. Similar to water source and pond water, *cml*A and *sul*1 were the predominant ARGs in shrimp intestinal tract. The bacterial community in the shrimp intestinal tract changed greatly from juvenile to adult. The results of the present study indicated that the abundances of ARGs in aquaculture varied temporally during the rearing period. Water source was an important medium disseminating ARGs to the aquaculture environments and reared organisms. *Sul*1 could be used as a potential indicator for ARGs in both water and sediment in aquaculture in the estuary of the Pearl River Delta, South China. This study represents a

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case study for the temporal variation of abundance and dissemination of ARGs in aquaculture and is a reference for potential risks to food safety and human health.

1. Introduction

Antibiotic resistance has become a major global public health issue in recent years (Wright, 2010). Consequently, as emerging environmental contaminants, antibiotic resistance genes (ARGs) have attracted increasing attention (LaPara et al., 2011; Pruden et al., 2006). The presence of ARGs has been widely detected in hospital wastewater (Durham et al., 2010; Vinue et al., 2010), wastewater treatment plants (LaPara et al., 2011; Zhang and Zhang, 2011), broiler feedlots (He et al., 2014), swine farms (He et al., 2016; Xia et al., 2010), beef farms (Hoyle et al., 2006), river water and sediment (Pei et al., 2006; Storteboom et al., 2010a; Storteboom et al., 2010b). Water is the main vector of contaminants and serves as a conduit of antibiotic resistant bacteria (ARB) and ARGs flow in the environment (Xi et al., 2009). As a consequence of incomplete removal, ARB and ARGs in the wastewater treatment plants will eventually enter the receiving water (Gao et al., 2012b; Munir et al., 2011).

ARB and ARGs have been shown to be prevalent in the aquaculture environments (Huang et al., 2017; Igbinosa, 2016; Lin et al., 2016; Muziasari et al., 2016; Stalin and Srinivasan, 2016; Tomova et al., 2015). Previous studies have reported that *Vibrio* isolates containing various resistance genes recovered from fish farms and shrimp farms pond water showed multiple drug resistance to ciprofloxacin, penicillin G, rifampicin, and vancomycin (Igbinosa, 2016; Lin et al., 2016; Stalin and Srinivasan, 2016). Significantly more plasmid-mediated quinolone resistance genes per bacterium and significantly higher numbers of *qnr*B genes in quinolone-selected bacteria were found in sediment samples from aquaculture sites than in those from non-aquaculture sites (Tomova et al., 2015). Muziasari et al. (2014) showed that ARGs persisted in the sediments of fish farms at very low antibiotic concentrations during a 6-year observation period from 2006 to 2012, but were less prevalent in the surrounding sediments. The sediment resistomes in fish farm were enriched in ARGs encoding resistance to antibiotics tetracycline, sulfonamide, trimethoprim and florfenicol, which had been used to treat fish in the farms. Aminoglycoside resistance genes were also enriched in farm sediments despite these farms not having used aminoglycosides (Muziasari et al., 2016). Di Cesare et al. (2013) found that aquaculture may influence the abundance and spread of benthic enterococci, and that farm sediments can be reservoirs of dormant antibiotic-resistant bacteria, including enterococci. Both culture-dependent and culture-independent methods did not reveal significant differences in ARB and ARG levels between the studied sites. The fish farm increased the diversity tetracycline-resistance genes in the receiving river (Harnisz et al., 2015). Tamminen et al. (2011) reported that two medium-scale fish farms in shallow water had a distinct composition of phylogenetic clusters compared with a small farm, a medium farm in open water and two pristine sites. Medium-scale fish farming in shallow water therefore seems to have a greater impact on sediment bacteria than fish farming in open water. A recent study found that integrated culture (duck–fish ponds) showed lowest absolute abundance of ARGs in water and the highest in sediment (Huang et al., 2017). Monoculture ponds (duck ponds and fish ponds) showed higher relative abundances of ARGs in both water and sediment. *Tet*A was suggested to be a potential indicator for the abundance of tetracycline resistance genes in these aquaculture modes in the Pearl River Delta (Huang et al., 2017). Harnisz et al. (2011) discovered that tetracycline-resistant bacteria are reliable indicator of antibiotic resistance. Sample collection in the previous studies has been performed using grab sampling or single sampling, or long-term monitoring for sediment in the aquaculture ponds. However, the information about the temporal variation in the abundance of ARGs during the entire rearing period and the dissemination of ARGs among water, sediment, and reared organisms in aquaculture need further study. It is necessary to understand the temporal variation in ARGs during the aquaculture season and the dissemination of ARGs in aquaculture. Thus, our hypotheses are 1) the abundance of ARGs in aquaculture environments varies temporally, 2) water source is an important medium for disseminating ARGs to aquaculture, 3) the ARGs in aquaculture environments may be disseminated to the reared organisms.

The objective of this study was to investigate the temporal variation and contamination profiles of ARGs and bacterial community during the entire rearing period in shrimp aquaculture environments and shrimp intestinal tract. The results of this study can provide a better understanding of the temporal variation in the abundance and dissemination of ARGs in aquaculture.

2. Materials and methods

2.1. Study sites and sample collection

The aquaculture zone selected as the study site is located in the estuary of the Pearl River Delta in Guangzhou (113.636154°E, 22.625284°N), South China. The aquaculture zone covers an area of 2350 ha. The selected shrimp farm is 6.7 ha in size and includes nine ponds. White leg shrimp (*Litopenaeus vannamei*) was the predominant reared organism, which was polycultured with 100 grass carp (*Ctenopharyngodon idellus*) in each pond. Water used in the aquaculture ponds was obtained from a nearby river. There is no water exchange during the rearing period after drawing water into the ponds.

The river used as the farming water source and three ponds, named A1, A2 and A3, were selected for sampling at monthly intervals during the rearing period between April and October in 2016. Water samples (0.5 L) from the water source and three ponds were collected from 30 cm below the surface using a stainless-steel sampler and transferred to sterile polyethylene bottles. Sediment samples (100 g) from a depth of 0–10 cm were collected using a grab sampler and then mixed well in sterile polyethylene bottles. Shrimp samples (100 juvenile shrimps and 50 adult shrimps) were collected using sterile plastic bags. Farming input samples, including feed (100 g), photosynthetic bacteria (PSB) product (0.5 L), *Bacillus* powder product (100 g) and lactic acid bacteria (LAB) product (0.5 L) were also obtained. All samples were collected in three replicates, stored in a portable ice box and transported to the laboratory for pretreatment within 24 h.

2.2. Environmental factors analysis

The dissolved oxygen (DO), temperature, pH and salinity of water samples were determined using YSI ProPlus meter. The total nitrogen (TN) and total phosphorus (TP) of water samples were determined with ultraviolet and visible spectrophotometer in accordance with the Chinese national standard methods of China HJ 636-2012 and GB 11893-1989, respectively. Similarly, the TN and TP of sediment samples were determined with ultraviolet and visible spectrophotometer according to the Chinese national standard methods of China HJ 632-2011 and LY/T 1228-1999, respectively.