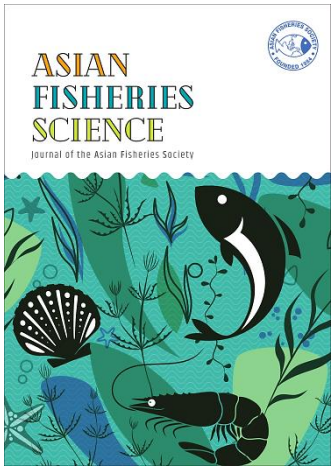

Antimicrobial Use and Antimicrobial Resistance in Aquaculture in the People's Republic of China

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Abstract

With the rapid development of aquaculture in the People's Republic of China, along with the increase of intensive aquaculture, there have been frequent disease outbreaks resulting in financial losses amounting to millions of dollars. Medication with antimicrobial agents is common practice in the treatment of bacterial infections, and thus there are nine antimicrobial agents permitted for use in Chinese aquaculture: doxycycline, neomycin, thiamphenicol, florfenicol, enrofloxacin, and four types of sulfonamide. Previous studies have demonstrated that freshwater animals can serve as reservoirs for *Aeromonas* containing multiple resistance genes. In this study, 1143 *Aeromonas* isolates were collected from fish and the aquatic environment in Guangdong Province, P.R. China from 2014 to 2016. Antimicrobial susceptibilities were determined by the micro broth dilution method. Moderate resistance to sulfamonomethoxine and nalidixic acid was found in *Aeromonas* strains, and most strains were highly sensitive to broad-spectrum antibiotics, such as β-lactams, fluoroquinolones, and aminoglycosides. Bacterial resistance mechanism studies have shown that various resistance genes are harboured in integrons and contribute to multiple resistances. In this study, class 1 integrons carrying various cassettes were determined in 50 *Aeromonas* strains, and displayed multiple resistance to trimethoprim, chloramphenicol, or ciprofloxacin. Data from the present study suggest that surveillance for antimicrobial resistance (AMR) of aquatic animal origin and the prudent and responsible use of antimicrobial agents are necessary.

Keywords: antimicrobial resistance, integron, AMR investigation, aquaculture

Introduction

The People's Republic of China is the world's largest producer, consumer, processor, and exporter of fish. It contributes to nearly 60 % of global aquaculture volume and roughly half of the global aquaculture value (FAO, 2020). The annual harvest from capture fisheries has been decreasing since 1999, but the yield from aquaculture production is continually increasing. In 2018, the total production reached nearly 65 million tonnes and total aquaculture production was about 50 million tonnes (FAMA, 2019). There is a great diversity of species cultured in Chinese aquaculture, including finfish, crustaceans, shellfish, reptiles, and seaweeds. More than 70 species are cultured, such as *Carassius auratus* (Linnaeus, 1758); *Ctenopharyngodon idella* (Valenciennes, 1844); *Cyprinus carpio* Linnaeus, 1758; *Oreochromis niloticus* (Linnaeus, 1758); *Penaeus vannamei* Boone, 1931; *Portunus trituberculatus* (Miers, 1876) and *Ostrea* sp. (FAMA, 2019).

With the development of aquaculture in P.R. China, especially the increase of intensive aquaculture, disease outbreaks frequently occur, causing millions of dollars in losses. As a result, the prevention of aquatic animal disease is essential for the betterment of the aquaculture industry, the improvement of farming production, and the increase in aquatic resources. Because of the complexity of their environment, aquatic animals are highly susceptible to viral, bacterial, fungal, and parasitic infections. These infections can adversely affect growth and development and can cause mortalities. For example, bacteria can cause serious infectious diseases such

as enteritis, gill rot, erythema and septicemia (Gauthier, 2015). Most bacterial pathogens are opportunistic and have high adaptability to environmental change. These pathogens often show a preference for certain species and certain organs. The predominant bacterial pathogens in aquaculture include members of the genera *Aeromonas*, *Vibrio*, *Streptococcus*, *Edwardsiella*, *Flavobacterium*, and *Pseudomonas* (Gauthier, 2015). Although the concept of "prevention is better than treatment" is fundamental to good aquaculture, due to their convenience, effectiveness, and economic justification, antimicrobial antiparasitic agents, disinfectants, and herbal medicines are commonly used in Chinese aquaculture. There are currently nine antimicrobial agents permitted for use in Chinese aquaculture: doxycycline, neomycin, thiamphenicol, florfenicol, enrofloxacin, and four types of sulfonamide (MARA, 2010). The use of antibiotics is one of the most important factors influencing the emergence of resistance in bacterial pathogens. Most of the time, an adequate dose of antibiotic that is properly applied will kill the bacteria. However, by the selective pressure of antibiotics, the bacteria may become resistant, and these resistant strains survive in nature and transfer to other animals (Qiao et al., 2018; Santos and Ramos, 2018) . Furthermore, the presence of resistant bacteria in foods is a potential threat to human health because such resistance may be spread to bacterial pathogens of humans, impeding the treatment of illness (Boerlin and Reid-Smith, 2008). The transmission of antimicrobial resistance (AMR) among bacteria may attribute to the mobile genetic elements such as plasmids, integrons, and transposons (Boerlin and Reid-Smith, 2008). Of these, the mobile integron encoded integrases can recombine gene cassettes and are primarily involved in the spread of antimicrobial resistance genes which contributed to multidrug resistance (Mazel, 2006).

Misuse and overuse of antimicrobial agents in human medicine and the food production industry have put every nation at risk. In response to this crisis, the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE), and the World Health Organization (WHO) jointly proposed a long-term basis for international collaboration aimed at coordinating global activities to address health risks at the animal-human-ecosystem interfaces. In response to the Global Action Plan on Antimicrobial Resistance developed by WHO, the Chinese Government launched a National Action Plan on AMR and a National Action Plan on AMR from Animal Origin. In 2008, the Surveillance Program on AMR from Animal Origin was established by the Ministry of Agriculture and Rural Affairs (MARA), P.R. China. Twenty-four provinces were involved in this resistance surveillance programme. At the beginning of this programme, AMR from aquatic animal origin was not included, and little information was available on the susceptibility of pathogens isolated from aquaculture. In 2015, the National Fisheries Technology Extension Center of MARA launched an aquatic surveillance programme involving 12 provinces, with more than 14 fish species being monitored, including *C. auratus*, *C. idellus*, *C. carpio*, *Scophthalmus maximus* (Linnaeus, 1758) and *O. niloticus*. *Aeromonas* spp., *Vibrio* spp., and other pathogens were isolated from diseased fish and evaluated for resistance to 14 antimicrobial agents by susceptibility testing. The purpose of the present study was to determine the prevalence and antimicrobial susceptibilities of *Aeromonas* spp. isolated from aquatic animals and their environments in Guangdong Province, P.R. China and to examine the genetic determinants in these resistant isolates. This information will help to evaluate the potential of multidrug-resistant aeromonads in these animals as a public health risk.

Materials and Methods

Sample collection, isolation, and identification

A total of 1143 isolates from fish and the aquatic environment was collected from 20 farms of six districts in four cities of Guangdong Province, P.R. China. The fish investigated included *O. niloticus*; *C. idellus*; *Cirrhinus mrigala* (Hamilton, 1822); *Siniperca chuatsi* (Basilewsky, 1855); *Megalobrama amblycephala* Yih, 1955; and *Channa maculata* (Lacepède, 1801) ♀ × *C. argus* (Cantor, 1842) ♂. Gills and intestines were aseptically swabbed using sterile cotton buds and inoculated into Luria-Bertani (LB) broth for pre-enrichment at 28 °C ± 2 °C for 18 h~24 h. The enriched cultures were streaked on Rimler-Shotts agar and incubated at 28 °C ± 2 °C for 18 h~24 h. Yellow, oxidase-positive colonies were isolated and presumptively considered as *Aeromonas* species. One to three *Aeromonas* strains were selected from each sample. The presumptive *Aeromonas* colonies were further investigated by biochemical typing using ATB™ New System (BioMérieux, France). The identification was confirmed by polymerase chain reaction (PCR) amplification of 16S rRNA gene and *gyrB* genes, which was performed as described in previous studies (Borrell et al., 1997; Yáñez et al., 2003). Taxonomic identification of the sequences was performed using BLAST in GenBank (http://blast.ncbi.nlm.nih.gov/).

Antimicrobial susceptibility testing

All strains were evaluated for resistance to 14 antimicrobial agents by the micro broth dilution method. The antimicrobial agents tested are listed in Table 1 and *Escherichia coli* ATCC 25922 was used as a control organism. The minimal inhibitory concentration (MIC) results were interpreted in accordance to breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2006a, b).

PCR assays for detection of integrons and gene cassettes

Genomic DNA was extracted by the whole cell boiled lysate protocol (Deng, et al., 2014). PCR amplication of *intI1*, *intI2*, and *intI3* genes was performed with the template DNA of the *Aeromonas* isolates. All the *intI1*-positive strains were also analysed for *sull* and *qacEΔ1* fragments by PCR using primers described previously (Lévesque et al., 1995; Sandvang et al., 1997; Reyes et al., 2003).

Results

Identification of Aeromonas *spp.*

A total of 1143 *Aeromonas* isolates were identified to the species level by PCR amplification of 16S rRNA and *gyrB* genes. The dominant species were *Aeromonas veronii* (689, 60.28 %) and *Aeromonas jandaei* (207, 18.11 %). Other *Aeromonas* species included *Aeromonas sobria* (91, 7.96 %), *Aeromonas hydrophila* (81, 7.09 %), *Aeromonas caviae* (67, 5.86 %), *Aeromonas dhakensis* (4, 0.35 %), *Aeromonas simiae* (3, 0.26 %), and *Aeromonas schubertii* (1, 0.09 %).

Antimicrobial susceptibility of Aeromonas

Susceptibility testing of the *Aeromonas* isolates showed moderate resistance to sulfamonomethoxine and nalidixic acid. Most of the isolates were highly sensitive to 12 other tested drugs (Table 1).

Comparison of antimicrobial resistance profiles among *Aeromonas* species showed that the strains of *A. caviae* were multiple drug resistant (MDR) to 10 of the 14 tested antimicrobials, with the MDR rate of 25.37 %. The MDR rate of *A. hydrophila* isolates was 11.11 %. *Aeromonas sobria*, *A. jandaei*, and *A. veronii* were susceptible to more antibiotics, with the MDR rates of 2.20 %, 1.93 % and 1.74 %, respectively.

Detection and characterisation of integron and gene cassettes

Overall, 50 (4.37 %) *Aeromonas* isolates were detected with *intI1* genes (Table 2), among which 36 isolates (72 %) harboured gene cassettes. Ten types of gene cassette arrays were determined by sequencing, including *dfrA17* (GenBank accession no. KR067581.1), *dfrA12-orfF-aadA2* (GenBank accession no. KR067578.1), *dfrB4-catB3-aadA1* (GenBank accession no. KR067582.1), *catB8* (GenBank accession no. KR067580.1), *aac6(6')-Ib-cr-arr-3* (GenBank accession no. KR868994.1), *aac-Ⅱ-bla*$_{OXA-21}$*-catB3* (GenBank accession no. KR067583.1), *aar2-aacA4-drfA1-orfC* (GenBank accession no. KR067585.1), *aac(6')-Ib-cr* (GenBank accession no. KR868995.1), *dfrA15* (GenBank accession no. KR868993.1), and *dfrB4-catB3-bla*$_{OXA-10}$*-aadA1* (GenBank accession no. KR067584.1). Among carriers of gene cassettes, the strains harbouring *aac(6')-cr-aar3* and *aac(6')-cr* displayed more multiple resistance than others.

Discussion

Antimicrobial susceptibility of Aeromonas

The majority of antimicrobial agents used in aquaculture are also used in human or veterinary medicine. In Europe, no more than two or three antimicrobial agents are licensed for use in aquaculture in each country (Carvalho and Santos, 2016). However, there are many countries with significant aquaculture industries where there is little effective regulation of access to, or use of, antimicrobials (Smith, 2008). For example, there are a variety of agents that have been or are being used in Asia (Nhung et al., 2016).

Fluoroquinolone, tetracyclines and sulfonamides have been commonly used for the last two decades to prevent and control motile *Aeromonas* septicemia or ulcerative infections in fish (Serrano, 2005) . Although only a few antimicrobial agents have been licensed for use in Chinese aquaculture (MARA, 2010), the imprudent and abusive use of antimicrobials has lead to various antimicrobial resistance mechanisms being encountered in different cultured species. In the current study, the results showed a detailed pattern of sensitivity of the various *Aeromonas* isolates to a variety of antimicrobials and provided useful information in the context of selective isolation and phenotypic identification of the aeromonads. In general, most of the isolates were susceptible to fluoroquinolones, doxycycline, cefotaxime, chloramphenicol, and amikacin. These results are in agreement to those published previously (Ishida et al., 2010; Nagar et al., 2011; Aravena-Román et al., 2012).

Integrons in Aeromonas

Globally, integrons are of increasing concern, as they are gene acquisition systems contributing to expression and dissemination of resistance genes (Mazel, 2006). Integrons with gene cassettes have mostly been reported in Gram-negative bacteria, especially in Enterobacteriaceae (Su et al., 2011; Tomova et al., 2018; Cheng et al., 2019). Class 1 integrons are also found extensively in *Aeromonas* spp. isolated from aquatic animals and their aquatic environments, and are associated with a variety of gene cassettes (Piotrowska and Popowska, 2015). The prevalence of integrons was also assessed in the present study, wherein class 1 integrons were detected in 4.37 % of the isolates. This prevalence is comparable to what has been reported for fish and aquatic environments from other geographical locations (Lin et al., 2016; Hossain et al., 2018).

Bacteria acquire resistance to antimicrobial agents

Table 1. MIC_{50}, MIC_{90} and resistance rates of *Aeromonas* strains to 14 antimicrobial agents during 2014 to 2016 (mg.L^{-1}).

Antimicrobial agents*	2014 (n = 483)			2015 (n = 277)			2016 (n = 383)			Total (n = 1143)		
	MIC_{50}	MIC_{90}	Resistance rate (%)	MIC_{50}	MIC_{90}	Resistance rate (%)	MIC_{50}	MIC_{90}	Resistance rate (%)	MIC_{50}	MIC_{90}	Resistance rate (%)
NAL	≤0.5	>128	44.93	128	>128	59.93	≤0.5	128	39.43	1	>128	46.72
CIP	0.03	0.5	3.31	0.06	1	6.50	≤0.004	0.06	2.61	0.03	0.5	3.85
ENR	0.015	0.25	0.62	0.12	4	10.47	≤0.004	0.06	1.83	0.03	0.5	3.41
FFC	≤2	8	10.14	≤2	16	17.69	≤2	≤2	3.39	≤2	≤2	9.71
CHL	≤2	4	5.18	≤2	32	14.80	≤2	≤2	3.13	≤2	8	6.82
DOX	≤0.5	8	3.31	1	16	16.97	≤0.5	1	3.13	1	8	6.56
NIT	≤4	≤4	0.00	≤4	8	1.08	≤4	≤4	0.78	≤4	≤4	0.52
SMM	512	>512	52.59	>512	>512	80.87	>512	>512	82.77	>512	>512	69.55
SXT	≤9.5/0.5	>76/4	12.22	≤9.5/0.5	>76/4	23.10	≤9.5/0.5	38/2	7.83	≤9.5/0.5	>76/4	13.39
CTX	≤0.03	0.25	2.69	≤0.03	≤0.03	1.08	≤0.03	0.25	1.83	≤0.03	0.12	2.01
AMP	>128	>128	97.93	>256	>256	98.92	256	>256	96.87	>128	>128	97.81
NEO	≤1	2	0.83	≤1	2	3.25	≤1	4	0.00	≤1	4	1.14
AMK	1	2	0.00	4	4	0.00	2	4	0.26	1	4	0.09
GEN	0.5	1	0.21	2	4	0.72	2	8	0.52	1	4	0.44

*Note: NAL, nalidixic acid; CIP, ciprofloxacin; ENR, enrofloxacin; FFC, florfenicol; CHL, chloramphenicol; DOX, doxycycline; NIT, nitrofurantoin; SMM, sulfamonomethoxine; SXT, sulfamethoxazole/trimethoprim; AMP, ampicillin; NEO, neomycin; AMK, amikacin; GEN, gentamicin.

Table 2. Characterisation of 50 integron-positive *Aeromonas* isolates.

Strains	Sources	Molecular identification	Gene cassette arrays	Resistance phenotypes*
2F3-7	Fish	*A. veronii*	-	SMM/NAL/AMP/SXT
5S16	Sediment	*A. veronii*	-	SMM/NAL/CIP/AMP/SXT
6S2-3	Sediment	*A. sobria*	-	CHL/FFC/THI/SMM/DOX/NAL/AMP/SXT/CTX
2W12	Pond water	*A. caviae*	-	SMM/NAL/AMP/SXT
2W14	Pond water	*A. veronii*	-	CHL/FFC/THI/SMM/NAL/AMP/SXT
6F10-3	Fish	*A. veronii*	-	CHL/FFC/THI/SMM/NAL/NEO/AMP/CTX/SXT
6F6-2	Fish	*A. veronii*	-	FFC/THI/SMM/NAL/AMP/SXT
7F10-3	Fish	*A. caviae*	-	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F6-3	Fish	*A. caviae*	-	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F9-1	Fish	*A. caviae*	-	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F9-2	Fish	*A. caviae*	-	CHL/FFC/THI/SMM/TET/NAL/CIP/NOR/CN/AMP/CTX/SXT
5P1-5	Pond surroundings	*A. caviae*	-	SMM/NAL/AMP/SXT
5P3-2	Pond surroundings	*A. caviae*	-	THI/SMM/AMP/SXT
5P3-4	Pond surroundings	*A. simiae*	-	FFC/SMM/NAL/NEO/AMP/SXT
7F8-3	Fish	*A. caviae*	*aac(6')-Ib-cr*	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F2-2	Fish	*A. caviae*	*aac6(6')-Ib-cr - arr-3*	SMM/NAL/NOR/ENR/AMP/SXT
7F2-3	Fish	*A. caviae*	*aac6(6')-Ib-cr - arr-3*	CHL/FFC/THI/SMM/NAL/CIP/ENR/AMP/SXT
7F5-2	Fish	*A. caviae*	*aac6(6')-Ib-cr - arr-3*	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F6-1	Fish	*A. caviae*	*aac6(6')-Ib-cr - arr-3*	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F6-2	Fish	*A. caviae*	*aac6(6')-Ib-cr - arr-3*	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
6S1-3	Sediment	*A. caviae*	*aac6- II-bla*$_{OXA-21}$*-catB3*	CHL/FFC/THI/SMM/NAL/AMP/SXT

Table 2. Continued.

Strains	Sources	Molecular identification	Gene cassette arrays	Resistance phenotypes*
5P2-3	Pond surroundings	*A. caviae*	*aac6-II-bla*$_{OXA-21}$*-catB3*	SMM/AMP/SXT
5P3-3	Pond surroundings	*A. caviae*	*aac6-II-bla*$_{OXA-21}$*-catB3*	CHL/FFC/THI/SMM/NAL/NEO/AMP/SXT
6S2-2	Sediment	*A. hydrophila*	*aar2-aacA4-drfA1-orfC*	FFC/THI/SMM/NAL/CIP/AMP/SXT
5S33	Sediment	*A. veronii*	*catB8*	SMM/SXT
5W13	Pond water	*A. veronii*	*catB8*	FFC/THI/SMM/NAL/AMP/SXT
5W14	Pond water	*A. veronii*	*catB8*	CHL/FFC/THI/SMM/NAL/NEO/AMP/SXT
2F1-2	Fish	*A. veronii*	*catB8*	THI/SMM/NAL/AMP/SXT
2F5-3	Fish	*A. veronii*	*catB8*	THI/SMM/NAL/AMP/SXT
5S13	Sediment	*A. caviae*	*dfrA12-orfF-aadA2*	CHL/FFC/THI/SMM/NAL/AMP/SXT
2W11	Pond water	*A. veronii*	*dfrA12-orfF-aadA2*	CHL/FFC/THI/SMM/NAL
7W4-6	Pond water	*A. veronii*	*dfrA12-orfF-aadA2*	CHL/FFC/THI/SMM/NAL/CIP/NOR/AMP/CTX/SXT
2F2-4	Fish	*A. veronii*	*dfrA12-orfF-aadA2*	CHL/FFC/THI/SMM/NAL/AMP/SXT
2F7-2	Fish	*A. veronii*	*dfrA12-orfF-aadA2*	SMM/NAL/AMP/SXT
7F4-2	Fish	*A. veronii*	*dfrA12-orfF-aadA2*	FFC/THI/SMM/NAL/CIP/ENR/AMP/SXT
2F6-4	Fish	*A. veronii*	*dfrA15*	CHL/FFC/THI/SMM/NAL/AMP/SXT
2W05	Pond water	*A. veronii*	*dfrA17*	FFC/THI/SMM/NAL/AMP/SXT
2W09	Pond water	*A. veronii*	*dfrA17*	CHL/FFC/THI/SMM/NAL/AMP/SXT
2W13	Pond water	*A. veronii*	*dfrA17*	THI/SMM/NAL/AMP/SXT
2W19	Pond water	*A. sobria*	*dfrA17*	SMM/DOX/NAL/AMP/SXT
2F4-5	Fish	*A. veronii*	*dfrA17*	FFC/THI/NAL/AMP/SXT
2F4-6	Fish	*A. veronii*	*dfrA17*	THI/SMM/NAL/AMP/SXT
2F4-7	Fish	*A. veronii*	*dfrA17*	THI/SMM/NAL/AMP/SXT
2W15	Pond water	*A. veronii*	*dfrB4-catB3-aadA1*	SMM/NAL/AMP/SXT
2W18	Pond water	*A. veronii*	*dfrB4-catB3-aadA1*	CHL/FFC/THI/SMM/NAL/AMP/SXT
2F2-2	Fish	*A. veronii*	*dfrB4-catB3-aadA1*	SMM/NAL/AMP/SXT
2F5-5	Fish	*A. veronii*	*dfrB4-catB3-aadA1*	THI/SMM/NAL/AMP/SXT
2F7-1	Fish	*A. veronii*	*dfrB4-catB3-aadA1*	FFC/THI/SMM/NAL/AMP/CTX/SXT
2F8-1	Fish	*A. sobria*	*dfrB4-catB3-aadA1*	FFC/THI/SMM/NAL/AMP/SXT
5P2-1	Pond surroundings	*A. caviae*	*dfrB4-catB3-bla*$_{OXA-10}$*-aadA1*	THI/SMM/AMP

*Note: NAL, nalidixic acid; CIP, ciprofloxacin; ENR, enrofloxacin; FFC, florfenicol; CHL, chloramphenicol; DOX, doxycycline; NIT, nitrofurantoin; SMM, sulfamonomethoxine; SXT, sulfamethoxazole/trimethoprim; AMP, ampicillin; NEO, neomycin; AMK, amikacin; GEN, gentamicin.

mainly due to genetic resistance determinants. In this study, 44 % (22/50) of the integron-positive *Aeromonas* carried various types of dihydrofolate reductases genes and displayed resistance to sulfamonomethoxine and sulfamethoxazole/trimethoprim. Chloramphenicol acetyltransferase genes were carried by 30 % (15/50) of the strains, and 66 % (10/15) of strains were resistant to florfenicol or chloramphenicol. The *aac(6')-Ib-cr* gene was identified in six isolates and displayed resistance to ciprofloxacin and enrofloxacin. Among these, 17 (85 %) carried gene cassettes and displayed multiple drug resistance. From the above it is obvious that integrons play an important role in mediating gene transfer between bacteria.

Conclusion

The increasing number of infections caused by antimicrobial-resistant bacteria in aquaculture is an important emerging issue. The contribution of aquaculture and the aquatic environment to the emergence of antimicrobial-resistant infections needs to be determined, delineated, and addressed. This study has demonstrated that freshwater aquatic animals can serve as reservoirs of *Aeromonas* containing multiple resistance genes. This report suggests that surveillance for AMR of animal origin and the prudent and responsible use of antimicrobial agents are necessary.

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References

Aravena-Román, M., Inglis, T.J., Henderson, B., Riley, T.V., Chang, B.J. 2012. Antimicrobial susceptibilities of *Aeromonas* strains isolated from clinical and environmental sources to 26 antimicrobial agents. Antimicrobial Agents and Chemotherapy 56:1110–1112. https://doi.org/10.1128/AAC.05387-11

Boerlin, P., Reid-Smith., R.J. 2008. Antimicrobial resistance: its emergence and transmission. Animal Health Research Reviews 9:115–126. https://doi.org/10.1017/S146625230800159X

Borrell, N., Acinas, S.G., Figueras, M.J., Martínez-Murcia, A.J. 1997. Identification of *Aeromonas* clinical isolates by restriction fragment length polymorphism of PCR-amplified 16S rRNA genes. Journal of Clinical Microbiology 35:1671–1674. https://doi.org/10.1128/JCM.35.7.1671-1674.1997

Carvalho I.T., Santos, L. 2016. Antibiotics in the aquatic environments: a review of the European scenario. Environment International 94:736–757. https://doi.org/10.1016/j.envint.2016.06.025

Cheng, H., Jiang, H., Fang, J., Zhu, C. 2019. Antibiotic resistance and characteristics of integrons in *Escherichia coli* isolated from *Penaeus vannamei* at a freshwater shrimp farm in Zhejiang Province, China. Journal of Food Protection 82:470–478. https://doi.org/10.4315/0362-028X.JFP-18-444

Clinical and Laboratory Standards Institute (CLSI). 2006a. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. Approved guideline - M45-A ,Wayne, PA, pp. 12–13.

Clinical and Laboratory Standards Institute (CLSI). 2006b. Methods for broth dilution susceptibility testing of bacteria isolated from aquatic animals; approved guideline, Wayne, PA, pp. 6–18.

Deng, Y., Wu, Y., Tan, A., Huang, Y., Jiang, L., Xue, H., Wang, W., Luo, L., Zhao, F. 2014. Analysis of antimicrobial resistance genes in *Aeromonas* spp. isolated from cultured freshwater animals in China. Microbial Drug Resistance 20:350–356. https://doi.org/10.1089/mdr.2013.0068

Fisheries Administration of the Ministry of Agriculture, P.R. China (FAMA). 2019. China statistics year book. China Agriculture Press, pp. 21–25. (in Chinese).

FAO. 2020. The state of world fisheries and aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Gauthier, D.T. 2015. Bacterial zoonoses of fishes: a review and appraisal of evidence for linkages between fish and human infections. Veterinary Journal 203:27–35. https://doi.org/10.1016/j.tvjl.2014.10.028

Hossain, S., De Silva, B., Wimalasena, S., Pathirana, H., Dahanayake, P.S., Heo, G.J. 2018. Distribution of antimicrobial resistance genes and class 1 integron gene cassette arrays in motile *Aeromonas* spp. isolated from goldfish (*Carassius auratus*). Microbial Drug Resistance 24:1217–1225. https://doi.org/10.1089/mdr.2017.0388

Ishida, Y., Ahmed, A.M., Mahfouz, N.B., Kimura, T., El-Khodery, S.A., Moawad, A.A., Shimamoto, T. 2010. Molecular analysis of antimicrobial resistance in gram-negative bacteria isolated from fish farms in Egypt. Journal of Veterinary Medical Science 72:727–734. https://doi.org/10.1292/jvms.09-0538

Lévesque, C., Piché, L., Larose, C., Roy, P.H. 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrobial Agents and Chemotherapy 39:185–191. https://doi.org/10.1128/AAC.39.1.185

Lin, M., Wu, X., Yan, Q., Ma, Y., Huang, L., Qin, Y., Xu, X. 2016. Incidence of antimicrobial-resistance genes and integrons in antibiotic-resistant bacteria isolated from eels and aquaculture ponds. Diseases of Aquatic Organisms 120:115–123. https://doi.org/10.3354/dao03013

Mazel, D. 2006. Integrons: agents of bacterial evolution. Nature Reviews Microbiology 4:608–620. https://doi.org/10.1038/nrmicro1462

Ministry of Agriculture and Rural Affairs (MARA). 2010. Announcement No.1435 of the Ministry of Agriculture and Rural Affairs. The Ministry of Agriculture and Rural Affairs of the People's Republic of China. http://www.moa.gov.cn/govpublic/SYJ/201008/t20100823_1622639.htm. (Accessed 23 August 2010). (in Chinese).

Nagar, V., Shashidhar, R., Bandekar, J.R. 2011. Prevalence, characterization, and antimicrobial resistance of *Aeromonas* strains from various retail food products in Mumbai, India. Journal of Food Science 76:M486–M492. https://doi.org/10.1111/j.1750-3841.2011.02303.x

Nhung, N.T., Cuong, N.V., Thwaites, G., Carrique-Mas, J. 2016. Antimicrobial usage and antimicrobial resistance in animal production in Southeast Asia: a review. Antibiotics (Basel) 5:37. https://doi.org/10.3390/antibiotics5040037

Piotrowska, M., Popowska, M. 2015. Insight into the mobilome of *Aeromonas* strains. Frontiers in Microbiology 6:494. https://doi.org/10.3389/fmicb.2015.00494

Qiao, M., Ying, G.G., Singer, A.C., Zhu, Y.G. 2018. Review of antibiotic resistance in China and its environment. Environment International 110:160–172. https://doi.org/10.1016/j.envint.2017.10.016

Reyes, A., Bello, H., Domínguez, M., Mella, S., Zemelman, R., González, G. 2003. Prevalence and types of class 1 integrons in aminoglycoside-resistant Enterobacteriaceae from several Chilean hospitals. Journal of Antimicrobial Chemotherapy 51:317–321. https://doi.org/10.1093/jac/dkg083

Sandvang, D., Aarestrup, F.M., Jensen, L.B. 1997. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiology Letters 157:177–181. https://doi.org/10.1111/j.1574-6968.1997.tb12770.x

Santos L., Ramos, F. 2018. Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. International Journal of Antimicrobial Agents 52:135–143. https://doi.org/10.1016/j.ijantimicag.2018.03.010

Serrano P.H. 2005. Responsible use of antibiotics in aquaculture. FAO Fisheries Technical Paper. Rome. 21 pp.

Smith P. 2008. Antimicrobial resistance in aquaculture. Revue Scientifique et Technique 27:243–64. http://dx.doi.org/10.20506/rst.27.1.1799

Su, H., Ying, G., Tao, R., Zhang, R., Fogarty, L.R., Kolpin, D.W. 2011. Occurrence of antibiotic resistance and characterization of resistance genes and integrons in Enterobacteriaceae isolated from integrated fish farms in south China. Journal of Environmental Monitoring 13: 3229–3236. https://doi.org/10.1039/c1em10634a

Tomova, A., Ivanova, L., Buschmann, A.H., Godfrey, H.P., Cabello, F.C. 2018. Plasmid-mediated quinolone resistance (PMQR) genes and class 1 integrons in quinolone-resistant marine bacteria and clinical isolates of *Escherichia coli* from an aquacultural area. Microbial Ecology 75:104–112. https://doi.org/10.1007/s00248-017-1016-9

Yáñez, M.A., Catalán, V., Apráiz, D., Figueras, M.J., Martínez-Murcia, A.J. 2003. Phylogenetic analysis of members of the genus *Aeromonas*

based on *gyrB* gene sequences. International Journal of Systematic and Evolutionary Microbiology 53:875–883. https://doi.org/10.1099/ijs.0.02443-0