Occurrence of human pathogenic bacteria carrying antibiotic resistance genes revealed by metagenomic approach: A case study from an aquatic environment

Renjun Zhou¹, Shenzheng Zeng¹, Dongwei Hou¹, Jian Liu¹, Shaoping Weng², Jianguo He¹,², Zhijian Huang¹,*

¹. State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering, School of Marine Sciences, Sun Yat-sen University, Guangzhou 510275, China
². School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China

ARTICLE INFO

Article history:
Received 19 June 2018
Revised 4 January 2019
Accepted 4 January 2019
Available online 14 January 2019

Keywords:
Antibiotic resistance genes
Human pathogenic bacteria
Mobile genetic elements
Aquatic environment

ABSTRACT

Antibiotic resistance genes (ARGs), human pathogenic bacteria (HPB), and HPB carrying ARGs are public issues that pose a high risk to aquatic environments and public health. Their diversity and abundance in water, intestine, and sediments of shrimp culture pond were investigated using metagenomic approach. A total of 19 classes of ARGs, 52 HPB species, and 7 species of HPB carrying ARGs were found. Additionally, 157, 104, and 86 subtypes of ARGs were detected in shrimp intestine, pond water, and sediment samples, respectively. In all the samples, multidrug resistance genes were the highest abundant class of ARGs. The dominant HPB was Enterococcus faecalis in shrimp intestine, Vibrio parahaemolyticus in sediments, and Mycobacterium yongonense in water, respectively. Moreover, E. faecalis (contig Intestine_364647) and Enterococcus faecium (contig Intestine_364647) carrying efrA, efrB and ANT(6)-Ia were found in shrimp intestine, Desulfoaricina cetonica (contig Sediment_825143) and Escherichia coli (contig Sediment_188430) carrying mexB and APH(3’)-Ia were found in sediments, and Laribacter hongkongensis (contig Water_478168 and Water_369477), Shigella sonnei (contig Water_880246), and Acinetobacter baumannii (contig Water_525520) carrying sul1, sul2, ereA, qacH, OXA-21, and mphD were found in pond water. Mobile genetic elements (MGEs) analysis indicated that horizontal gene transfer (HGT) of integrons, insertion sequences, and plasmids existed in shrimp intestine, sediment, and water samples, and the abundance of integrons was higher than that of other two MGEs. The results suggested that HPB carrying ARGs potentially existed in aquatic environments, and that these contributed to the environment and public health risk evaluation.

© 2019 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.
Introduction

During the past few decades, antibiotics have been widely used in China as food additives and veterinary drugs in livestock farming to prevent diseases (Cheng et al., 2014). Approximately 25%–75% of antibiotics administered to animals are excreted via feces or urine, and then released into the receiving environment (Karthikeyan and Meyer, 2006; Zhou et al., 2018). Antibiotics that remain in the environment promote the enrichment of antibiotic resistance genes (ARGs). The World Health Organization (WHO) has identified antibiotic resistance as one of the largest threats to global health, food security, and development, and has indicated that new resistance mechanisms are emerging, threatening the ability to treat common infectious diseases (Fang et al., 2015).

Recent studies have indicated that ARGs are found in diverse environments, including water (Zhang et al., 2009), soil (Lin et al., 2016), sediments (Han et al., 2017), airborne particles (Hu et al., 2018), and livestock manure (He et al., 2014). Antibiotic resistance genes could be transferred among microorganisms via mobile genetic elements (MGEs), including integrons, insertion sequences, and plasmids (Ochman et al., 2000), with MGEs considered to be the main factor accelerating the dissemination of ARGs in aquatic environments (Ouyang et al., 2015). As a carrier of ARGs, MGEs have been found to be significantly correlated with ARGs in sediments of the South China Sea ocean (Chen et al., 2013) and waste water (Hu et al., 2016).

The pathogen Escherichia coli O157:H7, was isolated from a patient who exhibited cephalosporin resistance because of plasmid-mediated β-lactam resistance (Folster et al., 2014). Virulence genes have also been shown to be transferred from pathogens via horizontal gene transfer (HGT) (Friese et al., 2006; Maiques et al., 2006). In the United States, a seafood-associated pathogen Vibrio parahaemolyticus, which causes acute hepatopancreatic necrosis disease (AHPND) to shrimp (Tran et al., 2013), was also found to be highly resistant to ampicillin (Elmahdi et al., 2016). In Malaysia, V. parahaemolyticus, isolated from shellfish, also exhibited high resistance to ampicillin, amikacin, kanamycin, cefotaxime, and ceftazidime (Vengadess et al., 2015). Seafood-borne bacterial pathogens have the potential to threaten human health, especially when seafood is consumed in the raw, ready-to-eat form. Enterococcus faecalis and Enterococcus faecium, which are associated with the colonization of the human and animal gastrointestinal tract, have multidrug resistance (Jahan et al., 2015) and virulence factor genes in their genomes (Chuang et al., 2009; Nallapareddy et al., 2008). These organisms cause infectious endocarditis, urinary tract infections, and bacteremia (Schaber et al., 1991). Previous studies have investigated ARGs, MGEs, and bacterial pathogens from aquatic environments; however, only a few investigations have focused on the distribution of bacterial pathogens carrying ARGs in aquatic environments.

Shrimp are one of the most important aquatic products among fishery trading commodities worldwide (Lebel et al., 2010). Shrimp production in China accounts for 37.35% of the global production (FAO, 2015). However, recent outbreaks of shrimp disease have incurred severe losses to the shrimp trade. For example, an emerging bacterial disease known as early mortality syndrome (EMS) has led to serious economic losses to the shrimp industry since 2009 (Liu et al., 2016), and outbreaks of AHPND have been particularly devastating to shrimp in a number of countries, leading to global losses of more than 1 billion US dollars per year (Suong et al., 2017). In China, approximately 15%–20% losses of annual total aquaculture production were caused by bacterial infection (Arthur et al., 2002; Huang et al., 2016). To prevent diseases caused by bacterial infections, antibiotics are used as disinfectants in aquaculture activities. In China, 13 antibiotics have been authorized for use in aquaculture, while 234 antibiotic residue cases have been reported according to a previous survey (Liu et al., 2017). These antibiotics remain in the environment, forming selective pressure on bacteria leading to increased antibiotic resistance (Tello et al., 2012). However, to our knowledge, only a few studies have focused on bacterial pathogens carrying ARGs in shrimp farming environments.

Although important advances have been made to standardize the detection of microorganisms in isolates, studies of uncultured and unknown isolation conditions of most environmental bacteria are still difficult to conduct using traditional isolation methods (Berglund et al., 2015). Recently, next generation sequencing has been applied for wide-spectrum detection of species and their genetic diversity, including unculturable microbes (Martinez-Porchas and Vargas-Albores, 2017). Metagenomic methods based on high-throughput sequencing have been developed to provide an insight into the correlation between pathogens and ARGs in environmental media (Fang et al., 2015). For example, 20 instances of co-occurrence between human pathogenic bacteria (HPB) and ARGs were observed in sewage treatment plants using metagenomic analysis (Ju et al., 2016). Another approach was applied to observe co-occurrence between ARGs and other genes using metagenomics via the identification of ARG-carrying contigs and their ORFs (Ma et al., 2016). However, the distinct co-occurrence between HPB and ARGs in aquatic environments has remained unclear.

The present study was conducted to investigate the distribution of ARGs, HPB, MGEs, and the occurrence of HPB carrying ARGs in different media of aquatic environments including shrimp intestine, sediments, and water using a metagenomic approach. The results of this study suggest the potential for occurrence of HPB carrying ARGs in aquatic environment, which will help to prevent ARG contamination and evaluate public health risk.

1. Materials and methods

1.1. Sample collection

Shrimp intestine, water, and sediment samples were collected from a shrimp pond, without any use of antibiotics during shrimp culture, in Maoming, Guangdong Province, China (21.68°N, 110.88°E) in November 2015, with a surface area of approximately 2600 m² and a depth of 1.5 m. The shrimp body length was approximately 15 cm. Sample storage and pretreatment was conducted as previously reported (Hou et al., 2017; Hou et al., 2018; Zeng et al., 2017). The shrimp surfaces
sterilized with 70% ethanol, after which their intestines were aseptically dissected from the musculature and placed into a 15 mL sterile centrifuge tube containing 1 mL PBS buffer. One intestine was kept as one sample for the DNA extraction. Additionally, 1.0 L of water taken from the surface of the pond was kept as one sample and filtered through 0.22 \( \mu \)m filter membranes for the DNA extraction. Finally, 5.0 g sediment was placed into 15 mL centrifuge tubes and washed with 10 mL PBS buffer three times. All samples were stored at −80°C until DNA extraction.

1.2. Genomic DNA extraction and sequencing

Genomic DNA from intestines, sediments, and water was extracted using a PowerFacel DNA Isolation Kit (Mobio, USA), PowerSoil DNA Isolation Kit (Mobio, USA), and Water DNA Kit (Omega Bio-Tek, USA), respectively. The DNA extracted from five biological repetitions of each sample was pooled into one DNA sample to minimize any potential DNA extraction bias. The concentration and purity of genomic DNA were determined using a NanoVuePlus Spectrophotometer (GE Healthcare, USA). Next, a DNA library was constructed in an average length of ~350 bp DNA fragment using the 150 paired-end sequencing strategy from the Illumina HiSeq platform, conducted by Novogene Bioinformatics Technology Co. Ltd. (Beijing, China). Approximately 12 GB of metagenomic data were generated for each sample after sequencing. The raw reads (150 bp) of each data set were trimmed to discard reads that contained ambiguous nucleotides of more than 10 bp, low sequencing quality nucleotides and contaminated adapters, prior to further analysis. Finally, the sequencing quality of all the samples was controlled with the Q30 (reads’ quality value larger than 30) ≥85%.

1.3. Bioinformatics analysis

1.3.1. ARGs analysis

The comprehensive antibiotic resistance database (CARD, https://card.mcmaster.ca/, version 1.1.9) (Jia et al., 2017) contained 2381 high-quality reference ARG sequences. The DIAMOND program (version 0.8.2) (Buchfink et al., 2015) was applied to annotate ARG-like reads against CARD, with a cut-off E-value of \( 1 \times 10^{-7} \), sequence identity of 85%, and query cover of 70% (Ma et al., 2016).

1.3.2. 16S rRNA analysis

The Greengenes 16S rRNA database (version 13.5) was downloaded directly from the Greengenes website (http://greengenes.lbl.gov/) (McDonald et al., 2012). The metagenomic reads from each sample were searched against the Greengenes database using BLASTN with a threshold of E-value of \( 1 \times 10^{-20} \). The Greengenes 16S hit reads were extracted from the metagenomic datasets for HPB analysis.

1.3.3. HPB analysis

A HPB 16S rRNA database was constructed using bacteria 16S RNA sequences from NCBI reference genomes after extracting bacteria accession numbers from the virulence factors (VFs) database (http://www.mgc.ac.cn/VFs/) (Chen et al., 2016b). The HPB IDs and their corresponding bacteria, accession number and 16S rRNA information are listed in Appendix A Table S1. Human pathogenic bacteria were characterized using BLASTN program to analyze 16S rRNA-like reads extracted from each sample with the E-value \( <1 \times 10^{-20} \). The outputs were filtered with a query identity ≥99%, mismatch ≤1 bp and an alignment length ≥150 bp to annotate HPB based on strict criteria (Fang et al., 2015).

1.3.4. HPB carrying ARGs

Reads from metagenomic trimmed data were assembled using MEGAHIT (version 1.1.2) (Li et al., 2016), with a contig length of at least 500 bp. Contigs from all samples were predicted open reading frames (ORF) using MetaGeneMark (version 3.38) (Zhu et al., 2010) in the default parameters. All ORF sequences were searched against the CARD database with a cut-off E-value \( <1 \times 10^{-7} \) and query identity of 85% to extract ARG-carrying contigs. The remaining ORFs from ARG-carrying contigs were searched against the VFs database with an E-value \( <1 \times 10^{-7} \). Contigs with ARGs and VFs were extracted using customized Matlab scripts, then annotated to the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov) (Ma et al., 2016).

1.3.5. MGE analysis

MGEs were characterized using the INTEGRALL database (Moura et al., 2009) (1447 integrase genes and 8053 gene cassettes) and plasmid sequences were downloaded from the NCBI RefSeq database (2408 plasmid sequences) using the BLASTN program (version 2.6.0+) with an E-value \( <1 \times 10^{-20} \), sequence identity ≥95% and hit length ≥90 bp (Chen et al., 2016a).

1.4. Statistical analysis

To avoid bias due to different eukaryotic and prokaryotic DNA portions in environmental samples, the abundance of ARGs and MGEs was normalized against the bacterial cell number in the samples through the ARGs online analysis platform (ARGs-OAP) (Yang et al., 2016). The calculation of ARGs and MGEs abundance was as follows:

\[
\text{Abundance}_{\text{gene}} = \frac{\sum_{i} N_{\text{gene-like sequences}} \times \text{L}_{\text{gene reference sequence}}}{N_{\text{cell number}}}
\]

In this equation, \( N_{\text{gene-like sequences}} \) are the number of the ARG- or MGE-like sequences annotated to one specific ARG or MGE reference sequence; \( \text{L}_{\text{gene reference sequence}} \) refers to the nucleotide sequence length of the corresponding specific ARGs or MGE reference sequence; and \( N_{\text{cell number}} \) represents the bacteria cell number in the sequencing data.

Data were analyzed using the customized MATLAB R2016a (MathWorks, USA) scripts, Microsoft Office Excel 2016 (Microsoft Corporation, USA) and OriginLab Pro Edition 2016 (OriginLab Corporation, USA). Sequence cluster analysis was conducted using Mega (version 6.0) (Tamura et al., 2013). Venn diagrams were drawn using the Venn diagrams tool (Bioinformatics & Evolutionary Genomics, website URL: http://bioinformatics.psb.ugent.be/webtools/Venn/).

Sequencing data from this study have been submitted to the NCBI Sequence Read Archive (SRA) under accession number SRP144257.
2. Results

2.1. Diversity and abundance of ARGs in the shrimp pond

A total of 19 classes of ARGs were characterized from the shrimp intestine, sediments, and water samples. Multidrug resistance genes were most dominant, being found in high abundance in the shrimp intestine and its surrounding environment. Specifically, these genes accounted for 39.0%, 37.2%, and 22.1% in shrimp intestine, sediments, and water samples, respectively. In shrimp intestine samples, the second most abundant class of ARGs was macrolides, followed by sulfonamides and aminoglycosides. Diaminopyrimidine resistance genes were the second most abundant ARGs found in pond sediment samples, while sulfonamide, macrolide, and diamino.pyrimidine resistance genes were dominant in pond water samples with multidrug resistance genes (Fig. 1). The total ARGs abundance of shrimp pond water was highest with 0.159 copies/cell, followed by 0.099 copies/cell in sediment and 0.072 copies/cell in shrimp intestine. The abundances of multidrug resistance genes were 0.028, 0.037, and 0.035 copies/cell in shrimp intestines, sediments, and water samples, respectively (Fig. 2). Generally, the ARGs resistance type in all three samples showed similarity in drug resistance diversity and abundance.

A total of 157, 104, and 86 subtypes of ARGs were identified in shrimp intestine, sediments, and water samples, respectively. Additionally, 34 subtypes of ARGs were shared among all samples, while 69, 30, and 20 subtypes occurred uniquely in the intestine, sediments, and water samples, respectively (Fig. 3, Appendix A Table S2). The abundance of the shared ARGs subtypes accounted for 38.7% in shrimp intestine samples, 81.6% in the sediment samples, and 61.4% in water samples, respectively (Table 1). The most abundant ARG subtype was the...
dfrE gene, which provided diaminopyrimidine resistance, followed by macrolide resistance gene ereA and sulfonamide resistance gene sul1 (Table 2).

### 2.2. HPB carrying ARGs

A total of 52 HPB has been found, 26 of which only occurred in 1 sample and 9 of which occurred in all the 3 samples (Appendix A Table S3), accounting for 45.1%, 79.1%, and 74.7% of the total (Table 3) in the intestine, sediments, and water samples, respectively (Fig. 4a). HPB_038 (V. parahaemolyticus, 28.8%) and HPB_303 (Brucella melitensis, 21.8%) were dominant in sediment sample, while HPB_354 (Mycobacterium yongonense, 34.0%) and HPB_168 (Mycobacterium gilvum, 17.9%) were dominant in water. However, HPB_333 (E. faecalis) only occurred in shrimp intestines with the highest abundance of 26.9%, followed by HPB_038 (V. parahaemolyticus, 13.6%) (Fig. 4b).

A total of 507,674, 718,429, and 618,607 contigs were generated via MEGAHIT, while 748,266, 1,266,154, and 1,004,119 ORFs were predicted from the intestine, sediments, and water metagenomic data, respectively, using MetaGeneMark. After alignment against the CARD database using DIAMOND, 69 ORFs were annotated as ARG-like sequences in shrimp intestines and its surrounding environment (Table 4). Eight contigs were found containing ARGs and VFs. The best hits from the bacteria annotations are shown in Fig. 5. Contigs Intestine_364647 and Intestine_80272 in the shrimp intestine were annotated as E. faecalis and E. faecium sequences, and contigs Sediment_825143 and Sediment_188430 in sediment were annotated as Desulfosarcina cetonica and E. coli sequences. Contigs Water_478168, Water_880246, Water_369477 and Water_525520 in water samples were annotated as Laribacter hongkongensis, Shigella sonnei and Acinetobacter baumannii sequences. In contig Intestine_364647, the efrA and efrB genes and a virulence factor

### Table 1 – Share antibiotic resistance genes (ARGs) relative abundances accounting for shrimp intestine, sediment, and water.

<table>
<thead>
<tr>
<th>Source</th>
<th>Shared ARGs percentage (%)</th>
<th>Intestine</th>
<th>Sediment</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine only</td>
<td>26.80</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sediment only</td>
<td>-</td>
<td>5.35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water only</td>
<td>-</td>
<td>-</td>
<td>3.57</td>
<td>-</td>
</tr>
<tr>
<td>Intestine-Sediment</td>
<td>19.01</td>
<td>11.91</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intestine-Water</td>
<td>15.54</td>
<td>-</td>
<td>31.78</td>
<td>-</td>
</tr>
<tr>
<td>Sediment-Water</td>
<td>-</td>
<td>1.14</td>
<td>3.21</td>
<td>-</td>
</tr>
<tr>
<td>Intestine-Sediment-Water</td>
<td>38.65</td>
<td>81.60</td>
<td>61.44</td>
<td>-</td>
</tr>
</tbody>
</table>

-: none.

### Table 2 – The 10 highest abundances of ARGs detected from shrimp intestine, sediment, and water.

<table>
<thead>
<tr>
<th>Class</th>
<th>ARGs</th>
<th>Abundance of ARGs (copies/cell)</th>
<th>Intestine</th>
<th>Sediment</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaminopyrimidine</td>
<td>dfrE</td>
<td>0.004074</td>
<td>0.024984</td>
<td>0.009541</td>
<td></td>
</tr>
<tr>
<td>Macrolide</td>
<td>ereA</td>
<td>0.008641</td>
<td>0.001086</td>
<td>0.018087</td>
<td></td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>sul1</td>
<td>0.002598</td>
<td>0.003820</td>
<td>0.021246</td>
<td></td>
</tr>
<tr>
<td>Multidrug</td>
<td>smeE</td>
<td>0.000474</td>
<td>0.014454</td>
<td>0.001063</td>
<td></td>
</tr>
<tr>
<td>Multidrug</td>
<td>mtrA</td>
<td>0.002289</td>
<td>0.003708</td>
<td>0.008343</td>
<td></td>
</tr>
<tr>
<td>Polymyxin</td>
<td>armA</td>
<td>0.000876</td>
<td>0.003609</td>
<td>0.008887</td>
<td></td>
</tr>
<tr>
<td>Multidrug</td>
<td>qacH</td>
<td>0.003163</td>
<td>0.000000</td>
<td>0.010054</td>
<td></td>
</tr>
<tr>
<td>Polymyxin</td>
<td>pmrA</td>
<td>0.000822</td>
<td>0.004973</td>
<td>0.004446</td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>mfd</td>
<td>0.000480</td>
<td>0.005027</td>
<td>0.002158</td>
<td></td>
</tr>
<tr>
<td>Macrolide</td>
<td>mphD</td>
<td>0.002663</td>
<td>0.000000</td>
<td>0.003611</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>0.046241</td>
<td>0.038724</td>
<td>0.071454</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3 – Shared human pathogenic bacteria (HPB) relative abundances accounting for shrimp intestine, sediment, and water.

<table>
<thead>
<tr>
<th>Source</th>
<th>Shared HPB percentage (%)</th>
<th>Intestine</th>
<th>Sediment</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine only</td>
<td>43.59</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sediment only</td>
<td>-</td>
<td>9.93</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water only</td>
<td>-</td>
<td>-</td>
<td>7.31</td>
<td>-</td>
</tr>
<tr>
<td>Intestine-sediment</td>
<td>6.92</td>
<td>9.19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intestine-water</td>
<td>4.36</td>
<td>-</td>
<td>17.35</td>
<td>-</td>
</tr>
<tr>
<td>Sediment-water</td>
<td>-</td>
<td>1.77</td>
<td>0.66</td>
<td>-</td>
</tr>
<tr>
<td>Intestine-sediment-water</td>
<td>45.13</td>
<td>79.11</td>
<td>74.68</td>
<td>-</td>
</tr>
</tbody>
</table>

-: none.

### Table 4 – Summary of assembled contigs in metagenomic sequences.

<table>
<thead>
<tr>
<th>Item</th>
<th>Sample number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contigs</td>
<td>507,674, 718,429, 618,607</td>
</tr>
<tr>
<td>ORFs</td>
<td>748,266, 1,266,154, 1,004,119</td>
</tr>
<tr>
<td>ARGs like ORFs</td>
<td>35, 8, 26</td>
</tr>
<tr>
<td>ARGs carrying contigs</td>
<td>32, 8, 19</td>
</tr>
</tbody>
</table>

ORFs: open reading frames.

Fig. 4 – The diversities and relative abundances of HPB detected in shrimp intestine, sediment, and water (a) diversity of HPB and (b) relative abundance of HPB.
gene encoding rab2 interacting conserved protein A were found, which was annotated as E. faecalis with 100% sequence coverage and 99% identity. Moreover, the ereA, qacH and OXA-21 genes and a virulence factor gene encoding hypothetical enhanced entry proteins were found in contig Water_369477, which was annotated as L. hongkongensis with 42% sequence coverage and 99% identity. In addition, L. hongkongensis was also found to include the sul1 gene and virulence factor gene encoding carbon storage regulator in water sample in contig Water_478168. Contig Intestine_80272 contained aminoglycoside resistance gene ANT(6)-Ia and virulence factor gene encoding methyltransferase associated with the glycopeptidolipid locus. In sediment sample, contig Sediment_825143, which contained the multidrug resistance gene mexB and a virulence factor gene encoding drug efflux protein, was annotated as D. cetonica. Contig Sediment_188430 was annotated as E. coli and found contain multidrug resistance gene APH(3′)-IIa and a virulence factor gene encoding lac family transcriptional regulator. In pond water samples, S. sonnei were found to include the sul2 gene and virulence factor encoding phosphoglucomutase in contig Water_880246. A. baumannii was found to contain the mphD gene and virulence factor encoding transposase.

### 2.3. Abundance of MGEs

A total of 0.046, 0.085, and 0.256 copies/cell MGEs were detected in the shrimp intestine, sediments, and water samples, respectively. The abundance of integron genes was highest among all MGEs, with 0.234 copies/cell in water, 0.062 copies/cell in shrimp intestine, and 0.036 copies/cell in sediments. The abundance of insertion sequences was 0.005, 0.002, and 0.014 copies/cell in the shrimp intestine, sediments,
and pond water samples, respectively, and the abundance of plasmid was 0.017, 0.008, and 0.010 copies/cell (Fig. 6).

3. Discussion

With the widespread use of antibiotics, ARGs have been found to be enriched in many environments, where they exert selective pressure (Zou et al., 2011), threatening public health and raising the costs associated with treatment of bacterial infections (Golkar et al., 2014). This study characterized HPB and ARGs distributed in shrimp intestine, pond water, and sediments using a metagenomic approach, and the results suggested that HPB carrying ARGs potentially occurred in aquatic environments, which will be helpful in evaluating the environmental and public health risks.

In shrimp intestine, pond water, and sediment samples, the most abundant ARG in sediment was dfrE encoding dihydrofolate reductase, which was found in the E. faecalis chromosome (Coque et al., 1999). The second most abundant ARGs in water samples was ereA, encoding erythromycin esterase, which hydrolyzed the lactone ring of macrolide drugs and was associated with gram-negative bacteria (Morar et al., 2012). Sul1, majorly located in plasmids (Martinez et al., 2007) and found to be linked to other resistance genes of class 1 integrons (Marquez et al., 2008), was prevalent in shrimp pond water samples, with a high level of integrons. Aminoglycoside resistance genes were found in high abundance in estuary water, where they comprised 6.9%–26.1% (Zhu et al., 2017). In the present study, the aminoglycoside resistance genes exhibited a low detection frequency with 4.7%, 8.7%, and 9.1% in the shrimp pond environment. The relative abundance of the aminoglycoside resistance gene may be related to different environmental sources.

Mycobacterium sp., which is associated with lung disease (Alexander et al., 2017), has been found in snakehead fish (Channa argus), usually dwelling in river catchments (Densmore et al., 2016). In the present study, M. yongonense and M. gilvum were most abundant in shrimp pond water samples. V. parahaemolyticus, which is a seafood-borne bacterial pathogen that was previously found in shellfish (Vengadesh et al., 2015) and freshwater shrimp (He et al., 2016), was present in high levels in shrimp intestine, pond water, and sediment samples in the present study. E. faecalis, which has a naturally high level of antibiotic resistance (Ryan et al., 2004), only occurred in shrimp intestine samples in the present study. This may have been because of its facultative anaerobic and commensal properties, allowing it to exist in animal gastrointestinal tracts.

A previous study explored the indirect correlation between HPB and ARGs by examining their abundance (Ju et al., 2016). In the present study, a direct correlation was demonstrated between HPB contigs carrying VF genes and ARGs. There were eight contigs found to have high similarity with pathogen bacteria. EfrA and efrB in Intestine_364647 were heterodimeric ABC transporter efflux pumps that conferred resistance to ciprofloxacin, erythromycin, rifampicin, and quinupristin (Jia et al., 2017), which have been found in E. faecalis (Lavilla Lerma et al., 2014; Lee et al., 2003). Similarly, in our study, efrA and efrB were also annotated in contig Intestine_80272 of E. faecalis. Contig Intestine_80272 was annotated with E. faecium, which contains the aminoglycoside resistance gene ANT(6)-Ia. E. faecium possessing ANT(6)-Ia has been reported to be isolated high frequently (Kobayashi et al., 2001). Sul2, which provides sulfonamide resistance, was found in S. sonnei (Chang et al., 2011), and is seen to cause shigellosis and mortality after oral ingestion (Pitisuttithum et al., 2016). These findings were concordant with our result from contig Water_880246. Overall, these results may provide an insight into the potential occurrence of HPB carrying ARGs in aquaculture organisms and culture environments, and are expected to facilitate environmental and public health risk evaluations.

4. Conclusions

The present study investigated the ARGs, HPB, and MGEs in shrimp intestine, pond water, and sediment samples. Overall, 19 classes of ARGs were found in shrimp intestine, sediment, and water samples, and these were dominated by multidrug resistance types. Additionally, 34 subtypes of ARGs were shared in all samples. The most abundant HPB was V. parahaemolyticus, followed by M. yongonense. Enterococcus faecalis was detected only in shrimp intestine. Moreover, eight contigs were annotated as HPB carrying ARGs in all the samples. Overall, results of this study indicate that HPB carrying ARGs likely exist in aquatic environments.

Acknowledgment

This work was financially supported by the China Agriculture Research System (No. CARS-48), the Guangzhou Science Technology and Innovation Commission Project (No. 201510010071), and the Guangdong Ocean and Fishery Bureau Project (No. 20164200042090023).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.01.001.

References

genes and plasmids of Shigella sonnei isolates from outbreaks and sporadic cases in Taiwan. J. Med. Microbiol. 60, 197–204.


FAO (Food and Agriculture Organization), 2015. Fishery and Aquaculture Statistics Rome, Italy.


