



Food and Agriculture Organization
of the United Nations

Understanding Antimicrobial Resistance in Aquaculture



ASIAN FISHERIES SOCIETY

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Through an agreement between FAO and the Asian Fisheries Society (AFS) signed in November 2020, it was mutually agreed to publish 17 papers contained in this special volume based on technical presentations that were delivered during the three workshops implemented under the auspices of the above-mentioned project. This volume addresses a wide range of topics that will assist in better understanding antimicrobial resistance (AMR) in aquaculture.

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Trends of Aquaculture Production and Trade: Carp, Tilapia, and Shrimp

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Abstract

Carp, tilapia, and shrimp are the most commonly cultured aquatic animals in meeting domestic and international demand for aquatic animal food and contributing to the local and national economies. These species groups accounted for nearly 50 % of the total production of farmed aquatic animals in 2018. Globally, carp remains the most important group of farmed aquatic animals, both in terms of quantity and total value. Carp are produced traditionally for domestic consumption but play an insignificant role in international trade. Globally, tilapia is one of the most popularly farmed aquatic animals. Production of cultured tilapia increased rapidly between 1998 and 2018, making it the second-most important group of farmed aquatic animals by quantity. Tilapia has become an important internationally traded aquaculture commodity, although the majority of its production is still consumed domestically. Shrimp has a relatively high market value and between 1998 and 2018, the general trend in production of farmed shrimp has been upwards. Unlike carp and tilapia, farmed shrimp are primarily destined for the international markets. Shrimp exports followed a general growth trend between 1997 and 2017. Production of farmed carp and tilapia will continue to grow, largely because of their importance in national food security and nutrition. Increasing incomes and improving living standards are likely to increase the demand for shrimp both domestically and internationally. However, although both production and international trade of shrimp are expected to continue to grow, they may face uncertainties related to limiting factors such as natural resource constraint and climate change.

Keywords: review, quantity, value, export, global, domestic market, prospect

Introduction

Fish and other aquatic animals are important sources of animal protein and other important nutrients in peoples' diets. Between 1961 and 2016, the average annual increase in global food fish consumption (3.2 %) outpaced population growth (1.6 %) (FAO, 2018). As a result, food fish consumption increased to 20.5 kg.capita⁻¹ in 2017 from 9 kg.capita⁻¹ in 1961. This increase in per capita fish consumption has been largely attributed to the rapid development of global aquaculture since the 1980s. Aquaculture currently supplies 50 % of food fish for direct human consumption globally.

Aquaculture is a complex food production sector that

includes a great diversity of aquatic animals and plants. Different groups of farmed aquatic animals and plants play different roles in food and nutritional security. Meanwhile, some groups of farmed aquatic animals and plants are also important commodities in international trade. This paper focuses on the three most important groups of farmed aquatic animals in terms of global production quantity and value, namely: carp, referring species in the Family Cyprinidae, such as grass carp (*Ctenopharyngodon idella* (Valenciennes, 1844)), silver carp (*Hypophthalmichthys molitrix* (Valenciennes, 1844)), common carp (*Cyprinus carpio* Linnaeus, 1758), bighead carp (*Hypophthalmichthys nobilis* (Richardson, 1845)), catla (*Gibleon catla* (Hamilton, 1822)), *Carassius* spp. and rohu labeo (*Labeo rohita* (Hamilton, 1822)); tilapia, referring species in the

Family Cichlidae such as Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)), blue tilapia (*Oreochromis aureus* (Steindachner, 1864)) and Mozambique tilapia (*Oreochromis mossambicus* (Peters, 1852)); and shrimp, referring the species included in the International Standard Statistical Classification of Aquatic Animals and Plants (ISSCAAP) “shrimp, prawn” group (which excludes freshwater prawn), such as whiteleg shrimp (*Penaeus vannamei* Boone, 1931), giant tiger prawn (*Penaeus monodon* Fabricius, 1798), kuruma prawn (*Penaeus japonicus* Bate, 1888), fleshy prawn (*Penaeus chinensis* (Osbeck, 1765)) and banana prawn (*Penaeus indicus* De Man, 1888). These three species groups play different roles in ensuring national food and nutrition security, meeting international market demand, and contributing to rural livelihoods. Understanding the trends in aquaculture production and international trade of carp, tilapia, and shrimp can help in shaping the future development of the subsectors and creating the strategies needed to support their development. All the production data used in this paper is from or generated from “Fishery and Aquaculture Statistics. Global aquaculture production 1950–2018 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2020”. All the trade data used in this paper is from or generated from “Fishery and Aquaculture Statistics. Global Fisheries commodities production and trade 1976–2017 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2019”.

Global Production of Farmed Carp

Carp are fish species in the Family Cyprinidae, a large family that includes cultured species such as common carp, Chinese carp, Indian major carp and *Carassius* spp. that have the longest history of aquatic farming in the world. Carp farming practices were recorded as early as 2,500 years ago. Carp is also one of the most commonly cultured groups of aquatic animals worldwide. According to Food and Agriculture Organization of the United Nations (FAO) Fisheries and Aquaculture Statistics, 123 countries or regions reported cultured carp production to FAO in 2018. The global farmed carp production reached 28.9 million tonnes in 2018, which is historically high.

Production trend of farmed carp

Carp is the most important group of aquatic animals species being cultured around the world in terms of both production quantity and value. Carp accounted for 25.2 % of global aquaculture production in 2018, which is a significant decline from the highest production of 35.1 % in 1997. Carp accounted for 35.2 % of global cultured aquatic animal production in 2018, which is significantly lower than the highest production, which was 45.8 % in 1997. Carp also accounted for 53.2 % of global cultured finfish production in 2018, which is a drastic decline from the 71.1 % contribution seen in 1996.

The total value of farmed carp reached 61.6 billion US dollars in 2018, which accounted for 23.4 % of total global aquaculture output value for that year. The share of total aquaculture output value contributed by farmed carp has significantly declined from the peak of 29.9 % achieved in 1985. Since 1950, when Member Countries first began reporting relevant aquaculture production statistics to FAO, the lowest share contributed by farmed carp to the total global aquaculture output value was 21.4 % in 2006. This decreased contribution to the global aquaculture output value is largely due to the reduced share of carp in the global aquaculture production.

During the three decades from 1989 to 2018, farmed carp production had an average annual growth of 6.0 %, ranging between 1.3 to 18.7 % throughout the period (Fig. 1). The fastest growth of production took place from 1992 to 1996, averaging 16.3 % annually. Since 1997, the annual production growth has dropped to 1.3 to 6.7 %, except for 8.0 % in 2004.

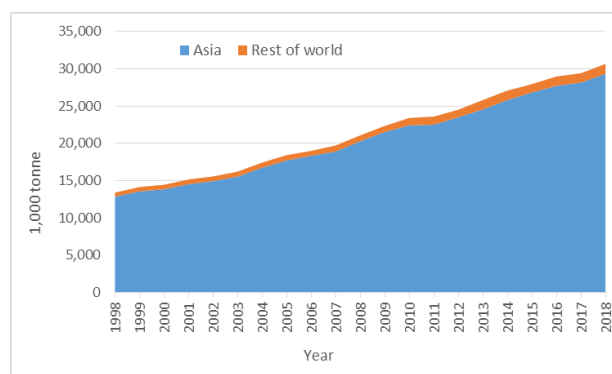


Fig. 1. Production of cultured carp in the world.

Global producers of farmed carp

Although carp are globally cultured, their production has been dominated by Asia (Table 1). The top-seven producers of farmed carp are all Asian countries, which together contributed 96.3 % of the world production in 2018. China has always been the major producer of farmed carp. However, its share in the world production has declined to 68.1 % in 2018 from 78.9 % in 1998, which can be attributed to both faster growth in aquaculture production of non-carp species in China and the reduced share of China in the world aquaculture production.

Globally, 51 species or species groups of carp are included in the FAO statistics for farmed carp production reported by the member nations. There were 29 farmed carp species with an individual production of over 100 tonnes in 2018. In 2018, 12 carp species contributed individually to over 1 % of the world production of farmed carp (Table 2). In 2007, grass carp surpassed silver carp to become the most important carp species. However, its share in the world production of farmed carp declined from 21.1 %

Table 1. Major producers of cultured carp in the world (individual production above 50,000 tonnes in 2018).

Country	Production (1,000 tonne)		% in global production	
	1998	2018	1998	2018
China	10,074	19,668	78.86	68.14
India	1,551	4,646	12.15	16.10
Bangladesh	402	1,175	3.15	4.07
Myanmar	80	999	0.62	3.46
Viet Nam	-	550	-	1.91
Indonesia	144	606	1.13	2.10
Pakistan	17	156	0.14	0.54
Russia	55	122	0.43	0.42
Iran	27	187	0.21	0.65
Egypt	51	181	0.40	0.63

Table 2. Global production of 12 major cultured carp species.

Species	Production (1,000 tonne)		% of total carp production	
	1998	2018	1998	2018
Grass carp (<i>Ctenopharyngodon idella</i> (Valenciennes, 1844))	2,698	5,704	21.12	19.76
Silver carp (<i>Hypophthalmichthys molitrix</i> (Valenciennes, 1844))	3,007	4,789	23.54	16.59
Common carp (<i>Cyprinus carpio</i> Linnaeus, 1758)	2,185	4,190	17.10	14.51
Bighead carp (<i>Hypophthalmichthys nobilis</i> (Richardson, 1845))	1,424	3,144	11.15	10.89
Catla (<i>Gibeleon catla</i> (Hamilton, 1822))	555	3,041	4.35	10.54
<i>Carassius</i> spp.	926	2,772	7.25	9.60
Roho labeo (<i>Labeo rohita</i> (Hamilton, 1822))	660	2,017	5.17	6.99
Wuchang bream (<i>Megalobrama amblycephala</i> Yi, 1955)	403	784	3.16	2.71
Black carp (<i>Mylopharyngodon piceus</i> (Richardson, 1846))	138	692	1.08	2.40
Cyprinids net* (Cyprinidae)	43	654	0.33	2.27
Mrigal carp (<i>Cirrhinus cirrhosus</i> (Bloch, 1795))	500	501	3.91	1.74
Silver barb (<i>Barbonymus schwanenfeldii</i> (Bleeker, 1854))	69	380	0.54	1.32

*not elsewhere included.

in 1998 to 19.8 % in 2018. The share of silver carp, the former top farmed carp species in the world by production, declined from 23.5 % in 1998 to 16.6 % in 2018. The production of farmed catla increased nearly five fold between 1998 and 2018, its share in the world production of farmed carp increasing to 10.5 % in 2018 from 4.3 % in 1998. The share of *Carassius* spp. and rohu labeo in the world production of farmed carp increased to 9.6 % and 7.0 % in 2018 from 7.3 % and 5.2 % in 1998, respectively. The share of mrigal carp (*Cirrhinus cirrhosus* (Bloch, 1795)) declined from 3.9 % in 1998 to 1.7 % in 2018.

Global Production of Farmed Tilapia

Production trend of farmed tilapia

Tilapia surpassed salmonids to become the second-most important farmed finfish group by quantity in 2005, when its farmed production first reached 2 million tonnes. By 2018, the world production of farmed tilapia reached 6.03 million tonnes, which further consolidated the position of tilapia as the second-most important farmed finfish species group. It also became the second-most important species

group of cultured aquatic animals in 2014 in terms of quantity, when its production first exceeded 5 million tonnes.

Farmed tilapia accounted for 5.27 % of global aquaculture production in 2018, which was more than double the 2.38 % in 1998. The share of farmed tilapia in the world production of farmed aquatic animals reached 7.34 % in 2018, an increase of 133 % over 1998. In 2018, tilapia increased its share in the global production of farmed finfish to 11.11 % from 4.85 % in 1998.

In 2018, the total estimated value of farmed tilapia reached 11.2 billion US dollars, accounting for 4.5 % of the total value of all cultured aquatic animals, a significant increase from 2.8 % in 1998, but lower than the peak of 5.2 % in 2012.

The tilapia-farming sector has achieved much faster growth than the overall aquaculture industry and most farmed aquatic species groups in the past two decades (Fig. 2). The average production growth of farmed tilapia reached 10.1 % between 1999 and 2018. The most rapid production growth of farmed tilapia took place from 1999 to 2013, with an average annual production growth rate of 11.8 %. From 2014 to 2018, the growth of farmed tilapia production slowed down to 5 % per year on average, fluctuating between 1.6 and 8.9 %, with the lowest growth of 1.6 % occurring in 2018.

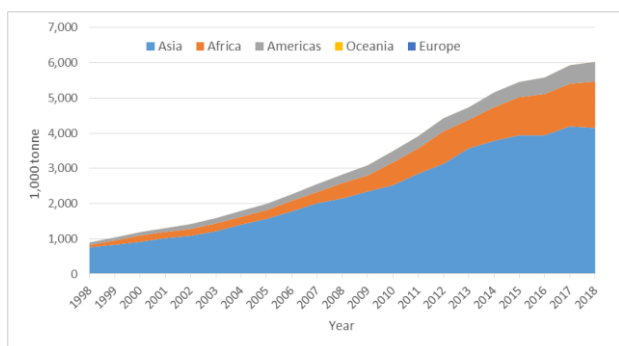


Fig. 2. Production of cultured tilapia in the world.

Producers of farmed tilapia in the world

Tilapia is currently the most popularly cultured aquatic animal group in the world, with 145 countries or regions reporting cultured tilapia production to FAO in 2018. Although tilapia originated in Africa, Asia has dominated its production ever since the fish was introduced to aquaculture. Asia produced 4.2 million tonnes of farmed tilapia in 2018, which accounted for 68.8 % of the world total. However, Asia's share of in the world production has declined significantly compared with 84.4 % in 1998. In contrast, Africa, the original source of tilapia, has successfully increased

its share from 7.5 % in 1998 to 21.8 % in 2018. The share of the Americas slightly increased to 9.3 % in 2018 from 8.1 % in 1998.

China remained the largest producer of farmed tilapia in 2018, with a production of 1.62 million tonnes (Table 3). However, its share in the world production declined from 52.3 % in 1998 to 26.9 % in 2018. Meanwhile, in 2013, Indonesia surpassed Egypt to become the world's second-largest producer of farmed tilapia. It produced 1.22 million tonnes of farmed tilapia in 2018, which accounted for 20.3 % of the world total. In 2018, Egypt was the only African country among the top-ten producers of farmed tilapia, contributing 17.4 % of the world total. Thailand and the Philippines used to be among the top-four producers of farmed tilapia, however, their shares in the world production of farmed tilapia declined to 3.5 % and 4.6 %, respectively in 2018.

Cultured tilapia production by species

Globally, 23 species and species groups in the family Cichlidae were included in FAO aquaculture production statistics by 2018. Among these, Nile tilapia has dominated global farmed tilapia production (Table 4). World production of farmed Nile tilapia reached 4.53 million tonnes in 2018, which accounted for 75 % of the total farmed tilapia production. However, the share of Nile tilapia in total production of farmed tilapia has declined significantly from 83.4 % in 1998. The production of tilapia that is not elsewhere included accounted for 17.1 % in the total farmed tilapia production, which suggests a significant proportion of farmed tilapia production could be reported down to species level. In 2018, production of the hybrid of blue tilapia and Nile tilapia reached 0.41 million tonnes and accounted for 6.7 % of total farmed tilapia production. The production is primarily from China, where technology has been adopted to take advantage of all male offspring (>95 %) from the hybridisation of blue tilapia and Nile tilapia without hormone manipulation. The share of Mozambique tilapia in the total farmed tilapia production significantly declined to less than 1 % in 2018.

Production of Cultured Shrimp

Production trend of farmed shrimp

Shrimp is the most important species group cultured worldwide in terms of international trade. The world production of farmed shrimp reached 6 million tonnes in 2018 (FAO, 2020) which is an historical record. Shrimp is also been the most important species group of farmed crustaceans globally, contributing 64.0 % of the world production of farmed crustaceans in 2018, the lowest since the peak share of 91.9 % in 1992. The share of shrimp in total farmed crustaceans has fluctuated between 64.0 % and 73.8 % from 1999 to 2018. The share of farmed shrimp in the world

Table 3. Major producers of cultured tilapia in the world (individual production above 50,000 tonnes in 2018).

Country/Region	Production (1,000 tonne)		% in global production	
	1998	2018	1998	2018
China	471.8	1,624.5	52.59	26.93
Indonesia	65.9	1,222.7	7.35	20.27
Egypt	52.8	1,051.4	5.88	17.43
Bangladesh	-	344.8	-	5.72
Brazil	24.1	317.1	2.68	5.26
Philippines	72.0	277.0	8.03	4.59
Viet Nam	-	260.0	-	4.31
Thailand	73.8	211.4	8.23	3.51
Colombia	17.7	77.9	1.97	1.29
Ghana	1.4	70.6	0.15	1.17
Uganda	0.2	70.1	0.02	1.16
Taiwan POC	36.1	62.6	4.03	1.04
Mexico	5.4	52.7	0.60	0.87

Table 4. Global production of ten major farmed tilapia species in 2018 (individual farmed production above 1,000 tonnes).

Species	Production (tonne)		% of total tilapia production	
	1998	2018	1998	2018
Nile tilapia (<i>Oreochromis niloticus</i> (Linnaeus, 1758))	748,040	4,525,431	83.38	75.03
Tilapias nei (Cichilidae)	103,564	1,030,004	11.54	17.08
Blue-Nile tilapia, hybrid	0	406,048	0.00	6.73
Mozambique tilapia (<i>Oreochromis mossambicus</i> (Peters, 1852))	40,652	53,754	4.53	0.89
Shire tilapia (<i>Oreochromis shiranus</i> Boulanger, 1897)	0	5,036	0.00	0.08
Blue tilapia (<i>Oreochromis aureus</i> (Steindachner, 1864))	844	3,182	0.09	0.05
Three spotted tilapia (<i>Oreochromis andersonii</i> (Castelnau, 1861))	2,689	2,147	0.30	0.04
Redbreast tilapia (<i>Coptodon rendalli</i> (Boulanger, 1897))	839	1,903	0.09	0.03
Longfin tilapia (<i>Oreochromis macrochir</i> (Boulanger, 1912))	207	1,800	0.02	0.03
Tanganyika tilapia (<i>Oreochromis tanganyicae</i> (Günther, 1894))	0	1,690	0.00	0.03

aquaculture production is rather small, only 5.2 % in 2018. However, the share has significantly increased since 1998, when it was only 2.6 %, with the most rapid increase (from 3.0 % to 5.0 %) between 2002 and 2006.

In 2018, the total value of farmed shrimp reached a historical high of 38.4 billion US dollars, contributing 14.6 % to the world aquaculture output value. The share of shrimp in the world aquaculture value in 2018 is the second highest between 1999 and 2018, just next to the share of 15.2 % in 2006. The share of farmed shrimp in world aquaculture by value is nearly three times the share by quantity. The percentage share fluctuated between 12.6 % and 15.2 % from 1999 to 2018. In 2018, shrimp accounted for 7.3 % of the

total production of farmed aquatic animals, more than double the 3.5 % contributed in 1998.

During the period 1998 to 2018, farmed shrimp production maintained higher annual growth than that of global aquaculture, with an average annual growth of 9.7 % in quantity (Fig. 3) vs an average annual growth in total global aquaculture production of only 5.7 % during the same period. The most rapid production growth of farmed shrimp took place during 2001–2006 when the average annual growth in production was 19.0 %. However, the production growth of farmed shrimp dropped to a moderate 5.0 % in 2018, which is still significantly higher than the world aquaculture growth of 2 % in that year.

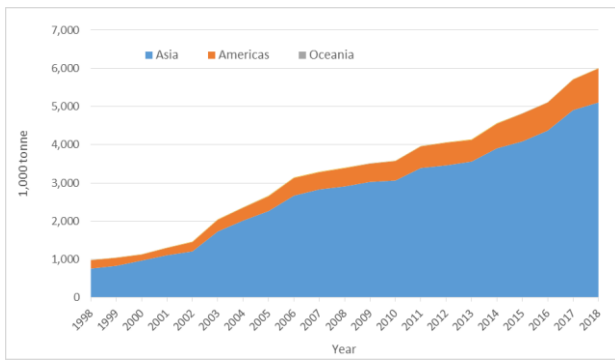


Fig. 3. Production of cultured shrimp in the world.

Producers of farmed shrimp

In 1988, 41 countries and regions reported their production of farmed shrimp to FAO. By 2018, the number of reporting countries and regions had increased to 82, covering all the continents. Farmed shrimp production has always been dominated by Asia and the Americas, which contributed 85.0 % and 14.8 % of the world production in 2018, respectively. However, the share of the Americas in the world production had declined significantly from 22.3 % in 1998. The share of Europe, Africa, and Oceania in the world production of farmed shrimp has always been negligible, dropping to a low of 0.2 % in 2018.

China is currently the largest producer of farmed shrimp (Table 5), and has significantly increased its share in world production from 13.2 % in 1998 to 34.2 % in 2018. The shares of Thailand and Ecuador, the former top-two producers of farmed shrimp in the world, declined from 25.6 % and 14.6 % in 1998 to 6.0 % and 8.5 % in 2018, respectively. Viet Nam, India, and Indonesia have significantly increased their shares in the world production of farmed shrimp from 1998 to 2018.

Species of farmed shrimp and their contribution to the total production

Currently, FAO Fisheries and Aquaculture statistics include 25 species and species groups of farmed shrimp. The giant tiger prawn (*Penaeus monodon*) used to be the dominant species of farmed shrimp (Fig. 4), contributing 51.0 % of the world production in 1998. However, its position as the top farmed shrimp species was taken over by whiteleg shrimp (*P. vannamei*) in 2003, and its contribution to world production had dropped to 12.5 % in 2018. The share of whiteleg shrimp in the world production of farmed shrimp increased from 20.4 % in 1998 to 82.7 % in 2018. The average annual production growth of farmed whiteleg shrimp was 20.6 % during 1999–2018, with the maximum annual growth of 103% in 2003, which led to the historically high production of 5.0 million tonnes in 2018. However, the growth in production has fluctuated throughout the period, largely due to disease problems in farming, such as the outbreaks of acute hepatopancreatic necrosis disease (AHPND) from 2012 to 2014.

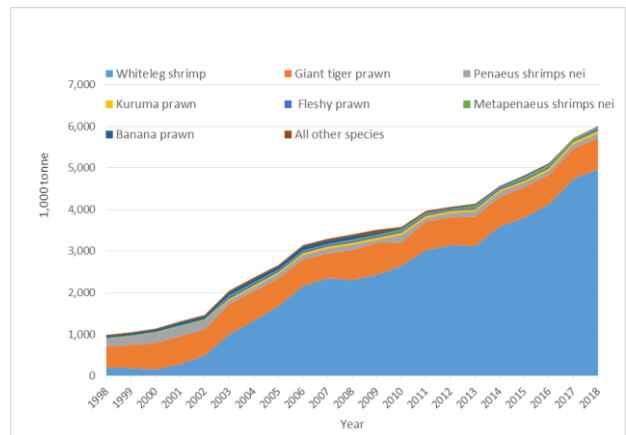


Fig. 4. Global production of major farmed shrimp species.

Table 5. Major producers of cultured shrimp in the world (individual production above 50,000 tonnes in 2018).

Country	Production (1,000 tonne)		% in total production	
	1998	2018	1998	2018
China	130	2,052	13.2	34.2
Indonesia	118	908	12.0	15.1
Viet Nam	52	775	5.3	12.9
India	83	682	8.4	11.4
Ecuador	144	510	14.6	8.5
Thailand	253	363	25.6	6.0
Mexico	24	158	2.4	2.6
Bangladesh	56	71	5.7	1.2
Brazil	7	62	0.7	1.0
Philippines	38	60	3.8	1.0
Saudi Arabia	2	56	0.2	0.9

International Trade of Carp, Tilapia, and Shrimp

Of all animal protein commodities, fish and fish products are among the most traded in terms of value and the most subject to competition from imported products (FAO, 2018). In 2017, the total volume of exported fisheries and aquaculture commodities reached 40.1 million tonnes, which is equivalent to 65 million tonnes in live weight (FAO, 2019a). The total value of exported fisheries and aquaculture commodities reached 156.5 billion US dollars. The total quantity and value of exported fisheries and aquaculture products increased by 44.2 % and 192.9 % respectively between 1997 and 2017 (FAO, 2019a). This implies that the average unit value of exported fisheries and aquaculture commodities is doubled compared with 1997. However, if inflation (around 52 % for the US dollar between 1997–2017) (Inflation Tool, 2020) is taken into consideration, the unit value of exported shrimp in 2017 increased by some 50 % only when compared with the unit value in 1997.

Carp, shrimp, and tilapia are among the most important groups of farmed aquatic animals globally in terms of contribution to people's animal protein supply. They are all traded internationally, although the volumes of international trade for individual groups are not in proportion to their farmed production.

Global trade of carp

Although carp is the most important group of aquatic animals farmed in the world, its contribution to international trade of aquatic products has been very small. The total quantity of exported carp products remained less than 20,000 tonnes until 2011 except for 2006 and 2007, when it was around 0.1 % of the total production (Fig. 5). In 2012, there was a sharp increase in the export of carp products globally, with a 3.7-fold increase in quantity over the previous year. The total annual exported volume of carp in the world has remained above 100,000 tonnes since 2012. However, the share of carp in the global export of aquatic products is still very small, being 0.32 % by quantity and 0.25 % by value in 2017.

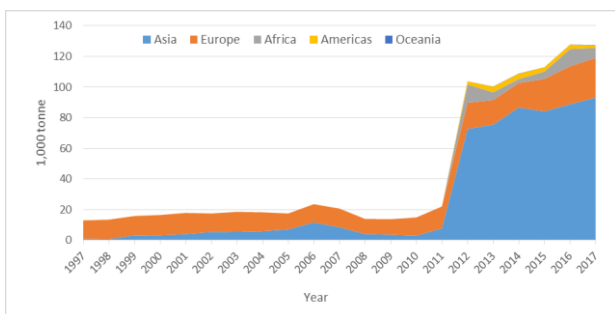


Fig. 5. Global carp exports.

Asia and Europe are the two major exporters of carp globally, having contributed 73.1 % and 20.3 %, respectively, to the world export of carp in 2017. Unlike with the international trade of shrimp and tilapia, the major carp-exporting regions are also the major carp-importing regions. Asia and Europe contributed 68.2 % and 25.7 % of the world carp import, respectively, in 2017, which suggests that the international trade of carp is more within the regions than global.

The major carp-exporting countries in 2017 included China (46,504 tonnes), Thailand (11,972 tonnes), Indonesia (11,817 tonnes), Czech Republic (10,755 tonnes), Turkey (8,034 tonnes), and Myanmar (7,421 tonnes). The largest importer of carp is Hong Kong SAR of China, which imported 41,153 tonnes in 2017. Other major carp importers in 2017 included Iraq (9,178 tonnes), United Arab Emirates (7,117 tonnes), Poland (5,216 tonnes), United Kingdom (4,928 tonnes), Macao SAR of China (4,085 tonnes), and the Kingdom of Saudi Arabia (4,034 tonnes).

Global trade of tilapia

Along with the rapid growth in tilapia farming globally, tilapia has become one of the most important internationally traded freshwater fish and is now the most widely cultured aquatic animal in the world. Although wild catch also contributes significantly to tilapia production, because of the quality requirements for aquatic commodities that are traded on the international markets, tilapia traded internationally originates mainly from aquaculture.

In 2017, the total quantity of exported tilapia products reached 766,438 tonnes in live weight (FAO, 2019a), having increased 22.4 fold between 1997 and 2017. The total value of exported tilapia products reached 1.66 billion US dollars in 2017 (FAO, 2019a), an increase of 39 fold between 1997 and 2017. The average unit price of exported tilapia products was 1.27 USD.kg⁻¹ and 2.17 USD.kg⁻¹ in 1997 and 2017, respectively (FAO, 2019a). The significantly increased unit price (71 % higher) has contributed greatly to the rapid growth in the value of exported tilapia, and also reflects the added value of the products. However, if inflation is taken into consideration, the increase in unit value of exported tilapia becomes far less significant.

The quantity of exported tilapia commodities demonstrates a rapid growth trend from 1997 to 2017 (Fig. 6). Meanwhile, the growth in the quantity of exported tilapia also fluctuated significantly from 1998 to 2017, ranging from -19.5 % (2012) to 72.3 % (2002), with an average annual growth of 16.8 % during this 20-year period (FAO, 2019b). However, the quantity of exported tilapia showed a trend of slight decline in the most recent years (by an average of 0.7 % per year from 2014 to 2017).

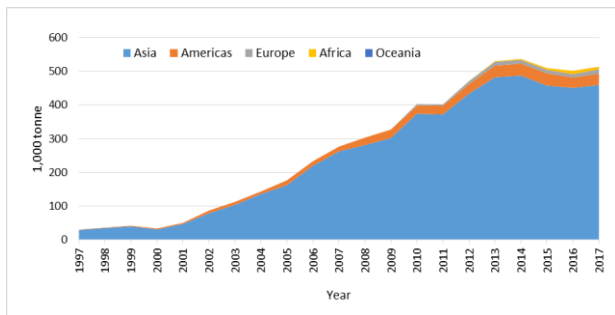


Fig. 6. Global tilapia exports.

In terms of regional contribution to international trade of tilapia, Asia has dominated the export of tilapia products from 1997 to 2017. The share of Asia in the world export of tilapia products was 89.4 % in 2017, which is a slight decline from 97.5 % in 1997. The shares of the Americas, Europe, and Africa increased to 6.5 %, 2.6 %, and 1.4 % in 2017 from 2.5 %, 0 %, and 0 % in 1997, respectively. Although over 70 countries or regions reported the export of tilapia in 2017, China has dominated the global exports since becoming the top exporter of tilapia in 2003. In 2017, China supplied 406,862 tonnes of tilapia products to the global market, which accounted for 79.2 % of the world's tilapia export. In the same year, the individual shares of the other nine top tilapia exporters ranged from 1 % to 4.4 %.

The United States of America is the largest tilapia importer in the world, importing 184,210 tonnes in 2017, which accounted for 38 % of total tilapia imports globally. Mexico is the second-largest tilapia importer, with 13.3 % of the world's tilapia imports in 2017. Côte d'Ivoire is another important importer of tilapia, having imported 35,484 tonnes in 2017, which represented 7.3 % of the world's tilapia imports. Another 13 countries imported over 5,000 tonnes tilapia each in 2017.

International trade of shrimp

Shrimp are heavily traded commodities and represent the second main group of exported species in value terms (FAO, 2018). The international trade data for fisheries and aquaculture commodities are not disaggregated by the source in the FAO Fisheries and Aquaculture Statistics. Although wild shrimp catches contribute large volumes to total supply, most internationally traded shrimp today is farmed (FAO, 2018).

In 2017, the total quantity of exported shrimp products reached 4.4 million tonnes in live weight (FAO, 2019a), an increase of 157.4 % from 1997. The total value of exported shrimp products reached 27.3 billion US dollars in 2017 (FAO, 2019a), an increase of 160.5 % from 1997 value. The average unit price of exported shrimp products was 6.18 USD.kg⁻¹ and 6.26 USD.kg⁻¹ in 1997 and 2017, respectively (FAO, 2019a).

The price of shrimp in the international market has hardly responded to the significant increase in the production cost of farmed shrimp resulting from higher input costs and the stricter requirements for product safety, quality, and farming practices imposed by the importing countries.

The quantity of exported shrimp commodities followed a general growth trend during 1997–2017 (Fig. 7). However, the growth in exported quantity was not steady, ranging from -8.5 % (2012) to 12.3 % (2017), with an average annual growth of 4.8 % during the 20-year period (FAO, 2019b). The sharp decline seen in the export of shrimp in 2012 was due to production losses of farmed shrimp caused by severe outbreaks of AHPND in several major Asian producers.

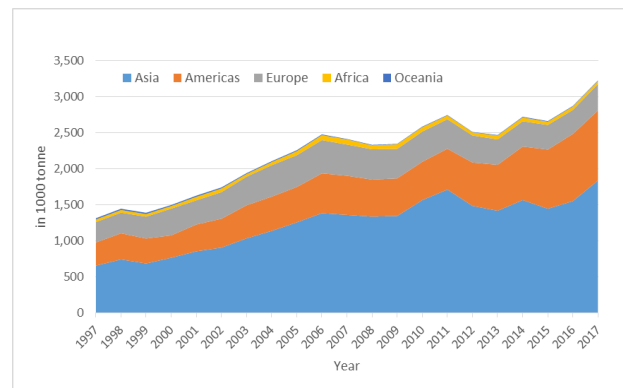


Fig. 7. Global shrimp exports.

In terms of regional contribution to international trade of shrimp, Asia, the Americas, and Europe have remained the major exporters from 1997 to 2017. However, the share of Asia and the Americas in the world shrimp export increased from 50.1 % and 24.5 % in 1997 to 57.0 % and 30.1 % in 2017, respectively, whereas, the share of Europe declined from 21.5 % in 1997 to 11.4 % in 2017. This can be attributed to the increasing share of farmed shrimp in the world shrimp export that has originated from Asia and the Americas.

Thailand was the largest shrimp exporter in 1997, when its share in the global shrimp export was 16.2 % (Table 6). In 2017, its position was replaced by India, and its share in the global shrimp export dropped to 6.7 % and ranked fifth in the world. Viet Nam and Ecuador have significantly increased their shares in the global shrimp export market during the past 20 years. Most of the countries that have significantly increased their share in global shrimp exports have benefited from the growth of farmed shrimp production.

Europe, the Americas, and Asia have remained the major importers of shrimp products from 1997 to 2017. While the share of the Americas in the world shrimp import increased to 30.7 % in 2017 from 26.8 % in

Table 6. Major exporters of shrimp products in the world.

1997			2017		
Country	Quantity (tonne)	% in world total	Country	Quantity (tonne)	% in world total
Thailand	212,399	16.2	India	576,153	17.9
India	111,295	8.5	Ecuador	439,660	13.6
Ecuador	109,521	8.3	Viet Nam	398,450	12.4
Indonesia	80,850	6.2	China	214,954	6.7
Denmark	74,655	5.7	Thailand	214,573	6.7
Viet Nam	67,072	5.1	Argentina	183,292	5.7
China	62,886	4.8	Indonesia	181,842	5.6
Netherlands	44,707	3.4	Denmark	85,208	2.6
Iceland	44,116	3.4	Netherlands	76,553	2.4
Greenland	42,820	3.3	Honduras	66,931	2.1
Mexico	36,848	2.8	Canada	62,200	1.9
Bangladesh	31,514	2.4	Greenland	54,183	1.7

1997, the shares of Europe and Asia declined from 37.4 % and 34.2 % in 1997 to 35.4 % and 30.7 % in 2017, respectively (FAO, 2019b). The decreased share of Asia in the world shrimp import was caused by the significantly reduced shrimp import by Japan, which was not be adequately offset by increased shrimp imports by China and other Asian countries.

The United States of America has remained the world's largest shrimp importer from 1997 to 2017. Its share in the global shrimp import (by volume) increased to 26.2 % in 2017 from 22.9 % in 1997. Japan has remained the second-largest importer; however, its share has declined to 9.2 % in 2017 from 22.8 % in 1997. Spain remained the third-largest importer of shrimp in 2017, with a share of 6.7 %, a slight decline from 6.2 % in 1997. In 2017, China was the fourth largest shrimp importer and exporter in the world, its share having increased to 4.7 % in 2017 from 1.1 % in 1997.

Future Prospects

Aquaculture production of carp, tilapia, and shrimp

Among aquatic species, carp supply the bulk of animal protein and other important nutrients in people's diets in many Asian countries. Despite the slow growth rate and the declining share in the global production of aquatic animals, the position of carp as the staple fish in domestic markets will remain unchanged in most Asian countries due to its low production cost, relatively high productivity, and the simple production technology. More importantly, carp farming has a relatively low carbon footprint because most carp species can utilise natural food to various extent and carp have low animal protein requirements in their commercial feed. Carp are highly tolerant to a wide range of temperature and environmental conditions.

Therefore, promoting carp farming can be an effective strategy for climate-change mitigation and impact adaptation in aquaculture. Besides, carp farming can be well integrated with other agricultural activities such as horticulture, crop farming, and animal husbandry, thereby contributing to more resilient food systems. The potential for further development of carp farming is quite promising. On the other hand, changes in consumer preference could be a limiting factor to the anticipated increase in market demand for carp. Development in carp processing and storage technology and modification of marketing strategies can help the carp-farming sector to better meet the changing requirements of consumers caused by sociodemographic changes, such as smaller family size, urbanisation, and faster life pace.

Tilapia is also a tough fish with a strong ability to adapt to environmental change. As a tropical fish, it can potentially benefit from global warming. Tilapia can be produced with simple technology and at relatively low cost. It can significantly contribute to local food security and nutrition. Tilapia fillet is a product that is well accepted by urban consumers due to its convenience in cooking and boneless nature. Thus further growth in farmed tilapia production can be anticipated.

Shrimp is generally a high-end aquaculture product globally. In general, increasing income and improving living standards can positively influence the demand for shrimp in both international and domestic markets. The governments of many countries are making good efforts to promote farmed shrimp production for the international market. On the other hand, shrimp farming is highly vulnerable to the impacts of climate change and disease. Shrimp farming, particularly when intensive or super intensive, relies on a high level of quality protein in the

feed and a high energy input in the farming operation. Thus it has a relatively high carbon footprint. Major innovations in shrimp feed and health management are essential to the realisation of anticipated growth in farmed shrimp production.

International trade of carp, tilapia, and shrimp

Consumer's preferences cannot be easily changed over a short period. Carp will remain a small player in the international trade of aquatic products. It is hoped that recognising the advantage of carp farming in climate-change mitigation could influence the choice of consumers. Innovation in carp processing and storage may improve the acceptance of consumers in major fish-importing countries. The development of niche markets may significantly increase the international trade of carp, although it may not fundamentally change the role of carp in the international trade of aquatic products.

After a rapid increase in international trade of tilapia for nearly two decades, the world export of tilapia showed a slight decline in 2016 to 2017. This decline may be only a short-term fluctuation instead of an actual trend. Along with the rapid increase in the quantity of internationally traded tilapia, the number of tilapia-importing countries has expanded significantly in the past decade. Added with other factors, such as consumer preference, affordability, and production feature, it is anticipated the global trade of tilapia will further expand.

Being a major internationally traded aquatic commodity, the volume of exported shrimp has increased constantly with some fluctuation during the past two decades. As a highly valued commodity, economic development will positively influence the international trade of shrimp. However, several factors may affect the direction of the shrimp trade. The foreseeable economic downturn caused by the Covid-19 pandemic may have an immediate impact. On the other hand, traditional Western markets for shrimp may have reached saturation after development for decades. Added to the increasingly stringent food safety and social standards for imported shrimp, it is hard to anticipate a significant increase of shrimp exports to the Western markets. On the other hand, economic growth in the developing world may be a good driver for further growth in the international trade of shrimp. For instance, China and Viet Nam significantly increased their shrimp imports from 54,698 and 17,605 tonnes in 2012 to 118,974 and 51,642 tonnes in 2017. This can make people more optimistic about the future of international trade of shrimp.

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Fish Waste Management: Turning Waste into Healthy Feed with Antimicrobial Properties

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Abstract

Fish processing results in a high volume of by-products that often goes to waste if not converted into value-added products. This review paper aims to present ideas on how to convert these by-products into healthy feed. As a result of fish processing, between 20 and 80 % of the whole fish is not used for direct human consumption. Bigger industrial fish processing units usually process the by-products into fishmeal and fish oil. For small-scale processing units, however, investing in a fishmeal plant is not economically viable unless at least 8 tonnes of raw material is available daily. The preservation of the raw material by acid silage is a simple and inexpensive alternative. Fish silage consists of minced fish by-products or minced whole fish not suitable for human consumption with an added preservative, usually an organic acid such as formic acid, to stabilise the mixture. Fish silage technology can also be used to treat dead fish to prevent the spread of diseases. Fish silage processing methods based on heat treatment at ≥ 85 °C for ≥ 25 minutes at $\text{pH} \leq 4.0$ will inactivate fish pathogens such as *Salmonella* and *Clostridium perfringens*. This treatment will also degrade DNA and inactivate genes potentially encoding antibiotic resistance. Furthermore, formic acid and the free amino acids and small-chain peptides in the fish silage mixture have antimicrobial properties. Thus, it can be used to reduce the use of antibiotics and promote healthy immune systems of fish. Fish waste can be converted into healthy feed through simple and inexpensive fish silage processing.

Keywords: aquaculture, fish silage, fish processing, value-added products, by-products

Introduction

The utilisation of world fish production for human consumption has increased significantly in recent decades, from 67 % in the 1960s to 87 % in 2014, equivalent to more than 146 million tonnes. The remaining 21 million tonnes were used for non-food products, 76 % of which was converted into fishmeal and fish oil, while the rest was used for different purposes including raw materials for direct feeding in aquaculture. In 2014, 46 % (67 million tonnes) of fish for human consumption was in the form of live, fresh, or chilled fish, 12 % (17 million tonnes) in dried, salted, smoked, or other cured forms, 13 % (19 million tonnes) in prepared and preserved forms, and 30 % (about 44 million tonnes) in frozen form (FAO, 2016).

Fish processing involves several steps: stunning,

grading, slime removal, deheading, washing, scaling, gutting, cutting of fins, filleting, and meat bone separation. During processing, the amount of waste generated ranges from 20 to 80 % depending on the level of processing and type of fish (Ghaly, 2013). This residue should not be seen as waste, but as raw material for a range of by-products. With looming food shortages, full utilisation of all resources is a moral and economic imperative. Some by-products containing meat, like heads, frames, and belly flaps, and some parts of the viscera, like liver and roe, can be used for human consumption. They are good sources of high quality proteins and lipids with long-chain omega-3 fatty acids. Furthermore, they are also rich in micronutrients such as vitamins A, D, riboflavin, and niacin and minerals like iron, zinc, selenium, and iodine. All too often, however, the by-products are regarded as low-value items that are best used as feed

for farmed animals, as fertiliser or discarded (Olsen et al., 2014).

This paper aims to develop awareness of the large amount of fish waste generated from processing and the existing technologies to utilise it. It introduces fish silage technology as one way to convert fish waste into a value-added product. Aside from being a potential source of income, the use of the fish silage process to treat dead fish can also reduce pathogens and prevent the spread of diseases.

Fish Waste Utilisation

Fish processing by-products can be used for the production of value-added products such as fishmeal, fish oil, fish protein hydrolysates, collagen, biodiesel, and even fish leather. The use of by-products to make fishmeal and fish oil is also an indirect way of providing healthy food since the expanding aquaculture sector is by far the largest user of these products (FAO, 2012). In a report by the World Bank (2013), it is projected that the use of by-products from processing will increase from 5.7 million tonnes under the baseline case to more than 10 million tonnes in 2030. There is an overall increasing trend in the amount of fishmeal and fish oil being obtained from by-products, concluding that the whole fish will be increasingly directed to direct human consumption and marine ingredients will need to be sourced more from by-products (Jackson and Newton, 2016).

Fish proteins can be found in all parts of the fish and can be extracted using chemical and enzymatic processes. Fish proteins can be used as a functional ingredient in many food items due to desirable properties, namely good water holding capacity, oil absorption, gelling activity, foaming capacity, and emulsifying properties. They are often used in food products as milk replacers, bakery substitutes, and in soups and infant formulas. Fish proteins are rich in amino acids which can be used in animal feed and fish sauce or can be used in the production of different pharmaceuticals (Ghaly, 2013).

Fish Silage

Fish silage is a liquid product produced from whole fish or fish parts to which acids, enzymes, or lactic-acid producing bacteria are added resulting in the liquefaction of the mass. This is activated by the action of enzymes from the fish (Babu et al., 2005; Borghesi, et al., 2009; Ferraz De Arruda et al., 2007; Mousavi et al., 2013). The fish silage process converts fish waste into a liquid mix of hydrolysed proteins, lipids, minerals, and other nutrients that are easily digested and absorbed by terrestrial and aquatic animals. The production of fish silage also offers economic advantages as it requires simple and independent scaling technology and low-cost materials (Haider et al., 2015; Vieira et al., 2015; Toppe et al., 2018).

There are two known methods of fish silage production, using either acid or fermentation. Fish silage can be produced by fermentation using lactic acid bacteria such as *Lactobacillus plantarum* as a starter culture (Ramirez-Ramirez et al., 2008). The addition of a carbohydrate source like molasses or fruit processing waste along with a lactobacillus culture converts sugars into lactic acid (Olsen et al., 2014; Carmen Ramirez Ramirez et al., 2016).

Acid preservation is a simple and inexpensive way to preserve by-products from processing. A combination of organic acids like formic acid and propionic acid can be used to turn fish processing by-products into fish silage. Formic acid is considered the best choice because silage made using formic acid is not excessively acidic and therefore, does not require neutralisation before being used (Tanuja et al., 2014). Mineral acids can also be used, however, the product should be neutralised before including in the feed (Pagarkar et al., 2006; Vieira et al., 2015; Olsen and Toppe, 2017). The main disadvantage of this method is the complete destruction of tryptophan and cysteine and the partial destruction of tyrosine, serine, and threonine (Ghaly, 2013).

In the production of fish silage, the material must be fresh and raw. It is important to include fish viscera to ensure sufficient enzymes for hydrolysis. The raw material is minced to a maximum particle size of one millimetre to ensure that acid can penetrate all cells. Then, 2 to 3 % (w/w) of formic acid is added and the mixture maintained at pH 3.5, regularly mixing to maintain the pH and to prevent mould growth (Toppe et al., 2018).

The maturation process of fish silage is affected by temperature. In tropical climates, it will take only 2 to 4 days depending on the amount of viscera. In colder temperatures, the process is longer, maybe a few weeks. The mature fish silage can be stored for years and can be used directly as feed, as a feed ingredient, or as a fertiliser (Toppe et al., 2018).

Benefits of Fish Silage

As healthy feed

According to Ferraz De Arruda et al. (2007), fish silage has long been produced and used in countries like Poland, Denmark, and Norway on a commercial scale. Experimental work using silage as a feed ingredient has been undertaken in several countries. Several studies on the use of fish hydrolysates from acid silage are mentioned by Olsen and Toppe (2017). Other studies include the use of fish silage as a potential protein supplement for growing lambs (Barroga et al., 2004) and pigs (Salas and Ornelas, 2010). Other studies concluded that fish silage could replace fishmeal as a protein source for aquafeeds (Ramasubburayan et al., 2013; Barreto Curiel et al., 2016; Jasim, et al., 2016;). Furthermore, in a study by

Carmen Ramírez Ramírez et al. (2016), the use of fish silage in broiler feed had no significant change in carcass yield, chemical composition, and sensory quality attributes of broiler meat. These studies revealed that low or moderate quantities of hydrolysate might be used in feed for improved feed intake, growth, and other performance indicators. These positive effects can be attributed to the presence of free amino acids and low molecular weight peptides (Ramasubburayan et al., 2013; Espe et al., 2015; Olsen and Toppe, 2017).

During fish silage production, the enzymes present in the acidic medium breaks down fish proteins into peptides, while the acidic environment helps to speed up their activity and prevent bacterial spoilage (Ghaly, 2013). The review of Harnedy and FitzGerald (2012) summarises the protein-derived bioactive peptides identified in marine processing waste, which have different functional properties such as antioxidant and antimicrobial.

The use of organic acids in fish silage has some additional benefits. Organic acids have antimicrobial properties, acting both as bacteriostats and bacteriocides (Suiryanrayna and Ramana, 2015). Each acid has its own antimicrobial potential (Rurangwa et al., 2014). Organic acids are also one of the most efficient feed additives for mould prevention (Coskuntuna et al., 2010). Organic acids in the diet can have beneficial effects on the performance of poultry by decreasing pathogenic bacteria Khan and Iqbal (2016). Given the weak acid nature of short-chain organic acids in their undissociated forms, they can easily diffuse through the cell membranes of the microorganisms. Once internalised in the neutral pH of the cell's cytoplasm, the organic acids dissociate into anions and protons and lower intracellular pH, thus affecting the enzyme-catalysed reactions and transport systems (Ricke, 2003; Olsen and Toppe, 2017).

Organic acids, when used as acidifiers in poultry feeds, can improve nutrient digestibility and stimulate natural immune response. Furthermore, organic acids also enhance apparent total tract digestibility and improve growth performance in pigs (Suiryanrayna and Ramana, 2015). The actions of organic acids include stimulating the secretion of pancreatic enzymes, lowering gastric pH, inhibiting pathogens, acting as an energy source during GI-tract intermediary metabolism, improving mineral utilisation, enhancing the apparent total tract digestibility, and improving growth performance (Suiryanrayna and Ramana, 2015).

The maturation process of fish silage is influenced by the amount of viscera and temperature. The high storage temperature results in faster maturation, usually 2 to 4 days in tropical climates. With the use of simple and unsophisticated equipment, the fish silage produced is rich in amino acids and peptides that have

antimicrobial properties. Furthermore, the presence of organic acids in the fish silage can improve nutrient digestibility, stimulate immune response, and improve growth performance when used as a feed ingredient for livestock.

Prevention of spread of disease

Fish silage can also be used to treat and prevent the spread of pathogenic microorganisms found in dead fish or fish killed for disease control due to the antimicrobial properties of the organic acids. The fish silage processing method (FSPM) in Norway is based on mincing to a ≤ 10 mm particle size, ensiling at pH 4.0 with formic acid, and heat treatment at ≥ 85 °C for ≥ 25 minutes. The process has been assessed for its potential to reduce the microbial risks of category 2 and 3 animal by-products of fish origin. Category 2 includes dead and clinically ill fish with external signs of disease and fish killed for disease control purposes. Category 3 includes animal by-products originating from the slaughtering of fish for human consumption (Norwegian Scientific Committee for Food Safety, 2010).

An ad hoc group appointed by the Norwegian Scientific Committee for Food Safety concluded that the FSPM would inactivate non-spore forming bacteria, *C. perfringens*, moulds, *Saprolegnia*, parasites, and viruses. Furthermore, *Clostridium botulinum* and preformed toxins of type E can be destroyed by the method. The FSPM can also degrade DNA and thus can inactivate the genes that encode antibiotic resistance. The method will not inactivate mycotoxins and prions. These are unlikely, however, to pose any hazard to animal or human health. The fish silage produced using this method can be safely used as agricultural fertiliser, biofuels, and feed for fur, zoo, pet, or circus animals (Norwegian Scientific Committee for Food Safety, 2010).

The Norwegian Institute of Food, Fisheries, and Aquaculture Research (Nofima) later verified the method, concluding that the process will adequately reduce the risks related to pathogens present in fish by-products from aquaculture. The process can inactivate *Salmonella*, *Enterobacteriaceae*, *C. perfringens* and *Clostridium sporogenes* spores (Nygaard, 2013).

Conclusion

Fish processing leads to a high volume of by-products that can be converted into value-added products like fish hydrolysates, fish collagen, fish sauce, fish oil, fish biodiesel, and fish leather. Most importantly, fish silage technology, using 2 to 3 % (w/w) of formic acid to treat fish processing by-products, can convert potential waste into valuable feed ingredients, thus transforming fish waste into profit. The resulting products also have antimicrobial properties, making

the process advantageous for the treatment of diseased fish.

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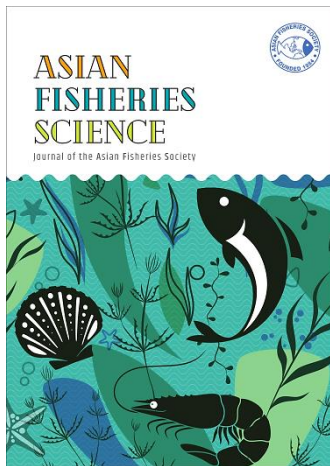
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Complexities Involved in Source Attribution of Antimicrobial Resistance Genes Found in Aquaculture Products

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Abstract

Aquaculture is contributing to nearly half of food fish production and the growth of the sector is the main contributor to the increase in fish production for the last three decades. Detection of antibiotic resistance in food-associated bacteria including those associated with products of aquaculture has been causing great concern. Often, this is directly linked to antimicrobial use in aquaculture. However, attributing the source of antimicrobial resistance in bacteria found in aquaculture products is complicated. In this study, we look at the origin, evolution and spread of antibiotic resistance documented in literature. The results indicate that antibiotic resistance observed in aquaculture environment has multiple sources. Antibiotic resistance is a natural phenomenon and existed in bacteria before the human use of antibiotics. Some bacteria have intrinsic resistance to certain antibiotics. Resistance to antibiotics is found in bacteria in the deep sea and in high seas, where it is unlikely to have any exposure to antibiotics. However, extensive use of antibiotics in different sectors has contributed to the enrichment of antibiotic-resistant bacteria in hospital environments, animal farms, and the aquatic environment. Aquatic bodies receive effluents from hospitals and animal farms that carry antibiotic-resistant bacteria from these sectors. These bacteria may end up in aquaculture farms and in fish harvested from these farms. Distinguishing resistance that has been selected in different sectors is extremely difficult and caution is needed while trying to attribute the source of antibiotic resistance in bacteria in the aquatic environment.

Keywords: AMR, antibiotics, fish production, aquaculture

Introduction

Aquaculture is one of the fastest-growing food production sectors in the world according to FAO estimates (FAO, 2020), aquaculture had an annual growth rate of 7.5 % since 1970, compared to only 0.9 % for capture fisheries and 2.6 % for terrestrial farmed meat production systems over the same period.

Global aquaculture production (including aquatic plants) in 2018 was reported to be 114.5 million tonnes by volume with a value of USD263.6 billion. Aquaculture contributes to about half of the global food fish production. Interestingly, 57.8 % of the total volume and 58.6 % of the total value of aquaculture production comes from the People's Republic of China; other top producers for 2018 were Indonesia,

India, Viet Nam, Bangladesh, Philippines, South Korea, Egypt, Norway and China (FAO, 2020). Fish is also one of the highly traded commodities and, in 2018, 67 million tonnes of fish (live weight equivalent) were traded internationally for a total export value of USD164 billion. While developing countries in Asia are major producers of aquaculture products, developed countries constitute the major market of consumers. European Union accounted for 34 % of global seafood imports in 2018, while the United States accounted for 14 % and Japan, 9 %.

There are very stringent regulations in the EU, USA, and Japan regarding the use of antimicrobials in aquaculture and as per the World Trade Organization (WTO) Sanitary and Phytosanitary (SPS) Agreement, imported products also need to comply with these

regulations. The EU has an elaborate system of verifying, whether outside countries exporting to their member countries have regulations on par with EU regulations. Additionally, the EU Food and Veterinary Office (FVO) conducts inspections to verify whether the Competent Authorities in fish exporting countries can provide guarantees regarding compliance with these regulations. These measures have led to significant improvements in aquaculture practices in producing countries. Nevertheless, there is a perception among some consumers and other non-governmental organisations, and also in some members of the scientific community, that there is heavy use of antibiotics in the aquaculture sector. Cabello (2006) indicated that the heavy use of prophylactic antibiotics in aquaculture is a serious problem for humans, animal health, and the environment. But many improvements have been made over the last decade, and more recent studies indicate that only 3 % of shrimp grow-out farmers in Viet Nam used antibiotics from 2011 to 2012 and that prophylactic treatments are rare (Rico et al., 2013). Antibiotic usage per ton of product in many species produced by aquaculture is much lower than that of other food-producing animals, even in developed countries. Usage in shrimp production in Viet Nam was 1.44 g.ton⁻¹ of production, and the corresponding figures for China and Thailand are 1.67 and 4.53 g.ton⁻¹ respectively. For tilapia culture, antibiotic usage was 1.32 g.ton⁻¹ of production in China and 7.31 g.ton⁻¹ of production in Thailand. The highest usage of antibiotics was 92.9 g.ton⁻¹ of Pangasius production in Viet Nam. This is still lower than the antibiotic usage of 144 g.ton⁻¹ of food-producing animals in the EU (ECDC/EFSA/EMA, 2015).

Issues Associated with Antimicrobial Use in Aquaculture

The Joint FAO/OIE/WHO Expert Meeting on Antimicrobial Use and Antimicrobial Resistance in Aquaculture identified that the two hazards to be considered are antimicrobial residues and antimicrobial resistance (AMR) (FAO/OIE/WHO, 2006).

While residues found in animal tissues may be directly related to the use of antimicrobials in the respective sector, the issue of AMR is more complicated in the case of aquaculture as illustrated in Figure 1 (Karunasagar, 2012). Detection of antimicrobial residues (e.g. chloramphenicol, nitrofurans, malachite green) in internationally-traded shrimp has resulted in the slowdown of imports, causing economic losses among producers and governments. This has also led to tightened national regulations on the use of antibiotics and the implementation of national residue control programs in many countries. As a result of these tightened regulations, the number of cases of rejections and alerts related to seafood due to antimicrobial residues has drastically decreased in recent years, although some cases of alerts and rejections still occur for some exporting countries. At the international level, the maximum residue limit (MRL) acceptable is set by the Codex Alimentarius Commission based on the scientific evaluation of the drugs by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Currently, there are MRLs for only a few antimicrobials in Codex. There are also additional national and regional MRLs in seafood importing countries, but these may or may not be consistent between countries. Hence, there is a need to have Codex MRLs for antimicrobials that are approved for use in aquaculture.

It is to be recognised that antibiotic-resistant genes are often carried on mobile genetic elements that can be transferred to other bacteria and that there are no phylogenetic or geographical barriers for such transfer. (FAO/OIE/WHO, 2006) Furthermore, because animal farm and hospital effluents reach the aquatic environment, antibiotic resistance determinants selected in other environments can also be found in the aquatic environment and may reach products of aquaculture. Rowe et al. (2016) demonstrated that the abundance of AMR genes (ARGs) in effluents entering a river catchment area is higher than that of the receiving environment. This review discusses the various aspects that need to be considered while making any conclusions about AMR detected in aquaculture products.

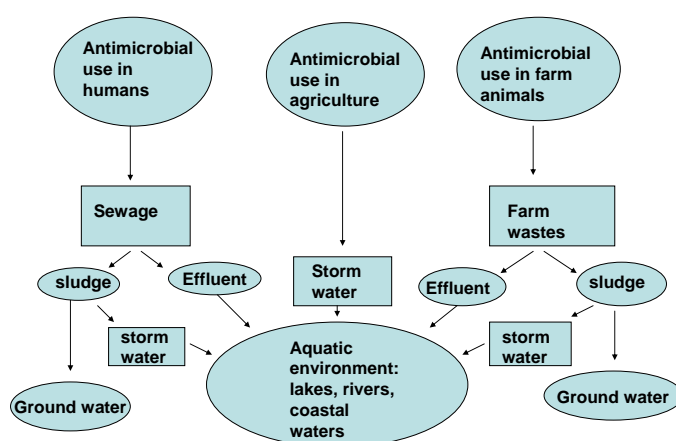


Fig. 1. Pathways for the spread of antimicrobial residues and resistant bacteria in the aquatic environment (Karunasagar, 2012).

Antibiotic Resistance is Natural, Ancient, and Predates Anthropogenic Use of Antibiotics

It is now well accepted that antibiotic-resistant genes are of ancient origin and can be found in bacteria from the pre-antibiotic era. D'Costa et al. (2011) noted that genes encoding resistance to β -lactam, tetracycline, and glycopeptide antibiotics could be found in bacteria from 30,000-year-old Beringian permafrost sediments. Viable multidrug-resistant bacteria were cultured from the Lechuguilla Cave in New Mexico, USA that was isolated for more than 4 million years (Bhullar et al., 2012). These bacteria were resistant to at least one antibiotic and often seven or eight antibiotics, including β -lactams, aminoglycosides, and macrolides, as well as newer drugs such as daptomycin, linezolid, telithromycin, and tigecycline. It has been estimated that the earth is inhabited by 5×10^{30} bacterial cells, with only about 1 % of these culturable and most of them are non-human pathogens (Monier et al., 2011); hence, we know very little about environmental bacteria and the genes that they carry. At least some of the resistance determinants presently circulating among human pathogens have been thought to originate from environmental bacteria. For example, *qnr* genes encoding quinolone resistance and found in plasmids of *Escherichia coli* and *Salmonella* might have originated from aquatic organisms like *Shewanella* or members of aquatic *Vibrionaceae*, where the *qnr* gene is found in the chromosome (Poirel et al., 2012). The plasmid-borne extended-spectrum beta-lactamase (ESBL) gene of CTX-M group (*bla*CTX-M) found in *E. coli* is thought to have originated from the environmental organism *Kluyvera* (Poirel et al., 2012). Thus, many environmental bacteria harbour resistance determinants that may not be related to the exposure to the antibiotics.

Recent molecular biological studies on antibiotic resistance genes provide interesting insights into the evolution and ecology of antibiotic-resistant genes. Tetracycline resistance is mediated by ribosomal protection proteins (RPPs) in Gram-positive and Gram-negative bacteria. Kobayashi et al. (2007) noted that RPPs are derived through duplication and divergence of GTPase before the divergence of the three superkingdoms, Bacteria, Archaea, and Eucarya. This suggests the extant function of RPPs occurred even before the evolution of *Streptomyces* which produce tetracyclines. They propose that RPPs evolved independently of tetracyclines and possibly serve a function other than antibiotic resistance. β Lactamases are enzymes involved in resistance to the penicillin group of antibiotics. Fevre et al. (2005) provided evidence to show that β lactamase genes in *Klebsiella oxytoca* were evolving for over 100 million years in this host, without concomitant evolution of an AMR phenotype. In addition to being involved in the hydrolysis of β lactam ring, metallo- β lactamases are involved in various basic cellular processes such as

hydrolysis, DNA repair, and RNA processing, and these enzymes can be found in all the three domains of life, i.e. Bacteria, Archaea, and Eucarya (Garau et al., 2005). The ancient evolution of antibiotic-resistant genes is further supported by the observation of antibiotic resistance in bacteria trapped in deep Greenland glacier ice cores for at least 120,000 years ago (Miteva et al., 2004).

Dutta and Hughes (1983) studied conjugative plasmids in enteric bacteria isolated before the medical use of antibiotics and compared them with plasmids found in more recent isolates. They noted that the 'pre-antibiotic' plasmids belonged to the same groups, as defined by incompatibility tests (Inc groups), as modern R plasmids. This suggests that the acquisition of antibiotic resistance in the past 50 years seems to have been by the insertion of new genes into existing plasmids rather than by the spread of previously rare plasmids.

Need to Differentiate Intrinsic Resistance and Acquired Resistance

Many Gram-negative bacteria have intrinsic resistance to the beta-lactam group of antibiotics. *Aeromonas* spp., commonly found in freshwater aquaculture environments, have been reported to have intrinsic resistance to ampicillin and amoxicillin and possess at least four chromosomally borne beta-lactamase genes (Janda and Abbot, 2010). This intrinsic resistance has been the basis for the development of the starch-ampicillin agar medium for quantitative detections of *Aeromonas* in foods (Palumbo et al., 1985). *Aeromonas hydrophila* strains possess the Ahe ABC efflux pump that contributes to intrinsic multidrug resistance (Hernould et al., 2008). Therefore, when attributing resistance of *A. hydrophila* isolated from aquaculture environments, it would be important to focus on the acquired resistance. Antibiotic-resistance genes have been reported to be present in mobile genetic elements such as plasmids, transposons, and associated with integrons (Piotrowska and Popowska, 2015).

When microorganisms that were once sensitive to an antimicrobial agent become resistant to a particular antibiotic, the resistance is acquired. The acquired resistance could be due to genetic changes such as mutations or to the acquisition of genes contributing to resistance through horizontal gene transfer. Antibiotic resistance genes may be transferred through mobile genetic elements such as plasmids, transposons, bacteriophages, genomic islands, or integrons. Though integrons are not self-mobile, they contain gene cassettes that are mobile.

Mechanisms of AMR

Bacteria resist the action of antimicrobial agents through different mechanisms (Alekhun and Levy,

2007; Blair et al., 2015). Some of the common mechanisms are:

- **Inactivation of the drug:** Bacteria acquire gene encoding enzymes that inactivate the antibiotic before it can reach the target, e.g. beta-lactamases inactivate beta-lactam antibiotics like penicillins and cephalosporins; carbapenemases inactivate carbapenems; aminoglycoside modifying enzymes such as N-acetyltransferases, O-adenyltransferases and O-phosphotransferases modify the antibiotics of this class.
- **Prevention of drug access to targets in bacterial cells:** Reduced access can happen due to: (a) reduced permeability to the drug: e.g. carbapenem resistance due to reduced or altered porin production by mutations. It has been reported that selective pressure due to carbapenems favours the emergence of mutations in porin genes or in genes that regulate porin expression; and/or (b) increased efflux: bacterial efflux pumps actively transport antibiotics outside the cell. Some efflux pumps may show narrow substrate specificity, e.g. Tet pumps, but others show broader activity such as multidrug efflux pumps.
- **Modification of drug targets:** Antibiotics act on specific bacterial targets (e.g. ribosomes) and modification of drug targets would render the antibiotic ineffective. For instance, the erythromycin ribosome methylase (*erm*) family of proteins methylate 16SrRNA and alter binding targets for macrolide antibiotics. The *qnr* family of genes encodes pentapeptide repeat proteins that bind to and protect topoisomerase IV and DNA gyrase from the lethal action of quinolones. Polymyxin antibiotics like colistin bind to lipopolysaccharides in Gram-negative bacteria and the antibacterial activity are due to the disruption of the cell membrane by the hydrophobic chain. Overexpression of *pmrC* in colistin-resistant bacteria leads to the addition of phosphoethanolamine to lipid A, leading to decreased binding of colistin.
- **Bypass targets:** Methicillin resistance in *Staphylococcus aureus* is due to the acquisition of chromosomal cassette *mec* element. The *mecA* gene encodes the β -lactam-insensitive protein PBP2a that enables cell wall biosynthesis to occur despite the native PBP being inhibited in the presence of the antibiotic. Resistance to sulphonamide and trimethoprim could be due to bypass mechanisms. These drugs interfere in different steps in de novo synthesis of tetrahydrofolic acid, an essential precursor for several amino acids and nucleotides. Bacterial resistance could be due to the production of drug-resistant

dihydrofolate reductase or dihydropteroate synthesis. Plasmid borne *sul1* and *sul2* genes in sulphonamide resistant bacteria encode drug-resistant dihydropteroate synthase.

Genetic Mechanisms of AMR

Detected phenotypic AMR could be due to different genetic mechanisms. Tetracycline is commonly used in the treatment of diseases in aquatic animals. When resistance to tetracycline is detected, it could be due to: (a) overproduction of efflux proteins, (b) production of ribosomal protection proteins, or (c) production of tetracycline inactivating proteins (Chopra and Roberts, 2001). Therefore, to understand the emergence and spread of AMR in aquaculture and the relation between antimicrobial use (AMU) and AMR in different sectors, it is important to have information on the genetic determinants related to the resistance.

In addition, information on the genetic type of resistance determinants in the study of the epidemiological aspects of the spread of AMR is also an important consideration. Extended-spectrum beta-lactamases could be of different molecular types and the genes encoding these show considerable variations. There are four classes based on molecular types and these different types are based on functions where some are inhibited by clavulanic acid while others are not. Some are serine-based enzymes and others are metalloenzymes.

AMR in Bacteria Associated with Aquaculture

AMR in pathogens of aquatic animals has been reported from different systems. In shrimp hatcheries, the presence of antibiotic-resistant luminous bacteria has resulted in mass mortalities. (Karunasagar et al., 1994). Acquired resistance in *Aeromonas salmonicida* causing furunculosis in temperate waters has been reported from various countries (FAO/OIE/WHO, 2006). Several mobile genetic elements such as plasmids, transposons, and integrons carrying AMR genes have been detected in *Aeromonas* spp. from aquaculture sites in different parts of the world (Piotrowska and Popowska, 2015). Over 80 % of *Vibrio harveyi* from finfish aquaculture systems in Italy showed resistance to amoxicillin, ampicillin, and erythromycin, while 76 % of strains of *Vibrio* spp showed resistance to sulphadiazine (Scarano et al., 2014). AMR in pathogens of aquatic animals could impact disease management in these systems and the resistance determinants could be transferred to human pathogens in aquatic systems.

Though AMR is observed in aquatic bacteria associated with aquaculture systems, it is difficult to find a direct link between the resistance profile and AMU. Culture-independent studies in the Baltic Sea show the presence of resistance genes encoding

resistance to sulphonamides, trimethoprim, tetracycline, aminoglycoside, and chloramphenicol. In addition, genes encoding multidrug efflux pumps were discovered in sediments below fish farms, even though some antibiotics like tetracyclines, aminoglycosides, and chloramphenicol are not used in this area (Muziasari et al., 2017). Some of these might represent a natural reservoir of resistance genes in the aquatic environment. Antibiotic-resistant marine bacteria have been found as far as 522 km offshore and in the deep sea at depths of 8,200 m (Aminov, 2011).

Public Health

From a public health perspective, AMR in aquatic bacteria of zoonotic potential would be significant. Studies done in Korea show that all *Vibrio parahaemolyticus* isolated from oysters were resistant to ampicillin and vancomycin and half of the number of isolates exhibited resistance to cephalothin, rifampin, and streptomycin (Kang et al., 2016). However, there was no linkage to the use of these antibiotics in aquaculture. There may be geographical variation in the prevalence of resistance. Studies in China with isolates from crustaceans and shellfish showed much higher (over 90 %) resistance to rifampin and 78 % showed resistance to streptomycin (Hu and Chen, 2016). Most *Vibrio vulnificus* strains isolated from Dutch eel farms showed resistance to cefoxitin, even though this antibiotic was not used in eel aquaculture (Haenen et al., 2014). Thus, the detection of antibiotic resistance in bacteria isolated from aquaculture cannot be directly linked to the use of antimicrobials in aquaculture. Therefore, the detection of antibiotic resistance in aquaculture systems needs to be interpreted with caution, considering that resistance determinants are naturally present in these environments and that ARGs also come from other sectors. But often in literature, we can see the simplistic linking of any resistance found to AMU in aquaculture, even when there is no use of antimicrobials in that system. For example, Akinbowale et al. (2006) attributed resistance found in aquaculture environments in Australia to significant off-label use. Implementation of an integrated surveillance program within the framework of One Health, which includes the study of AMU and ARGs in different sectors (human, agriculture, veterinary, aquaculture), could improve our understanding of the drivers leading to the selection and spread of AMR in the aquatic environment.

AMR in Bacteria Associated with Products of Aquaculture in Retail Markets

When farmed fish are handled and processed, there can be significant changes in the microflora. Therefore, bacteria found in farmed fish at the retail level may not represent the microflora coming from

the aquatic environment. For example, farmed fish such as Vietnamese catfish and tilapia are filleted before reaching the market. Aquacultured shrimp are handled and processed (beheaded, gutted, peeled) before being sent to export markets. Uddin et al. (2013) examined the microflora of raw-cultured and wild-caught shrimp imported into Denmark and concluded that the flora changes considerably during processing. They suggested that it is not possible to pick up any indicator bacteria representative of the aquaculture environment at this stage. This calls for caution in the interpretation of AMR found in fish and shrimp at the retail level or import control points and in linking resistance found in bacteria at this stage and AMU in aquaculture. Data from aquatic products at the primary production stage is essential to understand any linkage between AMU and AMR in aquaculture.

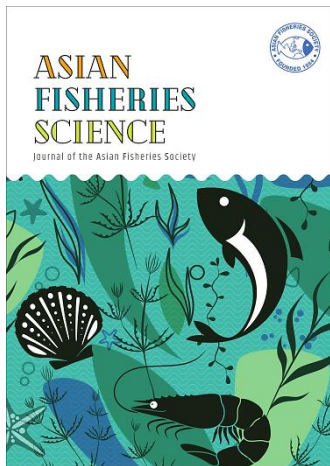
Conclusion

Antibiotic resistance is a natural phenomenon and exists in bacteria from environments even without exposure to antibiotics. Nevertheless, extensive use of antibiotics in different sectors has led to the selection and spread of multidrug resistant bacteria. However, it is very difficult to link AMR in bacteria found in aquaculture systems with the use of antibiotics in this sector, since the aquatic environment receives effluents from hospitals and terrestrial animal farms, consequently leading to the spread of resistant bacteria from all sectors through aquatic systems. The processing of fish leads to change in microflora and resistant bacteria found in processed fish may not be derived from aquaculture. Therefore, there is a need to be cautious while drawing conclusions about the source of antibiotic resistance in bacteria associated with aquaculture.

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Critical Review of Methods Used in Published Studies of Susceptibility of *Vibrio* spp.; Lessons to Be Learnt

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Abstract

The World Health Organization, the World Organisation for Animal Health, and the Food and Agriculture Organization of the United Nations recognises that the threat posed by the emergence of resistance to antimicrobials must be addressed using a One Health approach. To quantify the contribution of aquaculture to this global problem, it is essential that we generate data on the antimicrobial susceptibility of bacteria isolated from aquatic animals that is of sufficient quality. This paper presents a review of the quality of the data presented in 182 published papers on the antimicrobial susceptibility of various non-cholera *Vibrio* species isolated from aquatic environments. This review revealed that serious shortcomings either in the performance of the susceptibility tests or in the reporting of those tests occurred with an alarmingly high frequency. The majority of studies failed to provide sufficient details of the testing protocols they used and only a small percentage of the studies provided explicit evidence that they had used standardised susceptibility protocols. Although 203 studies reported frequencies of resistance in the isolates they studied, 185 of them either did not provide the criteria they used to determine resistance, used criteria that had not been validated or used criteria that were inappropriate. As a result of these shortcomings, it is difficult or impossible to compare the data that these papers have presented. It is argued that adopting a few simple rules in the design and reporting of susceptibility studies would, at little cost or effort, result in the production of papers that could make a significant contribution to our understanding of the issues involved in the use of antimicrobial agents in aquaculture.

Keywords: aquaculture, AMR, detection, analysis, CLSI

Introduction

In-vitro antimicrobial susceptibility tests are not robust and the quantitative data they produce is critically dependent on the details of the experimental protocols adopted in the performance of those tests (Smith, 2019). It is, therefore, essential that any report of susceptibility data is accompanied with a complete description of the testing protocol used to generate that data. When a report also includes an interpretation of the meaning of susceptibility data, it is also essential that the criteria and the sources of the data are provided. However, reviews of published studies of antimicrobial susceptibility of isolates from humans (Turner and Ashley, 2019) and terrestrial animals (Schwarz et al., 2010) have demonstrated that

shortcomings in the descriptions of the testing protocol and/or the interpretive criteria applied occur in these studies with a disturbing frequency.

In addition to these requirements the World Organisation for Animal Health (OIE) Aquatic Animal Health Code (OIE, 2018) has argued that, in order to ensure maximum international comparability, any programme for monitoring or surveillance of antimicrobial susceptibility of isolates from aquatic animals should be designed so that the data generated can be compared with data produced in other laboratories. Smith et al. (2013) have argued that to ensure maximum international comparability required by the Code, it is essential that standardised and internationally harmonised susceptibility test protocols be used to the greatest extent possible.

They further recommend that the meaning of the quantitative data should be determined using internationally harmonised, consensus-based interpretive criteria when these are available.

This paper presents an analysis of the published literature on the antimicrobial susceptibility of non-cholera *Vibrio* isolates. The analysis was performed to investigate how consistent the methods used in these studies were with current best-practice guidelines.

Materials and Methods

Collection of the relevant published literature

The first step in collecting papers that had been published on the susceptibility of non-cholera *Vibrios* was to use relevant keywords in Google Scholar. This identified an initial list of relevant papers. The second step was to examine those in the initial list for the papers they cited and the papers that cited them. Iteration of this process generated 190 papers. Copies of the full text were accessed for 182 of these. Some of these papers reported more than one study and in total the 182 papers provided details of 207 studies.

The papers originated from 39 different countries with the majority (55 %) originating from Asia, 20 % from Europe, 15 % from America, and 8 % from Africa. The majority of the papers (91 %) had been published in the last 20 years and 62 % published in the period 2008 to 2017. The fact that the median citations that the papers received were 16 indicated that, generally, they had been widely consulted.

We do not believe that this process identified all relevant papers, but we do suggest that those accessed provide a reasonable overview of current practices in this field.

Results and Discussion

General observations

An observation that remains after reading 207 studies is that, for many of them, the methods used are inadequately or incompletely described and, on some occasions, incorrectly or inappropriately referenced. This may suggest that the authors have not given sufficient weight to the fact that the quantitative values generated from such tests are protocol dependent. Without full details of the methods used to generate them, it is impossible to establish the meaning that can be given to the quantitative data generated. The poor quality of the description of the methods used had a consequence for the analysis presented here. For many papers, it was not possible to determine how the quantitative data presented was obtained.

A second general observation is that the term

'resistant' was more or less universally used in the papers examined but its meaning was rarely defined. Silley (2012) has argued that much confusion has been generated by the variation in the meanings given to this word by different authors. It is strongly argued that, in order to improve communication, all workers in this field should adopt the use of the terminology suggested by Silley (2012).

Which species were studied?

In the 207 studies, five species were investigated most frequently. There were 88 studies that reported on the susceptibility of *Vibrio parahaemolyticus*, 72 on *Vibrio alginolyticus*, 50 on *Vibrio harveyi*, 27 on *Vibrio vulnificus*, and 19 on *Vibrio anguillarum*. In addition, 66 studies either reported the susceptibility of various other species of *Vibrio* or did not provide a species-level classification of the *Vibrio* isolates they studied.

What media were used?

Over 90 % of the studies examined reported using either unmodified Mueller-Hinton agar (MHA), cation-adjusted Mueller-Hinton broth (CAMHB), or these media supplemented with various concentrations of NaCl. Analysis of the media used in these studies (Table 1) showed that more than half had used media without additional NaCl. This would suggest that the supplementation of MHA or CAMHB is not necessary for susceptibility testing of the majority of the *Vibrionaceae*.

What incubation temperatures were used?

Of the studies that reported the incubation temperature, the majority reported using temperatures at ≥ 28 °C for susceptibility testing of their *Vibrios* (Table 1). This would suggest that protocols that specify incubation at 28 °C provided in VET03-A (CLSI, 2006) and VET04-A2 (CLSI, 2014) would be suitable for these bacteria. Four of the most commonly studied *Vibrio* species (*V. alginolyticus*, *V. harveyi*, *V. parahaemolyticus*, and *V. vulnificus*) were reported as being capable of infecting humans. For these species, not surprisingly, a significant number of studies reported testing at ≥ 35 °C. Thus, the protocols specifying incubation at 35 °C provided in M02-A12 (CLSI, 2015) and M07-A10 (CLSI, 2014) could be used for testing their susceptibility. It is, however, unlikely that *V. anguillarum* could be tested at this temperature.

What methods were used?

Most papers reported using either disc diffusion methods or minimum inhibitory concentration (MIC) methods, although a minority reported using both. In all of the papers collected, 150 studies that used disc diffusion methods, 50 that used MIC methods, and seven that used the hybrid E-test were reported.

Table 1. Summary of the media and incubation conditions used in studies of the antimicrobial susceptibility of various *Vibrio* species.

Species	Media ^a	Temperature ^b	
	No added NaCl	≥35 °C	≥28 °C
<i>V. alginolyticus</i>	56 %	44 %	80 %
<i>V. anguillarum</i>	40 %	0 %	76 %
<i>V. harveyi</i>	48 %	22 %	84 %
<i>V. parahaemolyticus</i>	68 %	65 %	95 %
<i>V. vulnificus</i>	76 %	35 %	85 %
<i>V. spp</i> ^c	60 %	25 %	78 %

^apercentage of those studies that used Mueller-Hinton media.

^bpercentage of those that reported their incubation temperature.

^c*V. spp.* includes unclassified isolates and miscellaneous species.

Were international standard testing protocols used?

In 103 of the 207 studies examined, insufficient details were provided to allow any identification of the source of the testing protocols used. Furthermore, 29 cited various books or papers as their source. However, 69 studies cited a Clinical and Laboratory Standards Institute (CLSI) document as the source of the protocol they used and an additional six papers stated that they used a CLSI protocol but provided no reference to a specific document.

Therefore, only 33 % of the studies reported using an internationally standardised testing protocol and that approximately 50 % failed to provide sufficient details of the source of the protocol they used, which is somewhat alarming. It is clear, however, that in the studies that did use a standard protocol, there was a clear preference for those published by CLSI.

How many strictly adhered to the CLSI protocol they claimed to use?

Of the studies that employed a disc diffusion method, 51 claimed to have used a standardised CLSI protocol. A detailed reading of the methods used in these studies revealed that 14 used a temperature other than that specified in the CLSI protocol and 16 used media with additional NaCl. Consequently, only 31 (20 %) of the 150 disc diffusion studies explicitly presented evidence that they used and adhered to a standard test protocol.

A similar situation was observed in the MIC studies. Of the 18 studies that claimed to have used a CLSI protocol, nine have modified the temperature or time. Therefore, only nine (18 %) of the 50 MIC studies explicitly presented evidence that they used a standard test protocol.

How many studies reported compliance with the quality control criteria?

Of the 31 disc studies that presented evidence of having used a standard CLSI protocol, 16 reported the use of a recommended reference strain as a quality control measure. Of these 16, only three reports cited an appropriate CLSI document as a source of the acceptable ranges for the reference strain they used. Additionally, there was one study that specified their results with the reference strain were within the acceptable range.

Of the nine MIC studies that presented evidence of having used a standard CLSI protocol, six reported the use of a recommended reference strain as a quality control measure. Of these six, only there was one report that cited an appropriate CLSI document as a source of the acceptable ranges for the reference strain they used. None reported the results they obtained with the reference strain they used.

Thus, very few of the studies provided evidence of their compliance with the quality control requirements of the standard protocol they used. It has to be borne in mind, however, that some authors may have considered that the statement where a standard protocol was used was sufficient to imply that the quality control measures specified in that protocol were also performed. Thus, full compliance with quality control may be more common than the examination of the relevant texts suggests.

How many studies reported their raw quantitative data?

Of the 207 studies examined, only four presented their quantitative data and five studies with histograms of these data. For 198 (96 %) studies, no quantitative data was made available. All the studies present estimates of the frequency of resistance, but only a minority reported the interpretive criteria used

to categorise the isolates as resistant and hardly any provide the quantitative data to which these criteria were applied. The consequence of this is that it is not possible to recalculate the frequencies of resistance provided in these studies using the current international, consensus-based criteria.

How many studies applied international consensus-based interpretive criteria?

Interpretation of the meaning of raw quantitative data can be made by applying either epidemiological cut-off values (ECVs) or clinical breakpoints (CBs) to them. Both ECVs and CBs are species-specific and protocol-specific but differ in the data needed to set them and the meanings that can be given to the categories they delineate.

Setting ECVs is relatively easy. It requires only in-vitro susceptibility data of adequate quantity and quality. The application of an ECV allows the categorisation of an isolate as a fully susceptible member of their species (wild type or WT) or as manifesting a reduced susceptibility when compared to other members of its species (non-wild type or NWT). In contrast, setting CBs is difficult. As they aim to categorise isolates based on the most probable clinical outcome of specific therapy of a specified infected host their setting requires very extensive microbiological, clinical, and pharmacodynamics data. Very little of these data are available for aquatic infections. It has been argued that the time and effort needed to generate these data would mean that CBs, relevant to any aquatic animals, will not be available for some time (Smith, 2008).

At present, no ECVs have been set by CLSI for susceptibility data generated for any *Vibrio* species by any standard protocol. Concerning CBs, CLSI has published some breakpoints applicable to data for *Vibrio* species tested at 35 °C on unmodified Mueller-Hinton media (CLSI, 2016). There are, however, two reasons why these breakpoints should be treated with caution.

The first is that there is little empirical evidence for them. The forward of the guideline that presents them states that "Users of the guideline should be aware that the very extensive microbiological, clinical, and pharmacodynamics databases normally used for setting breakpoints by CLSI do not exist for the collection of organisms described in this document". The CBs for *Vibrio* species presented in this document are, in fact, simply copied from the CBs presented for the *Enterobacteriaceae* in M100-A27 (CLSI, 2017), and very few cited papers provided evidence of their validity when applied to the *Vibrio* species isolated from aquatic animals.

The second follows the fact that of their nature, CBs are host specific. Those presented in the guideline

M45-A3 (CLSI, 2016) relate only to the prediction of the clinical outcomes of therapies of humans. They cannot, with any legitimacy, be applied to predicting the clinical outcome of infections of aquatic animals.

Of the 31 disc studies that are assumed to have used standardised CLSI test protocols, 17 cited an appropriate CLSI document as a source of the CBs they used to interpret the meaning of their data. One of these 17 was concerned with mortalities of abalone and, therefore, these CBs were not relevant to that study. Of the nine MIC studies that are assumed to have used appropriate CLSI test protocols, only two reports cited an appropriate CLSI document as a source of the CBs they used to interpret the meaning of their data.

Overall, only 18 (9 %) of the 207 studies examined explicitly provided evidence that standard test protocols had been used and relevant internationally harmonised interpretive criteria had been applied to the in-vitro data generated.

The Way Forward

It is possible to offer a few, relatively simple, recommendations that, if implemented, would go a long way to improving the cost-benefit ratio of studies of the susceptibility of bacteria isolated from aquatic animals.

1. Standard testing protocols are available for a large percentage of the bacterial species isolated from aquatic animals. When they are available, they should be used, and their procedures should be strictly adhered to. Compliance with the quality control procedures of these standard test protocols is an absolute requirement.
2. Data generated by susceptibility testing should be interpreted using internationally harmonised and consensus-based criteria when these are available. When they are unavailable, the meaning of the data should be established using ECVs calculated by an objective and statistically based method. For MIC data sets, two automatic validated statistical methods are available (ECOFFinder @ clsi.org/meetings/microbiology/ecoffinder/ and NRI @ <http://www.bioscand.se/nri/>). For disc data, there is only one automatic statistical method (NRI @ <http://www.bioscand.se/nri/>).
3. Reports of any susceptibility study should provide an accurate citation for the source of the test protocols used and present evidence of compliance with the quality control requirements of that protocol. They must also include or provide access to the unprocessed quantitative data generated in the study.

A more comprehensive treatment of these recommendations has been provided by Smith (2020).

Conclusion

Improving our understanding of antimicrobial resistance (AMR) in aquaculture will require the consideration and comparison of a large number of susceptibility data sets collected from diverse environments. If these comparisons are to be made, the data sets must be commensurate (Smith et al., 2013). The 207 studies of the antimicrobial susceptibility of non-cholera *Vibrio* species analysed here have generated a truly vast amount of data from a geographically diverse set of environments. Due to either the lack of information provided about how they were obtained or variations in the protocols and interpretive criteria used to generate them, however, very little of these data could be considered as commensurate. Therefore, it would seem to be an inescapable conclusion of the analysis presented here that very considerable time and effort have been expended but the gain in our understanding of AMR is disappointingly small.

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Correct Diagnostics: Prerequisite for Prudent and Responsible Antimicrobial Administration

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Abstract

Since bacterial diseases have an adverse impact on the profitability of aquaculture, causing direct and indirect losses, this review paper is assessing the importance of accurate diagnostics in prudent and responsible administration of antimicrobials. Diagnostics and treatment of bacterial diseases in aquaculture are inevitable factors in their responsible management and consequently contribute to the reduction of antimicrobial use (AMU) and prevention of antimicrobial resistance (AMR) development. To mitigate and prevent the losses, fast and accurate recognition and detection of bacterial pathogens are the main prerequisites. Monitoring programmes in all stages of production, from broodstock to fattening units, are needed to avoid long diagnostic processes and enable fast commencement of diagnostic procedures and responsible AMU. Moreover, preventive measures to reduce the risk of bacterial infection includes good aquaculture practices (GAP) and biosecurity measures, in the absence of specific immunoprophylaxis, or vaccination, against endemic bacterial diseases. Antimicrobial use may be considered as therapeutic, metaphylaxis, prophylaxis, and growth promotion. Antimicrobials are most often administered through bio-enrichment of fish larvae or shrimp post larvae and medicated feed. The efficacy of the treatment via medicated feed depends on the rapid diagnosis and commencement of treatment, selection of appropriate antimicrobials, proper dosage, and duration of treatment. To prevent possible mistakes in AMU, it is necessary to avoid prophylactic use of antimicrobials, medication of viral infections, and repeated use of the same medicines.

Keywords: AMR, AMU, aquaculture, disease management

Introduction

Diseases have become the main constraint to aquaculture growth, impacting both economic and socio-economic development in many countries (Subasinghe et al., 2001; Bondad-Reantaso et al., 2005). The annual economic losses due to diseases are estimated to be billions of dollars. Among causative agents, bacteria can survive in the aquatic environment independently of their hosts and became the major obstacle to the cultivation of freshwater and marine fish species as well as crustaceans. The global economic impact of bacterial diseases on the aquaculture sector likely ranges from hundreds of millions to billions of dollars annually (Subasinghe, 2005) due to direct (mortalities, diagnostics and treatment costs) and indirect losses (cost of wasted

feed, removal of dead animals, retarded growth and lower feed conversion ratio (FCR).

Very rarely it is possible to control bacterial diseases in aquaculture by eradication and targeted antimicrobial chemotherapy remains vitally important for the treatment of some bacterial diseases (Smith et al., 2003). Hence, diagnostics and treatment of bacterial diseases in aquaculture are inevitable factors to its responsible management. The effective control and treatment of bacterial diseases require rapid, reliable, and highly sensitive diagnostic methods (Haenen and Zrnčić, personal communication). Clinical aspects of the outbreak, post-mortem examination, and histopathology are the primary methods used in diagnostics, but they often lack specificity and the pathogen is difficult to detect in animals without

clinical signs of the disease. Cultivation of pathogenic bacteria is a widely used method, but it is time-consuming and there are some non-cultivable, fastidious bacterial pathogens. Thus, it takes almost 10 days, from the occurrence of first signs of disease to the end of diagnostics procedure and sensitivity testing. However, this period is too long, and the losses may become enormous (Buller, 2004).

Generally, bacterial diseases are controlled by feeding infected fish with antibiotic-medicated feed, based on sensitivity testing. However, this practice may be ineffective because sick fish lose appetite. In addition, frequent use of antimicrobial compounds has led to the development of resistance to antimicrobial compounds in pathogens, posing serious challenges to both aquatic animal health and human health (Cunha, 2000).

Appropriate use of antimicrobials will cure some sick animals, speed up the recovery of the population, improve the welfare of treated animals, and prevent the spread of the bacterial infection to other animals (Kemper, 2008). Appropriate use of antimicrobials is dependent on the proper diagnosis, based not only on the detection of the pathogen but also on information such as farm history and outbreak or event history, followed by a visual examination of the aquatic animals with and without clinical signs before taking samples for laboratory tests. Prudent and responsible use of antimicrobials to minimise the risk of resistance is a challenging duty for aquatic animal health experts.

This paper emphasises the contribution of diagnostics for the responsible management of bacterial diseases in aquaculture. Moreover, the administration of antimicrobial compounds should be based on accurate diagnostics and carried out in a way that enables effective treatment and consequently promotes the reduction of antimicrobial use (AMU) and the prevention of the development of antimicrobial resistance (AMR).

Impact of Bacterial Diseases on the Profitability of Aquaculture

Bacterial disease outbreaks have an adverse impact on the profitability of the aquaculture facility, regardless if the disease occurs in the hatchery or on-growing facilities. Direct losses caused by the disease are mortalities, which vary according to the pathogen and category of the affected population. The fry and juveniles are usually more susceptible to bacterial infections and mortalities, which may reach up to 35 to 40 % of the population whereas mortalities in the older population may reach 15 to 25 % (Varvarigos, 2003). Costs for antimicrobial substances and diagnostic procedures should be included in the direct cost, as well as losses caused by disease re-occurrence. Indirect losses include the adverse effects on growth, which are expected to be severe due to the prolonged loss of appetite and the long and

drastic reduction of the feeding rate as a management response that will eventually increase the FCR. Additional indirect costs comprise of labour for the daily removal, transport, and sanitary disposal of dead fish. Extra costs for additional disinfection in the hatchery and of the equipment used for feeding, removal of dead fish, may also be added to the indirect costs due to the disease outbreak. The labour cost and time required to prepare the medicated feed daily is yet another additional cost caused by a bacterial disease outbreak. There is also the significant, but unquantifiable, psychological burden on the fish farmers.

Role of Diagnostics in Bacterial Disease Management

Immediate management of an outbreak on the aquaculture farm

When there is an outbreak of bacterial disease in the aquaculture facility, the main prerequisite for mitigation of losses is fast and accurate recognition and detection of the bacterial pathogen. Sensitive and specific methods for the detection of the bacterial pathogen are very important factors of the health monitoring program. Diagnostic skills should be continuously improved upon regardless of diagnostic capacity. Different actors in the disease recognition process have different diagnostic capacities (Bondad Reantaso et al., 2001) – level 1 consisting of environmental changes determination (water temperature, pressure, oxygen saturation, eutrophication, etc.), gross signs observation (changes in behaviour of the aquatic organism, pattern of the feeding, external signs,) and necropsy findings; level 2 consisting of general bacteriological and histological methods; and level 3 consisting of DNA based methods and spectrophotometric methods – are equally important for an accurate diagnosis.

Standard diagnostic procedure for immediate disease control begins when the farmer notices changes in the appearance of the farmed shoal and informs health specialists. They then commence the diagnostic procedure by identifying changes in the environment, clinical appearance of the affected population, and, together with results of the necropsy, inform the choice of bacteriological media and the procedure to be performed (Christofilogiannis, 2013). After obtaining pure bacterial cultures, sensitivity testing to approved and indicated antimicrobials should be carried out. Results of the testing using standardised protocols will advise on the choice of antimicrobials. The next step is an evaluation of the affected biomass that will enable the quantification of the required medicated feed. The farmer will order the medicated feed from an approved feed mill or, under the supervision of a health specialist, will prepare the medicated feed on

the farm. Treatment against the bacterial disease should last at least 10 days. The diagnostic procedure starts with notifying the first signs of the disease, followed by sampling sick fish and seeding the bacterial plates. The time needed to complete the diagnosis and susceptibility testing which is the basis for appropriate use of antimicrobials mixed in medicated meal lasts at least 6 to 7 days or even longer, in the case of slow-growing or fastidious bacteria (Buller, 2004).

Health management plans (HMP)

As it is obvious from the above described bacterial disease management procedure, that losses may rise to the level where they begin to pose a serious threat to the sustainability of the production. To mitigate the losses caused by a disease outbreak, reduce the risk of propagation of the pathogens, and to allow improvement of the treatment efficacy, it is useful to create and implement a health management plan, which includes several requirements:

1. Knowledge of the technical procedure including optimal ecological condition and normal appearance and behaviour at all stages of the farmed species during all steps of the cultivation – from broodstock to market size fish.
2. Knowledge of the ecological and environmental conditions favourable for a disease outbreak.
3. Knowledge of the clinical appearance of the particular bacterial disease, post-mortem signs, and histopathological changes in affected tissues, as primary methods for diagnostics often lack specificity and it is difficult to detect the pathogen in the animals without clinical signs of the disease. It is important to recognise the first changes to set up suspicion.
4. Effective control and treatment of bacterial diseases require rapid, reliable and highly sensitive and specific diagnostic methods; therefore, the health expert needs to choose the most appropriate diagnostic procedure. Cultivation of pathogenic bacteria is a widely used method, but time-consuming and there are some hardly cultivable, fastidious bacterial pathogens. If there is a possibility to implement immunological, protein-based, and molecular methods, all mentioned limitations might be solved.
5. In order to start the diagnostic procedure timely, it is most important to notify the suspicions of the disease quickly.

A health management plan should consist of several equally important components (Le Breton and Sourd, 2011):

1. Reducing bacterial pathogen pressure by implementing the following:
 - 1.1. Good aquaculture practices (GAPs) including the separation of generations, favourable stocking densities, proper feeding, feed quality, reducing stress by providing farmed animals appropriate light, protection from the predators, water exchange.
 - 1.2. Sanitation practices implementing appropriate cleaning and disinfection of farming units, equipment, containers, boats, nets.
 - 1.3. Biosecurity measures which include an introduction of certified stocks, awareness of the disease history on the farm, control of animal movement between and within farms, movement of people and vehicles, control of birds, predators, removal of dead fish.
2. Health monitoring programmes, which aim for early detection of the pathogen in all stages of the production. This should start with broodstock health monitoring and although biosecurity measures have been implemented, the vertical transmission of different bacterial diseases should be considered. For example, bacterial kidney disease caused by *Renibacterium salmoninarum* can be transmitted through fertilised eggs in salmonids (Pascho et al., 2002), or *Photobacterium damsela* subsp. *piscicida* (Romalde et al., 1999) in marine fish. Health monitoring in the hatchery should be carried out because sometimes biosecurity measures can fail and an infection may appear. Regular testing of fry, the most susceptible life stage, is needed. It should be kept in the mind that transport from the hatchery to on-growing units is a lot of work and the latent infections could occur after transportation. Disease monitoring, clinical inspection, and sampling followed by diagnostic testing should be performed during the on-growing period based on the knowledge of predisposing factors/periods for endemic diseases occurrence. In the absence of specific immunoprophylaxis (vaccination against endemic bacterial pathogens), losses can be mitigated only by prudent and responsible use of antimicrobials, and the key to successful treatment is continuous monitoring and early diagnosis.

Administration of Antimicrobials

Antimicrobial compounds are defined as substances that can kill or inhibit the growth of microorganisms (Romero et al., 2012). According to the document

issued jointly by FAO/OIE/WHO (WHO, 2004), the use of antimicrobials can be divided into (1) Therapeutic, antimicrobial treatment of established infections; (2) Metaphylaxis, a term used for group-medication procedures aimed to treat sick animals while medicating others in the group to prevent disease; (3) Prophylaxis, the preventative use of antimicrobials in either individuals or groups to avoid the development of infections; and (4) Growth promotion use, when an antimicrobial agent is used as a feed supplement in food animals to promote growth and enhance feed efficiency. When applying this scheme of antimicrobial use in aquaculture, it should be emphasised that the majority of antibiotic treatments in aquaculture are administered to populations (Smith, 2012). In fish farms and crustacean grow-out facilities, antibiotics are most often administered orally through a medicated feed. Prophylactic treatment is an administration of antibiotics to the population without observed clinical symptoms of the disease. Largely, therapeutic treatment is administered to the population where not all specimens are infected and uninfected specimens are treated prophylactically. However, the terms used for individual treatment cannot be correctly used for treatments of populations. When treatments are given to populations that contain infected individuals, it should be classified as metaphylactic.

Methods for Application of Antimicrobials

There are six different application methods (Austin and Austin, 2007) comprising of baths and dips, topical application, injection, and oral application via medicated feed or through bio-enrichment. Each method has its advantages and disadvantages depending on the targeted use, as well as potential environmental impacts (Rodgers and Furones, 2009):

1. Baths and dips are not as effective as some other treatment methods, particularly for systemic infections due to poor absorption of the antimicrobials used.
2. Topical application is usually necessary only for more valuable individual fish, such as ornamental varieties or broodstock, to treat ulcers or injuries.
3. Injection is more effective than using medicated feed but practically they are usually used only for valuable individuals. Injections are stressful and, before administering in the sick fish, anaesthesia is required. Injection application may be intraperitoneal or intramuscular.
4. Administration through bio-enrichment of live feed organisms, most often *Artemia* or rotifers, is done either directly or indirectly for fish larvae and shrimp postlarvae.

5. Medicated feed is a preferred method for antimicrobial administration, more often commercially prepared as sinking or floating pellets.

Many bacterial diseases of fish or crustaceans can be successfully treated with medicated feed. Medicated feeds are prepared by the incorporation of antimicrobial substances into the feed via powdered premix containing active ingredients and carriers (up to 5 %) in the form of sinking or floating pellets. The feed and antimicrobial substances have to be mixed thoroughly to be evenly distributed in the pellets. Medicated feed should be always administered according to a veterinary prescription. The choice of antimicrobials should be based on good diagnosis and sensitivity testing. The dosage of the antimicrobial compound is determined by the ratio of the active ingredient and biomass of fish being treated, as well as on the daily feeding rate. Medicated feeds need to be stored under the appropriate conditions, otherwise, it will deteriorate, and the antimicrobial compound may lose its efficacy. A vitally important prerequisite for effective treatment is a fast commencement of the medicated feed administration after the first clinical signs of the disease. For instance, if treatment of vibriosis in sea bass starts on the first day after the appearance of symptoms, overall mortalities are about 1.5 % of the fish in the facility compared to mortalities of 16 % if the treatment is delayed by one week (Zarza, 2012).

There are a few antibacterial compounds licensed for use in aquaculture but their approval varies from country to country. To treat aquaculture animals efficiently and avoid the development of AMR, it is very important to avoid sub-dosing, taking into consideration several very important facts such as correctly calculating the active ingredient concentration, appropriately identifying biomass to treat, and the daily feeding ratio. The treatment should last at least 3 days after cessation of the symptoms of the disease but not less than 7 days. Treated fish should not be harvested for human consumption before expiring the withdrawal period.

When medicated feed is used on a farm it is necessary to follow several rules aiming to foster the efficacy of treatment. The aquaculture animals to be treated should be starved before medicated feed administration. The daily feeding ratio of medicated feed should be reduced to ensure that most of the animals eat it and it should be offered as the first daily meal or adapted to the age and number of daily meals. It is preferred to administer the medicated feed manually or through small air cannons in big cultivation units like off-shore cages with high biomass per unit.

Conclusion

Aquaculture is the fastest growing food-producing

sector globally and there are many health challenges associated with this growth (Brun, 2016). Climate change, movement of aquatic commodities, and industrialisation are facilitating the spread of diseases and making them a primary constraint to the cultivation of many aquatic species. Health management programmes including biosecurity measures, disease notification and reporting, vaccination, and appropriate disease treatment should be implemented. There is good availability of commercial vaccines for bacterial diseases and they have resulted in the reduced use of antimicrobial agents, although there is still a need for improvements in delivery methods and efficacy (Rodgers and Furones, 2009). However, the lack of commercially available vaccines for the global fish culture means that there is a perpetual reliance on antibiotics to treat bacterial infections (Crumlish, 2017). Furthermore, farmed shrimp species cannot be conventionally vaccinated as they lack the appropriate immune system (Rowley and Pope, 2012). This leads to the demand for antimicrobial use. To minimise the risk of AMR development, it is necessary to improve knowledge on how and when to use antimicrobials, to enforce better regulation and policy, and support capacity building in all aspects of the aquaculture production chain.

The role of rapid and accurate diagnostics in AMR prevention should be emphasised as it enables the prudent use of antimicrobials to better treat infection, slowing the rise of drug resistance by reducing the unnecessary use of the particular antibiotic. Ultimately, implementation of appropriate diagnostics is changing our approach to treat bacterial infections through targeted and precise therapy.

It is imperative to engage efforts in avoiding all possible mistakes in the use of antimicrobials, namely to start the treatment too late, administer inadequate dosage or select improper medicine, implement too short of a duration of treatment, use antimicrobials prophylactically, use of antibiotics for viral infections, or repeatedly use the same medicine.

Ultimately, we should be aware that continuous monitoring and early diagnosis is a key to successful treatment and that prevention is always better than cure.

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Understanding Antibiotic Treatment Failures in Salmon Aquaculture

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Abstract

Antibiotic treatment failure can occur due to several reasons. In this paper, we summarise our research in Chile and review relevant literature to identify the issues that result in antibiotic treatment failure. The four basic issues we have found for explaining treatment failure include misdiagnoses, resistance, subtherapeutic antibiotic tissue concentrations in target organs, and insufficient treatment time for the elimination of the pathogen at the individual and population levels. Our hypotheses are based on salmonid aquaculture systems but likely apply to other aquaculture industries that use in-feed antibiotic treatments for bacterial infections. It is important to better understand the specific causes of treatment failure as they result in repeated treatments and increased pathogen exposure to subtherapeutic antibiotic levels. Both of these phenomena could increase the risk of antibiotic resistance over time.

Keywords: biosecurity, AMR, *Piscirickettsia salmonis*, Chile

Introduction

It is anticipated that by 2050, the world's population will be over 9.8 billion (United Nations, 2017) and the demand for food will increase accordingly. As aquaculture industries grow to meet global market demand, farms are becoming larger and more densely clustered which increases the potential for transmission of host-dependent pathogens, such as bacteria, and makes it more difficult to control the spread of pathogens within and between facilities. It is, therefore, critical to prevent infections and control outbreaks early in the disease process.

To prevent bacterial diseases on fish farms, producers can increase their biosecurity to minimise the likelihood of pathogen introduction and improve the fish's resistance to infection to increase the likelihood that fish do not succumb to disease if the biosecurity fails. Because failure to prevent pathogenic bacteria from infecting fish on a farm can result in heavy losses, farmers often use antibiotics to prevent losses and curtail the spread of infection within a farm. As

farms increase in size, the quantity of antibiotics required to control bacterial disease outbreaks also increases, which raises the risk of developing antimicrobial resistance (AMR). Judicious use of antibiotics is of critical importance, and ensuring they are administered in a way that effectively treats the entire population will reduce the risk of developing antibiotic resistance and optimise treatment efficacy. Identifying and addressing issues associated with treatment failure is essential for improved antibiotic use and for the development of good practice guidelines. The objective of this paper is to identify issues that may account for antibiotic treatment failure, describe mitigation strategies to resolve failures, and improve the efficacy of antibiotic treatments in salmon aquaculture based on recent research conducted in Chile.

Antibiotic Use in Salmonid Aquaculture

The amount of antibiotics used annually in aquaculture industries varies significantly, depending

on the region. Norway, for example, uses orders of magnitude less than other countries (NORM/NORM-VET, 2016). Antibiotic use per kilogram of fish produced in this country has declined dramatically since 1992 (NORM/NORM-VET, 2016). In 2016, the Norwegian industry reported antibiotics usage at approximately 0.13 g.tonne⁻¹ of fish harvested (NORM/NORM-VET, 2016). The predominant antibiotics used in Norway are florfenicol and oxolinic acid (NORM/NORM-VET, 2016). Chile, on the other hand, uses mostly oxytetracycline and florfenicol, but reports between 300 and 650 g.tonne⁻¹ of fish harvested, depending on the year (Lozano et al., 2018). Scotland is reporting a decline in the usage of antibiotics in salmon, but the industry is still using between 3- and 100-fold more than Norway, depending on the year (Burrige et al., 2008). The USA and Canada do not report antibiotic use in aquaculture. A publication in 2008 suggested the Canadian industry used antibiotics at approximately 115 g.tonne⁻¹ of salmon harvested (Burrige et al., 2008, 2010). In the United States, a modelling study conducted by Benbrook (2002), for the Northwest Science and Environment Policy Center, estimated the use of antibiotics in the salmonid industry comparable to the usage in Chile, although this was not confirmed with empirical data. With exceptions of the Norwegian and Scottish industries, the use of antibiotics per kilogram of salmon, across the industry globally, is on par, or in some cases higher, than other food production industries (Van Boeckel et al., 2015). Given the market is moving towards antibiotic-free products, it is essential to reduce the use of antibiotics without the negative impacts on animal welfare. The judicious use of antibiotics is also important for slowing down the development of AMR (Anderson and Hughes, 2014).

Scenario for This Assessment

Currently, the Chilean salmon aquaculture industry is one of the most developed in terms of tracking antibiotic use and treatment efficacy. All antibiotic treatments in the Chilean aquaculture industry are conducted under the supervision of veterinarians and must have laboratory bacterial confirmation before treatment. These are federal regulatory requirements and the data on antibiotic usage is maintained by the government.

The dominant reason for antibiotic use in salmon in Chile is *Piscirickettsia salmonis* infections during the saltwater grow-out phase of the production cycle (Rozas and Enríquez, 2014). Despite veterinary oversight, treatments are often not effective at reducing mortality associated with this intracellular bacterial pathogen. The poor responses to treatments have resulted in farmers treating crops of fish multiple times with antibiotics. Although antibiotic resistance is one of the possible reasons for treatment failure, a recent study suggests that most

isolates of *P. salmonis* are susceptible to the two most common antibiotics used in this industry: florfenicol and oxytetracycline (Henriquez et al., 2016).

Investigating Antibiotic Treatment Failure

In the salmon industry, similar to other aquaculture industries, almost all antibiotic treatments are administered as in-feed metaphylactic treatments. That is, antibiotics are administered at a population level once a bacterial disease is identified in a cage of fish or on a farm. Antibiotics are given to the entire population, which may include infected and not-yet-infected animals. Metaphylactic treatments are the preferred treatment method in aquaculture for several reasons. First, it is not possible to treat only animals that are infected in a population because all the animals are housed together and are not handled individually. Second, the uninfected animals share the same environment as the infected animals, so in cases of pathogens that are transmitted via the water, it is very likely that at least some subclinical fish are already infected by the time the treatment is administered, and that uninfected animals will become infected if they are not treated. Third, having multiple unsynchronised treatments on a farm, where pathogens are shared between cages, maintains pathogens within the area and may increase pathogen exposure to subtherapeutic levels of antibiotics when the fish have finished treatment and are metabolising the drugs. Last, the automated feeder systems used in large saltwater salmon farms do not easily permit the customisation of feed regimes to individual cages. Therefore, although metaphylactic treatments increase the overall use of antibiotics, they may be the only practical way to effectively target all sources of infection in aquatic animal populations.

The reason metaphylactic treatments fail on salmon farms is likely multi-factorial. We have identified four primary conditions when this could happen for *P. salmonis* treatments, but the rationale may apply to many other bacterial treatment scenarios as well (Fig. 1). First, antibiotic treatments will not work if there is a misdiagnosis and the fish are not infected with a bacterial pathogen. Antibiotics will also not perform well if the fish have a concurrent non-bacterial infection. This may result in the successful treatment of the bacterial issue, but the fish will continue to experience mortality due to the untreated co-infection. There are anecdotal reports of this in the salmon industry when fish are infected with both infectious pancreatic necrosis virus and *P. salmonis*. In these cases, antibiotic treatment failure is likely due to the viral co-infection.

It is also possible that the bacteria have a natural or acquired resistance to the antibiotic. AMR is on the rise globally in all hosts, including humans (Center for Disease Dynamics, Economics & Policy, 2015). In the

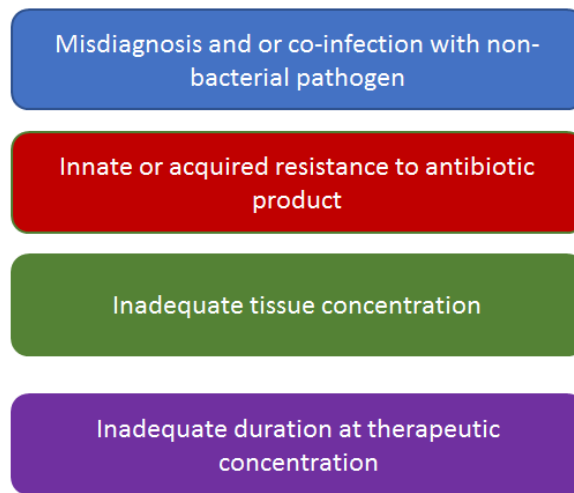


Fig. 1. Conditions for antibiotic treatment failure.

case of *P. salmonis*, AMR has been reported, but it tends to be the exception (Henriquez et al., 2016). Bacteria may also appear tolerant of an antibiotic if they are in a slow-growing or stationary growth phase (Pletnev et al., 2015). Products such as florfenicol and oxytetracycline inhibit peptidyltransferase reaction and protein synthesis respectively (Sekkin and Kum, 2011), so they require the bacteria to be replicating in order to be effective.

The other explanations for treatment failure are associated with sub-therapeutic tissue concentrations and insufficient contact time. The level of antibiotics in treated animals may not be adequate to be therapeutic, and/or the duration of the treatment may not be sufficient to eliminate the bacteria.

Recent studies have confirmed that the level of antibiotics in subclinical fish in treated pens is not always above the minimum inhibitory concentration (MIC) for *P. salmonis*, even on the last day of treatment (Price et al., 2018). Several reasons could account for low levels of antibiotics in tissues (Fig. 2). First, there could be insufficient levels of medication in the feed which could happen due to a feed mixing issue or a biomass miscalculation. The latter could happen because of the variation in fish weights. Some fish may also not be eating enough to receive the appropriate dose. Using in-feed medication to treat a population assumes that all animals will consume feed at a specific proportion of their body weight. Otherwise, it could result in an inadequate drug consumption and subtherapeutic tissue concentrations. This issue would be more problematic for drugs that have a short half-life, such as florfenicol (Martinsen et al., 1993), because they do not accumulate in tissues throughout the treatment. In the Price et al. (2018) study, the proportion of fish with levels of an antibiotic below the MIC was lower for florfenicol than for oxytetracycline.

Fish may also not consume sufficient feed to achieve a therapeutic dose because they are sick and reduce their feed consumption. If it takes a long time to diagnose and treat fish, then a higher proportion of the population will be off fed as the disease progresses. Although farmers in the salmon industry in Chile examine all the fish that die daily, it can take up to two weeks before the farmer initiates an antibiotic treatment on a farm after the initial suspicion of disease. This is due to the delay in obtaining a diagnosis from a laboratory and the time required to manufacture and deliver medicated feed to the farm, some of which are quite remote.

Fish may also not have adequate antibiotic tissue concentrations after treatment because they don't have access to sufficient feed, which can arise if the feeding strategy favours dominant fish. A wide variation in the size of fish from the same year class is one indication there may be hierarchical behavioural issues in a population. Addressing this behavioural issue when it first starts, before the occurrence of health issues, will reduce the negative impact of antibiotic consumption within the population.

The concentration of antibiotics may be sufficient in some tissues but inadequate in others, which could lead to poor treatment success. For example, in the case of florfenicol, the brain and skin often have lower concentrations than the visceral organs (Armstrong et al., 2005). This means that if these are the target organs for a pathogen, which is the case in chronic cases of piscirickettsiosis, then inadequate levels of antibiotics may occur even with a dose that provides therapeutic levels in visceral organs and the bacteria may not be effectively treated. In the case of *P. salmonis*, bacteria also hide within the tissue in granuloma-like lesions, which can further reduce exposure to therapeutic levels of antibiotics.

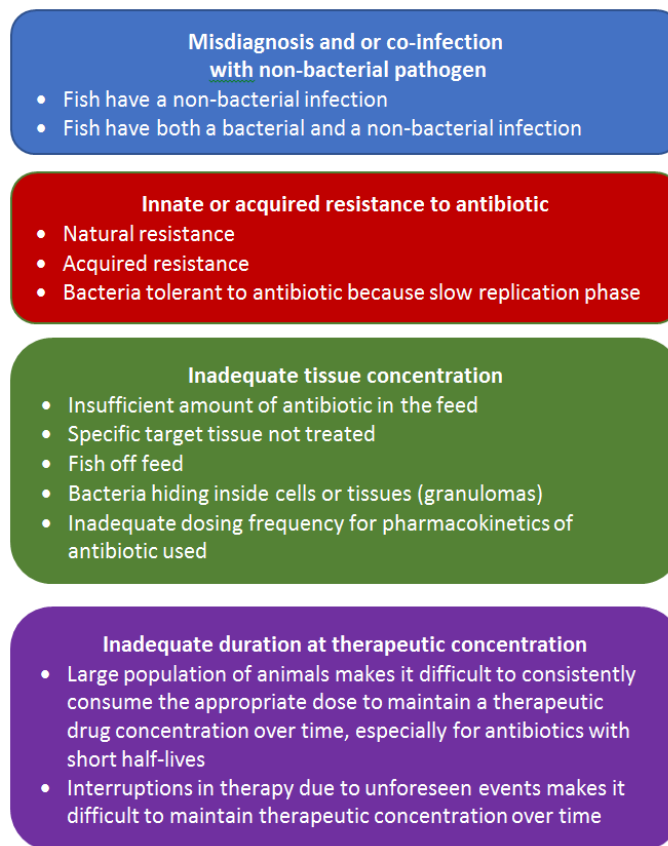


Fig. 2. Specific scenarios that could result in conditions leading to treatment failure.

Finally, even if a fish acquires adequate tissue concentrations of antibiotics, some products, such as oxytetracycline and florfenicol, are time-dependent antibiotics (Sekkin and Kum, 2011) and require drug concentrations to be maintained above the MIC of the bacteria to maximise efficacy. When treating individual animals, it is possible to achieve this contact time by ensuring the dose is taken at specific intervals based on the pharmacokinetics of the drug. For drugs with a short half-life that require frequent dosing, however, it may be difficult to ensure that all fish in a large population feed at precisely the correct interval to maintain tissue concentrations at or above the therapeutic dose. Additionally, the water temperature can further complicate the issue by affecting the pharmacokinetic properties of the antibiotic. No advice is provided on antibiotic labels to adjust for the effect of the water temperature on the tissue concentration and required contact time of products. Not considering this information may be another reason why some treatments are terminated before the entire population has been treated adequately.

On open net-pen farms, there are also instances when treatments are interrupted due to unforeseen reasons resulting in a drop in antibiotic tissue concentration. Reasons for treatment interruptions range from algal blooms to sea lice treatments, storms, predator attacks, and other events that can occur frequently. All these issues provide sources of

variation for antibiotic treatments that can lead to inadequate therapeutic levels of drugs in all or a portion of a population.

Consequences of not treating the entire population

The first consequence of the inadequate treatment of the population is treatment failure. If infected fish receive inadequate treatment and remain in the population beyond the treatment period, they can serve as a source of re-infection for the other successfully treated fish on the farm. This is less so the case with acute bacterial diseases where fish succumb to infection within a week of exposure to the pathogen, as moribund fish that are not adequately treated would not remain in the population long enough to serve as a significant source of infection for other fish. Ensuring infected fish are either treated appropriately or removed from the population before the completion of the antibiotic treatment will reduce the frequency of treatment failure on farms.

As well as increasing the chances of treatment failure by inadvertently treating fish at subtherapeutic concentrations, producers may also be increasing the risk of AMR (Van Houweling and Gainer, 1978). If there is a large proportion of fish that do not achieve therapeutic levels of antibiotics for a sufficient period during treatment, then the bacteria may be directly exposed to subtherapeutic drug levels. Perhaps more

significantly, if a pathogenic bacteria is maintained within the fish population because of inadequate treatments and infected fish do not succumb to the disease before the end of the treatment, the infected fish can act as a source of bacteria to re-infect fish while they are metabolising the antibiotic at the end of their treatment period. The re-infection of fish during this critical period could be very significant on a salmon farm with a large number of fish. The period when antibiotic drug concentrations are low, but still detectable, would depend on the half-life of the product and could range from a few hours to days or weeks.

Addressing issues with antibiotic treatments in fish populations

The most effective way to address the issues surrounding metaphylactic antibiotic treatments in aquaculture is to reduce their use through good disease prevention strategies. When treatments are unavoidable, it is critical to ensure that the delivery of antibiotics, both dose and dosing interval, is closely monitored, and that the fish in the population achieve therapeutic concentrations at the site of the target organs for the required period to successfully eliminate the pathogen. Starting treatments early in the disease process, while the fish are still on feed, will improve treatment effect. Farmers should consider hierarchical behaviours within cages and should take into account the pharmacokinetics of different products so that all fish receive an adequate dose during feeding. A study presented at an industry meeting in Chile suggested the frequency of meals may play an important role in distributing feed more evenly within salmonid populations (unpublished). Finally, ensuring that there are no sources of bacteria in the population (i.e. infected fish) once the treatment has ended is key to reducing the exposure of pathogenic bacteria to subtherapeutic levels of drugs and re-infection. The latter requires consideration of population-level pharmacokinetics when determining the duration of treatments in large fish populations.

Conclusion

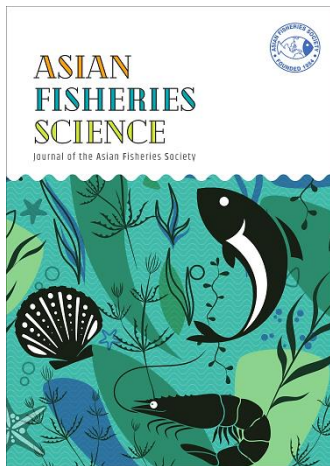
The long-term impacts of metaphylactic antibiotic treatments on bacterial communities, including pathogens, and animal health in aquaculture are unknown. The fact that the salmon industry, one of the most sophisticated and heavily regulated aquaculture industries globally, has issues with antibiotic treatment failures suggests that this problem is likely to exist in other aquaculture industries and that better guidelines for the use of antibiotics are required. Identification of specific reasons for the treatment failures for different scenarios, as well as factors associated with these failures, will enable veterinarians to take corrective measures. It will also help identify circumstances or practices leading to treatments that may increase the

risk of AMR. It is imperative to investigate the practices that maximise the efficacy of antimicrobial treatments while minimising AMR if we are to provide effective guidelines for practitioners and producers to mitigate this growing problem.

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Contact-Zoonotic Bacteria of Warmwater Ornamental and Cultured Fish

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Abstract

In this small review, the most important contact-zoonotic bacteria and the diseases they cause in fish and humans are described. Especially, warmwater ornamental and fish culture professionals, owners, and processors are at risk in acquiring infections by *Vibrio vulnificus*, *Photobacterium damsela* subsp. *damsela*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Mycobacterium marinum*, *Streptococcus iniae*, or *Streptococcus agalactiae*, transmitted from their fish and fish water, in freshwater to marine environments. The chance of acquiring such a zoonosis is low, unless humans are immuno-compromised, and in case their skin is injured. These zoonoses are under reported, as in most countries they are non-notifiable. Strict hygiene for humans having direct contact with these fish in various fish holding and rearing facilities, and regular screening and health checks of imported warmwater ornamental fish at airports are recommended to avoid zoonosis and spread of potentially zoonotic, and often multiresistant bacteria.

Keywords: fish health, zoonosis, disease, antibiotics, antimicrobial resistance

Introduction

At a global scale, numerous warmwater fish species may be cultured as food or as ornamental fish in tropical countries, cultured indoors in warm water as food fish (FAO, 2018), or kept in warmwater aquaria as tropical ornamental fish; there are more than 800 ornamental species, mostly farmed in Asia (OFI, 2018). Warmwater fish may carry or be infected with zoonotic bacteria which may be harmful to humans via contact, i.e. potentially contact zoonotic (Lehane and Rawlin, 2000; Haenen et al., 2013). Moreover, as warmwater ornamental fish are often treated with various antibiotics before transport, and as multiresistant bacteria are proven to be present (Chanda et al., 2011; Weir et al., 2011), this implies a risk of transmission of multiresistant bacteria to humans.

Risks apply to fish handlers in the country of origin as well as in the importing country, in the transfer port in case water of the fish bags is refreshed, and in importing countries where live warmwater fish are

unpacked to enter the chain of ornamental fish trade as pets. Groups at risk are all individuals in direct skin contact with live fish, residues, and fish water. This includes professionals in all segments of the ornamental fish business (aquaculture and fisheries), inspections and, to a lesser extent, persons keeping an aquarium at home and recreational fish anglers. Most countries, including the European Union (EU), don't have legislation on prevention for these potential human health risks in place at international border inspections posts.

The principal pathogens causing contact zoonoses from either handling fish through spine puncture or open wounds are *Vibrio vulnificus*, *Photobacterium damsela* subsp. *damsela*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Mycobacterium marinum*, *Streptococcus iniae*, and *Streptococcus agalactiae* (Dryden et al., 1989; Lawler, 1994; Weinstein et al., 1997; Lehane and Rawlin, 2000; Chotmongkol et al., 2004; Oliver, 2005; Haenen et al., 2013). In exceptional cases, *Vibrio parahaemolyticus* may also cause contact

zoonosis (Ralph and Currie, 2007).

Most contact-zoonotic bacteria grow well at water temperatures above 25 °C, and these bacteria may, therefore, pose a risk in subtropical and tropical regions or indoor warmwater aquaculture systems. These pathogens are all indigenous to the aquatic environment and have also been associated with disease outbreaks in food fish. Although most fish-associated wound infections are self-limiting, more serious cases are mostly associated with an underlying immune deficiency or incompetence in the patient, infection with highly virulent strains, contact with a large inoculum, depth of penetration of the skin, or a combination of these factors. Patients may develop mild to severe infections that, in exceptional cases, may prove lethal, as in the case of *V. vulnificus* (Haenen et al., 2013).

Fish Diseases Caused by Common Zoonotic Bacteria

Vibrio vulnificus may cause haemorrhagic disease in eels. Fish may show redness of the flanks of the body and tail (Austin and Austin, 1999). In advanced cases, pathological changes may be observed in the gills, heart, liver, spleen, and gastrointestinal tract (Miyazaki et al., 1977). Clinical signs in European eels (*Anguilla anguilla* (Linnaeus, 1758)) differed between the non-zoonotic ST 140 strain (Fig. 1), which showed open ulcers, and the zoonotic strain ST 112 (Fig. 2), which showed muscle boils that burst open (Haenen et al., 2014). In diseased tilapia, haemorrhages around the fin bases, exhaustion in swimming behaviour, and stiffness of muscles were observed as a chronic condition and resulted in a gradual death of 10 to 20 % of the pond fish (Sakata and Hattori, 1988). *Vibrio vulnificus* is found in warm coastal and estuarine environments and can be associated with even healthy aquatic animals. Infection by *V. vulnificus* may happen due to contact with seawater or estuarine water.

Photobacterium damsela spp. *damsela* is a normal inhabitant of the marine environment (Hawke, 2014), and causes a chronic bacterial infection characterised by skin ulceration that may progress to haemorrhagic septicaemia. This occurs in a wide variety of marine fish including Japanese amberjack (*Seriola quinqueradiata* Temmuck and Schlegel, 1845), gilthead seabream (*Sparus aurata* Linnaeus, 1758), European seabass (*Dicentrarchus labrax* (Linnaeus, 1758)), Senegalese sole (*Solea senegalensis* Kaup, 1858) common sole (*Solea solea* (Linnaeus, 1758)), striped bass (*Morone saxatilis* (Walbaum, 1792)), hybrid striped bass (*M. saxatilis* x *M. chrysops* (Rafinesque, 1820)), and white perch (*Morone americana* (Gmelin, 1789)) (Romalde, 2002; Rivas et al., 2013). Fish affected by systemic infection show fatty liver with petechiae, abdominal swelling caused by splenomegaly, and ascites (Labella et al., 2011).



Fig. 1. *Vibrio vulnificus* infection in eel by the non-zoonotic ST 140 strain (Haenen et al., 2014).



Fig. 2. *Vibrio vulnificus* infection in eel by the zoonotic ST 112 strain (Haenen et al., 2014).

Aeromonas hydrophila is considered ubiquitous in fresh and brackish water and is a facultative pathogen of various cold- and warm-blooded animals. It may cause motile aeromonad septicaemia, haemorrhagic septicaemia, motile aeromonad infection, red pest, red sore ulcerative disease, and furunculosis, especially in intensively cultured warmwater fish such as Indian major carps, catfish, cyprinids, goldfish, etc. (Austin and Adams, 1996; Camus et al., 1998; Austin and Austin, 2007). A highly virulent pathotype of *A. hydrophila* (vAh) is emerging in the United States of America, causing high losses in the channel catfish industry (Hossain et al., 2014).

Edwardsiella tarda is considered one of the most important bacterial pathogens in aquaculture worldwide (Kodama et al., 1987; Castro et al., 2006; Xu and Zhang, 2014). It has been reported as a causative agent of edwardsiellosis from over 20 fish species across five continents (Plumb, 1999; Mohanty and Sahoo, 2007). It has also been isolated from reptiles, birds, and mammals (Sharma et al., 1974; Tan and Lim, 1977; Leotta et al., 2009; Wang et al., 2012). Its major economic impact is in disease outbreaks in both freshwater and brackish water cultured fish. The disease includes small cutaneous ulcerations on the sides and caudal peduncle that can progress into deep abscesses in the musculature with gas (Meyer and Bullock, 1973).

Streptococcus iniae may cause haemorrhagic disease in various freshwater and marine warmwater fish species, like channel catfish (*Ictalurus punctatus* (Rafinesque, 1818)), Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)), barramundi (*Lates calcarifer* (Bloch, 1790)), European seabass, gilthead seabream, bastard

halibut (*Paralichthys olivaceus* (Temminck and Schlegel, 1846)), white-spotted spinefoot (*Siganus canaliculatus* (Park, 1797)) and red porgy (*Pagrus pagrus* Linnaeus, 1758)), but also in rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) and some ornamental fish (Kitao et al., 1981; Kusuda, 1992; Perera et al., 1994; Eldar et al., 1995). Haemorrhagic meningoencephalitis often accompanied by blindness is typical for this disease. At necropsy, pale and/or haemorrhagic liver and kidney, swollen spleen, and occasionally ascites are seen (Soltani et al., 2005; Salati, 2011).

Streptococcus agalactiae is an important fish pathogen and causes warmwater streptococcosis. It has been isolated from humans, various warm-blooded animals, and various freshwater, brackish, and marine farmed, wild and ornamental fish species (Evans et al., 2008; Amal and Zamri-Saad, 2011; Bowater et al., 2012; Delannoy et al., 2013). The disease affects fish species like Nile tilapia, hybrid striped bass, gilthead seabream, golden shiner (*Notemigonus crysoleucas* (Mitchill, 1814)), hardhead sea catfish (*Aropius felis*), squeteague (*Cynoscion regalis* (Bloch and Schneider, 1801)), and flathead grey mullet (*Mugil cephalus* Linnaeus, 1758), among others (Evans et al., 2002; Garcia, 2007). However, *S. agalactiae* may be a member of the normal bacterial flora for common carp (*Cyprinus carpio* Linnaeus, 1758) (Buller, 2014) and North African catfish (*Clarias gariepinus* (Burchell, 1822)) (O.L.M. Haenen, unpublished).

Mycobacterium marinum may cause chronic mild to severe disease in many freshwater and marine fish species, especially in warmwater (ornamental and edible) fish culture (DeCostere et al., 2004; Gauthier and Rhodes, 2009). Mycobacteriosis should be suspected when a typically chronic and progressive condition resulting in weight loss is seen. The external clinical signs in affected fish include scale loss and dermal ulceration, exophthalmos, abnormal behaviour, pigment changes, spinal defects, emaciation, and ascites (Gauthier and Rhodes, 2009). Some infected fish may develop fin and tail rot. Internal clinical signs of mycobacteriosis include enlargement of kidney, liver, and spleen, nodular skin lesions, abdominal distension, and haemorrhages (Chinabut, 1999). Some infected fish do not show clinical signs. *Mycobacterium fortuitum* and *M. chelonae* are also considered as causing fish tuberculosis in various species (Thoen and Schliesser, 1984; Stoskopf, 1993; Noga, 1995; Sanders and Swaim, 2001). *Mycobacterium fortuitum* is also considered a zoonotic pathogen (Nigrelli and Vogel, 1963).

Diseases Common Zoonotic Bacteria May Cause in Predominantly Immuno-Compromised Humans

Vibrio vulnificus may cause wound infections when a

person with skin injury comes into contact with infected seawater, fish, or shellfish. This human skin infection may develop into *fasciitis necroticans* and, in exceptional cases, even full sepsis and death (Oliver, 2005; Ralph and Currie, 2007; Jones and Oliver, 2009; Austin, 2010). Immuno-compromised patients suffering from liver diseases are at risk. Mortality after wound infection may reach up to 25 %. After sepsis, mortality may reach up to 55 %, mostly within 48 hours of the first appearance of clinical signs (Haenen et al., 2013). Diagnostics, including ribotyping and genotyping of *V. vulnificus* can discriminate potential zoonotic strains from others (Arias et al., 1995, 1997; Rosche et al., 2005; Cohen et al., 2007; Sanjuán et al., 2009; Haenen et al., 2014).

Photobacterium damsela subsp. *damsela* may cause wound infections in humans (Dryden et al., 1989) and in exceptional cases necrotic fasciitis (Barber and Swygert, 2000), especially when humans are severely immunocompromised. An extreme variant of a highly severe necrotising fasciitis where antibiotic administration proved unable to control the progression of fatal infections was reported in some cases (Rivas et al., 2014). Few extremely serious infections with fatal outcome where patients infected by *Ph. damsela* subsp. *damsela* developed multiple organ failure very soon after occurrence of initial symptoms despite therapy and surgical debridement of infected tissues were reported (Yamane et al., 1993)

Aeromonas hydrophila may cause local skin infections and occasionally, diarrheal disease (Lehane and Rawlin, 2000). Several large-scale retrospectives or prospective investigations on bacterial diarrhoea indicate that aeromonads are associated with stools of 0.5 to 16.9 % of ill persons and 0 to 10 % of controls (Janda and Abbot, 2010), but the role of *A. hydrophila* in causing diarrheal diseases is still debated.

Edwardsiella tarda may cause extra-intestinal infections through puncture wounds in adults with underlying disorders such as hepatobiliary disease, diabetes, malignancies, and other immune-compromising conditions (Lehane and Rawlin, 2000). It also causes gastrointestinal infections in children. *Edwardsiella tarda* is an important zoonotic pathogen, and is one of the principal causes of human infections acquired from fish, including from ornamental fish (Vandepitte et al., 1983; Javier, 2012; Haenen et al., 2013). Clinical disease in humans may include necrotic skin lesions, gastroenteritis, and in severe cases, septicaemia leading to osteomyelitis, meningitis, or cholecystitis (Gilman et al., 1971). At present, the zoonotic potential of *E. anguillarum* and *E. piscicida* is unknown.

As a consequence of fish handling, *S. iniae* may cause severe disease, including septicaemia, endocarditis, arthritis, meningitis, fever, and abdominal distension and pneumonia, especially in elderly humans with underlying conditions such as chronic rheumatic

heart disease, osteoarthritis, duodenal ulcer, gallstones, diabetes mellitus, hepatitis, liver cirrhosis, alcoholism, hypertension, and hypothyroidism (Evans et al., 2009a). Soft-tissue infections and acute discitis have been reported by Fuller et al. (2001), and Koh et al. (2004). In humans, infection is clearly opportunistic, usually associated with direct infection of puncture wounds during the preparation of contaminated fish, and generally seen in immunocompromised individuals (Haenen et al., 2013). In 1995, an epidemic occurred in Toronto, Canada in patients handling live or freshly killed tilapia (Weinstein et al., 1997).

Streptococcus agalactiae may cause bacteraemia, septicæmia, meningitis, pneumonia, endophthalmitis (Chotmongkol et al., 2004), endocarditis (Kannan et al., 2001), spondylodiscitis (Sijpkens et al., 1997), and osteomyelitis (Bauer et al., 1997) in immune-compromised patients. In these cases, however, there were no links with fish as the source of infection. Infections in humans related to fish bacterial strains are scarce and were mostly opportunistic, associated with direct infection of puncture wounds during the preparation of contaminated fish, and generally in immunocompromised individuals (Haenen et al., 2013). The infection may be transmitted from a pregnant woman to her newborn child (Glaser et al., 2002). A link was proven between a fish strain and human neonatal meningitis infections in Japan (Evans et al., 2008, 2009b). Additionally, Liu et al. (2013) showed that a sequence type 7 (ST 7) strain from diseased cultured tilapia in the People's Republic of China showed a close genomic relationship with the human strain A909, although no related zoonosis was published. Verner-Jeffreys et al. (2012) isolated *S. agalactiae* from diseased warmwater red garra (*Garra rufa* (Heckel, 1843)) used for pedicure immediately after importation from Indonesia. This means that we need to be aware of the potential of *S. agalactiae* from fish to cause contact-zoonotic infections in humans.

Mycobacterium marinum may cause granulomatous inflammation and nodular or diffuse granulomas of the skin, subcutaneous tissues, and tendon sheaths of fingers and hands, and is referred to as "swimming pool granuloma", "fish tank granuloma", "fish handlers disease", "fish fanciers disease" or "fish TB" of man (Lawler, 1994; Lewis et al., 2003; Petrini, 2006; Haenen et al., 2013). *Mycobacterium marinum* and *M. fortuitum* are a potential occupational hazard for workers in the aquaculture and subtropical aquarium fish industries, as well as for private tropical pet fish owners who have direct contact with their fish when cleaning their aquaria. Piscine mycobacteria may cause morbidity and mortality in fish, but also have documented zoonotic potential for humans, especially for immunocompromised individuals. Occasionally, piscine mycobacteria can also spread to internal organs of the human body and have been isolated from pulmonary lesions, synovial fluid, and

muscles (Blacklock and Dawson, 1979; Chinabut, 1999). Diagnosis in humans is set up by examining a biopsy of the suspected lesion. A history of any aquatic-related activities should be informed to the doctor. A Ziehl Neelsen stain of the biopsy is made and isolation should be done at 30 °C (and not at 37 °C, as is often done at hospitals, because at this temperature no colonies of *M. marinum* will appear) (Haenen et al., 2013). For quick results, polymerase chain reaction (PCR) based techniques should be performed from the biopsy sample. Corticosteroids should never be given to patients infected with mycobacteriosis, as it is contraindicated. Precautionary principles such as education about the health risks, with hygiene and care to prevent contracting the disease must be applied to personnel involved in this industry and private aquarium owners, since mycobacteria may be serious zoonotic organisms. *Mycobacterium fortuitum* and *M. chelonae* may cause local skin infections, but also pulmonary inflammation (Tanaka et al., 1992).

In general, fish-related contact zoonoses are under reported, as in most countries they are non-notifiable (Haenen et al., 2013). In the United States of America, however, for instance, *Mycobacterium marinum* zoonosis is notifiable (Lahey, 2003), apart from *Vibrio* zoonosis (CDC, 2020).

Conclusion

Warmwater live fish culture, tropical fish, and their trade may pose a risk to professionals and fish hobbyists because of direct contact with potential contact-zoonotic bacteria in the infected fish or the transport water. Current border inspections for import control do not consider these risks, nor do import centres, or retail shops selling tropical fish. Awareness-raising about these risks among warmwater fish culturists, slaughter professionals, ornamental fish traders, warmwater fish hobbyists, veterinarians, medical practitioners, and governmental authorities is important.

To prevent contact zoonosis, good hygiene is a must. Hand and skin washing with soap after contact with warmwater fish and their holding water at fish farms, zoos, ornamental import sites, tropical aquaria, or warmwater fish processing facilities are a must. Regular screening for potential zoonotic bacteria in warmwater fish and their water is of utmost importance. Besides, monitoring of the health status of ornamental fish should be implemented on a global level, as its transboundary movement may act as a source of zoonotic infection and antimicrobial resistance for aquatic animals and humans.

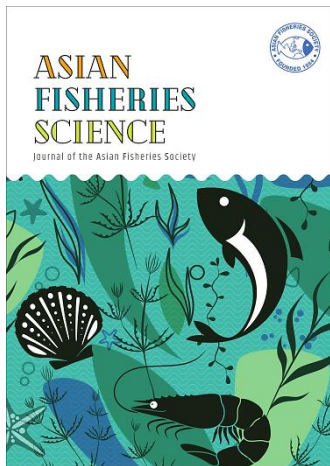
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Potential Transfer of Antimicrobial Resistance and Zoonotic Bacteria Through Global Ornamental Fish Trade

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Abstract

There is an impressive global trade in live tropical freshwater ornamental fish. These consignments may contain potentially harmful bacteria and contaminants of therapeutics, a potential public health risk when professionals have direct contact with fish and transport water. In 2014–2015 we sampled and tested fifty consignments from 13 countries outside Europe at arrival in the Netherlands. Potential zoonotic bacteria were detected in 11 of 50 ornamental fish consignments. *Aeromonas* spp. (n = 59) isolated from fish showed resistance to oxytetracycline (85 % of strains), flumequine (53 %), trimethoprim-sulphamethoxazole (30 %), neomycin (34 %), florfenicol (9 %), and to nitrofurantoin (17 %). Isolates from fish consignments from Singapore and Congo showed multi-resistance against various antibiotics. In total 11 *Escherichia coli* isolates suspected of ESBL (extended-spectrum beta-lactamase)-production were found in 2 of 50 freshwater ornamental fish and 9 of 50 transport water samples, from Singapore (4×), Indonesia (2×), Congo (2×), Thailand (1×), and Hong Kong Special Administrative Region of the People's Republic of China (HKSAR) (1×). OXA-48-like carbapenemase gene variants of limited public health risk were frequently found in *Shewanella* spp. Forty-nine of fifty water samples contained residues of one or more antibiotics, mostly tetracyclines and fluoroquinolones, but also chloramphenicol and nitrofurans, and of malachite green. Our findings are of concern since the current EU border inspections for import control do not consider these human health risks. It is therefore recommended to regularly screen consignments from more countries for the presence of antimicrobial resistant bacteria, residues of antibiotics, and potential zoonotic bacteria.

Keywords: AMR, aquaculture, diseases

Introduction

Over one billion live ornamental fish are transported globally each year, and these numbers are increasing over time (OFI, 2011). The Netherlands is a relatively important transfer and importing country of ornamental fish. Yearly, around 3000 consignments of live ornamental fish are imported into the Netherlands from approximately 40 third countries, i.e. from outside the European Union (EU). Fifty per cent of these consignments originate from South East Asia, and 80 % of these are freshwater cultured fish. In 10 % of all Dutch households, 11 billion ornamental fish are kept in aquaria (Dibevo, 2015).

In the international trade of live ornamental fish,

antibiotics are frequently added to the transport water as a prophylactic measure to prevent the occurrence of disease during transport, in most cases tetracycline (for instance at 5–20 ppm), and less frequently nifurpirinol (Furanace[®]) (for instance at 0.05–0.2 ppm) (Cole et al., 1999). This may pose a risk for humans: Groups at risk are individuals in direct skin contact with live fish, residues, and transport water containing bacteria. It includes professionals in all segments of the ornamental fish business (aquaculture and fisheries) and inspections, and to a lesser extent, persons keeping an aquarium at home.

The risk for humans may be direct, as the antibiotic may enter the body of humans via skin or ingestion when fish trade professionals do not wear protective

clothing. The bacteria of humans may become multiresistant when exposed to these antibiotics. Moreover, the fish trade professionals may be directly exposed to bacteria which are already multiresistant against antibiotics, and this imposes a risk for transfer of resistance to other bacteria, which, in case of causing disease in humans cannot be treated anymore with the antibiotic (WHO, 2020).

Another risk for humans is the fact, that tropical freshwater fish may be carriers or be clinically infected by potential zoonotic bacteria, which may be harmful to humans via direct contact (Lehane and Rawlin, 2000; Haenen et al., 2013). The risks of contracting fish-borne contact zoonoses may be associated with the country of origin, the transfer port, if transport water is refreshed, and the target countries where live tropical fish are unpacked to enter the chain of ornamental fish trade as pets. Current EU legislation does not imply checking for parameters related to these potential human health risks at the EU border inspections posts.

Antimicrobial Resistance (AMR) is a recognised worldwide risk for human health that can spread from animals to humans by direct contact or via the food chain and environment (WHO, 2014). As a consequence of a One Health approach (McEwen and Collignon, 2018), AMR monitoring in animals should include not only food-producing animals, but also pet animals and other animals like ornamental fish. Carbapenems are broad-spectrum beta-lactam antimicrobials, which are used as last-resort options for treatment of community-acquired and healthcare-associated infections caused by multidrug-resistant (MDR) Gram-negative bacteria (Sheu et al., 2019). Since carbapenem resistance results in resistance to nearly all beta-lactam antibiotics, it narrows the therapeutic options dramatically.

Carbapenemases are extended-spectrum beta-lactamases (ESBLs) that hydrolyse carbapenems, last-line therapeutics to treat multidrug-resistant Gram-negative infections (Queenan and Bush, 2007). Carbapenemase-producing microorganisms are increasingly reported in livestock and fish products (Fischer et al., 2013; Stolle et al., 2013; Köck et al., 2018; Nadimpalli et al., 2019) and the environment (Woodford et al., 2014; Tacão et al., 2015). Non-European countries are increasingly reported in recent years as sources of multidrug-resistant bacteria and associated antimicrobial resistance genes (Gerzova et al., 2014; Verner-Jeffreys et al, 2009, WHO, 2019).

To test for parameters related to the public health risks associated with the import of live, freshwater tropical ornamental fish and their transport water from third countries, a study was conducted in the Netherlands by Wageningen Bioveterinary Research (WBVR), in close cooperation with The Netherlands Food and Consumer Product Safety Authority (NVWA) and the Wageningen Food Safety Research (WFSR).

The project was entitled "Investigation into Extended-Spectrum Beta-Lactamase (ESBL)-, Carbapenemase Producing (CP)- bacteria and potential zoonotic bacteria in ornamental fish imported into the Netherlands, and residues of antibiotics and anti-parasitics in the transport water". In this study, the NVWA selected and sampled consignments of live, freshwater tropical ornamental fish imported into the Netherlands from November 2014 to February 2015 from so-called third countries (outside the EU). The purpose was to investigate the presence of potentially zoonotic and ESBL-producing *Escherichia coli* and carbapenemase genes in imported freshwater tropical ornamental fish and transport water, and residues of antibiotics and leuco malachite green in the transport water, in consignments imported from outside the EU.

Materials and Methods

Consignments and sampling

The NVWA selected 50 consignments of various imported tropical freshwater ornamental fish species from countries outside Europe, mainly from Asia and South America, subsampled (2 live fish randomly per consignment, in their original transport water and bag), and sent the subsamples directly after arrival at Schiphol Airport, Amsterdam, to WBVR for analysis. At WBVR, the original transport water and the two fish per batch were sampled. All fish and water were analysed directly as given below, but part of the transport water was frozen at -20 °C upon analysis by WFSR.

Bacteriology

The Fish Disease Laboratory of WBVR tested both ornamental fish per consignment directly for the presence of potential zoonotic bacteria, especially *Edwardsiella tarda*, *Streptococcus iniae*, *Streptococcus agalactiae*, *Vibrio vulnificus*, *Mycobacterium marinum*, and other mycobacteria, and multidrug-resistant *Aeromonas* and *Vibrio* spp. In short, fish were euthanised with an overdose of 2-phenoxyethanol, and necropsied. Specimens for bacteriology were taken from the skin and internal organs of each fish. Skin specimens were inoculated onto sheep blood and thiosulfate-citrate-bile salts-sucrose (TCBS) agars. The liver was inoculated onto sheep blood agar, after which the plates were incubated at 22 °C for three days. From the isolated multi-bacterial cultures, colonies were purely cultured onto sheep blood agar for three days at 22 °C and identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) (Singhal et al., 2015).

In parallel necropsy, a fresh smear of the liver of each fish was made, air dried, fixated, and Ziehl Neelsen (ZN) stained and read for the presence of mycobacteria (Sheehan et al., 2015). The livers were stored at -80 °C and, if ZN were positive, were used for mycobacteria identification by an in-house

combination real-time PCR for three mycobacteria, *Mycobacterium haemophilum*, *Mycobacterium ulcerans* and *M. marinum* (Inoue et al., 2011). The species was further identified by a second specific TaqMan real-time PCR developed in-house by WBVR (R. Ruuls, 2019, pers. comm.).

Antimicrobial resistance testing

After preliminary visual identification from isolated multi-bacterial cultures, colonies suspected of being *Aeromonas* and *Vibrio* spp. were purely cultured and identified with MALDI-TOF (Biotyper, Bruker). These bacterial isolates were stored in Buffered Peptone Water with 30 % glycerol at -80 °C upon collective antibiogram testing. Susceptibility was tested for tetracycline, flumequine, trimethoprim + sulfamethoxazole, neomycin, florfenicol, and nitrofurantoin using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (document VET03-A, CLSI, 2006). *Shewanella* spp. strains were sent to the AMR laboratory for further analysis.

During necropsy, the gut of each fish was directly inoculated on MacConkey-agar to isolate indicator *E. coli*, and the plates were transferred to the antimicrobial resistance (AMR) laboratory of WBVR for inoculation at 37 °C. Also, a piece of the gut was placed into a tryptone soy broth (TSB) as a non-selective enrichment step to culture possible ESBL-producing *E. coli* at 37 °C. Antimicrobial susceptibility testing of commensal indicator *E. coli* and ESBL-suspected *E. coli* was performed with broth microdilution according to ISO standards (ISO document 20776-1) using pre-defined panels of dehydrated antimicrobials (Sensititre plates EUVSEC and EUVSEC2, Thermo Fischer) according to EFSA guidelines (EFSA, 2012).

The AMR laboratory of WBVR tested the gut of the ornamental fish and the original transport water for the presence of ESBL-producing *E. coli* by inoculating 10 µL of the incubated TSB on MacConkey agar plates with 1 mg.L⁻¹ cefotaxime. In addition, purified DNA from the TSB enrichment was tested for the presence of carbapenemase genes using a commercial RT-PCR (CarbaCheck MDR RT, CheckPoints, Wageningen, NL). From a random set of 12 samples tested positive for *bla*_{OXA-48} with PCR, additional selective culturing was performed by inoculating selective media with 10 µL TSB for isolation of carbapenemase-producing Enterobacteriaceae (Chrom ID Carba en ChromID OXA plates) and *Shewanella* (heart infusion agar plates with 5 % sheep blood (HIS) and 0.125 mg.L⁻¹ ertapenem) as described earlier by Ceccarelli et al. (2017). Suspected *Shewanella* isolates were pure cultured and identified with MALDI-TOF (Singhal et al., 2015). Susceptibility of *Shewanella* was determined with broth microdilution using the same antibiotic panels as used for testing *E. coli*. The presence of *bla*_{OXA} genes was confirmed by PCR and Sanger sequencing. Conjugations and

transformation experiments were performed to test for possible transfer of the *bla*_{OXA} genes. As all transmission experiments showed negative results, the suspected chromosomal location of the genes was confirmed by performing pulse-field gel electrophoresis (PFGE) of I-CEU1 digested total DNA followed by Southern blot hybridisation. To exclude potential spread of the *bla*_{OXA} genes, isolates were screened for the presence of transposon IS1999 with PCR. This transposon is strongly linked to the presence of *bla*_{OXA-48} genes on IncL/M plasmids in humans.

Residue testing

WFSR analysed the original transport water samples for residues of antibiotics. A microbial screening method based on the Nouws antibiotic test (Pikkemaat et al., 2008) was used to determine whether residues of tetracyclines, sulfonamides, macrolides, fluoroquinolones, β-lactams, aminoglycosides, florfenicol, and colistin were present. For the determination of chloramphenicol, an immunochemical screening method (EIA) was used. The principle of this method is based on an antigen-antibody reaction followed by a photometric determination of chloramphenicol. For the confirmation of suspect samples for antibiotics and chloramphenicol (results from the screening tests) and for the determination of nitrofurans and leuco malachite green, instrumental methods based on liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) were used (Verdon et al., 2007; Hurtaud-Pessel et al., 2011; Berendsen et al., 2015).

Results

Consignments and sampling

The list of consignments included in the study and countries of origin is given in Table 1. Consignments arrived in boxes at Schiphol Airport (Fig. 1), containing bags with live fish. Thirty six different fish species were sampled at WBVR, predominantly *Corydoras* spp. (2× a batch), *Corydoras paleatus* (Jenyns, 1842) (2×), *Xiphophorus maculatus* (Günther, 1866) blue (2×), *Poecilia reticulata* Peters, 1859 (guppy, 5×), and *Carassius auratus* (Linnaeus, 1758) (8×). Only one of 50 consignments had diseased fish (Fig. 2), two consignments had fish with light mechanical injuries, and one consignment had anorexic fish.

Bacteriology

After 2 to 3 days incubation of the inoculated agar plates at 22 °C, only one plate out of 50 consignments showed no bacterial growth. From the other 49 fish consignments, 321 pure bacterial cultures were retrieved, mostly from the skin; these were sub-cultured again for 3 days and identified by MALDI-TOF. This resulted in 49 unidentifiable bacterial isolates, 55

Table 1. Imported consignments of live, freshwater tropical ornamental fish (consisting of 36 different species) from countries outside Europe. Per consignment, two live fish and their original transport water were tested.

Country of origin of the fish	Number of consignments of fish of this study
Brazil	2
China	1
Colombia	2
Congo	2
Hong Kong (China)	3
Indonesia	8
Israel	6
Peru	1
Singapore	18
Sri Lanka	3
Thailand	4
Grand total	50



Fig. 1. Ornamental fish containers at arrival at Schiphol Airport with prescribed labelling, and filled with bags with live fish.

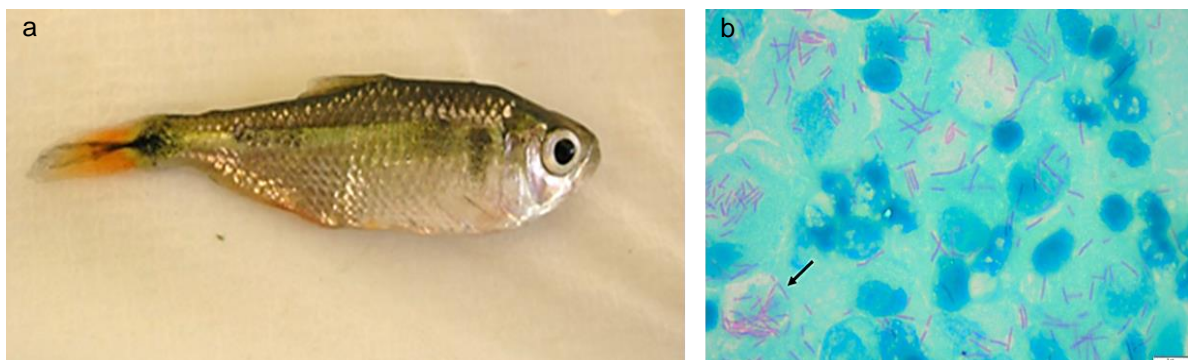


Fig. 2. *Mycobacterium haemophilum* was detected in one Buenos Aires tetra (*Hemigrammus caudovittatus*) from Indonesia: (a) The fish did not show clear clinical signs, except for a congested tail; (b) Ziehl Neelsen stain of a liver smear of the fish exhibiting many bright pink acid fast rods (indicated by the arrow).

bacterial isolates identifiable up to genus level, and 217 bacterial isolates identifiable up to species. Of these, 59 *Aeromonas* spp. and three *Vibrio* spp. were selected for an antibiogram. *Shewanella* spp. were isolated 24x in total, in 12 consignments of 50, mostly from the skin of fish, and were analysed by the AMR laboratory, as given below.

Regarding potential zoonotic bacteria, *Elisabethkingia meningoseptica* was found in nine fish consignments, of which five consignments were from Singapore (2 *C. auratus* (goldfish), 1 *C. paleatus*, 1 *Corydoras aeneus* (Gill, 1858) longfin, and 1 *Hyphessobrycon bentosii* Durbin, 1908, three consignments from Sri Lanka (1 *Poecilia sphenops* Valenciennes, 1846, 2 *Poecilia reticulata* (guppy)), and one batch from Brazil (*Otocinclus* spp.). *Mycobacterium haemophilum* was detected by PCR in one consignment from Indonesia (1 *Hemigrammus caudovittatus* (Eigenmann, 1907)) (Fig. 4). In one consignment from China (1 *C. auratus*) a *Mycobacterium* spp. was detected. By our PCRs this bacterium appeared not further identifiable to species. It was not *M. marinum*, *M. ulcerans*, nor *M. haemophilum*. Overall, regarding potential contact zoonotic bacteria in our monitoring, in 50 consignments of tropical freshwater ornamental fish we detected 9 *E. meningoseptica* positives, and 2 mycobacteria positives. No *M. marinum* (known to cause fish tuberculosis) was detected, and no *V. vulnificus*, *E. tarda*, *S. iniae*, nor *S. agalactiae* were isolated from the imported fish.

Antimicrobial resistance testing

In *Aeromonas* spp., 85 % of the 59 isolates showed resistance against tetracycline, 53 % against flumequine, 30 % against trimethoprim + sulfamethoxazole, 34 % against neomycin, 9 % against florfenicol, and 17 % against nitrofurantoin. One of the *Vibrio* spp. (n = 3) showed resistance to oxytetracycline, and only one also to neomycin. Furthermore, the 3 *Vibrio* spp. were sensitive to the fore-mentioned antibiotics. *Aeromonas* species from fish originating from Singapore and Congo showed the highest levels of resistance.

Commensal indicator *E. coli* were identified in 10 out of 50 consignments by non-selective isolation on MacConkey agar. In three consignments, both fish and transport water samples were positive for *E. coli* indicative of faecal contamination. *E. coli* isolates exhibited relatively high resistance percentages for ampicillin (80 %), chloramphenicol (53 %), quinolones (47 %), sulfonamides (87 %), trimethoprim (93 %) and tetracyclines (100 %). On top of that 53 % of the isolates showed a specific quinolone resistance phenotype indicative for the presence of plasmid-mediated quinolone resistance (PMQR) exhibiting resistance to ciprofloxacin, but not to nalidixic acid. The presence of these PMQR genes was not confirmed by molecular analysis.

ESBL-suspected *E. coli* isolates were detected in 11 of the 50 consignments (18 %), often exhibiting a multiresistant profile (resistant to 3 or more antimicrobial classes). Molecular analysis revealed the presence of different ESBL/AmpC-genes (*bla*_{CMY-2} (n = 4), *bla*_{DHA-2} (n = 1) *bla*_{CTX-M-1} (n = 1), *bla*_{CTX-M-14} (n = 1) and *bla*_{CTX-M-15} (n = 4)). In addition, PMQR genes were identified in four isolates. One isolate harboured *qnrB4* coinciding with *qnrS1*, two isolates solely *qnrS1* and one isolate *aac(6)-1b*.

All samples of fish and transport water were tested negative for the carbapenemase genes *bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, and *bla*_{VIM}. However, in 41 out of 50 consignments (82 %) variants of *bla*_{OXA-48} were identified. Susceptibility testing of 16 *Shewanella* isolates from 12 consignments revealed high levels of resistance to quinolones (69 %), tetracycline (69 %), sulfonamides (56 %) and trimethoprim (56 %) and to a lesser extent to chloramphenicol (31 %). In addition, resistance to colistin was detected in two isolates (13 %) and to azithromycin in only one isolate (6 %). In a subset of seven *Shewanella* isolates originating from seven different consignments *bla*_{OXA-48}-like genes (predominantly *bla*_{OXA-48b}) were confirmed to be chromosomally located.

Residue testing

Forty nine of 50 (98 %) water samples tested at WFSR contained one or more types of antibiotics, as depicted in Table 2. Most frequently found were oxytetracyclines, in concentrations from 7 to 6300 µg.L⁻¹. Furthermore, regarding quinolones, ciprofloxacin and enrofloxacin were found in 32 of 50 transport water samples, in concentrations between 12 to 13000 µg.L⁻¹. Additionally, oxolinic acid was detected only in 4 water samples, all from Singapore, in concentrations of 5.3 to 13 µg.L⁻¹. However, none of the 50 water samples contained detectable flumequine, nor other fluoroquinolones. Results also showed 26 of 50 water samples contained chloramphenicol, 34 nitrofurans, and 7 the non-licensed malachite green. There was no relation between the country of origin or fish species and the detected antibiotic.

Table 2. Number of antibiotics/(leuco) malachite green positive transport water consignments of 50 imported tropical freshwater fish consignments from countries outside Europe imported into the Netherlands with range of concentrations.

Drug	# of positives of 50	Range (µg.L ⁻¹)
(Oxy)tetracycline	27	7 to 6300
Quinolones ¹	32	12 to 13000
Chloramphenicol	26	0.2 to 40.0
Nitrofurans	34	0.1 to 39
(Leuco)malachite green	7	0.01 to 0.17

¹Only ciprofloxacin, enrofloxacin, and oxolinic acid were detected.

Discussion

Bacteriology of imported tropical ornamental fish and risk of zoonosis

The potential zoonotic bacteria found have been reported to cause human disease in scarce cases, particularly in immunocompromised patients: *E. meningoseptica* has been incidentally associated with meningitis in immunocompromised patients, especially in neonatal intensive care, and seldom causes nosocomial pneumonia, endocarditis, post-operative bacteraemia, and other infections (Tuon et al., 2007). *Mycobacterium haemophilum* may cause skin ulcers and arthritis in immunocompromised humans and is seldom associated with lung inflammation, and, in healthy children, cervical and perihilar lymphadenitis (Lindeboom et al., 2011).

Overall, from this study, the risk of infection with zoonotic bacteria is estimated as low if standard hygiene is practised. At a minimum, this includes washing hands, arms, face, and other exposed areas of skin with soap and warm water after contact with ornamental fish or their water.

Antimicrobial resistance

The presence of *E. coli* in 20 % of the consignments is indicative for faecal contamination. However, the origin of these bacteria remains unclear. It could either be caused by human contamination or could belong to the endogenous flora of the fish. Nevertheless, the high level of resistance for the antibiotics tested ranging from 47 % to 100 % is noteworthy. These resistance percentages are substantially higher than those reported in livestock in the Netherlands in the same period (MARAN, 2015). ESBL/AmpC-producing *E. coli* were identified in 22 % of the consignments. The molecular typing of the responsible resistant genes revealed the presence of ESBL/AmpC genes commonly present in animals sources in Europe (*bla_{CTX-M-1}* and *bla_{CMY-2}*). Other genes found are more commonly present in humans in South-East Asia (*bla_{CTX-M-14}*) or considered to be pandemic in humans (*bla_{CTX-M-15}*), but were also identified in animals. In addition, Plasmid Mediated Quinolone Resistance (PMQR) genes, which are incidentally found in *E. coli* in livestock in Europe, were frequently identified. The combined presence of ESBL and PMQR genes are indicative of a high selective environment.

All samples were negative for carbapenemase families *bla_{KPC}*, *bla_{NDM}*, *bla_{IMP}* and *bla_{VIM}*. However, a high percentage (82 %) of the consignments was tested positive for *bla_{OXA-48}*-like genes due to the presence of *Shewanella* spp. *Shewanella* are commonly present in aqueous environments and frequently harbour OXA-48-like genes on their chromosomes. For this reason, these genes are considered to be non-transferable (Ceccarelli et al., 2017). Consequently, *Shewanella*

harbouring OXA-48-like genes are not considered to be a public health risk. Still, a high percentage of the *Shewanella* was multidrug resistant which is another indication of the selective environment.

Residues

Forty nine of 50 water samples contained residues of one or more types of antibiotics, with tetracyclines and fluoroquinolones detected most frequently. These antibiotics are registered in the EU to use for animal husbandry animals. For the tetracyclines, particularly tetracycline and oxytetracycline, concentrations comparable to therapeutic use (Yanong, 2019) (>1000 µg.L⁻¹) and much higher were detected. The most common fluoroquinolones detected were enrofloxacin and its metabolite ciprofloxacin, with concentrations reaching 13000 µg.L⁻¹. Since fluoroquinolones are very potent, these concentrations are within the therapeutic range for fish (Reimlinger et al., 1990).

Remarkably, a large number of samples contained the EU-banned antibiotics chloramphenicol (36 %) and nitrofurans (68 %) (EU, 2010), and (14 %) the non-licensed malachite green (EU, 2004). Although the level of residues of the aquaculture-banned (EU, 2004) antiparasitic and antifungal drug leuco malachite was below the minimum required performance level (MRPL), these drugs are assigned as teratogenic and mutagenic, and therefore, any contact with humans needs to be avoided (Culp et al., 2002). Since 1983, (leuco) malachite green has been banned in food-related products by the United States Department of Agriculture (Andersen et al., 2005).

A positive correlation was found between high concentrations of tetracyclines or fluoroquinolones in certain water samples and resistance to these antibiotics in *Aeromonas* and *Vibrio* spp. isolated from fish which had been transported in those water samples. This suggests that resistant bacteria may have been selected by inappropriate use of antibiotics at ornamental fish farms and centres exporting the tropical ornamental fish. Therefore, antibiotic use should be reduced to a minimum, and if used at ornamental fish farms, export centres, and during transport (in transport water), only in a responsible and prudent way (World Health Organisation for Animal Health (OIE), 2020).

Awareness about these risks for the ornamental fish branch, fish hobbyists, veterinarians, medical practitioners, and governmental authorities is important. Hygienic measures must be in place to avoid contact with zoonotic infections. If antibiotics are needed, there should be responsible and prudent use of antibiotics, and the addition of antibiotics to transport water should be avoided, to reduce the likelihood of AMR selection. Regular screening for potential zoonotic bacteria and antimicrobial resistance of bacteria from imported ornamental fish

is important. At import, bags with live ornamental fish and small volumes of transport water are diluted with freshwater directly after arrival at the import sites of ornamental fish. This will result in fast dilution and disposal of antibiotics via the drain. Therefore, it is recommended to screen more samples in international trade of ornamental fish, and from more countries, for the presence of antibiotic resistant bacteria, residues of antibiotics, and potential zoonotic bacteria. This will be instrumental in developing international guidelines to protect human health related to the import of live tropical ornamental fish, as these may pose a risk to human health during direct contact with fish and transport water. Lastly, good hygiene practices should be in place at transfer centres of ornamental fish to reduce the risk of zoonosis and AMR transfer. This should include good communication of the best hygiene practices via educative flyers and on-site demonstrations.

Conclusion

In summary, this study showed that almost all tropical freshwater fish transport water samples of imported consignments into Schiphol contained low to high concentrations of residues of authorised and non-authorised antibiotics. Malachite green was also detected, a carcinogenic substance to animals and man, which is of concern. Most fish consignments did not show any signs of fish disease, although some potential zoonotic bacteria were detected such as *Elisabethkingia meningoseptica* and *Mycobacterium* spp. Immunocompromised humans may be at risk. In addition, multidrug-resistant bacteria were found in almost all consignments included in this study. This indicates a selective environment caused by the high use of antibiotics at ornamental fish farms sector from outside the EU during farming and transport.

However, when good hygiene is practised in the ornamental fish transport chain, the risk to humans is estimated as low. Fish mostly carried opportunistic *Aeromonas* spp., which were mainly resistant against oxytetracycline. Moreover, fish bacteria from Singapore and Congo showed relatively high levels of multi-resistance to antibiotics. Fish imports may pose public health risks especially because of direct contact with infected fish (zoonotic infections) or because of direct contact with possible AMR bacteria in the transport water. Current EU border inspections for import control do not consider these risks.

Therefore, we recommend to regularly screen consignments from various countries for the presence of antimicrobial resistant bacteria, residues of antibiotics, and potential zoonotic bacteria, and implement standard hygiene throughout the global ornamental fish transport chain.

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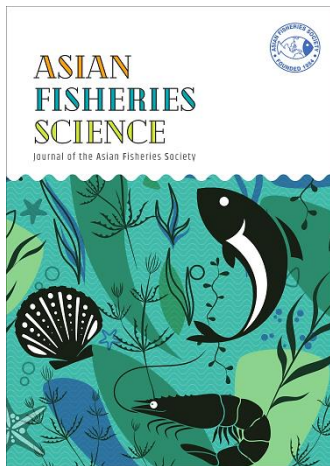
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Practical Management of Bacterial Diseases in Finfish Aquaculture to Minimise Antimicrobial Resistance

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Abstract

The development of drug-resistant strains of bacterial pathogens of fish threatens the efficacy of limited aquaculture approved drugs. Development of antimicrobial resistance (AMR) is a natural process in resident bacteria in the environment. Any time antibiotics are used in an aquaculture facility it provides a competitive advantage for pathogens with AMR. This results in a build-up of drug-resistant fish pathogens. Aquaculturists can minimise the build-up of AMR pathogens by reducing the frequency of antibiotic applications and by making sure the antibiotic is properly applied it is when needed. Management practices that reduce antibiotic use are the most important strategies to avoid the build-up of AMR. Disease prevention is a continuous process in all stages of planning and all phases of production. It involves site and strain selection, managing the environment and handling to minimise stress, controlling the feed, using effective vaccines, and applying biosecurity. An effective antibiotic treatment regimen must provide the therapeutic dose and persistence needed to kill the bacteria. When using medicated feed, the fish must still be eating well and the incorporated antibiotic must be of good quality and at the proper dose determined by the weight of the fish. The antibiotic must be provided for the prescribed time even after fish mortality has stopped. Management to reduce the persistence of AMR pathogens also assures that antibiotics will be effective when needed.

Keywords: antibiotics, disease prevention, disease treatment, disease avoidance

Introduction

Aquaculture is the best option for reducing protein deficiencies and relieving the world's demand for seafood. However, the aquaculture environment is not a natural environment for the fish and crustaceans being reared. This environment makes infectious diseases more common than in the wild. Furthermore, the higher density of a single species of fish or crustacean allows for rapid spread of infectious disease once it starts resulting in devastating losses. Some of the most damaging and insidious diseases in aquaculture are caused by bacterial pathogens. Often, the use of antibiotic medicated feed is needed to control the losses, but excessive use of antibiotics can select for antibiotic-resistant strains of bacteria. When this happens, the medicated feeds are no longer effective. Also, the buildup of bacteria that carry antibiotic resistance genes is of concern to human

medicine because aquatic pathogens could transfer these genes to human pathogens. There are management protocols to prevent diseases thus reducing the need for using antibiotics. This helps assure that the antibiotics will be effective when they are truly needed.

With a focus on disease prevention, this paper addresses management methods that may result in reducing antibiotic use. Also, because antibiotics are critical tools for reducing losses when needed, this paper discusses the proper and safe use of antibiotics.

Disease Prevention Strategies to Reduce the Need for Antibiotics

It is widely understood that antibiotics should not be used to treat non-infectious diseases and diseases

caused by viruses, fungi, or parasites. However, animals are more susceptible to bacterial diseases when another pathogen or predisposing factor weakens their defenses. Often, the predisposing factor is subclinical, not causing disease on its own. For example, it is often seen that moderate levels of external parasites on fish facilitate columnaris disease or focal *Aeromonas* infections. The breaks in the mucus layer caused by the external parasites or by the fish rubbing their skin on submerged structures allow these common opportunistic bacteria to gain a foothold and cause disease. Viral infections also weaken the immune system. Often, virus infected fish will display a variety of bacterial diseases that are secondary pathogens. If a thorough diagnostic evaluation is not done, the producer will focus on treating the bacteria and not recognise the underlying viral infection. Prevention of viral diseases through biosecurity and vaccine use reduces the need for antibiotics. General disease prevention should be part of any comprehensive aquatic animal health plan.

In large operations, developing this plan requires a coordinated team effort. The team must include aquaculturists and aquatic animal health practitioners. Personnel in the aquaculture facility must be trained and continuously encouraged to understand the importance of following the plan and improving the biosecurity of the facility. The components of the plan should include biosecurity, disease recognition, and disease response.

In small operations, a similar plan is needed as well, but this plan may be carried out upon the advice of experts outside of the operation. This may include extension personnel, industry representatives (feed, agriculture suppliers, and/or processing plants), and university experts. With online and email resources, experts outside of the area can be accessed for help. It is important to have this team identified in advance so that their expertise, helpfulness, and availability can be confirmed. Then, when a disease outbreak does occur, the response and management steps can be implemented quickly enough to resolve the problems before it becomes unmanageable.

Disease Prevention by Managing the Environment

Environmental factors that contribute to disease are often recognised as stressors (factors that cause a stress response) in the aquatic animals. Stress is a physiological response of the aquatic animal to a perceived unhealthy environment and this response causes the animal to be predisposed to infectious disease. Also, unhealthy environmental factors may reduce the fish's ability to combat infections without directly causing a stress response. The predisposing factors can work together to greatly suppress the fish's defenses. For example, the combination of marginal water chemistry and marginal water temperature can greatly increase the susceptibility of

the fish to infectious diseases. When evaluating potential predisposing environmental factors, the aquaculturist should aim for optimal conditions, not just conditions that do not directly harm the fish. Feeding activity is often a good indication of optimal conditions. When conditions are less than optimal, feeding activity is usually impacted.

Optimising the environment should be done in every phase of an operation and should be a major focus during the planning stages. The temperature range and salinity in the region should be the optimum for the selected species and strains of fish. The site should be free of pollution, distant from agrichemical use and run-off, and located in an area that is not heavily populated by predatory birds and located to avoid natural roosting sites. The site for ponds should provide the optimal alkalinity and hardness for the species of animals cultured. Also, if the ponds are constructed on agricultural lands where organochlorine pesticides, such as dichlorodiphenyltrichloroethane, hexachlorocyclohexane, aldrin, and dieldrin, have been used, the soil must be tested because these compounds can bioaccumulate. These pesticides can cause chronic health effects in animals and humans. Also, transplacental or transmammary transfer can possibly cause disease and negative developmental effects in children that were not directly exposed. Sites for cage culture should avoid areas that have industry discharges, fish processing plants, sewage outfall, and other substantial human activities. The location of shops and traffic areas should be considered to reduce the impact of human activity. Also, site selection in large water bodies should avoid areas that toxic algal blooms are known to occur. The facility design can also be important. Pond shape and size can influence temperature fluctuations, light exposure, and wind exposure.

The water chemistry must be managed to ensure an optimised culture environment. The soil and water source will usually dictate the alkalinity, hardness, and salinity. These parameters are very stable and can be monitored yearly. Alkalinity is the buffering capacity of water. High alkalinity (<50 ppm CaCO₃) in freshwater ponds helps maintain a stable pH and provides a more stable microbial community. Hardness indicates the amount of calcium and magnesium present in the pond. These ions are important especially during periods when fish are not actively eating so there is no external nutrient source. Both hardness and alkalinity are usually provided by the limestone content of the soil, but it can be supplemented by the addition of agricultural limestone if natural levels are low. Salinity is an indication of the total ion content of the water. Most freshwater fish can tolerate very low salinity but will thrive in elevated salinities (1 ppt). Likewise, most marine fish can tolerate salinities as low as 10 ppt. Production is influenced by the tolerance of the fish and the tolerance of the pathogens. In some freshwater fish production

systems, maintaining elevated chloride levels is done by adding salt (NaCl) because this protects against nitrite toxicity.

The more transient critical components of water chemistry are dissolved oxygen, pH, ammonia, and nitrite. These parameters should be routinely monitored in systems that have high stocking densities. Dissolved oxygen and pH can fluctuate rapidly over the day in ponds. Dissolved oxygen tolerance of aquatic animals can vary considerably based on underlying health parameters that influence gills, blood transport, and tissue oxygen demand through physical activity or digestion. It is important to maintain oxygen levels well above the level that causes stress in the fish. If this is not possible it is important to reduce physical demands on the fish and withhold feed during periods of low oxygen. Although pH in aquaculture systems is rarely a stress factor, it is a critical component in determining the toxicity of ammonia and several other less common stressors such as hydrogen sulphide. The ammonia levels change slowly over days, but pH can vary substantially from early morning to afternoon due to photosynthesis. In a pond with an algal bloom, the pH will be highest in the afternoon and high pH makes ammonia more toxic.

Ammonia and nitrite levels are related to feeding activity and the microbial community in the ponds. These parameters should be monitored once or twice a week in systems with high feeding rates. When ammonia levels are on the rise, feeding levels should be reduced. In nitrite-sensitive species, such as members of the catfish family and salmonids, nitrite toxicity and stress can be prevented by adding salt to maintain at least a 10:1 chloride to nitrite ratio. Proactive water chemistry management and routine monitoring are a critical part of an aquatic animal health management plan (Svobodova et al., 1993).

Disease Prevention by Managing the Host

Managing the host physiology is critical for minimising the impact of diseases. This involves careful choice of the genetics of the aquatic animals and optimising handling, feeding and immune status of the fish.

Like managing the environment, host-based disease management starts in the planning stages. The aquaculture operation should consider disease susceptibility when selecting strains and species to culture. Often, one strain or species, or a hybrid, is much more resistant to an important disease that is endemic in the region. Using this strain may not only reduce losses to the specific disease but may also reduce secondary infections and increase growth because subclinical infections of the causative agent are also reduced. As a caution, many strains of aquatic animals are bred for growth, appearance, or carcass yield (the percent of the body weight that is

meat). If disease resistance is not part of the selection process, these varieties may be more susceptible to one or more infectious diseases. Furthermore, some select strains are more resistant to one pathogen, but becomes more susceptible to another. As a rule, it is important to use fish that are not highly inbred. These should be from large breeding populations where parent-offspring or sibling-sibling breeding is avoided, and no known genetic bottlenecks have occurred (Tave, 1998).

Feed management is another important factor in disease prevention. The fish must be provided an adequate amount of good quality feed to allow a strong immune system. Generally, commercial feeds from a reputable source are properly formulated for the target fish species. These feeds, generally, are supplemented with essential minerals, fatty acids, and vitamins to produce healthy fish (Tacon, 1987). However, these feeds must be fresh and stored in cool and dry places. Vitamins C and E and essential fatty acids are quickly lost due to oxidation. Furthermore, rancid fats and toxins produced by mould can also negatively impact the health of the fish. Spoiled or expired feed should be discarded.

Feed pellet size and structure can also be important. The size of the pellets should be small enough for all fish in the population to consume. If dry pellets that are too large are being used, the pellets will cause small abrasions to the mouth and may predispose the fish to bacterial infections. Furthermore, large pellets provide the bigger fish in the population a competitive advantage and this will result in a wide size distribution in the population.

Feeding activity and management have important impacts on fish health and disease management. They not only affect the nutritional status of the fish, but they also impact the physiological needs during digestion, provides excess nitrogen to the water affecting water quality, and affects the shedding and uptake of pathogens. Each fish must receive enough food for optimal immune system functioning. Feed management should be optimised so there is minimal size variation and that there are no malnourished fish in the population. Malnourished fish, often called runts or pinheads, have weak immunity, and pave the way for a pathogen to get a foothold and start a disease outbreak. Feeding should be done in a dispersed manner to near satiation so that all fish have an opportunity to feed. If there is a need to restrict feeding because of concerns about water quality or to restrict growth, the feeding frequency can be reduced, but continue to feed near satiation whenever they are fed.

The timing of feeding can affect the sensitivity of the fish to marginal oxygen levels. The fish need higher oxygen levels to digest the feed, therefore, aquaculturists often prefer to feed in the morning so the feed is digested during the day when oxygen

levels are high. Also, the frequency of feeding can affect the transmission of certain pathogens. If fish are fed before their gut is cleared from the last feeding, they will defecate while feeding this time. This situation promotes the spread of certain pathogens by faecal-oral transmission. If one of these diseases is problematic in an aquaculture facility, the use of alternating day feeding may reduce outbreaks during periods when the disease is most prevalent (Wise and Johnson, 1998).

Another critical factor for optimising host defenses is careful handling of the fish. Whenever possible, handling, moving, and stocking fish should be done during periods when disease problems are less severe. Any handling during an outbreak will likely worsen losses. Any physical handling of the fish causes breaks in the mucus layer which is the main barrier to pathogens. Bringing them in close contact with each other, especially during netting, facilitates pathogen spread and induces the stress response resulting in suppressed immunity.

To minimise skin damage, fish must be transferred in a cushion of water whenever possible and nets must not be overloaded. To minimise pathogen spread, avoid holding the fish in crowded tanks or net pens for a prolonged time as this allows pathogen amplification. The fish must be held off-feed for at least one day before handling to reduce defecation, pathogen shedding and the associated water fouling. The use of an oxidiser such as potassium permanganate before release from holding tanks may help reduce parasites and external bacteria. To reduce the stress response and the negative effect on physiology, the fish, especially valuable ones such as brooders, must be sedated during handling. Proper vigilance in maintaining high levels of dissolved oxygen throughout the handling process must be made as well as making sure the water temperature does not change over 2 °C during the entire process. The osmolarity of the handling water must be adjusted to minimise osmotic stress. For example, the use of 1 to 5 ppt salt in freshwater fish is usually beneficial. Other measures that can reduce stress is to avoid sudden noises and impacting the sides of holding tanks, causing shock waves that startle the fish. In general, it is important to minimise activity around the tanks and cover them with opaque lids so that the fish are not startled by people moving around. Fish are inherently afraid of overhead activity likely because of fear of predatory birds.

Vaccination is also widely used to make the fish resistant to specific pathogens. The use of vaccines must be evaluated for a particular operation. Vaccines are very effective in reducing losses due to certain bacterial or viral diseases. However, in aquaculture, they are rarely so effective that they eliminate the disease risk. The use of vaccines is generally somewhat expensive and the process of vaccinating generally causes some stress to the fish. Vaccines

should only be used on healthy fish that are immune-competent. They must be of sufficient age and physical status to develop a good immune response and the water temperature must be in the range where the immune system is functioning well. Also, the fish need to be vaccinated at least 2 to 3 weeks before the risk of being exposed to the target pathogen so that the immunity can be established.

Disease Prevention by Pathogen Avoidance

Effective biosecurity is a critical management tool that reduces the outbreak of infectious diseases. Biosecurity is especially recognised for preventing the introduction of new diseases to the aquaculture system. Moreover, it can also prevent the introduction of more virulent or persistent variants or pathogens or antibiotic-resistant strains of bacteria. Effective biosecurity requires a facility-specific plan that minimises the opportunity for the introduction of pathogens. Components of the biosecurity plan should include preventing pathogen introduction and spread within the facility through cultured animals, water, equipment, personnel, feed, and animals especially predators and scavengers.

The most common sources of pathogens on a facility are either introduced pathogens from the newly stocked fish or resident pathogens being passed down from previous stocks. All efforts should be made to receive healthy stocks and, if possible, stock fish that are certified free of specific pathogens of concern for the species under culture. If there are not certified specific pathogen-free stocks available, it is good to receive fish from a known reliable source that has no disease problems and have a health check performed on the fish before the purchase. The health check can screen infectious diseases and parasites, as well as general health conditions, such as condition factor (length-weight factor), swimming activity, presence of deformities, injuries or signs of previous disease events. A more sophisticated health check may also include a serological evaluation by screening the antibodies present in the fish to determine if the fish have been previously exposed to certain pathogens. The availability, pathogen list, and practical level of inspecting fish stocks should be done with the aid of an aquatic animal health professional that is familiar with the regional industry and fish health resources. It is important to be vigilant when starting an operation because once a pathogen is introduced into a system, it is difficult to be eliminated. An additional disease prevention measure is by employing quarantine by holding the arriving fish stocks in an isolated location and observing them for 10 to 14 days before releasing them into the facility. After the quarantine period, it is good to keep the fish from different sources separated in different ponds, tanks, and circulation systems. Any fish brought on to the facility can be given a prophylactic chemical treatment to remove external parasites. Commonly

used treatments include formalin or potassium permanganate (Piper et al. 1982). Although popular in the past, the use of antibiotics for prophylaxis is not recommended and is illegal in many countries because this promotes the build-up of AMR pathogens.

Preventing the transmission of pathogens from one generation to the next is also critical. This involves batch culturing by completely harvesting and disinfecting culture systems between production cycles. If the facility produces fry or larvae, the eggs must be separated from the parents and disinfected before being moved into the hatchery. In salmonids, the use of a buffered solution (pH 6 to 8) containing 100 mg L⁻¹ of iodine for 10 minutes after the eggs have water hardened is recommended (OIE Aquatic Animal Health Code, 2019a). A similar treatment procedure can be used in other production systems. Furthermore, brood fish must be from a source that is free from pathogens that can be vertically transmitted from eggs or sperm to the offspring.

The water source and feed are also important sources of introduced pathogens. Water should ideally come from wells or springs that have no exposure to aquatic animals. This includes species of fish other than that the species being cultured. Often, wild fish can be carriers of infectious diseases. The feed is safe if it is a dry extruded pellet due to the heat involved in producing it. However, moist pellets or unprocessed feeds must be pasteurised to kill any potential pathogens. In rare cases where live feed must be used, the aquaculturist should try to produce the feed organisms under specific pathogen-free conditions. The fish should never be fed unpasteurised fish bycatch or live feed obtained from the wild.

Prevention of spread within a facility includes restricting the movement of animals, water, or equipment between rearing tanks and ponds. It is best to have dedicated equipment for each pond, tank, or raceway or a designated portion of a facility. If this is not possible, the equipment should be cleaned and disinfected before being moved from one water body to the next. Disinfection requires the use of both the proper concentration of disinfectant and exposure time to the disinfectant (OIE Aquatic Animal Health Code, 2019b).

Wild animals moving between water bodies on a facility is especially problematic. Reptiles, amphibians, mammals, and birds are common in outdoor facilities. Birds are especially a problem because they often fly from one facility to another. Predators and scavengers that eat infected fish can efficiently transfer many fish pathogens through their faeces. Also, they are often the source of parasites that have predators as the final life stage (trematodes and nematodes). Active programs for minimising the presence of predators and scavengers on the facility is important.

Another obvious step to minimise the spread within and between culture systems is to quickly remove dead and sick fish from the system. Dead and sick fish are often cannibalised by other fish in the system and this results in the rapid spread of the pathogen. Also, scavengers and predators physically drag dead fish between production systems as well as consume infected fish then defecate the pathogens in other production systems. By removing and sanitarily discarding dead and dying fish, pathogens are directly reduced to ease the burden on the facility. Dead fish can be incinerated, securely buried away from the facility, or composted.

Biosecurity and AMR

The availability and use of antibiotics are critical for minimising the losses of fish when bacterial disease outbreaks occur. However, these tools are only effective if there is a proactive component of the fish health management plan to minimise the build-up and persistence of AMR. Any time antibiotics are used on a facility, it provides a selective advantage to microbes that are resistant to that antibiotic. A proactive plan to minimise AMR not only strives to minimise the need to use antibiotics, but also actively uses biosecurity to minimise the introduction, spread, and persistence of AMR pathogens. Such a program should avoid the introduction of AMR pathogens from other sources by either producing the fish on-site or by receiving fish only from facilities that have active an active program to minimise AMR. The health plan should include active surveillance and routine diagnostic procedures to investigate, even minor mortality events. This should include bacterial culture and antibiotic sensitivity testing. The presence of antibiotic-resistant pathogens should be noted in the farm health records and enhanced biosecurity should be implemented on the population of concern to avoid spreading the resistant strain of pathogen.

When and How to Safely Use Antibiotics

The proper use of antibiotics requires a detailed plan on its use as a component in the facility's aquatic animal health plan. The plan must include how managers and aquaculturists will respond to a mortality event, and how the antibiotic will be obtained and used rapidly enough to be of value. The fish health professional must be identified in advance so an accurate disease diagnosis can be made quickly. The diagnosis would include identifying the primary pathogen as well as environmental factors contributing to the disease. As stated earlier, antibiotics should only be used when treating a bacterial disease and when the use of the antibiotic can be provided in an effective dose. Furthermore, it is important to minimise the use of antibiotics. It is best to not use the antibiotic if losses are not expected to be substantial or if another management procedure can effectively reduce the losses. The

selected antibiotic should be safe and effective for the treatment of the known disease. If the antibiotic is being used in a fish that will be marketed for food, it must be safe and legal to be used in the species of food fish being produced. Antibiotics are primarily provided to fish using a medicated feed. Therefore, to be effective, the fish must be actively feeding so that the feeding rate can be closely estimated. The fish health professional will consider all these parameters before making a recommendation for the use of an antibiotic. In many countries, the use of the antibiotic must be prescribed by a licensed professional. The prescription will indicate the dosage (antibiotic per kg of fish), the formulation (antibiotic per kg of feed), the feeding rate (kg of feed per day), the duration of the treatment, and the withdrawal time, which is the minimum number of days the fish must be off the medicated feed before they can be harvested for market. If a prescription is not required in the country, the same information should be provided by the fish health professional's recommendation. These parameters are critical for getting effective treatment and minimising AMR because the dose and duration must be sufficient to kill the bacteria. If the dose and duration are below the desired level, there will likely be a selection for bacteria that have intermediate sensitivity and these bacteria can then further evolve into resistant strains. The withdrawal period is critical for the safety of consumers. This prevents antibiotic residue in the fish meat, therefore, reducing the possibility of AMR development in the consumer and prevents other potential health risks.

In the diagnostic evaluation, the diagnostician should culture the bacterial pathogen and run an antibiotic sensitivity test to evaluate AMR, although this process would usually take several days. A treatment may already be initiated before the results are obtained. It will indicate the likelihood that the antibiotic will be effective and will provide possible alternatives for treating the current and future outbreaks on the facility.

In some cases, a pre-made medicated feed can be purchased. However, many times the medicated feed must be made by the producer. If the fish are being fed dry pellets, the antibiotic can be coated on the outside of the pellet using a binder. If the fish are fed a moist feed it can be directly mixed into the ingredients. It is important to use protective clothing, latex gloves, and dust masks when working with the antibiotics. Some antibiotics have toxic effects on humans or their unborn children. Also, exposure to antibiotics may cause a build-up of AMR pathogens in the human body system that may make it hard to treat a disease. To coat the feed, the powdered premix is first mixed into a binder (5 % gelatine solution, vegetable oil or fish oil, or a commercially available binder). Then, this mixture is mixed thoroughly with the feed. The antibiotic must be evenly distributed on all the pellets. Some producers use cement mixers when mixing large amounts of feed. If a water-based

binder such as gelatine is used, the coated feed should then be spread out to air dry. The feed can then be used or stored under proper conditions.

The aquaculturist must feed only the medicated feed to the fish for the entire duration of the treatment (do not mix the medicated feed with non-medicated feed). This action must be done for the entire recommended treatment time, even if the fish stopped dying. The aquaculturists must minimise contact with the feed and avoid breathing small feed particles or dust. Feeding must not be rushed to make sure the fish will eat the medicated feed relatively soon after it hits the water because the antibiotic will leach out of the pellet. Also, it must be assured that as many fish will eat the medication as possible and will not leave a lot of uneaten medicated feed in the water.

Conclusion

Preventing disease through management is much more effective than treating diseases after they start. Effective management requires a formal aquatic animal health plan. This plan must identify the availability and roles of the experts, resources and actions to be used during all phases of the aquaculture operation to minimise all diseases and health associated losses, and should include detailed action plans that can quickly be implemented when disease situations arise. The proactive process of managing diseases is important for minimising the need for antibiotics. This reduces costs and minimises the build-up of AMR pathogens and allows the effective use of antibiotics when they are needed. Proactive efforts in minimising introductions of AMR pathogens and preventing the persistence of AMR pathogens on the facility are important components to assure the sustainability of the operation.

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Review of National Residue Control Programme for Aquaculture Drugs in Selected Countries

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Abstract

Residues of drugs in aquaculture-raised products could potentially cause health hazards for consumers. Most seafood importing countries have regulations on maximum residue limits (MRL) for veterinary drugs in aquaculture products. National MRLs are generally based on Codex and where there are no Codex recommendations, countries may develop MRLs based on risk assessments. Most importing countries have regulations that require aquaculture-producing countries to demonstrate compliance by implementing a National Residue Monitoring Programme (NRMP). To understand the regulations and implementation of NRMP in seafood exporting and importing countries, an analysis was made on the regulations in Canada and EU and NRMP implementation in four major exporting countries; China, Viet Nam, Malaysia and Philippines. Data source were from websites of seafood inspection agencies in the countries and reports of inspection from EU Food and Veterinary Office (FVO). All seafood exporting countries have harmonised their regulations with that of EU and data on the implementation of NRMP is available from these countries. The regulatory pressure from the importing countries seems to drive NRMP implementation in the exporting countries.

Keywords: ASEAN, EU, maximum residue limit, regulatory

Introduction

The importance of aquaculture in meeting the growing demand for fish cannot be overemphasised. Presently, nearly half of global fish consumption comes from aquaculture (FAO, 2020). The rapid growth of aquaculture during the last two decades has not been without challenges. Mortality due to disease has been one of the greatest challenges and this has frequently been accompanied by the overuse of chemicals and drugs. The selection and spread of antibiotic resistance due to indiscriminate use of antibiotics in various sectors, including aquaculture, have been drawing the attention of agencies involved in public health as food safety regulators and consumers. The FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance identified the following hazards associated with antimicrobial use in aquaculture (a) antimicrobial residues and (b) antimicrobial resistance (FAO/OIE/WHO, 2006). This paper mainly deals with

antibiotic residues and risk management measures associated with this hazard.

Monitoring food commodities for the presence of chemical contaminants at a certain level is an important risk management measure that has been adopted by many countries for a long time. Modern food safety control programs are based on the principles of risk analysis. The Codex Alimentarius Commission (CAC) has guidelines for performing food safety risk analysis (CAC, 2018a). According to these guidelines, risk analysis has three major components: risk assessment, risk management, and risk communication. At the national level, national authorities are responsible for risk management. Generally, risk assessment requires a team of multidisciplinary scientists and data on the hazard and toxicological information. Risk management starts with risk evaluation, which includes identification of food safety issues and the development of risk profiles.

In the case of microbial hazards, a food safety issue may be brought to the attention of risk managers due to an outbreak of foodborne infection where the most adverse effects are acute and the result of a single exposure event (e.g. a meal of contaminated food). The level of the microorganism may go up or down in the food chain and contamination may even take place at various stages of the food chain. Whereas, chemical hazards such as residues of veterinary drugs, pesticides, and heavy metals, can cause adverse health effects due to the cumulative effect of multiple exposures. They are typically present at the primary production stage and their levels are not altered along the food chain. Therefore, when performing chemical risk evaluation, it is important to have information on the presence of the chemical hazard at the primary production stage.

Control of microbial hazards involves the implementation of measures in the food chain and the responsibility lies with those involved in the handling and processing of food. Conversely, control of chemical hazards involves the identification of fish farms where levels of hazards are above acceptable limits. This generally involves monitoring, testing, and implementing control measures to minimise the public health risk which is generally the responsibility of the national regulatory agencies.

Seafood industries have been using Hazard Analysis Critical Control Point (HACCP)-based food safety management. Certain antibiotics, such as chloramphenicol and nitrofurantoin, have been banned for use in food production animals. Detection of any residue of such banned antibiotics suggests a violation of the regulations. Certain antibiotics like tetracycline may be permitted for use for the treatment of bacterial diseases in aquaculture. The CAC has recommended maximum permissible limits for residues of such as antibiotics (CAC, 2018b). If any fish processing industry is using aquaculture products as raw material, antibiotic residues should be included in the list of possible hazards during the step of hazard identification. In the HACCP process, the critical control point would be at the reception of raw material to ensure that no contaminated fish enters the production chain. Data from farm monitoring would be helpful in sourcing raw material free of unacceptable residues.

Legislative Requirements

At the international level, the responsibility of providing advice on risk management concerning veterinary drug residues lies with the CAC and its subsidiary body, the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). The primary responsibility for risk assessment lies with the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The CCRVDF determines the priorities for consideration of residues of veterinary drugs and JECFA provides independent scientific advice by

evaluating the available data on veterinary drugs prioritised by CCRVDF. The Risk Assessment Policy for setting of MRLs in food established by the CAC, defines the responsibilities of CCRVDF and JECFA and their interactions. For the establishment of the priority list, CCRVDF identifies, with the assistance of member countries, the veterinary drugs that may pose a consumer safety problem and/or have a potentially adverse impact on international trade.

The JECFA uses a risk assessment process to establish an acceptable daily intake (ADI) and maximum residue limits (MRLs). Veterinary drugs that are toxic or have carcinogenic potential are not evaluated by JECFA and therefore no ADI or MRLs are established. Chloramphenicol and nitrofurans, compounds that caused disruptions in the trade of aquaculture products, belong to this category and are banned for use in food-producing animals in most countries. Presently, there are Codex MRLs only for chlortetracycline/oxytetracycline/tetracycline in fish and shrimp and flumequine in trout. The Codex MRLs exist for therapeutic agents used against parasites in salmon and trout aquaculture (e.g. deltamethrin, emamectin)(CAC, 2018b).

However, there are national/regional MRLs for several other antimicrobial agents. In the European Union (EU), the Commission Regulation (EC) No. 37/2010 establishes MRLs for veterinary drugs in foods of animal origin, including aquaculture products. The lack of Codex MRLs for veterinary drugs could be a problem for many developing countries that adopt Codex MRLs as national MRLs. This situation has led FAO/WHO (2004) to recommend that veterinary drugs which have been evaluated by national governments and are legally used in many countries, a comprehensive approach should be adopted to expedite harmonisation. The JECFA evaluation of substances may be constrained by the lack of data from companies that market the drug. FAO/WHO (2004) recommended that with the assistance of JECFA and based on national/regional MRLs, an initial list of temporary/operative MRLs could be adopted by CCRVDF. This list could be made permanent by CAC if the national/regional risk assessments are not questioned or if JECFA could establish the ADI using the data collected by the country/region to propose MRLs. Substances that do not fulfil these requirements could then be moved to the list of compounds not to be used in food animals. The CCRVDF has been working on a list of MRL needs of the member countries (what countries) and developed a database of MRL needs. The CCRVDF in its 23rd Session, held in Houston, Texas in October 2016, concluded that the Global Survey Database on MRL needs to be maintained and updated. The Committee established an Electronic Working Group to identify priority veterinary drugs and information gaps for a successful and comprehensive assessment by JECFA. The 85th meeting of JECFA, in Geneva, Switzerland from 24 October to 2 November 2017, re-

evaluated ampicillin and amoxicillin. Based on this evaluation, Codex has established MRL for amoxicillin and ampicillin in finfish fillet and muscle (CAC, 2018b).

For veterinary drugs without an ADI/MRL, regulatory authorities generally adopt a zero-tolerance approach. In this situation, as the analytical capability improves, the levels that were not detectable by earlier technology become detectable and hence reportable. Therefore, independent of any toxicological risk posed by the food product, the residues would attract regulatory action. The countries taking a zero-tolerance approach argue that the products are not acceptable because they have evidence of the use of a banned drug in animal production and therefore represent a violation of regulations. For example, in the EU, the misuse of banned antimicrobials is monitored using an analytical method that has a prescribed Minimum Required Performance Limit (MRPL). Liquid chromatography and tandem mass spectrometer (LC-MS/MS) are used to detect residues and the MRPL for chloramphenicol is 0.3 ppb and 1.0 ppb for metabolites of nitrofurans (EU regulation No EC 181/2003). A national residue control programme needs to be in place as per Council Directive No 93/26/EC and external countries wanting to export to the EU need to follow a sampling frequency based on the volume of production. The sample should consist of one or more fish depending on the size and the requirement of the analytical method. The minimum number of samples should be one per 100 tonnes of annual production.

In accordance with the EU guidelines, the substances to be monitored are divided into two groups: Group A includes substances having anabolic effects and unauthorised substances such as chloramphenicol and nitrofurans. Group B comprises of antibacterial substances, such as sulphonamides and quinolones, other veterinary drugs like anti-parasitic agents, and other substances and environmental contaminants including dyes, pesticides, and polychlorinated biphenyls (PCBs). Aquaculture products need to be monitored for the following groups of substances:

Group A: Substances having an anabolic effect and un-authorised substances:

- A1: Stilbenes, stilbene derivatives, their salts and esters
- A3: Steroids
- A6: Unauthorised substances. These include pharmacologically active substances for which no maximum limits can be fixed (chloramphenicol, nitrofurans)

Group B: Veterinary drugs and contaminants:

- B1: Antibacterial substances such as sulphonamides
- B2a: Antihelmintics
- B3a: Organochlorine compounds including PCBs
- B3c: Chemical elements
- B3d: Mycotoxins
- B3e: Dyes

One-third of the total samples are tested for Group A substances and two-thirds for Group B substances. The regulation further specifies that for Group A substances, samples should be taken at the farm level, at all stages of production, including fish that are ready to be placed on the market. For Group B substances, sampling should be carried out at the farm level, on fish ready to be placed on the market for consumption, either at the processing plant or at the wholesale level, and on fresh fish, on the condition that in the event a positive sample is detected, the sample can be traced back to the farm.

Table 1 presents information on the veterinary drugs considered by the Canadian Food Inspection Agency (CFIA) for residue monitoring. Drugs are grouped into approved (A) or banned (B). The fish species and the tissue in which the residue is to be monitored are specified. The residue levels at which would action would be taken are also indicated in Table 1.

Table 2 indicates the Canadian guidelines for malachite green in fish. As a minimum performance level of laboratory testing for Malachite Green (MG) or Leucomalachite Green (LMG), the laboratory must have a limit of quantification (LOQ) of at least 0.5 ng.g⁻¹ for MG or LMG. When the level exceeds 0.5 ng.g⁻¹ but is below 1.0 ng.g⁻¹, the importers have the option of presenting evidence that there has been no deliberate use.

Gentian violet (GV) is not permitted in Canada for use during any part of the aquaculture fish production life cycle. Guidelines on regulatory action when the residue of GV or leucogentian violet is detected above 0.5 ng.g⁻¹ is indicated in Table 3.

The Association of Southeast Asian Nations (ASEAN), a regional organisation comprising of ten southeast Asian countries, have agreed on guidelines for the use of chemicals in aquaculture and measures to eliminate the use of harmful chemicals (ASEAN, 2013). Table 4 presents the regulatory status with respect of antibiotics in selected ASEAN countries.

Table 1. Residue monitoring in aquaculture products (Canadian Food Inspection Agency - CFIA).

Class name	Substance name (Marker residue / metabolite)	Use status	Species	Tissue	Action level (ppm)	Action level (ppb)
Amphenicols	Florfenicol	A	Salmonids	Muscle	0.8 ^a	800 ^a
	(Florfenicol amine)					
	Chloramphenicol	B	All	N/A	DTC	DTC
Avermectins	Thaimphenicol	NA	All	N/A	DTC	DTC
	Emamectin benzoate	A	Salmonids	Muscle	0.1*	100*
Benzoylureas	Ivermectin	NA	All	N/A	DTC	DTC
	Teflubenzuron	A	Salmonids	Muscle Skin	0.3 3.2	300 3200
Fluoroquinolones	Ciprofloxacin, Danofloxacin, Enrofloxacin, Sarafloxacin	NA	All	N/A	0.001 ^b	1.0 ^b
Macrolides	Erythromycin	EDR	Fish Crustacean	Muscle	0.03 ^c	30 ^c
Nitrofurans	Furazolidone (AOZ), Furaltadone (AMOZ), Nitrofurantoin (AHD), Nitrofurazone (SEM)	B	All	N/A	DTC	DTC
Nitroimidazoles	HMMNI, IPZ, MNZ, RNZ, DMZ	B	All	N/A	DTC	DTC
Quinolones	Flumequine Oxolonic acid	B	All	N/A	DTC	DTC
Sulphonamides	Ormetoprim	A	Salmonids	Edible tissue	0.1*	100*
	Sulphadiazine					
	Sulphadi-metoxine					
	Trimethoprim	A	Salmonids	Muscle	0.1	100
Sulfonamides	Sulfacetamide	NA	All	N/A	DTC	DTC
	Sulfadoxine					
	Sulfachloro-pyridazine					
	Sulfaquanadine					
	Sulfamerazine					
	Sulfamethazine					
	Sulfamethiazole					
	Sulfamethoxazole					
	Sulfamethoxy-pridazine					
	Sulfamono-methoxine					
	Sulfamoxole					
	Sulfanilamide					
	Sulfapyridine					
	Sulfaquinoxaline					
	Sulfathiazole					
Sulfisoxazole						
Tetracyclines	Oxytetracycline	A	Salmonid Lobsters	Muscle	0.2	200
	Chlortetracycline	NA	All	N/A	DTC	DTC
	Tetracycline					
Steroids	Boldenone	NA	All	N/A	DTC	DTC
	(17 beta-boldenone)					
	Methyl-testosterone					
	(17 alpha-methyl-testosterone)					
	Nandrolone					
	(17 beta-19-nor-testosterone)					
	Epi-boldenone					
(17 alpha boldenone)						
Stilbenes	Epi-nandrolone					
	(17 alpha 19 nor-testosterone)					
	Dienestrol	NA	All	N/A	DTC	DTC
Triphenyl-methane dyes	Diethyl-stilbesterol					
	Hexestrol					
	Gentian violet (Leucogentian violet)	NA	All	N/A	See footnotes	See footnotes
	Malachite green (Leucomalachite green)					

A: Approved, B: Banned, NA: Not accepted to be used, N/A: Not applicable, DTC: Detected above the reporting limit, AHD: 1-Aminohydantoin hydrochloride, AMOZ: 3-amino-5-morphinomethy-oxazolidine-2-one, AOZ: 3-amino-2-oxazolidinone, DMZ: Dimetridazole, HMMNI: 2-Hydroxymethyl-1-methyl-5-nitroimidazole, IPZ: Iprnidazole, MNZ: Metronidazole, RNZ: Ronidazole, SEM: Semicarbazide.

* - Administrative maximum residue limit (AMRL), ^a - Fish will be considered rejected when the sum of florfenicol (parent drug) and florfenicol amine (metabolite) detected in the sample exceeds the florfenicol MRL, ^b - As a minimum performance level of the laboratories testing for fluoroquinolones, the laboratory must have a limit of quantification (LOQ) of at least 1.0 ng.g⁻¹ for fluoroquinolones, ^c - Interim action level set by Health Canada.

Table 2. Interim guidelines for product acceptability criteria for imported and domestic fish products (Health Canada and CFIA).

MG or LMG levels	Product action
$\leq 0.50 \text{ ng.g}^{-1}$ (interim LOQ for MG or LMG)	No regulatory action
$> 1.0 \text{ ng}$ for MG or LMG	Product unacceptable. Importers have the option of gathering information to provide evidence of non-deliberate use. On a case-by-case basis, CFIA will take regulatory action
$> 0.5 \text{ ng.g}^{-1}$ to $< 1.00 \text{ ng.g}^{-1}$ for MG or LMG	Gathering of information required to determine deliberate use. The product is unacceptable unless a review of information shows there has been no deliberate use. Appropriate regulatory action will be taken as required

Table 3. Interim guidelines for the presence of gentian violet (GV) and leucogentian violet (LGV) as therapeutants and as possible contaminants (Health Canada).

GV or LGV levels	Product action
$< 0.5 \text{ ng.g}^{-1}$ for GV and /or LGV (interim LOQ for GV or LGV)	No regulatory action
Sum GV and LGV $> 1.0 \text{ ng.g}^{-1}$	Product unacceptable
GV $< 0.5 \text{ ng.g}^{-1}$ and LGV $\geq 0.5 \text{ ng.g}^{-1}$ and $< 1.0 \text{ ng.g}^{-1}$ OR GV $\geq 0.5 \text{ ng.g}^{-1}$ and $< 1.0 \text{ ng.g}^{-1}$ and LGV $< 0.5 \text{ ng.g}^{-1}$	This result will trigger a follow-up investigation for possible therapeutant use before making a decision
GV $\geq 0.5 \text{ ng.g}^{-1}$ and LGV not detected at reporting level	This result will trigger a follow-up investigation for possible postharvest contamination before making of decision

Table 4. Regulations for antibiotics in selected ASEAN member countries.

Antibiotic/ Chemotherapeutic Agent	Malaysia	Philippines	Viet Nam
Tetracycline	Permitted	Permitted	Not used
Oxytetracycline	Permitted	Permitted	Permitted
Doxycycline	No data	Permitted	Permitted
Chlortetracycline	Permitted	Permitted	Not used
Nitrofurans	Prohibited	Prohibited	Prohibited
Chloramphenicol	Prohibited	Prohibited	Prohibited
Oxolonic acid	Permitted	Permitted	Not used
Erythromycin	Permitted	Permitted	Not used
Sulfonamides	Permitted	Permitted	Permitted
Sulfamerazine	Permitted	Permitted	Permitted
Amoxicillin	Permitted	Permitted	Not used
Enrofloxacin	No data	Permitted	Prohibited
Florfenicol	No data	Permitted	Permitted
Norfloxacin	No data	Permitted	Not used
Rifampicin	No data	Permitted	Not used
Ciprofloxacin	No data	No data	Not used
Sarafloxacin	No data	No data	Not used
Ormethoprim	No data	No data	Permitted
Sulphadimethoxin + Ormethoprim	No data	No data	Permitted
Sulphadimethoxin + Trimethoprim	No data	Permitted	Permitted
Metronidazole/ Dimetronidazole	Prohibited	Prohibited	Prohibited
Acriflavine	Permitted	No data	Not used
Trichlorofon	Permitted	Permitted	Not used
Trifluralin	Not used	Permitted	Prohibited
Cypermethrin	Not used	Permitted	Permitted
Praziquantel	Permitted	Permitted	Permitted
Levamisole	Not used	Not used	Permitted

Implementation of the National Residue Control Plan (NRCP) in Selected Countries

China

Since China is a major exporter of aquaculture products to the EU, the NRCP in China is largely harmonised with that of the EU. The EU Food and Veterinary Office (FVO) has been carrying out audits of the fish inspection system being implemented in China and reports of FVO audits provide information on the implementation of the NRCP (FVO, 2006, 2009, 2013). The Ministry of Agriculture and Rural Affairs (MARA) and the General Administration of Quality Supervision, Inspection, and Quarantine of the People's Republic of China (AQSIQ) are involved in planning, supervision, and follow-up of the annual NRCP. The AQSIQ is responsible for all the exported commodities while the MARA is principally responsible for the control and supervision of the domestic market. The MARA is also involved in sampling and follow-up of non-compliant results on farms that are approved under the Export Oriented System (EOS). The AQSIQ officials collect the majority of the samples in EOS farms. Each year AQSIQ and MARA hold three coordinating meetings for the planning of the prospective NRCP. The information on the NRCP results, experiences obtained during the previous year, and suggestions are sent from the MARA and China Inspection Quarantine (CIQ) provincial authorities to their respective Central Authorities at the beginning of the year. The MARA and AQSIQ each develop a separate NRCP, taking into account the input of their respective local authorities. The combined NRCP for the year is finalised by the end of March and includes the two different plans of the MARA and AQSIQ. China has harmonised its NRCP with EU requirements and sampling is planned based on usage data (Fig.1).

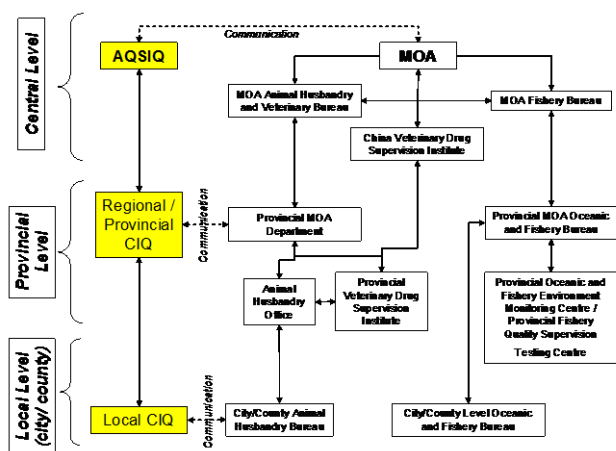


Fig. 1. Organisational structure of general administration of quality supervision, inspection, and quarantine at the Ministry of Agriculture of the People's Republic of China.

Follow-up procedures are issued from AQSIQ to the provincial CIQs via its annual NRCP. These procedures communicate that samples should be analysed and reported within 30 working days by the laboratory. In the event of a non-compliant screening result, the analysis is required to be confirmed within a week. The final confirmed result is transmitted to the sample submitter who will inform the sample taker and the farm/establishment of origin within 48 hours. An investigation should be carried out on the farm with two additional follow-up samples, which should be analysed within 10 days. If this result is non-compliant, the approval for the export of this establishment is revoked and corrective measures must be taken.

General follow-up instructions are also issued from MARA to their provincial authorities via its annual NRCP. The procedure communicates that five additional samples should be taken in case of a non-compliant result and that an investigation must be performed.

Some of the AQSIQ laboratories have been designated National Reference Laboratories (NRLs) for certain substances. NRLs are responsible for one or more substance groups that may carry out confirmatory analysis when the routine laboratory has no method for confirmation and are required to organise proficiency tests and give training and technical advice to the control laboratories within their respective networks. All AQSIQ laboratories are accredited to ISO 17025 by the Chinese National Accreditation body (CNAL). The laboratories in the network are well equipped and their quality assurance systems generally contain the essential elements such as a quality manual, standard operating procedures, equipment calibration records, internal standards, and analytical standard management. CNAL is a member of the International Laboratory Accreditation Cooperation. According to the EU FVO audit report (FVO, 2009), there are a total of 106 laboratories administering the 2009 NRCP. Of these, 71 are in the MoA network (of which are reference laboratories) and 35 are within the AQSIQ network (8 of which are reference laboratories).

The Food Safety Law of the People's Republic of China, which was established on 1 June 2009, requires all food producers and traders to establish a food safety management system, to inspect and test foods produced including raw materials, food additives, and to allow the release of the products only after successful inspection. Some of the ISO 17025-accredited processing establishments may be using Enzyme-Linked Immunosorbent Assay (ELISA) for screening, but some establishments including feed manufacturing units have LC-MS/MS systems which can reach the required level of sensitivity. There is a comprehensive national legal framework governing the manufacture, authorisation, sale, and distribution of veterinary medicinal products in China. National

MRLs have also been established (MoA Order 235) and withdrawal periods for pharmacologically active substances are specified in the MoA Order 278 of 22 May 2003. Off-label use is not permitted in Chinese regulation. China has also banned the use of certain veterinary medicinal products (Article 39, Chapter 6 of the Regulation on the Administration of Veterinary Drugs). Lists of banned drugs have been published (MoA Orders No 193, 560, 176, 265, and Joint MoA/State Food and Drug Administration Order No 227).

According to the MoA, veterinary medicinal products may be distributed from manufacturers/importers to qualified and licensed veterinary drug practitioners and end-users (e.g. feed mills or farms with a veterinarian on-site). Veterinary medicinal products may also be sold by qualified and licensed vendors to end-users (e.g. farmers). The administrative department for veterinary medicine of the local people's governments at or above the county level (i.e. the Veterinary Livestock Bureau at County Level) administers the licensing system for veterinary medicinal product retailers. Licensed retailers must comply with the Good Sale Practice for veterinary medicinal products established by the administrative department for veterinary medicine of the State Council and comply with the measures for the administration of veterinary prescription drugs. Qualified veterinary drug practitioners must have competent technical personnel appropriate for their veterinary drug practice. They must have fixed premises, equipment, and storage facilities for business and must also have obtained a veterinary drug practitioner certificate and the business license issued by the industry and commerce authority. Not all veterinary medicinal products for use in food-producing animals are classified as "prescription only" since some are available over-the-counter (FVO, 2009).

Malaysia

The Fisheries Biosecurity Division of the Department of Fisheries of Malaysia is responsible for developing, monitoring, evaluating, auditing, and compiling records of the Aquaculture Residue Monitoring Programme (ARMP). As per EU regulation, the minimum number of samples to be collected is maintained at one per 100 tonnes of production. One-third of the total samples are tested for Group A substances and two thirds for Group B substances. Standard Operating Procedure for the ARMP is available with the Fisheries Biosecurity Division and this specifies that the selection of commodity-matrix-residue combination for inclusion in residue monitoring would be based on a risk profile that considers several factors including:

- Use of a particular chemical or veterinary drug;
- Likelihood of the occurrence of residue;

- Extent of use, usage pattern, and incentives for misuse;
- Extent to which the residue has been monitored in the past and the results of that monitoring;
- Specific market access requirements and the perception of the residue as a possible health hazard.

While the minimum samples to be collected are based on EU regulations, samples collected at the farm level cover a minimum of 10 % of registered farms. The number of samples for each will be taken/collected by staggering months and the Fisheries Biosecurity Division shall determine these timelines. Sampling is on a random basis and covering all registered farms, including those exporting products to the EU market and farm locations being monitored for sanitary and phytosanitary (SPS) compliance. Tables 5 and 6 provides data on number of samples of shrimp and finfish collected and number of samples analysed for different veterinary drugs. All samples are not analysed for all drugs and the Biosecurity Division uses the risk profile criteria mentioned above to decide on the veterinary drug to be analysed in a particular sample.

The following species are covered based on their annual production:

- Black tiger shrimp (*Penaeus monodon* Fabricius, 1798)
- Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931)
- Giant freshwater prawn (*Macrobrachium rosenbergii* (de Man, 1879))
- Seabass (*Lates calcarifer* (Bloch, 1790))
- Tilapia (*Oreochromis* sp.)
- Grouper (*Epinephelus* sp.)
- Snapper (*Lutjanus* sp.)
- Silver pompano (*Trachinotus* sp.), and
- River catfish (*Pangasius* sp.)

The laboratories performing analysis have ISO 17025 accreditation. The turnaround time in the laboratory is 14 days.

To improve food safety and aquaculture production that does not go to the EU market, Malaysia introduced the SPS programme in 2011. The number of samples analysed for residues of veterinary drugs from farms covered under the SPS programme was 60 in 2011, 325 in 2012, and 105 in 2013.

Table 5. Samples collected for residue monitoring under the aquaculture residue monitoring programme (ARMP) during 2008–2013.

Year	Shrimp	Finfish
2008	562	213
2009	505	299
2010	574	448
2011	806	599
2012	918	744
2013	710	770

Table 6. Number of shrimp and finfish samples analysed for various veterinary drugs under aquaculture residue monitoring programme (ARMP).

Parameter	2008	2009	2010	2011	2012	2013
Shrimp						
Chloramphenicol	79	45	60	80	100	70
Nitrofurans	67	45	55	80	100	80
Nitroimidazoles	22	35	55	70	100	80
Antibacterials	128	123	180	250	300	230
Anthelmintics	68	64	70	100	120	100
Dyes	68	25	25	40	45	35
Finfish						
Stilbenes	18	23	50	65	80	80
Steroids	16	23	50	65	80	80
Chloramphenicol	40	20	15	25	30	25
Nitrofurans	30	17	20	25	30	30
Nitroimidazoles	22	16	15	20	20	25
Antibacterials	84	74	150	200	240	250
Anthelmintics	39	29	60	80	100	100
Dyes	26	16	25	35	40	35

Philippines

The Philippines is implementing an NRCP that is in line with international market requirements despite several limitations. The Fish Health Management and Quality Assurance Section (FHMQAS) of the Bureau of Fisheries and Aquaculture Resources (BFAR) have the responsibility of implementing the NRCP in the Philippines. The NRCP in the country includes:

- Aquaculture farm registration system;
- Monitoring hygiene of production;
- Disease surveillance and reporting;
- Dissemination of information and education of aquaculture food chain operators on the need for aquatic animal feeds, veterinary drugs, and

product registration before their marketing and usage;

- Surveillance and monitoring of aquatic animal feeds, veterinary drugs, and products by the Aquatic Animal Feed and Veterinary Drug and Product Control Officers;
- Regulatory action on any violation of policies and guidelines on registration, manufacturing distribution, and use of veterinary drugs and aquatic animal feeds;
- Assistance in planning, directing, and supervising the national programme on aquatic feeds, veterinary drugs, and product control.

Many administrative orders and decisions form the legal basis for the NRCP. These include Fisheries

Administrative Orders (AO), Fisheries Office Orders (FOO), General Memorandum Order (GMO), and Department of Agriculture Administrative Order (DA-AO) which are indicated below:

- Fisheries AO No 210 series of 2001- Regulations for the exportation of fresh/chilled and frozen fish and fishery products.
- Fisheries AO No 212 series of 2001- Guidelines on implementation of HACCP systems.
- Fisheries AO No 21 series of 2003- Amendment of Fisheries Office Order 147-01, Series of 2001: Designation of Regional Fish Health Officers of BFAR.
- FOO No. 210 Series of 2003 - On-farm residue monitoring.
- GMO No 225 Series of 2004 - Continued implementation of Commission Decision 2003/858/EC by Fish Health Officers.
- Memorandum Circular Order No 01, Series of 2005- Sanitary and Phytosanitary requirements for exportation of aquaculture products for quality assurance and food safety.
- Special Order 310, Series of 2005 - Designation of Fish Health Section as the National Reference Laboratory for Veterinary Residues for Aquaculture Products.
- FOO No. 155, Series of 2005 - Creation of the Fish Inspection and Quality.

Assurance Service (FIQAS); FOO No. 152, Series of 2005 - Creation of Fishery Inspection and Quality Assurance Service: Residue Monitoring and Disease Surveillance;

- FOO No. 247, Series of 2006 - Powers and Functions of Regulatory Officers (Fish Inspectors, Fish Health Officers, Fisheries Quarantine Officers, and Certifying Officers) for Safety and Quality Assurance of Fishery and Aquaculture Products Intended for Human Consumption;
- DA-AO No. 24, Series of 2009 - Implementing Guidelines on the National Veterinary Drug Residues Control Program in Food according to Administrative Order No. 14, Series of 2006;
- DA-AO No. 14, Series of 2006 - Implementation of the National Veterinary Drug Residues Control Program and Creation of the Inter-agency Committee.

The NRCP implementation was also strengthened under DA-AO No. 14, Series of 2006, on the

implementation of the national veterinary drug residues control programme and the creation of an inter-agency committee, and DA-AO No. 24, Series of 2009, as its implementing rules and regulations. This defines the roles of the competent authority, farmers, and suppliers. The fish health officers of BFAR are deputised as Aquatic Animal Feed and Veterinary Drug and Product Control Officers through DA Special Order No. 23, Series of 2002 and Special Order No. 69, Series of 2004, to conduct inspection and sampling at aquaculture facilities, fish ports, fish processing plants, and markets to monitor the use of veterinary drugs and products in aquaculture. The application of restricted veterinary drugs requires a prescription by a duly licensed veterinarian and their use must comply with the applicable regulations, particularly for drugs requiring a minimum withdrawal period.

The following products have been banned through joint DOH and DA Administrative Orders (AOs):

- Beta-agonist: DA AO No. 14, Series of 2003 - Ban on the Use in Food Animals of Beta-agonist Drugs Used in Humans as Bronchodilators and Tocolytic Agents.
- Nitrofurans: DOH and DA Joint AO No. 2, Series of 2000 - Declaring a Ban/Phase-Out of the Use of Nitrofurans in Food-Producing Animals.
- Olaquinox and carbadox: DOH AO No. 4-A and DA AO No. 1, Series 2000 - The Banning and Withdrawal of Olaquinox and Carbadox from the Market.
- Chloramphenicol: DOH AO No. 91 and DA AO No. 60, Series of 1990 - Declaring a Ban on the Use of Chloramphenicol in Food-Producing Animals.

The designated fish health officer collects samples from farms supplying raw materials to accredited exporters. A representative sample of 1 kg (pooled sample) is collected from the farm. The sample label would contain information such as sample code, date of collection, name of the farm, pond number, days of culture, feed being used, and the analysis to be performed. Table 6 shows the limit of detection, limit of quantification and maximum detection limit for residues of chloramphenicol and metabolites of nitrofurans in the Philippines. Once the analysis is completed, copies of the results are sent to FIQAS for issuing a health certificate and another copy is given to the farmer. In the case of feed, duplicate samples of 250 to 500 g are collected from each representative bag and the sample label would contain information on the date, kind, brand name, and name of the miller. The samples are sealed and labelled in front of the manufacturer/distributor and a duplicate sample is given to the miller/manufacturer. A copy of the results of the analysis is also provided to the miller/manufacturer. In the case of products, 1 kg of the sample is collected and information on the farm

which produced the raw material and the country destination of the product is recorded on the label. The results of the analysis should be provided within 3 to 4 days of sample collection.

The Central Fish Health Laboratory is the National Reference Laboratory for residues of veterinary drugs. In addition to BFAR Regional laboratories, the services of two private laboratories in Manila and General Santos, are used for residue monitoring.

Viet Nam

In Viet Nam, the Department of Aquaculture (DOA) is responsible for controlling the production, distribution, and use of feeds. The Department of Animal Health (DAH) is responsible for controlling the production, distribution, and use of veterinary medicinal products. The National Agro-Forestry-Fisheries Quality Assurance Department (NAFIQAD) is responsible for the planning and implementation of residue control plan, including follow up. The legal basis for the residue control programme has been harmonised with the requirements of EU Directive 96/23/EC as recorded in the 2003 report of the audit by the EU FVO. Viet Nam has been implementing NRCP, while reviewing and improving the programme continuously. The FVO audit report of 2003 indicates that NAFIQAD has well-equipped laboratories and well-trained manpower to carry out residue monitoring as a requirement under the EU legislation. Some of the shortcomings noted in this report are inadequacy of legislation to ensure that veterinary medical products approved for other animal species are not used in aquaculture. In addition, rules need to be in place regarding the use of veterinary medicines through the feed. Regardless, the improvements made are evident from the fact that in 2001 Viet Nam received 20 Rapid Alert System for Food and Feed (RASFF) notifications for chloramphenicol (CAP) in crustaceans and received 34 RASFF notifications for CAP in crustaceans and two in fish in 2002. These RASFF notifications were reduced to three in shrimp and one in fish in 2003. Data in Table 7 indicates that while RASFF notifications for CAP have been subsequently low, new problems arose due to malachite green. There were eight RASFF notifications for this dye in 2004, which increased to 30 in 2005 and came down to eight in 2006 and four in 2007. The FVO audit report noted that further improvements have been made in NRCP and most of the deficiencies pointed out in the 2003 report have been addressed by NAFIQAD. The number of samples to be collected is based on the EU requirement of one sample per 100 tonnes of production. The NAFIQAD prepares a sampling plan based on data from the previous year, test reports from importing countries, substances authorised for use in aquaculture in the country, and information on the use of veterinary medicines. The Ministry approves the plan at the beginning of the year. During implementation, local authorities select sites based on production,

information on the use of veterinary medicines, harvest period, and occurrence of diseases. Decision No. 130/2008/QĐ-BNN of 31 December 2008 of the Ministry of Agriculture and Rural Development forms the main legal basis for the residue-monitoring programme in Viet Nam. Four appendices to Circular 15/2009/TT-BNN established which chemicals, drugs, and antibiotics are either prohibited or authorised for use in manufacturing and trading in aquaculture. Further amendments to the list have come through circulars e.g. Circular 20/2010/TT-BNNPTNT of 2 April 2010 adding trifluralin to the prohibited list, Circular 03/2012/TT-BNNPTNT of 16 Jan 2012 adding cypermethrin, deltamethrin, and enrofloxacin to the prohibited list. Commercial production of medicated feed is prohibited, though farmers may add veterinary medicinal products to the feed using their mixers.

The NAFIQAD website provides details of the NRCP plan and data from 2009 onwards. This is illustrated in Tables 8 and 9. Generally, the planned numbers of samples are collected. The numbers of samples that fail to meet the requirements are also indicated in Table 10. In addition to the NRCP, Viet Nam has been carrying out extensive pre-export testing for banned veterinary medicines, CAP, nitrofurans, and malachite green since 2005. A minimum of two samples per batch of aquaculture products are collected by local authorities and tested. These measures resulted in a significant drop in the number of RASFF notifications after 2005. The 2009 Mission Report of EU FVO noted that Vietnamese authorities make some adjustments in sample numbers based on cultural practices. For crustaceans farmed in intensive farms, sampling is conducted per EU regulations (i.e. one sample per 100 tonnes of production). But for semi-intensive farms, testing is focused on the contaminants in EU regulation (Group B3) and samples drawn are less than 1 per 100 tonnes (e.g. 1,740 samples tested from 245,908 tonnes of production). In the case of fish grown in super-intensive systems (300–500 tonnes.ha⁻¹), sampling is usually one per pond (of 500 tonnes of production), e.g. 1,751 samples taken from 915,082 tonnes of production. Group A6, which includes banned antimicrobials, is tested at all stages of production. The scope of pre-export testing was redefined through Decision No. 1471/QĐ-BNN-QLCL of 20 June 2012 to include enrofloxacin and trifluralin in addition to chloramphenicol, nitrofurans, malachite green, and leucomalachite green. Additionally, processors should make internal checks before procuring raw material and this may include chlorpyrifos and flumequine in the test panel (EU FVO Report 2012).

The NAFIQAD Branch No. 4 is the National Reference Laboratory for fisheries products and receives samples from other laboratories. This laboratory is well equipped and is accredited to ISO 17025 for all the analyses.

Table 7. Detection of nitrofurans and chloramphenicol using ELISA by the Philippine Bureau of Fisheries and Aquatic Resources.

Limit	Chloramphenicol		Nitrofurans	
	Fish and fishery products	Aquatic feeds	Fish and fishery products	Aquatic feeds
Limit of detection	0.05 ppb	0.2 ppb	0.1 ppb	10 ppb
Limit of quantification	0.15 ppb	0.6 ppb	0.3 ppb	30.0 ppb
Maximum detection limit	4.05 ppb	16.2 ppb	8.1 ppb	81.0 ppb

Table 8. Number of rapid alerts due to residues of antibiotics and dyes in aquaculture products from Viet Nam during 2001-2016.

	Number of rapid alerts during years during 2001-2016															
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Chloramphenicol	20	36	4	4	1	3	0	0	2	2	1	1	0	2	2	
Nitrofurans	0	12	0	2	1	1	1	1	0	2	5	0	0	5	5	5
Quinolones	0	0	0	2	2	1	0	0	0	0	0	0	0	0	0	0
Tetracyclines	0	0	0	0	0	0	0	0	0	0	0	0	2	22	4	6
Malachite green	0	0	0	8	30	8	4	1	2	2	2	0	1	0	3	0
Others	0	0	0	0	0	0	0	0	0	0	0	1	0	2	4	1

Table 9. Number of samples tested for residues of antimicrobial agents in Viet Nam.

Species	2009	2010	2011	2012	2013	2014	2015
<i>Pangasius</i> sp.		1474	1265	1378	1194	766	733
<i>Tilapia</i> (<i>Oreochromis</i> sp.)		60	98	211	175	181	168
<i>Anabas</i> (<i>Anabas</i> sp.)		26	45	49	20	12	4
<i>Channa micropeltes</i> (Cuvier, 1831)		52	34	69	46	56	71
<i>Penaeus vannamei</i> Boone, 1931		1338	731	829	1268	1430	1193
<i>Penaeus monodon</i> Fabricius, 1798		804	945	1300	1082	661	491
<i>Macrobrachium rosenbergii</i> (de Man, 1879)		35	24	22	15	13	17
<i>Scylla serrata</i> (Forskål, 1775)		12	13	13	16	15	18
Fishery raw material		134	141	161			
Hatchery water		140	202	192			
Featherback (<i>Notopterus</i> sp.)			20	9			4
Grass carp (<i>Ctenopharyngodon idella</i> (Valenciennes, 1844))			6	5			
Four-eyed sleeper fish (<i>Bostrychus sinensis</i> Lacepède, 1801)			7	3			14
Sea bass (<i>Lates calcarifer</i> (Bloch, 1790))							12
Total		4075	3531	4241	3830	3134	2719

Conclusion

Residue monitoring in most of the aquaculture producing countries is driven by international market requirements. As a single trading block, the EU accounts for over 60 % of imports, and the

regulations in EU member countries are consistent and uniform. Therefore, many aquaculture-producing countries strive to comply with EU requirements. For chemicals banned for use in aquaculture, the EU follows the approach of using the most sensitive method available for detection and the regulations

Table 10. Number of tested samples and non-compliant samples in Viet Nam.

Substance tested	MRL	2009	2010	2011	2012	2013	2014	2015
Diethylstilbestrol	ND	0/51	0/58	0/72	0/64	1/62	0/50	0/49
Methyltestosterone	ND	0/53	0/55	0/74	0/62	2/65	0/51	0/50
Chloramphenicol	ND	05/887	3/742	1/511	2/669	0/731	1/396	3/367
HMMNI, IPZ, IPZ-OH, MNZ, MNZ-OH, RNZ, DMZ		NT	NT	NT	NT	0/25	7/196	1/153
AOZ	ND	0/883	1/765	1/526	0/948	0/699	0/338	0/329
AMOZ	ND	0/883	3/765	0/526	0/948	0/699	0/338	0/329
AHD	ND	0/883	4/768	4/526	0/948	0/699	0/338	0/329
SEM	ND	07/888	3/766	3/526	0/948	0/699	0/338	0/329
Tetracyclines								
Chlortetracycline	100	0/258	0/185	0/56	0/165	0/215	NT	NT
Oxytetracycline	100	0/258	0/185	1/149	2/165	4/215	1/247	2/219
Tetracyclines	100	0/258	0/185	0/149	0/165	NT	0/247	0/219
Doxycycline	100	NT	NT	NT	NT	1/30	3/247	0/219
Sulphonamides								
Sulphadimethoxine	100	0/697	0/577	0/404	0/694	1/484	1/304	0/261
Sulphachloropyridazine	100	2/697	0/577	0/404	0/694	0/484	0/304	0/261
Sulphamethoxazole	100	1/697	1/577	0/404	1/694	0/484	0/304	1/261
Sulphamethazine	100	0/697	1/577	0/404	1/694	1/484	0/304	0/261
Sulphadiazine	100	0/697	0/577	0/404	1/694	0/484	2/304	1/261
Quinolones								
Ciprofloxacin/ Enrofloxacin	100	03/702	4/581	5/443	3/818	2/498; 14/498	4/307; 6/307	0/244, 14/244
Flumequine	600 in fish, 200 in crab, prawn	0/702	0/581	0/443	0/818		0/307	0/244
Difloxacin	300	00/702	0/581	0/62	0/818			
Sarafloxacin	30	0/702	0/581	0/443	0/818		0/307	0/244
Oxalonic acid	100	0/702	0/581	0/144	0/818			
Danofloxacin	100	0/702	0/581	0/62	0/818			
Florfenicol	1000	0/75	0/105	0/100	0/124		0/155	0/150
Trimethoprim	100	0/141	1/175	0/172	0/135		1/186	2/162
Neomycin							0/191	0/164
Trichlofon	ND	0/198	0/286	0/106	NT		1/300	1/266
Praziquantel	ND	NT	0/168	0/456	1/408		1/300	2/266
Trifluralin	ND	NT	22/222	9/456	3/429		1/300	0/266
Ivermectin							5/300	2/266
Malachite green/ Leucomalachite green	ND	0/364	1/289	0/255	0/232		5/264	1/246
Crystal violet/ Leucocrystal violet	ND	0/293	0/173	0/104	0/35			

1 - Number of non-compliant/Number of tested samples.

establish the minimum required performance limit for the method to be used. Most aquaculture producing countries have adopted these methods and the laboratories performing residue monitoring are accredited to ISO 17025. However, there are some

antibiotics, like tetracyclines, and antiparasitics, permitted in the EU. There is no uniformity in drugs permitted for aquaculture in many producing countries and there have been some instances of differences in MRLs and methodology used for

determining their levels. Overall, there has been a drastic reduction in import refusals and rapid alerts for veterinary drugs in aquaculture products.

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European Union's Action Plan on Antimicrobial Resistance and Implications for Trading Partners with Example of National Action Plan for Croatia

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Abstract

The European Union (EU) is addressing antimicrobial resistance (AMR) as a global challenge. A new EU One Health action plan against AMR was issued in 2017 with the goal of preserving the effective treatment of infections in humans and animals, providing a framework for continued, more extensive action to reduce the emergence and spread of AMR, and increasing the development and availability of new effective antimicrobial agents inside and outside the EU. The plan proposes measures to help member states (MS) the proper implementation of antimicrobial stewardship practices to ensure optimal use of antimicrobials and puts forth proposals for new regulations on veterinary medicinal products and medicated feed currently undergoing the ordinary legislative procedure. As a MS, Croatia is putting significant efforts into training and awareness-raising to those who prescribe and use antimicrobials in human and veterinary medicine. The national action plan (NAP) contains: (1) surveillance of AMR of bacteria and reporting to appropriate EU agencies; (2) tracking antimicrobial use (AMU); (3) promotion of responsible use of antimicrobial agents – antimicrobial stewardship; (4) controlling the spread of resistant bacterial strains; (5) awareness-raising on the adverse effects of excessive AMU; and (6) support for the scientific activities through research in AMR by the Ministry of Health, Agriculture and Sciences. An example of the monitoring of AMR in *Salmonella*, *Campylobacter*, and indicator *Escherichia coli* and *Enterobacter* in poultry, fattening pigs, and calves with regards to a set of antimicrobial agents proposed by the EFSA (European Food Safety Agency) is presented.

Keywords: One Health, aquaculture, antibiotics

Introduction

In recent years, antimicrobial use (AMU) in human and veterinary medicine has increased the threat of development and spread of antimicrobial resistance (AMR). The development of new drugs is insufficient while patient deaths, costs for health care and loss of productivity due to infections caused by resistant pathogens are increasing (EU, 2015). Furthermore, AMR also reduces the ability to safeguard animal health and welfare, with possible repercussions for public health, food safety, and food security (EU, 2016). The European Commission (in further text: Commission) recognised the importance and awareness of AMR in 2001 and the Community Strategy against Antimicrobial Resistance was set up as the first policy instrument addressing AMR at European level (CEC, 2001). This strategy was put in

place and is based on four key areas of action: (1) surveillance; (2) prevention and control; (3) research and product development; and (4) international cooperation (COM, 2011). In 2011, the policy was reinforced with the action plan against the rising threats from AMR using the “One Health” approach, covering both the human and animal health sectors. The evaluation of this action plan concluded that Commission had more possibilities to act on the animal than on human health (EU, 2016). That was reflected in the fact that legislative proposal in the veterinary field was created. The One Health action plan was focused on combatting increasing AMR by reducing infections caused by resistant microorganisms (EU, 2016). The drivers responsible for spreading and accelerating the resistant pathogen were defined as: (1) poor hygiene and preventive measures in healthcare settings and at the farm level;

(2) inappropriate or overuse of antimicrobial agents in human and veterinary medicine; (3) lack of new antimicrobial agents or alternatives; (4) transmission of resistant bacteria from animals to humans either directly or through the food chain; (5) spread caused by contaminated food via the environment, international trade, and travel; and (6) poor awareness and knowledge on AMR in professionals and the general public. To mitigate the adverse effect of these drivers, the action plan defined seven main objectives that were envisaged to be achieved through 12 actions related to human and animal health. The strategy was focused on: (1) prevention of microbial infections and their spread in humans and animals; (2) appropriate AMU in humans and animals; (3) the development of new effective antimicrobial agents or alternatives for treatment; (4) reinforcing research to develop the scientific basis and innovative means to combat AMR; (5) communication, education, and training; (6) strengthening the monitoring and surveillance systems in the human and veterinary fields; and (7) global aspects of AMR.

Evaluation of the EU Action Plan Against Rising Threats from AMR

In 2015, an evaluation analysis was performed to assess if the 12 key strategic actions (RAND, 2016) of the action plan were the most appropriate actions to combat AMR. The evaluation analysis assessed the relevance of the action plan objectives to the current needs in tackling AMR, and if the approach appropriately involved all sectors (the One Health approach) and aspects of AMR (human medicine, veterinary medicine, animal husbandry, agricultural, research, environment, and trade). The evaluation addressed the relevance, effectiveness, efficiency, internal and external coherence, and EU benefit. It analysed the role of the Commission, member states (MS), associated non-EU member countries so called "thirds countries", and international organisations such as Food and Agriculture Organisation (FAO), the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR), the Organization for Economic Co-operation and Development (OECD), the World Health Organization (WHO), and presented the views of independent experts, researchers, and innovation stakeholders.

The basis for the evaluation of the particular topics came from various documents on "Antimicrobial resistance surveillance in Europe 2011" "Microbial infections up to 2011" (ECDC, 2012; ECDC, 2013), and Antimicrobial agent consumption in the human sector reported in 29 EU/EEA MS (ECDC, 2015). Moreover, a report on antimicrobial agent consumption in the veterinary sector showed a slight decrease (Grave et al., 2014), but a notable decrease of consumption for antimicrobial agents with the highest priority for human medicine was seen. In the evaluation document it was concluded that no significant progress in the development of new antimicrobial

agents, their alternatives and diagnostic tools were seen due to the merging of pharmaceutical companies, and that according to European Food Safety Agency (EFSA) documents (2009) the transmission through food chain was not clearly scientifically evidenced and environmental spread caused by contaminated food and water systems is, despite several studies on the spread of the resistant microorganisms, still considered as a knowledge gap. Awareness and knowledge of AMR among participants in surveys varied considerably among MS and also across socio-demographic profiles (Eurobarometer, 2016).

Monitoring and surveillance were dynamic activities coordinated by the EU Commission agencies in the area of health (European Centre for Disease Prevention and Control, ECDC), food (EFSA), and pharmaceuticals (European Medicines Agency, EMA). Data regarding AMR are gathered through the European Antimicrobial Resistance Surveillance Network (EARS-Net) for seven microorganisms of major public health importance (EU, 2012). This network is coordinated and funded by ECDC and annually publishes a report. EFSA coordinated the Scientific Network for Zoonosis Monitoring Data which assisted in gathering and sharing the information on zoonosis in their respective countries. The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) was launched by EMA to harmonise, collect, and report data on the use of antimicrobial agents in animals. In 2004, the Commission established a EURL-AMR (European Reference Laboratory for AMR) to ensure the quality and standardisation of antimicrobial susceptibility testing.

In the international scene, AMR is a problem for developed and underdeveloped countries and is a major threat to diseases treatments. The Partnership for Pharmaceutical Policy was implemented in 78 African, Caribbean, and Pacific countries, aiming to support the development and implementation of essential medicinal strategies. The EU established bilateral cooperation with the United States of America through TATFAR in the areas of the appropriate therapeutic use of antimicrobial agents in human and veterinary communities, prevention, and strategies for improving the pipeline of new antimicrobial agents. The Commission participated in the preparation of the WHO's Global Strategy for Containment of Antimicrobial Resistance and cooperated with FAO and WHO on the Codex Alimentarius, or "Food Code". The Commission also contributed to the World Organisation for Animal Health (OIE) *ad hoc* AMR group in the development of Health Codes regarding AMR and animal health. The EURL-AMR collaborated with WHO in supporting activities of the Global Foodborne Infections Network to develop the global standards for monitoring of AMR and capacity building.

In 2002, MS adopted the Council Recommendation on the prudent use of antimicrobial agents in human medicine and the report on its implementation in 2010 showed that most EU/EEA MS had, or were close to, put in place, a national strategy to contain the problem of AMR (EC, 2002). All MS had implemented a surveillance system and had an action plan covering all topics listed in the Council Recommendation: surveillance of antimicrobial resistance, detection and control of outbreaks, prevention policy, education, and training of health professionals, general public information and research.

Main actions and outputs related to human and animal health are summarised in "Evaluation of the Action Plan against the rising threats from antimicrobial resistance" (RAND, 2016):

- Strengthened promotion of appropriate AMU in human medicine documented in the "Report on the implementation of the Council Recommendation on the prudent use of antimicrobial agents in human medicine" (EU, 2016a), the "Guidelines on prudent use of antimicrobials in human medicine" (ECDC, 2017), and in veterinary medicine documented in the "Guidelines on the prudent use of antimicrobials in veterinary medicine" in (EU, 2015).
- Strengthened regulatory framework on veterinary medicine and medicated feed and adopted the proposal on veterinary medicinal products and medicated feed.
- Strengthened infection prevention and control in healthcare settings documented in the "Report on the implementation of the Council Recommendation on Patient safety" (EU, 2014).
- Adoption of a proposal for an EU Animal Health Law in March 2016 (EU, 2016c).
- Promotion of collaborative research and development of new antimicrobial agents for human patients and promotion of efforts to analyse the needs of new antimicrobial agents in veterinary medicine.
- Development of mutual collaborative commitments for prevention and control of AMR in all sectors, including cooperation on the reduction of environmental pollution by antimicrobial agents particularly from production facilities. The EU contributed to the WHO Global Action Plan, the Global Infection Network, OIE standards, Guideline for Risk Analysis of Foodborne AMR, and TATFAR; and worked with OECD on economic impact. The EU is developing a strategy for pharmaceuticals in the environment.

- For strengthened surveillance systems on antimicrobial agent consumption in human medicine, the European Surveillance on Antimicrobial Consumption (ESAC) and EU AMR Surveillance Network data collection, as part of ECDC's activities, have been improved. At the same time ESCVAC has strengthened surveillance on antimicrobial consumption in veterinary medicine and monitoring and reporting has extended to zoonotic and commensal bacteria in food-producing animals and certain foods (EU, 2013). EFSA, EMA, and ECDC reported a first integrated analysis on antimicrobials consumption and resistance in humans and animals in 2015 (ECDC/EFSA/EMA, 2015).
- For reinforced and coordinated research efforts, a Joint Programming initiative for coordinating research activities on AMR has been set up. The Commission supports research activities focused on diagnostic tools, vaccines, and alternative treatments and understanding AMR.
- For communication, education, and training, the European Antibiotic Awareness Day and Eurobarometer on public knowledge on antibiotics were established in 2013 and 2016, respectively.

These actions and outputs were included in the document issued by the Directorate General for Health and Food Safety entitled "Evaluation of the EC Action Plan against the rising threats from antimicrobial resistance, Final Report" (RAND, 2016). This document was used as a background for the preparation of the recent EU action plan on AMR. Despite all undertaken plans and activities, incidences of infections resistant to multidrug therapies and last-resort treatments have significantly increased in the EU in recent years.

Purpose of the Paper

The purpose of this paper is to summarise the main activities envisaged by the EU action plan on AMR, present the role of the EU on the global plan and the relations, requirements, and implications of this plan for the trading partners/third countries, and give an overview of Croatia's Action Plan, with particular emphasis on the activities in the animal sector.

The European One Health Action Plan Against AMR

The new action plan was developed based on the 2011 One Health document and was launched in 2017 (EU, 2017). Its main goal is to preserve the possibility of effective treatment of infections in humans and animals by providing a framework for continued, more extensive action to reduce the emergence and spread

of AMR and to increase the development and availability of new effective antimicrobial agents inside and outside EU. Three pillars are defined as crucial in future activities:

Making the EU a best practice region

Within the EU, the situation concerning AMR varies across MS and differences are in the effectiveness of national policies to deal with AMU and AMR and occurrence of resistance. The Commission will continue to support the cooperation of all relevant EU scientific agencies (EFSA, EMA, and ECDC) to jointly take appropriate actions and to support and strengthen efforts on better One Health surveillance and AMR. The EU legislation on AMR monitoring and detection in humans, reporting diseases in humans, identification and assessment of zoonotic bacteria, and consideration of AMR monitoring in the environment will be reviewed and improved.

The vast evidence-based analyses will be used to provide a possible link between consumption of antimicrobials and the occurrence of AMR in humans and food-producing animals and to develop an assessment model of the economic burden of AMR and to estimate the cost-effectiveness of national policies to reduce it. The awareness-raising, public understanding and knowledge sharing on AMU should be highlighted through national awareness programs and contributing to the European Antibiotic Awareness Days.

The EU will promote implementation the EU rules on AMR by sharing information at MS and EU level, supporting One health NAPs, joint actions, increasing assistance of EU Health Security Committee and Commission Working group on AMR in veterinary and food safety area. In cooperation with WHO will help MS to develop and implement own One health NAPs, monitor implementation and organise training programs for competent authorities (CA) through Better Training for Safer Food (BTFS).

Activities in AMR prevention and control will be implemented by infection prevention through control measures in hospital environments, protection of vulnerable groups, promotion of vaccinations in humans, promotion of animal husbandry systems and supporting good animal health and welfare, promotion of prudent use of antimicrobial agents by reserving antimicrobials for human use, reporting the sales and use of antimicrobial agents, developing guidelines for prudent use in human medicine, assisting MS to implement EU guidelines for prudent use in veterinary medicine, and encouraging EMA to review information on benefits and risks of older antimicrobial agents. The EU will better address the role of the environment using the existing data from monitoring programs on pharmaceuticals presence and will reinforce the role of the Scientific Committee on Health and

Environmental Risks (SCHER).

The EU would become a best practice region by development of partnerships against AMR and better availability of antimicrobials by establishing collaboration among key stakeholders in the human and animal health, food, water, and environmental sectors.

Boosting research, development, and innovation on AMR

Detection, effective infection control, and surveillance should be improved by supporting research on the prevention of development and spread of AMR, its epidemiology and the development of new tools for early detection of resistant pathogens in humans and animals. A new eHealth solution should be implemented to improve prescription practices.

Development of new therapeutics and alternatives for humans and animals should be boosted by the support of Small- and medium-sized enterprises (SMEs) will be supported in research and development of innovative and alternative therapeutic approaches in the treatment and prevention of bacterial infections. European-wide sustainable clinical network will be established to share antimicrobial research data among stakeholders.

The development of new vaccines and new diagnostic tools, wider use of vaccination in medical and veterinary practice, should be fostered and supported. Economic models and incentives gathering evidence for understanding the societal costs and benefits of different strategies for fighting AMR and the development of therapeutics, vaccines, and diagnostics should be developed and explored by MS.

Research on the release of resistant microorganisms and their spread in the environment, monitoring and methods to degrade antimicrobial agents in wastewaters and the environment and risk assessment tools should be supported to close the knowledge gaps of AMR in the environment.

Shaping the global agenda

The EU and its MS are included in an intensive exchange of people and commodities around the world and policies implemented in one region can have significant impacts elsewhere. Based on the positive effects of EU interventions at the global level, the continued efforts are accentuated in the action plan.

The EU should contribute to the normative work of the WHO, OIE, FAO, and Codex Alimentarius on the development of international frameworks and standards/norms/guidelines related to AMR.

Technical cooperation in the WHO Global Action Plan on AMR will be reinforced and different international activities aiming to raise political attention to AMR Action in the G7 and G20 UN forums and collaboration within TATFAR (EU, USA, Canada, Norway), and promotion of international regulatory convergence between EMA, FDA, and the Japanese Pharmaceutical and Medical Devices Agency (PMDA) on plans for new antimicrobial agents will be supported.

The EU will consolidate bilateral partnerships by advocating the EU standards and measures for tackling AMR and its implementation by trade partners and their incorporation into cooperative arrangements in trade agreements with major global players and strategic countries (India, China, and Brazil). Capacity building and legislation implementation in candidate and neighbouring countries related to AMR will be supported.

Through cooperation with developing countries, the EU will contribute to reducing AMR by implementing programmes on the prevention and control of infectious diseases, organising workshops and training on capacity building to assist in the development of national strategy in food safety and animal health. The EU will also improve global coordination of research activities, support the establishment of virtual institutes, support sub-Saharan Africa in collaborative research, and foster international research collaboration on AMR in the animal health sector.

Strategies Regarding AMR in Aquaculture

The strategies regarding AMR in aquaculture are considered in the “Guidelines for the prudent use of antimicrobials in veterinary medicine” (EU, 2015) and the principle is that similar strategies used for reducing the AMU in terrestrial farm animals should also be used in aquaculture.

Actions to prevent and reduce AMU in aquaculture include implementation of good aquaculture practices that provides the appropriate environmental conditions (water quality, water flow rates, oxygen levels), appropriate feeding, antimicrobial sensitivity testing before treatment, development of specific disease surveillance programs to identify and help prevent possible outbreaks, implementing hygiene (cleaning and disinfection of units between production cycles, keeping separate equipment, boots, and clothes for each unit, etc.), and biosecurity measures (operating an “all-in all-out” system, single-bay management, fallowing between production cycles, quick removal of dead fish, development of systems to avoid disease spread by transport, restriction on access to the farm, etc.). Vaccination against some of the bacterial diseases has been demonstrated effective for reducing AMU and consequently AMR.

Implication of the EU Action Plan on Trading Partners

The new plan will propose measures to help MS implement antimicrobial stewardship practices to ensure optimal AMU. Furthermore, the Commission proposals for new regulations on veterinary medicinal products and medicated feed, currently undergoing the usual legislative procedure, contains a set of requirements for addressing the threat of AMR including provisions aimed at responsible use of veterinary antimicrobials.

The Commission is gathering information from the main EU trading partners on their policies regarding the fight against the rise of AMR. These include actions taken nationally to monitor the incidence of AMR in bacterial isolates from animals and meat. This information will be used to set the pathways for the Commission's future activities in this area.

The Commission will reinforce its engagement and collaboration with multilateral organisations, such as the WHO, OIE, and FAO, to contribute towards their normative work on the development of international standards related to AMR. This includes the work of the Task Force on AMR recently established by the Codex Alimentarius. The Commission will also promote the inclusion of AMR on the agenda in the next G20 Summit. Finally, to include the AMR issue in an all-new Free Trade Agreements is being systematically proposed and negotiated by the Commission.

National Action Plan on AMR – the Example of Croatia

Based on the recommendations of the WHO, ECDC, and EC in 2006, the Croatian government issued the decision on the establishment of the Intersectional Coordination Mechanism for the Control of Antimicrobial Resistance (ISKRA), an interdisciplinary session for the control of AMR (Tambić Andrašević, 2009). The first NAP on AMR was implemented from 2009 to 2014 and was prepared according to the EU Action Plan and regulations. Currently, the second NAP is in force from 2015 to 2020 in line with the Global Action Plan on antimicrobial resistance (WHO, 2015) and ECDC Multiannual Strategic Plan (2014).

According to EC Directive (2003b) on the monitoring of zoonoses and zoonotic agents and Decision 2013/652/EU (EU, 2013) on the monitoring and reporting of resistance of zoonotic and commensal bacteria to antimicrobial agents, MS are obliged to ensure monitoring which will provide comparable data on the occurrence of AMR in zoonotic agents and, as far as they present a threat to public health, other agents. General and specific requirements for monitoring are set in the aforementioned documents, including technical specifications on the harmonised monitoring and reporting of AMR in *Salmonella*,

Campylobacter, and indicator *E. coli* and *Enterobacter*. The manual for the notification of zoonosis, causative agents, and AMR proposes an obligation of reporting. EFSA (2012) issued specific technical specifications with instructions on the monitoring and reporting of AMR. This document precisely describes targeted bacteria, targeted animal species and populations, required samples and targeted antimicrobials, and recommends the modes and places of sampling (farms, slaughterhouses, etc.), testing methods, and interpretation of results. All these documents were used for the preparation of the national surveillance plan on AMR that has been implemented in Croatia since 2011 (Ministarstvo zdravlja RH, 2015).

The animals included in the surveillance are laying hens, broilers, fattening turkeys and pigs, calves younger than one year, sheep, goats, dairy cattle, and young bovine. The samples, swabs, or coecal splash should be collected on farms and in slaughterhouses to be analysed for the presence of *Salmonella*, *Campylobacter*, and commensal bacteria like *E. coli* and *Enterobacter*. Sets of antimicrobial compounds are proposed as well as methods for the determination of epidemiological cut-off values (ECOFFs), clinical breakpoints, and concentration ranges to be tested. Results of the surveillance are reported to the Ministry of Agriculture, Veterinary Directorate, and EURL-AMR where data from all MS are analysed, compared, and finally published on their webpage.

Conclusion

The Council of the EU has issued conclusions on the next steps under the One Health approach to combat AMR (EU, 2016b) that welcomes former activities, acknowledges many efforts, and highlights the future actions that are of the utmost importance.

They welcome the Global Action Plan on AMR developed by WHO, FAO, and OIE, the Resolution on Antimicrobial Resistance adopted in 2015 by the FAO, the Resolution combating Antimicrobial Resistance and promoting the prudent use of antimicrobial agents in animals by OIE, the Codex Alimentarius Commission initiative concerning the need to review and update standards, codes, and guidelines related to AMR and other international and regional initiatives such as the declaration by the G7 on AMR and the decision to put AMR on the agenda of the G20.

In the EU Council document, it is recalled that AMR is a cross-border health threat that cannot be sufficiently addressed by single MS, cannot be confined to a geographical region or MS, and hence requires intensive cooperation and coordination between MS. The document also acknowledges that several legislative and non-legislative measures have already been taken and are taken at the EU level to coordinate and ensure a common EU approach for reducing the risk of AMR in the veterinary sector.

These measures include regulation on additives for use in animal nutrition, prohibiting the use of antibiotics as growth promoters (EC, 2003a), Commission Implementing Decision on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (EU, 2013), Commission Decisions on modifications of marketing authorisations for products containing critically important antimicrobials to reflect specific measures against the development of AMR, and the Guidelines for the prudent use of antimicrobials in veterinary medicine (EU, 2015).

The Council expresses its concern regarding the estimation that about 700,000 deaths may be caused globally each year by AMR (Review on Antimicrobial Resistance, 2014). The economic impact associated with current rates of AMR in OECD countries may reach about 0.03 % of GDP in 2020, 0.07 % in 2030, and 0.16 % in 2050, resulting in cumulative losses of about USD 2.9 trillion by 2050.

The scientific opinions and reports on antimicrobial resistance published by the ECDC, EFSA, and EMA are acknowledged, as well as the ongoing work on the economic impact of AMR carried out by OECD and the World Bank.

Finally, they underline that in order to stimulate the development of new antimicrobial agents, alternative therapies, and rapid diagnostics, EU and global coordination and cooperation on research programs and incentives are needed.

Due to the complexity of the problem – its cross-border dimension and high economic burden – the impact of AMR goes beyond its severe consequences for human and animal health and has become a global public health concern that affects the society and requires urgent and coordinated intersectoral action based on the precautionary principle.

To make progress in the fight against AMR, the new EU action plan should contain measurable and clearly defined quantitative or qualitative goals, benchmarks, and effective measures to achieve these goals. The success of the fight against AMR relies heavily on the commitment and willingness of governments to take action to ensure the implementation of the initiatives under the One Health approach involving all relevant sectors and on the will of the EU MS to cooperate within the EU and at an international level.

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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* (Lacépède, 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 amplification of *int11*, *int12*, and *int13* genes was performed with the template DNA of the *Aeromonas* isolates. All the *int11*-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 *int11* 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 led 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. MIC₅₀, MIC₉₀ 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	<i>A. veronii</i>	-	SMM/NAL/AMP/SXT
5S16	Sediment	<i>A. veronii</i>	-	SMM/NAL/CIP/AMP/SXT
6S2-3	Sediment	<i>A. sobria</i>	-	CHL/FFC/THI/SMM/DOX/NAL/AMP/SXT/CTX
2W12	Pond water	<i>A. caviae</i>	-	SMM/NAL/AMP/SXT
2W14	Pond water	<i>A. veronii</i>	-	CHL/FFC/THI/SMM/NAL/AMP/SXT
6F10-3	Fish	<i>A. veronii</i>	-	CHL/FFC/THI/SMM/NAL/NEO/AMP/CTX/SXT
6F6-2	Fish	<i>A. veronii</i>	-	FFC/THI/SMM/NAL/AMP/SXT
7F10-3	Fish	<i>A. caviae</i>	-	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F6-3	Fish	<i>A. caviae</i>	-	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F9-1	Fish	<i>A. caviae</i>	-	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F9-2	Fish	<i>A. caviae</i>	-	CHL/FFC/THI/SMM/TET/NAL/CIP/NOR/CN/AMP/CTX/SXT
5P1-5	Pond surroundings	<i>A. caviae</i>	-	SMM/NAL/AMP/SXT
5P3-2	Pond surroundings	<i>A. caviae</i>	-	THI/SMM/AMP/SXT
5P3-4	Pond surroundings	<i>A. simiae</i>	-	FFC/SMM/NAL/NEO/AMP/SXT
7F8-3	Fish	<i>A. caviae</i>	<i>aad(6)-Ib-cr</i>	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F2-2	Fish	<i>A. caviae</i>	<i>aac(6)-Ib-cr - arr-3</i>	SMM/NAL/NOR/ENR/AMP/SXT
7F2-3	Fish	<i>A. caviae</i>	<i>aac(6)-Ib-cr - arr-3</i>	CHL/FFC/THI/SMM/NAL/CIP/ENR/AMP/SXT
7F5-2	Fish	<i>A. caviae</i>	<i>aac(6)-Ib-cr - arr-3</i>	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F6-1	Fish	<i>A. caviae</i>	<i>aac(6)-Ib-cr - arr-3</i>	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
7F6-2	Fish	<i>A. caviae</i>	<i>aac(6)-Ib-cr - arr-3</i>	CHL/FFC/THI/SMM/DOX/TET/NAL/CIP/NOR/AMP/CTX/SXT
6S1-3	Sediment	<i>A. caviae</i>	<i>aac6-II-bla_{OXA-21}-catB3</i>	CHL/FFC/THI/SMM/NAL/AMP/SXT

Table 2. Continued.

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

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Abstract

Antimicrobial resistance (AMR) is a serious and growing global public health threat. Given the grave importance of AMR, the United Nations General Assembly has called for the development and implementation of the national action plans (NAP) on AMR in each of its member countries. The Malaysia NAP was launched in February 2017 with collaborative approach from Ministry of Health, Ministry of Agriculture and Agro-based Industries [Department of Veterinary Services (DVS), Department of Fisheries (DOF), Department of Agriculture (DoA)], Department of Environment, Ministry of Higher Education, Ministry of Defence Hospitals, Private Healthcare Facilities, Community Pharmacist, The Animal Food Industry and Professional Organisations pertaining to Human And Animal Health) to address and mitigate AMR in respective sectors. This paper presents the aquaculture component of Malaysia's NAP on AMR lead by the DOF, Malaysia. The objective of this paper is to briefly present the outcomes of activities carried out by the DOF in relation to AMR and AMU in Malaysia which includes: i) the development of the aquaculture component of the NAP; ii) results of the AMU survey; iii) outcome of AMR surveillance; iv) AMR education and awareness; and iv) strengthening governance. The initial AMR results indicate that most of the *Escherichia coli* isolates were resistant towards erythromycin (90.7 %), cefepime (26.6 %), tetracycline (18.2 %), ampicillin (15 %), and chloramphenicol (10 %). On the other hand, the majority of the *Vibrio parahaemolyticus* isolates were resistant to ampicillin (72.3 %) followed by erythromycin (10 %), cefotaxime (4 %), and tetracycline (4 %).

Keywords: One Health, national action plan, fisheries, antimicrobial usage

Introduction

According to the World Health Organization (WHO), antimicrobial resistance (AMR) occurs when microorganisms (such as bacteria, fungi, viruses, and parasites) change when they are exposed to antimicrobial drugs (such as antibiotics, antifungals, antivirals, antimalarials, and anthelmintics). As a result, these medicines become ineffective and infections persist in the hosts, increasing the risk of spread to others. In 2016, Malaysia established a National Antimicrobial Resistance Committee (NARC) comprised of members from the human and animal health sectors under the One Health approach to develop the National Action Plan (NAP) on AMR. This was following the outcome of the 68th United Nations General Assembly in 2015 which urged all member

states to adopt the global action plan on AMR and to develop the country's NAP on AMR (WHO, 2015). The initial NAP framework outlines the views of stakeholders from across the sectors of human and animal health (including aquaculture) regarding the status, gaps, and solutions to address the AMR situation in Malaysia. This was continued with the development of the NAP, led by the Ministry of Health (MOH) and the Ministry of Agriculture and Agro-based Industries (MOA). The four main pillars of the NAP include: i) public awareness and education; ii) surveillance and research; iii) infection prevention and control; and iv) appropriate use of antimicrobials. The Malaysian NAP on AMR, or abbreviated as MyAP AMR, was officially launched by both the MOH and the MOA on 27 February 2018 in Putrajaya (Malaysian Action Plan on Antimicrobial Resistance (2017-2021), 2017). Among

the main roles of the Department of Fisheries (DOF) in the MyAP AMR are to: a) implement the survey on the status of antimicrobial usage (AMU) in the aquaculture sector; b) determine AMR properties of bacterial isolates obtained from fish, aquaculture facilities/ecosystems; and c) carry out awareness programs for the fish farmers on AMU and AMR.

The DOF's AMR aquaculture working group was established in April 2017 to carry out the implementation. At the same time, the DOF had also participated in the Project FMM/RAS/298/MUL: Strengthening capacities, policies and national action plans on the prudent and responsible use of antimicrobials in fisheries, which consists of workshops held in India (10–13 April 2017), Putrajaya (7–9 August 2017) and Singapore (12–14 December 2017) of the Food and Agriculture Organization of the United Nations (FAO). These workshops complemented the activities planned in MyAP AMR. The establishment of the governance mechanism for the NAP AMR (aquaculture component) in the DOF is represented in Table 1.

Status of Aquaculture in Malaysia

Aquaculture is now being promoted in Malaysia as an important engine of growth and a mainstay contributor to the nation's economy. Similar to many countries in the region, aquaculture in Malaysia serves to provide supplementary fish for national food security and production of high-value fish for foreign exchange earnings. Situated in a region with an abundant supply of land and water, two determinant factors for aquaculture activities, the Malaysia government has always strived to ensure that this sector is not sidelined in the country's development efforts.

According to the Annual Fisheries Statistics (2016), a total of 21,939 fish farmers and culturists were involved in the aquaculture industry. The majority (72.5 %) of the workforce was involved in the freshwater aquaculture sub-sector. The remaining 27.5 % of fish farmers/culturists were involved in the brackish-water aquaculture industry. In 2016, freshwater aquaculture contributed 103,348 tonnes valued at RM1,091,463 million. The main cultured species were freshwater catfish (*Clarias* sp.), black and red tilapia (*Oreochromis* sp.), riverine catfish (*Pangasius* sp.), and giant freshwater prawn (*Macrobrachium rosenbergii* Johnson, 1966). Brackishwater aquaculture production in 2016 contributed about 304,039 metric tonnes valued at RM2,509,717 million. The main cultured species were marine prawns (Tiger shrimp (*Penaeus monodon* Fabricius, 1798) and Whiteleg shrimp (*Penaeus vannamei* Boone, 1931)), cockles (*Tegillarca granosa* (Linnaeus, 1758)), grouper (*Epinephelus fuscoguttatus* (Forsskål, 1775)), red snapper (*Lutjanus argentimaculatus* (Forsskål, 1775)), and seabass (*Lates calcarifer* (Bloch, 1790)).

The DOF, Malaysia is the competent authority responsible for the implementation of the Fisheries Act (1985) which deals with the health of aquaculture animals and the prevention and control of aquatic diseases. In its role as the competent authority, the DOF carries out many functions which relate to the control of AMR including:

- Registration of aquaculture farms.
- Coordination of the national fish health surveillance programme.
- Public health monitoring programmes like the Sanitary and Phyto-Sanitary Programme for Aquaculture and the National Shellfish Sanitation Programme (NSSP).

Table 1. Steps taken in the establishment of a governance mechanism for Malaysia Action Plan on AMR (Aquaculture) in Malaysia.

Establishment of governance mechanism	Months in 2017											
	3	4	5	6	7	8	9	10	11	12		
a. Dialogue Session: AMR - Playing our part in combating the crisis (MOH, National Pharmaceutical Regulatory Agency, DVS, DOF, Federation of Livestock Farmers Association Malaysia)(Lead by MOH)		X										
b. Establishment of AMR (aquaculture) Working Group (DOF)		X										
c. Technical Working Groups (TWG) meetings		X	X									
d. AMR (aquaculture) Working Group Meeting (DOF)		X		X			X				X	
e. Present the NAP AMR (aquaculture) to the Department of Fisheries top management and endorsed (DOF)				X								
f. Co-ordination meeting of 4 TWGs on AMR and finalisation of National Action Plan on AMR (MOH)											X	
g. Development and formalisation of NAP AMR (which incorporates AMR (aquaculture))			X	X	X	X	X	X	X	X	X	X

- d. Development of fisheries biosecurity protocols.
- e. Assessment and management of imports, exports, and internal movements of live aquatic animals.
- f. Certification of fish farms – Malaysia Good Agricultural Practice(myGAP)(2014).
- g. Adoption of MS 1998:2007 – Good Aquaculture Practice (GAQP) – Aquaculture arm General Guidelines.
- h. Regular inspection on registered fish/shrimp farms by State Biosecurity Unit, DOF.
- i. Enforcing the Animal Feed Act (2009).

Purpose of the paper

The objective of this paper is to briefly present the outcomes of activities carried out by the DOF about AMR and AMU in Malaysia which includes: i) the development of the NAP AMR (aquaculture component) document; ii) results of AMU survey; iii) outcome of AMR surveillance; iv) activities on AMR education and awareness and iv) strengthening governance.

The Development of MyAP AMR

The MyAP AMR was officially launched by both the MOH and the MOA on 27 February 2018 in Putrajaya. The component on aquaculture is being included briefly in MyAP AMR. Hence the NAP AMR (aquaculture) is being prepared to include details specifically on AMR activities planned and implemented in the aquaculture sector in Malaysia. The first draft of the NAP AMR (aquaculture) is expected to be ready by June 2018. It is hoped that with this document, the activities regarding AMR will be more clearly defined, documented, and adhered to. The main activities carried out under the NAP AMR (aquaculture) are briefly presented below.

AMU Survey

Reports on AMU in Malaysia are very limited. The latest record is the report on the use of chemicals in aquaculture in Malaysia by Shariff et al. (2000), where a variety of antimicrobials used for treating fish and shrimp diseases in Malaysia are listed. Most of the antimicrobials used are generic imports from China and Thailand. Commonly used antibiotics include sulfonamides, tetracyclines, nitrofurans, chloramphenicol, oxolinic acid, and virginiamycin. Due to the long lapse of the available information in this area, a preliminary AMU survey was carried out by the DOF on 4th August 2017 at the Malaysia External Trade Development Corporation (MATRADE) during the AMR Awareness and Stakeholders Consultation organised together with the Malaysian Aquaculture Development Association (MADA). The session comprised of an awareness talk on AMR followed by an AMU survey. An AMU questionnaire adopted from FAO and the Network of Aquaculture Centers in Asia and the Pacific, with some modification, was used. A

total of 106 questionnaires were handed out to the participants. Out of this, only 46 respondents participated in filling the questionnaires which consisted of farmers (n = 28, 61 %), feed and drug suppliers (n = 12, 26 %), and aquatic animal health professionals (n = 6, 13 %). The results showed that only 8 out of 28 (28 %) farmers used antibiotics. Most of the farmers (19/28, 68 %) used other chemicals such as anti-helminthic and anti-parasitic drugs to treat fish infested with parasites. Commonly used antibiotics include oxytetracycline, erythromycin, and amoxicillin. Awareness of the approved or banned drugs, regulations on antimicrobial used in aquaculture, and guidelines for AMU in aquaculture were very limited among the farmers. The knowledge and experience gained during this exercise were used to improve the AMU surveillance program that continued until the end of 2018. The questionnaires have also been simplified. Face to face interviews will be adopted and more stakeholders (hatcheries, nurseries, grow out, ornamental fish farms, fish feed manufacturers) will be approached in future surveys.

AMR Surveillance

Under the MyAP AMR, the DOF focused on *Vibrio parahaemolyticus* and *Escherichia coli* as the target microorganisms to be studied for AMR surveillance. Both isolates are of importance to animal and public health. *V. parahaemolyticus* and *E. coli* isolates obtained from the DOF active surveillance program for the Sanitary and Phyto-Sanitary Programme (Aquaculture), the National Shellfish Sanitation Programme (NSSP), research and development undertakings, and diagnostic activities were used for AMR surveillance. The isolates were tested against 20 antibiotics of human and aquaculture interest. Bacterial isolation and antimicrobial susceptibility testing were carried out according to standard methods together with control culture in place. All the information regarding the sampling plan, sample preparation, bacterial isolation, and susceptibility testing methods are specified in the National Integrated AMR Surveillance Manual.

Bacterial isolation was executed at six DOF laboratories, namely: i) Fisheries Biosecurity Laboratory (FBL) Kuala Lumpur; ii) FBL, Bintawa, Sarawak; iii) FBL, Kuantan, Pahang; iv) FBL, Selangor; v) Fisheries Research Institute, Batu Maung, Penang; and vi) National Fish Health Division, Batu Maung, Penang. Antibiotic susceptibility pilot testing was done at the FBL in Kuala Lumpur. Table 2 provides the work plan for AMR surveillance that was carried out in 2017.

A total of 88 *E. coli* and 51 *V. parahaemolyticus* isolates, obtained from the DOF surveillance programmes from June to November 2017 were tested for antibiotic susceptibility testing. Details on the sources and origin of the isolates are given in Table 3. The initial results indicate that most of the

Table 2. Work plan for antimicrobial susceptibility testing conducted in 2017.

Activity/Month	Months in 2017									
	4	5	6	7	8	9	10	11	12	
a. Development of National Integrated AMR Surveillance Manual	X	X	X	X	X		2 nd Draft			
b. Preparation of laboratories to conduct AMR surveillance for <i>Vibrio parahaemolyticus</i> and <i>Escherichia coli</i>		X	X	X	X					
c. Bacterial isolation, identification, and confirmation		X	X	X	X	X	X	X		
d. Antibiotic susceptibility pilot testing in Fisheries Biosecurity Laboratory Kuala Lumpur							X		X	
e. WHONET* data entry and reporting to NARC									X	X

*WHONET is free Windows-based database software developed for the management and analysis of microbiology laboratory data with a special focus on the analysis of antimicrobial susceptibility test results.

Table 3. Sources of samples, type of samples, sampling location and number of bacteria isolates for AMR surveillance.

Bacterial isolates	Sources of samples (Jun–Nov 2017)	Type of samples	Sampling location	Number of isolates
<i>Escherichia coli</i> (n = 88)	Sanitary and Phytosanitary Programme (Aquaculture)	Catfish (<i>Pangasius</i> sp.) Tilapia (<i>Oreochromis</i> sp.)	Kedah, Melaka, Perlis, Kelantan, Negeri Sembilan	18
	National Shellfish Sanitation Programme (NSSP)	Green mussel (<i>Perna viridis</i> (Linnaeus, 1758)) Clams (<i>Geloina</i> sp.), Cockles (<i>Tegillarca granosa</i> (Linnaeus, 1758)), and freshwater bivalves (<i>Corbicula fluminea</i> (O.F. Müller, 1774))	Johor, Terengganu, Kelantan, Penang, Perak, Selangor, Kelantan	70
<i>Vibrio parahaemolyticus</i> (n = 51)	Sanitary and Phytosanitary Programme	Tiger shrimp (<i>Penaeus monodon</i> Fabricius, 1798)	Sarawak	4
	National Shellfish Sanitation Programme (NSSP)	Green mussel (<i>P. viridis</i>) Clams (<i>Geloina</i> sp.)	Sarawak	3
	R&D	Tiger shrimp (<i>P. monodon</i>) Whiteleg shrimp (<i>Penaeus vannamei</i> Boone, 1931)	Perak Selangor, Johor	8 14
	Diagnostic cases	Tiger shrimp (<i>P. monodon</i>) Whiteleg shrimp (<i>P. vannamei</i>)	Melaka, Kedah, Penang, Sabah, Perak, Sarawak	20
	Antibiotic Residue Monitoring Programme (ARMP)	Tiger shrimp (<i>P. monodon</i>)	Sarawak	2

E. coli isolates were resistant towards erythromycin (90.7 %), cefepime (26.6 %), tetracycline (18.2 %), ampicillin (15 %), and chloramphenicol (10 %). There was also a small percentage of resistance against colistin (7.3 %). On the other hand, the majority of the *V. parahaemolyticus* isolates were resistant to ampicillin (72.3 %). Resistance towards colistin was

the second-highest in *V. parahaemolyticus* isolates, followed by erythromycin (10 %), cefotaxime (4 %), and tetracycline (4 %). Susceptibility testing of *E. coli* and *V. parahaemolyticus* will be continued in 2018. The antimicrobial susceptibility testing of *E. coli* and *V. parahaemolyticus* isolates against colistin will be repeated with broth dilution assay method.

AMR Awareness and Education

One of the fundamental ways to address AMR in aquaculture is to ensure that fish farms adhere to the best practices for hygiene, biosecurity, and fish care and handling. This reduces the need for antimicrobials in the first place as does vaccinating fish to build their natural ability to withstand disease. To achieve this, the DOF must first improve the awareness and understanding of AMR through effective communication, education, and training. The first activity to initiate the campaign was to organise a series of awareness talks to the farmers, the extension staff of the DF, and the general public. The education and awareness programmes carried out by the DOF in 2017 are in Table 4 and Table 5.

In 2018, the DOF implemented more awareness talks and briefings to the farmers. In addition, the DOF prepared and printed simple, yet interesting posters on AMR and AMU distributed to farmers, drug suppliers and fish feed manufacturers in Malaysia. The DOF also published reports and articles relevant to AMR in fisheries and disseminated materials regarding the best practices.

Strengthening Governance Related to AMU and AMR in Food and Agriculture

The responsible use of veterinary medicines, including antimicrobials, in aquaculture in Malaysia, is governed by many regulations. The most relevant regulations are The Poison Act 1952 (Act No. 366) and Regulations, Poisons Regulation 1952, Feed Act 2009 (Act No. 968), Food Act (1983)(revision 2016) (Act No. 281), Fisheries Act (Act 317) (1985), Malaysian Quarantine and Inspection Services Act (Act 728) (2011), Feed Act (Act 968)(2009)[Section 53(2)(b), (c), (e), (f), (g) and (h)], Inland Fisheries and Aquaculture Enactment (2003) of Sabah State, Law of Sarawak, State Fisheries Ordinance (2003)[Chapter 54] and the latest being the Inland Fisheries Rules (Inland Aquaculture) 2017 (Section 29). Section 29 of the latest Fisheries Rules (Inland Fisheries Aquaculture) (2017), states that any licensee shall not use any aquaculture chemicals except with the approval of the Director-General of Fisheries and any licensee which contravenes this commits an offence.

Table 4. Education and Awareness programs conducted to develop awareness at the Competent Authority level.

Activity/Month	2017											
	3	4	5	6	7	8	9	10	11	12		
a. MyOHUN* National AMR Workshop, Putrajaya (6-9/3/17)	X											
b. FAO Workshop 1, Mangalore, India (10-13/4/17)		X										
c. MyOHUN National AMR Workshop, Faculty of Veterinary, University Putra Malaysia (UPM)(31/7-2/8/2017)							X					
d. FAO Workshop 2, Putrajaya, Malaysia (7-9/8/17)							X					
e. WHO Net Training (28-29/8/17)							X			X		
f. FAO Workshop 3, Singapore (12-14/12/17)												X

*Malaysia One Health University Network.

Table 5. Awareness building within the agriculture sector and activities undertaken to disseminate the information on AMR.

Activity/Month	2017											
	4	5	6	7	8	9	10	11	12			
a. Articles in Fisheries Bulletin (quarterly bulletin by DOF)			X					X				
b. A talk on AMR during Asia Pacific Aquaculture Conference 2017 (Farmers Day)(25/7/2017)				X								
c. A talk on AMR during Fisheries Biosecurity Strengthening Course (1-2 Aug 2017)							X					
d. AMR Awareness and Stakeholders Consultation (4/8/17)- Presented a talk and distributed a Fact Sheet on AMR							X					
e. Executive Talk: AMR in Fisheries							X					

In addition to the acts and regulations, there are also programmes such as the Fish Disease Surveillance Programme, Aquaculture Residue Monitoring Program (ARMP), Sanitary and Phytosanitary Aquaculture, and Malaysia Good Agricultural Practices (myGAP) Certification Programme that monitor, control, and encourage the prudent use of antimicrobials in fisheries.

The DOF also promotes the adoption of best practices in aquaculture, including the use of antimicrobials in aquaculture. This includes monitoring and improvement of protocols on the prudent use of antimicrobials and controlling the distribution of antimicrobials in aquaculture.

Research in vaccine development and on substitutes of antibiotics for use in aquaculture started in the year 2010. The Fisheries Research Institute under the DOF has invented a vaccine developed from a local *Streptococcus* isolate against *Streptococcus* infection in Tilapia (Ismail et al., 2016, Ismail et al., 2017, Sa'aidatun et al., 2018). This vaccine was named StrepToVax and filed as patent in 2015 (Certificate of Filing (COF) No: PI 2015702360). In addition, two herbal-based products SirehMAX (Patent No: MY-176273-A) and SitroPro (COF No: PI 20177031310) to treat bacterial infection and parasitic infestation in cultured marine fish have also been developed and are in the process of commercialisation.

Conclusion

The DOF still has a long way to go in assessing AMU and AMR status in Malaysia. The surveys need to be continued in obtaining data to ascertain the quantity of the antimicrobials used, their sources, and linkages between use and resistance frequencies observed. Participation in FAO workshops has given the DOF staff further information, knowledge, expert guidance, and some financial support to carry out preliminary AMU surveys. Furthermore, the DOF top management has been very supportive of the overall AMR planning and has given consent to proceed with AMR surveillance and training using the DOF's development fund. The activities carried out so far have managed to raise awareness on AMR at the fisheries/aquaculture sector level and enhance the knowledge and capacity of the competent authority.

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Status of Aquaculture Component of the Philippine National Action Plan on Antimicrobial Resistance

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Abstract

The Philippine Bureau of Fisheries and Aquatic Resources, in order to address the issue on antimicrobial resistance (AMR), actively participated in the efforts of the Department of Health in response to the call of the Tripartite Collaboration on AMR, i.e. the World Health Organization, the World Organisation for Animal Health and the Food and Agriculture Organization of the United Nations (FAO) to develop country National Action Plans on AMR using the One Health approach to promote best practices to avoid the emergence and spread of AMR. Thus, "The Philippine National Action Plan to Combat Antimicrobial Resistance: One Health Approach" (PNAP) has evolved and continuously being improved. At present, studies on AMR and antimicrobial use in the Philippine aquatic sector are very limited. The BFAR participated in the FAO project FMM/RAS/298 Strengthening Capacities, Policies and National Action Plans on Prudent and Responsible Use of Antimicrobials in Fisheries which enhanced the understanding of AMR in aquaculture and laboratory capacity. As part of the project, a survey on the use of antimicrobials in Philippine aquaculture was conducted. Eighty four respondents from the shrimp and tilapia farming sectors and two aquaculture product suppliers participated. Results showed that antibiotics are no longer applied in participating grow-out farms monitored for residues and there has been no detection of antibiotics in shrimp or fish meat from registered farms. About 77 % of the respondents know the regulations on the use of antimicrobials and the majority of them are aware of banned and regulated antibiotics in food animals.

Keywords: antimicrobial resistance, One Health, aquaculture

Introduction

Aquaculture in the Philippines has a long history and involves many species and farming practices in diverse ecosystems. Most of the production comes from the farming of the seaweed *Kappaphycus* spp., milkfish *Chanos chanos* (Forsskål, 1775), tilapia *Oreochromis niloticus* (Linnaeus, 1758), and the shrimps *Penaeus monodon* Fabricius, 1798 and *Penaeus vannamei* Boone, 1931. The Philippines used to be one of the top shrimp producing countries in the world, but after its decline in the late 1990s, mainly because of disease problems, shrimp production remains low. At present, the Philippines is the 11th largest producer of fish and fishery products due to government interventions and private-sector efforts to increase production.

The condition in many aquaculture systems, whereby animals are confined in a relatively small space during culture, causes stress and make them susceptible to diseases. Aquafarmers use antibiotics available in the market to cure disease. Previous studies by Baticados and Paclibare (1992), Primavera et al. (1993), Lacierda et al. (1996), Somga et al. (2012), Alday-Sanz et al. (2012), showed that farmers used antibiotics in aquaculture.

The imprudent use of antimicrobials in human health is recognised as a major contributor to antimicrobial resistance (AMR) in human pathogens. There are instances where antimicrobials used in both food-producing and companion animals, including aquatic animals are key contributing factors.

Governance

It is in this context that the Bureau of Fisheries and Aquatic Resources (BFAR), to ensure safety of fish food, currently implements an antimicrobial residue monitoring program that includes control of veterinary drugs in aquaculture and the prevention of movement of contaminated fish and fishery products. These are also required by trading partners of the Philippines.

The BFAR is the government agency responsible for addressing the issue of AMR in aquaculture. This authority is provided by Republic Act (RA) 10611- "Food Safety Act of 2013" and RA 8550 - "The Philippine Fisheries Code of 1998" and its revision, RA 10654 - "An Act to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated Fishing". The National Fisheries Laboratory Division (NFLD) is in charge of the development of plans and programs on AMR. Such programs are supported by a network of fish health officers (FHOs) deployed in various regions throughout the country.

In response to the World Health Organization's (WHO) endorsement of the One Health approach to combat AMR during the 2011 World Health Assembly, the Philippines developed the "Philippine National Action Plan to Combat Antimicrobial Resistance: One Health Approach" (PNAP), launched in 2015 during the First Philippine AMR Summit.

The PNAP outlined an integrated approach by concerned agencies to prevent the occurrence of AMR. BFAR identified seven key strategies relevant to the aquaculture sector. These are:

1. Commit to a comprehensive, financed national plan with accountability and civic society engagement
2. Strengthen surveillance and laboratory capacity
3. Ensure uninterrupted access to essential medicines of assured quality
4. Regulate and promote rational use of medicines, including in animal husbandry and ensure proper patient care
5. Enhance infection prevention and control across all settings
6. Foster innovations, research, and development
7. Development of a risk communication plan to combat AMR

The objective of this paper is to share information on two aspects, namely: 1) brief description of activities and achievements on AMR in aquaculture relevant to the four pillars of the Food and Aquaculture Organization's (FAO) Action Plan on AMR, i.e., governance, awareness, evidence, and best practice including impacts; and 2) outcome of the survey on antimicrobial use (AMU) in aquaculture.

Governance of AMU and AMR in aquaculture is a collaborative effort between the Food and Drug Administration of the Department of Health (FDA/DOH) and the BFAR of the Department of Agriculture (BFAR/DA). The scope of the FDA/DOH mandate includes licensing and registration of drug establishments, registration of products and evaluation, assessment and approval of veterinary drugs used in aquaculture. The BFAR is responsible for monitoring and surveillance activities pertaining to: 1) health status of cultured animals in aquaculture farms; 2) usage of drugs in the primary and post aquaculture farms; and 3) drugs incorporated in the aquafeeds. The monitoring and surveillance system is evolving and continuously being improved particularly on building capability on risk-based inspection as well as strengthening the existing manual of operation of the National Residue Control Program (NRCP).

Under the PNAP, an Inter-agency Committee on AMR (ICAMR) was created and composed of representation from different concerned government agencies including the NFLD. The ICAMR follows a coordinated approach in developing and implementing intervention strategies for AMR stewardship in the human and animal sectors.

The PNAP, which the BFAR is continuously refining, closely resembles the FAO's Action Plan on AMR in its focus on four pillars within the seven key strategies. It issued Fisheries Office Order 104, series of 2018, designating the AMR Regional Coordinators of BFAR for better farmer engagement and smoother implementation of programs and activities to address issues of AMR.

Several national laws give the BFAR authority and responsibility for AMR. The relevant provisions to ensure enforcement and management on the use of drugs and AMR are RA 10611 and RA 10654.

Republic Act 10611, the "Food Safety Act of 2013", states that the BFAR shall be responsible for the development and enforcement of food safety standards and regulations for fresh fish and other seafoods including those grown by aquaculture, in the primary production and post-harvest stages of the food supply chain. The BFAR shall be responsible for the registration of food businesses, licensing of fishery establishments, official certification of products and services, official accreditation of inspection and certifying bodies, and other official controls prescribed by the regulatory system and in compliance with the international commitments.

Section 62 of Republic Act 8550, known as the "Philippine Fisheries Code of 1998", as amended by RA 10654, known as "An Act to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated Fishing" states that all fish and fishery products for export, import and domestic consumption shall meet the quality grades/standards, labelling and information requirements set by the DA-Bureau of Agriculture and Fisheries Standards (DA/BAFS) and by the BFAR as well as international standards set by the Codex Alimentarius Commission (CAC).

BFAR also assists the BAFS in the development of Philippine National Standards (PNS) on codes of aquaculture practices. Examples of these are: PNS/BAFS (2014), PNS/BAFS (2017a), PNS/BAFS (2017b).

RA 10654 mandates BFAR to develop and implement a 5-year Comprehensive Fisheries Development Plan focusing among others to food security by increasing aquaculture production in key species. Major programs are implemented in order to provide safe and quality fish free from hazardous substances like antibiotics (BFAR, 2016).

Regional efforts also play an active role in responsible AMU. Guidelines for the Association of Southeast Asian Nations (ASEAN) was developed for the competent authority to regulate the use of veterinary drugs and chemicals in aquaculture (ASEAN, 2013). It also aims to develop measures to eliminate the use of harmful chemicals in aquaculture. The document includes the list of veterinary drugs and chemicals that are banned and those that are allowed to be used by ASEAN member countries.

Awareness

Several activities in the form of fora, symposia, technical assistance, training programs and other activities are regularly being conducted to ensure that plans, programs and modern technologies, and

information are imparted to various stakeholders including the use of drugs and good practices.

The BFAR actively continues to participate in several department-wide food safety activities of the DA. In these programs, BFAR provides information on food safety, farm registration, disease and residue monitoring, and surveillance programs to stakeholders at the local government unit level. At every opportunity, in the trainings and meetings that BFAR conducted, participants were made aware of the issue of AMR. These activities include:

- a) Food Safety Road Shows in the islands of Luzon, Visayas and Mindanao where policy, codes of practice like good aquaculture practice (GAqP), and the FAO project FMM/RAS/298 were discussed and disseminated to stakeholders for their information and compliance to the requirements;
- b) Industry Congresses, such as the National Tilapia Congress and National Shrimp Congress, provided awareness on food safety requirements and updates on fish health;
- c) Training on GAqP which updated the sector on prudent use of antimicrobials;
- d) Participation in the celebration of the World Antibiotic Awareness Week where invited stakeholders shared good aquaculture practice in shrimp farming in Negros province;
- e) National Planning Workshops of FHOs which discussed, among others, the implementation of the FAO project FMM/RAS/298 and shared the knowledge and experience gained from the participation of the Philippine delegates to the three regional workshops of said project.

Other details on training activities are in Tables 1 and 2.

Table 1. Trainings, workshops and public consultations held by Bureau of Fisheries and Aquatic Resources on antimicrobial resistance related topics in 2017.

	Title of activity	Date/ Location	Scope	Number of participants
1	Training Workshops on the Implementation of Philippine GAqP*	March 1- 4/ Metro Manila March 13-16, Cebu City March 21-24/ Davao City	Capacity building BFAR production and extension officers	50
2	Public Consultations			
	a. GAqP for Seaweeds and Soft-Shell Crab (SSC) Product Standard	June 5-7/Luzon	Stakeholders consulted prior to approval of the Standard	50
	b. GAqP for Seaweeds and Mollusks- Product Standard	June 13-15/Visayas		50
	c. GAqP for Mollusks and SSC Product Standard	June 19-21/Mindanao		50

*EU-TRTA-technical and funding assistance.

Table 2. Training/workshops on strengthening laboratory capability held by Bureau of Fisheries and Aquatic Resources in 2017.

Date	Title	Scope	Number of participants
May 15-19	Workshop on the Harmonisation of Central and Regional Fisheries Laboratories	ISO/IEC 17025:2005 Standards	60
June 27-30	Training on the Detection of the Tilapia Lake Virus (TiLV) and Hepatopancreatic Microsporidiosis (HPM) in Shrimp	Detection of tilapia lake virus (TiLV) using PCR Detection <i>Enterocytozoon hepatopenaei</i> (EHP), the causative agent of HPM in shrimp	35
September 4-8	Workshop on the Standardisation of the Analytical and Sampling Methods of the Central and Regional Fisheries Laboratories	Developed manual of operations, including standard procedures for laboratory analyses and sampling procedures	60
September 18-22 and 25-29	Training of the Central and Regional Fish Health Laboratory Analysts on Standard Fry Quality Assessment in Shrimp	Trained the BFAR fish health laboratory analysts on shrimp fry quality criteria including the physical, morphological and behavioural characterisations	50
November 6-10	Training in Histopathological Examination of Shrimp Tissues for, new and emerging significant diseases	Strengthened the capability of laboratory analysts in disease diagnosis by histopathological examination	15
November 20-24	Year-end review and Planning Workshop of the Network of Activities by the fisheries laboratories	National and regional plans targeting and harmonisation of monitoring surveillance inspection and reporting system	60
December 4-8	Lecture on Biotechnology 101: Basic Molecular Techniques in the Diagnosis of Diseases	Capability building of laboratory analyst	25

Evidence

To support PNAP's key strategy 2, several initiatives focused on surveillance and monitoring system for AMU and AMR in food-producing animals. These initiatives include:

1. National Residue Control Program

The NRCP aims to monitor drug use and its residues in fish and fishery products by conducting national sampling to detect residues in economically important aquaculture products, namely tilapia shrimp, and milkfish. Sampling is based on production statistics, i.e. one sample for every 100 tons of production. The yearly sample target depends on the regional production data based on risk and the result of the previous year's monitoring data. One sample can be subjected to one or more residue analyses.

The NRCP includes farm registration based on compliance with the minimum requirement of GAqP. Activities include inspection on farm hygiene, disease surveillance and residue monitoring, and risk-based sampling. Sampling is also conducted at the feed mills. The aquatic feed mills are regulated by the Bureau of Animal Industry (BAI); sampling activities are conducted

by FHOs who are deputised as Aquatic Animal Feed, Veterinary Drug and Control Officers.

The regulation on antimicrobials such as the banned antibiotics is issued through the Joint Administrative Order of the DOH and DA, and other antibiotics that are allowed for use but have maximum residue limits (MRLs).

There are five BFAR laboratories that conduct antibiotic residue analysis covering the whole country. The NFLD and Regional Fisheries Laboratories of Regions VI and VII are accredited with ISO/IEC 17025:2005 by the Philippine Accreditation Bureau. The Regional Fisheries Laboratories of Regions III and IV-A are currently preparing to apply for accreditation. Analysis can also be conducted by recognised third-party laboratories should the need arise.

A total of 2,130 analyses on residues from shrimp, milkfish, tilapia and feed samples from the monitoring activities were conducted in 2016. One sample of shrimp was detected to contain chlortetracycline above its MRL. Table 3 provides more details.

Table 3. Summary of sampling of fish tissues and feed done in 2016 and 2017 for analysis of antibiotic, chemicals and drug residues.

Analyses	2016				2017			
	Sample type				Sample type			
	Shrimp	Milkfish	Tilapia	Feeds	Shrimp	Milkfish	Tilapia	Feeds
a. Unauthorised substances								
Chloramphenicol	116	203	89	205	114	214	86	146
Furazolidone(AOZ)	122	205	89	-	113	211	86	-
Furaltadone (AMOZ)	122	205	88	-	113	211	86	-
Nitrofurantoin(AHD)	2	1	0	-	0	0	0	-
Nitrofurazone(SEM)	2	1	0	-	0	0	0	-
Diethylbestrol	0	28	0	-	0	36	0	-
Nitroimidazole	0	0	0	-	5	0	0	-
b. Antibacterials								
Chlortetracycline	23	41	4	8	49	66	5	91
Oxytetracycline	22	41	4	4	56	66	5	112
Sulfamethazine	21	41	4	4	47	66	5	26
Amoxicillin	6	4	1	-	5	5	0	-
Trimetoprim	6	4	1	-	5	5	0	-
Sulfadiazine	6	4	1	-	5	5	0	-
Oxilinic Acid	10	5	0	-	14	4	0	-
Erythromycin	10	5	0	-	14	4	0	-
Florfenicol	10	5	0	-	14	4	0	-
c. Anthelmintic								
Ivermectin	0	39	0	-	6	47	0	-
d. Mycotoxin								
Aflatoxin	0	0	0	93	0	0	0	144
e. Chemical elements								
Lead	17	25	2	-	36	30	7	-
Cadmium	17	25	2	-	36	30	7	-
Mercury	17	25	2	-	36	30	7	-
f. Organochlorine compound								
Organochlorines	15	21	0	-	25	22	4	-
g. Dyes								
Malachite Green	14	13	0	-	26	22	3	-
Leucomalachite Green	14	13	0	-	26	22	3	-
h. Ethoxyquin								
Ethoxyquin	0	0	0	3	0	0	0	10
Total	572	954	287	317	745	1100	304	529

The FDA, BFAR, and the BAI are now working on the best arrangements to prevent the irresponsible use of drugs and prevention of the occurrence of AMR.

2. Strengthening capacities, policies, and national action plans on the prudent and responsible use of antimicrobials in fisheries, and strengthening laboratory capability

The AMR surveillance plan in aquaculture developed during the first regional workshop of the FAO project

FMM/RAS/298, held in Mangalore, India is now integrated in another FAO project covering terrestrial and aquatic animals. Aquaculture is under the project's third component: AMR surveillance of bacterial pathogens from diseased aquatic animals specifically, tilapia, milkfish and shrimp. This runs parallel with the surveillance of patients who have been diagnosed to be infected with AMR-resistant bacteria. Its objective is to develop evidence-based clinical guidelines for veterinarians on the proper use of antimicrobials in aquatic animals.

The AMR surveillance plan was developed during the Mangalore workshop, with the following details:

- i. Target population include the following species, namely: tilapia (*O. niloticus*), milkfish (*C. chanos*) and shrimp (*P. vannamei* and *P. monodon*)
- ii. Study population: farms that are registered at BFAR
- iii. Samples sources: Luzon area
- iv. Sample size: this depends on case finding/syndromic surveillance on fish farms
- v. Target organisms: *Streptococcus agalactiae* and *Streptococcus iniae* in tilapia, *Vibrio parahaemolyticus* in milkfish and shrimp
- vi. Sampling strategy: 10 % of the BFAR registered farms in each identified region; farm with cases of mortalities and disease outbreaks
- vii. Logistics: sample collection by FHOs; bacterial isolation and identification, antimicrobial susceptibility test by the NFLD.

At present, implementation of above plan is continuing; in addition, laboratory analysts have undergone appropriate trainings on AMR detection conducted by BAI; methods for AMR detection are being optimised; and work is continuing on procurement of reagents and consumables for sampling and laboratory analysis.

Best Practice

Best practice is synonymous with the PNAP's key strategy N5. The BFAR's strategy included programs on infection prevention and control such as the implementation of GAqP and strengthening animal health. Significant resources and efforts were invested on the NRCP and control of the use of drugs in the fishery sector. While there are questions on the association between AMR and AMU, it is certain that AMR would not exist if antibiotics would not be used in the first place.

Farm registration is not mandatory. In the aquafarm registration program, farmers send their applications to the BFAR regional offices and must comply with the required documentation. They must also allow BFAR FHOs to conduct inspection and sampling. The registered farms are monitored on their compliance to minimum requirement to GAqP by the regional FHOs.

The Philippine National Standard (PNS) on good aquaculture practices for different farmed species include the following:

1. PNS/BAFS 135 Code of Good Aquaculture Practice (GAqP)(PNS/BAFS, 2014)

2. PNS/BAFS 196Code of GAqP for Milkfish and Tilapia(PNS/BAFS, 2017a)
3. PNS/BAFS 197foCode of GAqP for Shrimp and Crab(PNS/BAFS, 2017b)

The Code on GAqP and mechanism for implementation was developed with technical and funding assistance from the European Union through the Trade-Related Technical Assistance.

The BFAR-accredited processing plants source their raw materials only from registered farms as part of the Hazard Analysis and Critical Control Point (HACCP) food safety system. This requirement compels farmers to be registered and comply with minimum requirements on GAqP. Further, an explanatory brochure for GAqP is being developed by the BAFS in coordination with BFAR to make it easy to understand and comply with.

Survey of AMU in Philippine Aquaculture

One of the components of the FAO project FMM/RAS/298 is the conduct of a survey on the use of antimicrobials in selected areas of the Philippines from November to December 2017. The survey aimed to understand the current status of the use of antimicrobials and other products in tilapia and shrimp aquaculture in the Philippines.

Survey structure

A survey questionnaire was developed with five sections, briefly described below:

1. Farm Information (owner, address, species, farm area, type of operation system based on stocking density, length of production cycle, number of production cycles per year)
2. Use of Antimicrobials (type of antimicrobials and other chemicals, mode of application, dosage, purpose of use, duration of use, withdrawal period, source of antimicrobial, veterinary prescription required, disposal of antimicrobials, monitoring of residue)
3. Disease Occurrence (major disease problem during culture operations, production losses, reporting the outbreak(s) to Competent Authority, use of antimicrobials during disease outbreak, efficacy)
4. Market Information (domestic or export market, sales through a middleman or direct to a processing plant)
5. National Regulations (awareness of national regulations on the use of antimicrobials, adoption of recommended management practices on the prudent use of antimicrobials, on-farm inspection by FHOs)

Since the use of antimicrobials is a sensitive matter because of its implications to product-market access, respondents were kept anonymous.

Distribution of the questionnaire

The survey targeted tilapia and shrimp farmers and distributors of aquaculture products. Face-to-face and telephone interviews with 86 respondents were conducted by 11 FHOs in 21 provinces throughout the country.

Results and Discussion

Survey on the use of drugs in aquaculture

The survey had a total of 84 respondents composed of 36 tilapia grow-out farmers, 48 shrimp hatchery and grow-out farmers, and additional two suppliers of aquaculture products.

The survey was conducted by the FHOs and provinces covered are indicated in Figure 1 below.

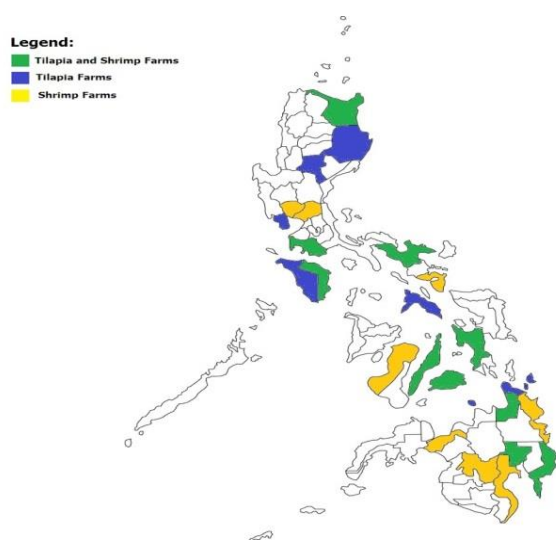


Fig. 1. Provinces in the Philippines where the survey on the use antimicrobials was conducted.

Use of antimicrobials

Among the farmer respondents (Table 4), 57 (68 %) were not using antimicrobials in their culture operation, while 27 (32 %) used probiotics, chemicals such as disinfectants and antibiotics. The antibiotic oxytetracycline is particularly used in shrimp hatchery operation. In grow-out systems, however, antibiotics were no longer applied since farms were being monitored for residues. There has been no detection of antibiotics in shrimp or fish meat from

registered farms.

The application of antimicrobials is usually resorted to upon the recommendation by either the farm manager, suppliers, technicians in neighbouring farms, or consultants. Antimicrobials are usually used as prophylaxis and seldom for treatment and growth enhancer. Most of the products were sourced either in the local market or distributors and from technicians of feed companies.

Disease occurrence

There were diseases, health problems and mortalities reported by the respondents in shrimp and tilapia culture (Table 5). According to 40 respondents (48 %), disease problems were encountered. Shrimp farmers in the Negros Province report and send samples to the Negros Prawn Producers Cooperative laboratory in Bacolod City. Many respondents did not respond to the disease section of the survey thus highlighting the sensitivity of collecting such information.

In other cases, some shrimp farmers continue to use probiotics with increased frequency of application and some farmers apply twice the required dose. According to them, these practices are effective in improving the health condition of the stock or treating disease problems. In tilapia farming, 25 respondents (69 %) mentioned that they had not experienced disease in their farms. In most cases, tilapia diseases are mixed infection of bacteria and parasites predisposed by poor environmental conditions brought by overstocking, improper feeding practices, and poor water quality.

Market information

Aside from the domestic markets, 13 farms (15 %) supply their harvest for export. Harvests mainly go to local markets within the provinces. Some shrimp farms sell through a middleman and some supply directly to the processing plants.

Awareness of national regulation on the use of antimicrobials

As to awareness of national regulations on the use of antimicrobials, 65 respondents (77 %) mentioned that they know the regulations. Sixty respondents (71 %) are also aware of banned antibiotics, particularly chloramphenicol, in food-producing animals including aquatic animals. Most of them are from registered farms. Respondents who were not yet aware of the regulations were provided with the information on banned antibiotics in the Philippines, and the prudent use of antimicrobials allowed for use in aquaculture.

There were 30 respondents (36 %) that followed the recommended practices provided by the FHOs,

Table 4. Chemicals and biological products used by 84 respondents that participated in the survey on use of antimicrobials in tilapia and shrimp aquaculture the Philippines.

Products used by respondents	Commercial name	Application	Withdrawal period
a. Probiotics			
	Sanolife Pro-2	Feed	N/A
	Sanolife Pro W	Water/soil	N/A
	Pond Plus	Soil	N/A
	Pond Dtox	Soil	N/A
	TOP S	Feed	N/A
	Super PS	Water/soil/feed	N/A
	EM-1	Water/feed	N/A
b. Disinfectant			
	PUR	Foot bath	7 days
		Hard surfaces and all equipment	7 days
		Water of grow-out pond for routine control of bacteria and viruses	7 days
		During bacterial challenge	7 days
	Virkon	Mixed in water	7 days
	Chlorine	Mixed in water	10 days at > 22 °C
	Lime	Soil/water	N/A
	Dolomite	Water/Soil	N/A
c. Immunostimulant			
	Beta Defense	Water/feed	N/A
d. Feed supplement			
	Aquamin	Feed	N/A
e. Antibiotics			
	Oxytetracycline	Feed	15 days at >22 °C

Table 5. Diseases and mortalities reported by 40 respondents who participated in a survey of tilapia and shrimp diseases in the Philippines.

Causes of disease and mortalities	No. of respondents
Shrimp	
Viral diseases	26
a. White Spot Disease	
b. Infectious Hypodermal and Hematopoietic Necrosis Virus	
Bacterial diseases	3
a. Luminous bacterial disease	
b. Acute Hepatopancreatic Necrosis Disease	
Parasite (<i>Enterocytozoon hepatopenaei</i>)	2
Tilapia	
Parasites	5
Bacterial disease	1
a. Streptococcal infection	
b. Infection with <i>Aeromonas hydrophila</i>	
Mortalities due to poor water quality, specifically low Dissolved Oxygen	17

extension officers, and salesmen from feed and aquatic product companies. The 54 respondents (64 %) that did not provide comments believe that their practices based on their experiences work out for them. The advocacy of the government in promoting the Code of GAqP and training for stakeholders will help them improve their practices.

As part of the NRCP, registered farms are inspected and monitored for both banned and regulated antimicrobials, chemical elements, and dyes such as malachite green. Seventy respondents (83 %) mentioned that they were regularly inspected by BFAR relative to the use of antimicrobials.

Conclusion

The survey on AMU provided the information on the common antimicrobials applied by fish farmers. Majority of the farmers interviewed, particularly those involved in shrimp farming use probiotics. Those that are registered with BFAR has knowledge on possible residue build-up of antibiotics and are advised to observe withdrawal periods in case of usage of antibiotics that are allowed for use in aquaculture. Using regulated antibiotics requires a prescription from a veterinarian and application should be according to the product label. Antimicrobials are being regulated by the FDA and registration of some antimicrobials for animals including aquatic animals is delegated to the BAI. Its use in aquaculture is monitored through the NRCP.

The level of awareness among the farmers on the regulations about antimicrobials and banned antibiotics can be attributed to the promotion of the GAqP, where requirements on the usage of drugs and chemicals are included. However, continuous information, education campaign on GAqP are needed so that more farmers would become aware and the explanatory brochure will make it easy to understand.

Although considerable accomplishments were achieved in the monitoring and surveillance of the use of antibiotics, there is a need to strengthen the capabilities of the BFAR laboratories to detect AMR in the fishery sector.

The FAO Project FMM/RAS/298 builds upon the previous projects on strengthening national aquatic animal health programs and has helped BFAR be a better policymaker when it comes to AMR. It also allowed BFAR to realise the relevance of AMR prevention in aquaculture to AMR. In addition, international standard-setting bodies like the FAO/WHO on Codex Alimentarius and the OIE on animal (terrestrial and aquatic) health standards and many relevant national, regional and international entities are providing the necessary push and assistance on activities related to AMR in aquaculture.

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Singapore's National Action Plan on Antimicrobial Resistance

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Abstract

The rapid emergence and spread of antimicrobial resistance (AMR) is a global threat, and international bodies have called for action against it. Studies show that Singapore is not spared from its effects. There was strong local political support for action and One Health agencies assembled a One Health AMR Workgroup to jointly develop the National Strategic Action Plan (NSAP) for Antimicrobial Resistance. The NSAP would set the framework for Singapore's response to AMR and would be aligned with the World Health Organisation's Global Action Plan on AMR with reference made to the standards and guidelines established by intergovernmental bodies such as the Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health. This paper describes existing initiatives to combat AMR, and lists priority areas for further action akin to the WHO Global Action Plan for AMR. These priority areas include education, surveillance and risk assessment, research, prevention and control of infection, and optimisation of antimicrobial use. Today, the One Health AMR Workgroup comprises of the Ministry of Health, the Health Promotion Board, the National Environment Agency, National Parks Board, PUB, Singapore's National Water Agency and the Singapore Food Agency.

Keywords: antimicrobial resistance, One Health, antibiotics, aquaculture

Introduction

The rapid emergence and spread of antimicrobial resistance (AMR) is a pressing threat to health globally (Robinson et al., 2016). As microbes become increasingly resistant to antimicrobial agents, the ability to treat infections are becoming increasingly compromised. Aside from prolonged illnesses and increased mortality, AMR can lead to decreased productivity, higher treatment costs, and economic losses (O'Neill, 2018). Singapore sees similar trends in the creeping costs of AMR. In a 2012 study on the financial burden of multi-drug resistant (MDR) Gram-negative infections in Singapore hospitals, excess hospitalisation costs attributable to MDR infection was shown to be about SG\$ 8,640 per person, and 62 % of the excess cost attributed to MDR infection was paid for by government subvention (Ng et al., 2012). Such infections were also associated with longer hospitalisation, with the excess length of stay

attributable to MDR infections of 6.1 days after adjustment for confounders (Lye et al., 2012).

AMR is a transboundary, inter-sectoral issue as it can spread across borders through international human, animal, and food movement. Resistance in each sector, e.g. human, animal, and environment, can have direct and indirect consequences on other sectors. A study in Korea showed that fishery workers exposed to antibiotic use in aquaculture had significantly higher resistance to cephalothin, tetracycline and trimethoprim-sulfamethoxazole compared to non-fishery workers (Shin and Cho, 2013). As Singapore is an international travel and transshipment hub that imports more than 90 % of its food, AMR poses a threat to Singapore's public and animal health and food security. Livestock farming in Singapore is very limited and predominantly chicken layer and ruminant dairy production. In contrast, Singapore has a small, but thriving and increasingly important food fish

aquaculture industry, which currently accounts for about 10 % of local food fish consumption (Lim et al., 2020), but has been rising through the years. As Singapore aims to produce 30% of the population's nutritional needs locally by 2030 (MSE, 2020), we envisage that the local aquaculture sector would continue to develop and expand in the long term.

Recognising the negative impact of AMR, Singapore joined the global call for action against AMR, beginning with the development of a multi-sectoral national plan to combat the threat of AMR. We describe here the development and implementation of Singapore's National Strategic Action Plan (NSAP) (OH AMRWG, 2017). The NSAP unifies and formalises the existing responses mounted across the animal, human, food, and environment sectors, while providing a roadmap to address existing gaps and prioritise future interventions.

Approach for Developing the NSAP

At the global level, the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), and the World Organisation for Animal Health (OIE) have agreed that addressing AMR requires a "One Health" approach (WHO, 2016). This concept of a tripartite collaboration on addressing health risks at the animal-human-ecosystems interfaces was formalised in 2010. In Singapore, the One Health Coordinating Committee (OHCC) was established in 2012 and provides strategic oversight including setting priorities, reviewing One Health policies and programmes, as well as promoting inter-agency coordination and collaboration. The OHCC convened a One Health AMR Workgroup in January 2017 that composed of representatives from the Ministry of Health (MOH), then-Agri-Food and Veterinary Authority (AVA), the National Environment Agency (NEA) and the PUB, Singapore's National Water Agency. From 1 April 2019, all food-related functions carried out by then-AVA, are carried out by a new statutory board, the Singapore Food Agency (SFA). In parallel, non-food animal and plant related functions of then-AVA are transferred to the National Parks Board (NParks). SFA is the lead agency for all matters related to food safety and security, and NParks is the lead agency for animal health, welfare, and management, as well as plant health. Both agencies continue to be part of the Workgroup. The One Health AMR Workgroup also included infectious diseases and public health professionals from public hospitals and the Saw Swee Hock School of Public Health, which were actively involved in AMR surveillance, research, and education. This One Health AMR Workgroup, led by the MOH, compiled and coordinated efforts to combat AMR, which included the identification of priority areas for action across human, animal, food, environment, and water sectors. In addition to the Workgroup, three multi-sectoral sub-working groups were formed to

help identify and coordinate cross-sectoral initiatives in education, surveillance, and research.

From the start, agencies agreed that the NSAP would set the framework for Singapore's response to AMR and would be aligned with the WHO Global Action Plan on AMR with reference made to the standards and guidelines established by intergovernmental bodies such as FAO and OIE. Following a series of meetings and discussions, the plan was launched on 01 November 2017. Today, the One Health AMR Workgroup comprises of MOH, the Health Promotion Board (HPB, the agency which oversees public education), NEA, NParks, PUB and the SFA.

The NSAP aims to reduce the emergence and spread of drug-resistant organisms through the five core strategies: a) education, b) surveillance and risk assessment, c) research, d) prevention and control of infection, and e) optimisation of antimicrobial use.

Under each of the five strategies, the NSAP describes existing AMR initiatives by the relevant agencies while articulating gaps and priority areas for further action, which were identified as strategic areas requiring attention. Initiatives were examined to see how cross-sectoral action and collective efforts with international partners and local stakeholders could be initiated, coordinated, or expanded. At the same time, the NSAP looks at specific initiatives for each key stakeholder group, which includes the public, professionals (e.g. doctors, veterinarians), and the industry (e.g. farmers, wholesalers, feed mill operators). While the overarching strategies to address AMR in the NSAP were designed with a long-term perspective, an interim time frame of five years was used for developing and implementing the initial programme and activities, with periodic review to ensure relevance and effectiveness.

Implementation of the NSAP

It was recognised in the early stages of development that a central body would be needed to coordinate the implementation of the NSAP and facilitate information sharing. In September 2018, the MOH established the AMR Coordinating Office (AMRCO) under the auspices of the National Centre for Infectious Diseases (NCID). Following the launch of the NSAP, a detailed multi-sectoral five-year work plan together with a reiterative monitoring and evaluation (M&E) framework, was laid out by the Workgroup to coordinate and track the progress of implementation across all sectors. The NSAP M&E framework identifies specific output and outcome indicators recommended for the monitoring of the Global Action Plan (WHO, 2015). Implementation of Singapore's NSAP is now underway; some of the initiatives under each of the five core strategies being implemented are described below.

Education

Education is essential to ensure that all stakeholders, including the public, have a correct understanding and perception of AMR's impact on health and society, as well as what they can do to combat AMR. Surveys have shown that the public has many misconceptions about antibiotics, including the manner that they should be used (Pan et al., 2016). Agencies agreed that a national strategy should be coordinated to improve awareness and understanding of AMR and to present consistent messages to the public and target audiences. In 2018 and 2019, public education campaigns were launched to educate the public on appropriate antibiotic use, that antibiotics do not work on the flu virus and does not hasten flu recovery. One of the mainstay messages for consumers is that AMR has an impact on all stakeholders along the food chain, and therefore food safety and good hygiene during handling, cooking, and storing food is important.

Targeted education and communication campaigns were established as a priority area for further action for the professional and industry groups. Veterinarians play a critical role in promoting and ensuring the responsible use of antimicrobials for animal health and welfare. NParks maintains active engagements with the veterinary professionals through current AMR awareness events that leverage on international events such as World Veterinary Day and World Antimicrobial Awareness Week. Through seminars and AMR articles in newsletters, NParks will also work on capability building and continuing professional education development programmes to help veterinarians exercise antimicrobial stewardship. For other industry stakeholders, it was recognised that educational efforts on infection and disease control must form a big part of the efforts to promote the prudent and responsible use of antimicrobials in animals. For the food aquaculture sector, SFA maintains a voluntary farm quality assurance scheme known as the Good Aquaculture Practices for Fish Farming (GAP-FF) (SFA, 2020), which will reduce the reliance on antimicrobials through the reduction of incidence of infections. In addition, as part of SFA's efforts to raise public awareness of AMR, SFA published an informative segment on AMR on its webpage and also developed an animation video to educate the public on the importance of AMR and what they can do to reduce the spread.

Surveillance and risk assessment

Detection of AMR through surveillance, coupled with risk assessment, enables timely and appropriate response to be mounted. However, monitoring of resistance trends will require surveillance data on AMR patterns, good epidemiological information of infection rates, antimicrobial usage, and health outcomes. AMR surveillance is also necessary to

measure, monitor, and evaluate the programme outcomes and overall impact of the NSAP.

A key objective of the NSAP is an integrated surveillance programme for antimicrobial resistance and utilisation across the human, animal, food, and environmental sectors. This would shed light on how specific resistance develops and spread between humans and animals, through food, water, and environment locally. As a first step towards integration, a joint report of existing surveillance data on resistance and utilisation has been published (OH AMRWG, 2019).

With respect to the food production and supply chain, Singapore monitors antimicrobial sales for agricultural use at the wholesalers' level, and utilisation based on antimicrobials stored at the farm. As Singapore imports most of its food, the National Centre for Food Science (NCFS), a designated OIE Collaborating Centre for Food Safety, focuses on surveillance for drug residues and monitoring of AMR bacteria in food, both locally produced and imported. Resistant pathogens monitored include multi-drug resistant (MDR) *Salmonella*, methicillin-resistant *Staphylococcus aureus* (MRSA), and extended-spectrum beta-lactamases (ESBL)-producing *Escherichia coli*. As one of the NSAP initiatives, existing surveillance for *Salmonella enterica* serovar Enteritidis in local chicken layer farms were recently extended to include all *Salmonella* species and *E. coli* in all local poultry and ruminant dairy farms. Singapore also conducts AMR testing for pathogens in diseased fish. AMR surveillance in food would subsequently be expanded to include retail food outlets, using a risk-based approach that takes into consideration antimicrobials of veterinary and human health importance in the OIE and WHO lists, respectively. The farm to fork surveillance in Singapore will better inform on the potential risk areas along the food supply chain, for targeted efforts to combat AMR.

Research

It was recognised that cross-sectoral AMR research was a gap that needed to be addressed as part of the NSAP. Consultations with the local research community had identified the following priority research areas for cross-sectoral collaborations:

- a) Transmission pathways between sectors (human, animal, food, and the environment) and the implications of cross-sectoral transmission on efforts to control AMR.
- b) Attitudes, practices, and knowledge of AMR, to determine how best to change behaviour to facilitate its control.
- c) Socio-economic impact of AMR.

A One Health AMR research grant is being established, which would specifically fund cross-sectoral research in these three focus areas.

Another priority area for further action in the aquaculture sector is to engage relevant stakeholders (local research groups, biomedical companies, feed producers, vaccine developers and academic institutions) and facilitate applied research into the development of viable alternatives to reduce the widespread use of antimicrobials in the aquaculture sector. Equally important is the collection of scientific evidence and cost-benefit studies to support and encourage the use of such alternatives. Of note is that a recently published qualitative study showed that the use of antibiotics for growth promotion was uncommon in Singapore food fish farms (Lim et al., 2020).

Prevention and Control of Infection

Every infection prevented means one less opportunity for antimicrobial use and for organisms to develop resistance. Thus, vaccination and other infection prevention and control measures are important to limit the development and spread of AMR.

The SFA and NParks currently implement both regulatory (e.g. licensing conditions, vaccine registration) and non-regulatory guidelines (e.g. good animal husbandry practices) for farms. In the future, local guidelines will be harmonised with the ASEAN (Association of Southeast Asian Nations) guidelines for Good Animal Husbandry Practices (GAHP) and Good Aquaculture Practices (GAP), developed by the ASEAN Sectoral Working Groups for Livestock and Fisheries, respectively. Local guidelines are actively promoted to the local farmers to improve animal health management practices. At the same time, Singapore will continue to highlight the benefits of vaccination and facilitate access to safe and effective vaccines of the farming industry. Several autogenous fish vaccines are currently registered and permitted for use in Singapore, and national guidelines on their use and production are being developed. In tandem, NParks is in the process of reviewing vaccine evaluation and pharmacovigilance processes.

Optimisation of Antimicrobial Use

The inappropriate (overuse and misuse) use of antimicrobials across animal and human sectors has been the biggest driver of the development of AMR in microorganisms. The NSAP explains the rationale for the recommendations for the proper regulation of health products and medicines, that is, to ensure that the right antimicrobials are used at the right time, in the right dose, and for the right duration.

The NSAP describes the various regulations, initiatives, and position statements on the regulation

and optimisation of the use of antimicrobials. This includes Singapore's position on the non-therapeutic use of antimicrobials, drug residues, and licensing conditions for farms and feed mills which ban specified antimicrobials. The NSAP highlights Singapore's commitment to establish a robust regulatory framework for the supply chain control of antimicrobials and to establish a roadmap to progress towards veterinary prescriptions for drugs used in all animal sectors and antimicrobial stewardship programmes. In this aspect, current regulations are in the process of being reviewed and strengthened to support these goals.

International Collaboration

Singapore's NSAP is not just focused on local initiatives, but it also identifies potential areas of collaboration with international partners. The inclusion of this strategy in the NSAP signals Singapore's commitment to global and regional efforts to minimise the emergence and spread of AMR, because AMR is a global risk that is beyond the capacity of any organisation or nation to manage or mitigate alone. This strategy is carried out in three ways:

- a) *International benchmarking*: Singapore is committed to participating in global surveillance networks such as the WHO global AMR surveillance system (GLASS) and OIE global database on the use of antimicrobial agents in animals. These networks and databases are important in establishing an international understanding of AMR and antimicrobial utilisation.
- b) *International partnerships*: Aside from participating in the implementation of the Global Action Plan, Singapore is a member of the ASEAN and supports member states to improve health systems by sharing technical expertise and experience. Singapore has committed to fighting AMR at the United Nations General Assembly in New York in September 2016 as part of the collective political declaration. Singapore, together with other ASEAN member states, also declared in November 2017, its commitment to combat AMR through a multi-sectoral and multidisciplinary approach within the framework of One Health, which is consistent with Singapore's NSAP. In addition, ASEAN has endorsed Singapore's initiative to coordinate and spearhead efforts to combat AMR in the livestock and aquaculture sectors in ASEAN at the ASEAN Ministerial Meetings on Agriculture and Forestry in 2016 (AMAF, 2016) and 2017 (AMAF, 2017). At the same time, Singapore is partnering with FAO to develop guidelines on monitoring guidelines of AMR in bacterial pathogens in aquaculture,

together with other international partners (FAO, 2018).

- c) *International research collaborations*: It is recognised that tapping on partnerships with international health research funding agencies will enable more efficient use of research funding and resources. Recognising that any investment in new medicines, vaccines and diagnostic tools to combat AMR would be for the long-term, Singapore will continue to foster industry partnerships and facilitate industry research and development.

Conclusion

Singapore's NSAP, which incorporates strategies for the aquaculture sector, was developed by parties in four different sectors: human, animal, food, and environment, and launched within a relatively short time. Some factors that enabled this was the strong political support received from the start, and strong foundation of One Health cooperation and collaboration. Agencies were familiar with one another, had a common purpose, and agreed early on the direction to take. Self-assessment and transparency between agencies were critical to facilitate this process. Decisive leadership and the commitment of team members also contributed to its successful development. The inclusion of infectious disease and public health experts in the AMR field provided current knowledge on antimicrobial utilisation and prescribing practices in human health, as public health was a key consideration in the NSAP objectives. Work is now underway to implement the initiatives outlined in the NSAP through a five-year multi-sectoral work plan, with crucial monitoring indices identified for evaluation. The NSAP expresses Singapore's commitment to the local and international community in recognising that AMR is a global problem and local efforts are part of a global solution. The NSAP would serve Singapore and the aquaculture sector well for the next five years until the next review to ensure the objectives and goals can be met.

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Status of Viet Nam's National Action Plan on Antimicrobial Resistance in Aquaculture

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Abstract

Antibiotic resistance has been considered as one of the biggest global concerns in terms of negative impacts on public health, resistant pathogens and environmental problems. This paper aims to review the present status in Viet Nam regarding Antibiotic Use (AMU) and Antibiotic Resistance (AMR) in aquaculture. To implement the National Action Plan (NAP) on Drug Resistance 2013–2020, the Vietnamese Government has established a national public health network that follows the “One Health Approach” and the National Steering Committee for Prevention and Control of Aquatic Animal Diseases, AMU and AMR in Aquaculture. The Government has also issued the NAP for Controlling AMU and AMR in Livestock Production and Aquaculture period 2017–2020. To promote awareness, national television programs and communication campaigns were conducted to increase awareness of AMR among farmers and other stakeholders. Farmers were also instructed on proper AMU and provided vital information on AMR. Pilot projects on the surveillance for AMR in cultured catfish and the use of antibiotics in shrimp and catfish aquaculture were carried out to gather evidence on AMU and AMR status in the country. In addition, households raising tilapia and traditional freshwater fish were interviewed for information on AMU and AMR in freshwater fish aquaculture. To promote best practices, programmes for aquatic animal disease control were established and trainings on good antibiotic use in aquaculture carried out. Best practices in the culture of shrimp, catfish, tilapia and other species have also been improved through the use of programmes such as VietGAP and GlobalGAP.

Keywords: survey, shrimp, catfish, traditional freshwater fish, *Aeromonas*, *Streptococcus*

Introduction

Antimicrobial resistance (AMR) is considered as one of the greatest threats to public health worldwide. At the international level, the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), and the World Health Organization (WHO) collaborating through a Tripartite Agreement have identified AMR as one of the three priority topics for joint actions (FAO/OIE/WHO, 2011) and have developed a Global Action Plan on Antimicrobial Resistance (WHO, 2015). Following a “One Health Approach”, the Global Action Plan provides a framework for national action plans (NAPs) to combat AMR.

In Viet Nam, the Ministry of Health (MoH) has initiated

national activities to tackle AMR with the publication of the National Action Plan on Combatting Drug Resistance from 2013 to 2020 (MoH, 2013). The overall objective of the plan is to promote the prevention of drug resistance, contributing to improving the quality and effectiveness of the prevention and control of epidemics, medical examinations and treatments to protect, care for, and improve people's health. It includes six specific objectives, one of which is the promotion of proper antimicrobial use in livestock, poultry, aquaculture, and cultivation.

Because of the rapid expansion in the aquaculture area and the use of increased stocking densities, Vietnamese aquaculture has been faced with serious disease outbreaks. Major diseases and pathogens of concern include white spot disease (WSD) and acute

hepatopancreatic necrosis disease (AHPND) in shrimp; *Edwardsiella ictaluri* and *Aeromonas hydrophila* infections in catfish *Pangasianodon hypophthalmus* (Sauvage, 1878); *Streptococcus* spp. and *A. hydrophila* infections in tilapia *Oreochromis niloticus* (Linnaeus, 1758); and *Aeromonas* spp. and *Pseudomonas* spp. infections in “traditional freshwater fish” such as common carp *Cyprinus carpio* Linnaeus, 1758, grass carp *Ctenopharyngodon idella* (Valenciennes, 1844), silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844), climbing perch *Anabas testudineus* (Bloch, 1792). As a result, antimicrobials play a critical role in the prevention and treatment of aquatic animal diseases. However, their imprudent use and overuse have been contributing factors in the spread of antimicrobial resistance (AMR). In this paper, we detail the status of the aquaculture component on the Vietnamese National Action Plan for AMR, in reference to the four pillars of the FAO Action Plan on AMR (FAO, 2016): i.e. governance, awareness, evidence or surveillance, and best practice.

Implementation of AMR Action Plan

Viet Nam is the first country in the World Health Organization’s (WHO) Western Pacific Region that has approved a NAP on combating antibiotic resistance. Viet Nam has also established a national public health network to implement the “National Action Plan on Drug Resistance 2013–2020”, according to Decision No. 2174/QĐ-BYT dated 21/6/2013 in which the Ministry of Health (MOH) is the lead agency and the Ministry of Agriculture and Rural Development (MARD) is one of the key implementing agencies (MoH, 2013). The main objective of the NAP is to improve people’s health care through preventing and controlling drug resistance and raising the quality and effectiveness of medical examination. The NAP consists of six main activities: (1) raising awareness of community and health staff about drug resistance; (2) strengthening and improving the capacity of the national surveillance system on AMU and AMR; (3) ensuring a supply of essential drugs of high quality; (4) promoting the safe, prudent and responsible use of drugs; (5) strengthening the control of infections; and (6) strengthening the prudent and responsible use of antibiotics in agriculture, livestock production, and aquaculture.

In order to further support this multisectoral approach to the control of AMR, in October 2016, the MoH established the National Steering Committee on Prevention of Antimicrobial Resistance for the period 2016–2020 (Decision 5888/QĐ-BYT dated 10/10/2016). The committee included 31 members from four ministries, as well as members of external partner institutions. AMR was identified as a key component of the Global Health Security Agenda of Viet Nam through which is a five-year plan to prevent and control the emergence and spread of AMR through the effective and rational use of antibiotics in humans

and animals was also established. By May 2018, several activities were conducted or were undertaken. These include: (1) organising an annual communication activity/meeting on AMU and AMR in November, since 2013; (2) developing regulations and technical guidelines on clinical pharmacological activity, drug use in treatment, and drug description; (3) preparing standardised professional materials and protocols related to disease diagnosis and treatment with antibiotics; (4) implementing an Aide Memoire on Multi-stakeholder Engagement to Combat AMR in Viet Nam (led by the Department of Animal Health (DAH) of MARD and the Drug Administration of Viet Nam of MoH) (MoH/MARD/MOIT/MONRE, 2015); (5) increasing awareness of AMU and the risks of AMR; and (6) issuing, by MARD, of Decision No. 2625/QĐ-BNN-TY dated 21/6/2017 on “National Action Plan (NAP) for Controlling Antimicrobial Use and Antimicrobial Resistance in Livestock Production and Aquaculture (2017–2020)” (Decision No. 2625/QĐ-BNN-TY dated 21/6/2017)(MARD, 2017a).

The main objective of this NAP is to mitigate the risk of antibiotic resistance in public health through controlling antibiotic use in livestock production and aquaculture (MARD, 2017a). Major activities of the NAP include (1) strengthening and consolidating the state management of AMU and AMR; (2) improving the legal basis for AMU and AMR management; (3) enforcing the regulations and technical guidelines currently in place; (4) increasing awareness of AMU and the risks of AMR; (5) implementing good treatment and husbandry practices in livestock feed manufacturing and livestock production and aquaculture; (6) monitoring AMU, AMR and antibiotic residues; and (7) strengthening inter-sectoral collaboration in AMR management. Under this NAP, MARD has established the National Steering Committee for Prevention and Control of Aquatic Animal Diseases, Antimicrobial Use and Antimicrobial Resistance in Aquaculture; issued 11 legal circulars on disease control, AMU and AMR; and issued more than 20 official letters to direct and enforce the control AMU and AMR. MARD has also organised several workshops and meetings between government agencies, companies, and associations to identify current problems, gaps, and difficulties in the control of AMU and AMR.

With regard to the control of veterinary drugs that are marketed in Viet Nam, MARD has requested the DAH and local agencies to inspect all importers (28 companies) for veterinary medicinal products and raw materials (especially for raw antibiotics) to identify how they are imported, used, and sold, and also to inspect veterinary drug shops to determine whether the antibiotic products that are sold are registered or non-registered. By law, all shops are now prohibited from selling raw antibiotic materials directly to farmers.

Awareness of Antimicrobial Resistance

To implement the activities of the NAP, the DAH has established collaborative programmes with the national television broadcasters (VTV1, VTV16) and newspapers to disseminate information on AMU and AMR, and has also conducted communication campaigns to increase awareness of AMR among farmers, drug sellers, and other stakeholders. Farmers have received instructions on proper AMU and key messages on AMR during surveys of AMU and AMR in shrimp, *Pangasius* catfish, tilapia, and traditional freshwater fish aquaculture. Technical staff, researchers, leaders, and managers have participated in national, regional, and international workshops and meetings on AMU and AMR to share and acquire experiences on how to improve awareness (such as participating in FAO project FMM/RAS/298/MUL and the Network of Aquaculture Centres in Asia-Pacific's (NACA) project on AMU in *Pangasius* catfish).

Surveillance

National programme on monitoring chemical/antibiotic residues on aquatic animals

MARD has approved, for every year since 2013, the national program on monitoring chemical/antibiotic residues in aquatic animals and their products. The monitored species include shrimp *Penaeus monodon* (Fabricius, 1798) and *Penaeus vannamei* (Boone, 1931), *Pangasius* catfish (*P. hypophthalmus*), tilapia *O. niloticus*, climbing perch *Anabas testudineus*, and snack-head fish *Channa striata* (Bloch, 1793). The parameters monitored include antibiotic and pesticide and chemical residues. Figure 1 shows the percentages of shrimp products that were positive for at least one antibiotic during the period 2013 to 2016.

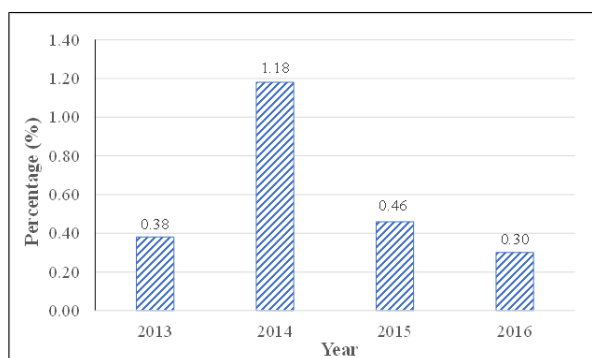


Fig. 1. Percentage (%) of shrimp products that were positive for at least one antibiotic during the period 2013–2016. No. of positive samples/no. of tested samples for each year were as follows: 2013: 09/2,365; 2014: 25/2,104; 2015: 08/1,751; and 2016: 06/1,692.

Pilot surveillance for AMU and AMR in *Pangasius catfish* aquaculture

During 2013–2014, MARD conducted pilot surveillance for AMR in *Pangasius* catfish aquaculture, sponsored by WHO. During this study, 75 catfish ponds belonging to six large catfish farms were sampled. The focus of the project was AMR in enteric (*Escherichia coli* and *Salmonella* sp.) and aquatic bacteria (*Aeromonas* spp. and *Vibrio* spp.) isolated from pond water, supply water, pond sediments, and catfish.

Survey on AMU in *Pangasius* catfish and shrimp aquaculture

During 2015–2016, through funding by the Government of Viet Nam, the DAH conducted a survey on AMU of 714 aquaculture households in three major *Pangasius* catfish production provinces (Ben Tre, Dong Thap, and An Giang provinces) and two major shrimp production provinces (Soc Trang and Bac Lieu provinces) (DAH, 2016). In 2017, DAH also carried out a survey on AMU in *Pangasius* catfish culture in Can Tho, An Giang, and Dong Thap provinces, sponsored by an FAO/NACA project.

Survey on AMU and AMR in tilapia and traditional freshwater fish

This survey was carried out by the DAH and Research Institute for Aquaculture No. 1 (RIA1) in 2017 under funding from project FAO/FMM/RAS/298. The objective of the survey was to assess the current status of AMU and AMR in the prevention and control of diseases in tilapia and traditional freshwater fish in two districts of Hai Duong Province (DAH, 2018). The survey design was developed using the principles and techniques of an epidemiological cross-sectional study and used a random multistage sampling method. Information and data on the production and disease situation in the culture of tilapia and traditional freshwater fish of Hai Duong Province were collected and used to develop the survey design. Two districts (Nam Sach and Ty Ky) having the highest density of tilapia production were selected for the survey (Fig. 2). A total of 60 households at six communes of these two districts were interviewed for information on AMU and AMR in November 2017.

Before implementing field activities, a one-day training course was organised in the surveyed province to provide local staff with background information on AMU and AMR. The training also included topics on the survey design, finalise the list of households to be interviewed, the standardised questionnaire, the methods for collection, management, and transportation of samples from the field to RIA1's laboratory; the detailed working plan and coordination of the field activities; and other logistic preparations. For each of the surveyed districts, a team was established to carry out field activities. At each selected household, the team

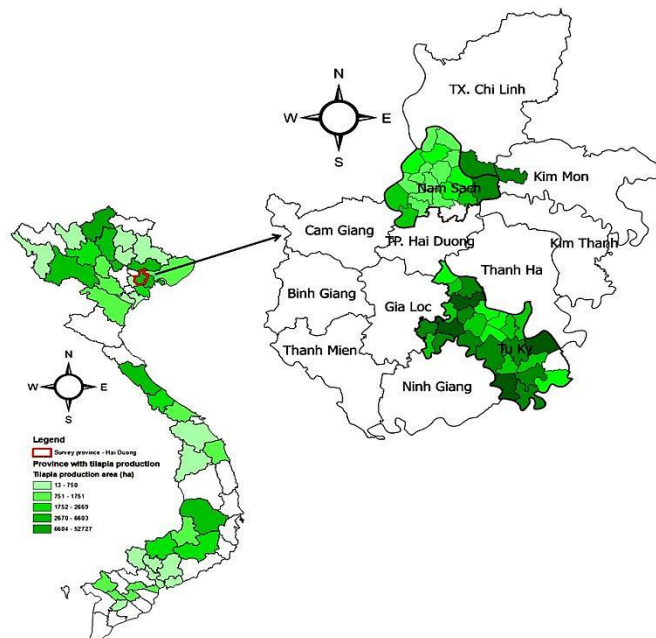


Fig. 2. Map of Nam Sach and Tu Ky districts in Hai Duong Province (red square) where the survey on the use of antibiotic and antibiotic resistance survey was conducted.

conducted a face-to-face interview with the owner to collect relevant information using 30 standardised questionnaire divided into three main parts: (1) baseline information about the household; (2) information on the household owner's knowledge on AMU and AMR; and (3) information on the owner's attitudes and practices regarding AMU and AMR.

A total of 177 samples (including 85 liver, 61 brain, 27 kidney and 4 mixes of liver and brain) were collected for isolation and identification of target pathogens (*Streptococcus* spp. and *A. hydrophila*). Also, in this study, a total of twenty *A. hydrophila* strains were further tested for AMR using 13 antibiotics (Table 1).

Table 1: Antibiotics used for antibiotic resistance testing of *Aeromonas hydrophila* strains.

No.	Antibiotic name
1.	Doxycycline (30 µg)
2.	Novobiocin (5 µg)
3.	Neomycin (30 µg)
4.	Rifampicin (30 µg)
5.	Florphenicol (30 µg)
6.	Chloramphenicol (30 µg)
7.	Trimethoprim/sulfamethoxazole (1.25/23.75 µg)
8.	Ciprofloxacin (5 µg)
9.	Oxacillin (1 µg)
10.	Erythromycin (15 µg)
11.	Streptomycin (10 µg)
12.	Tetracycline (30 µg)
13.	Vancomycin (30 µg)

For AMR analysis, isolates were categorised as wild type (fully susceptible, WT) or non-wild-type (NWT) using normalised resistance interpretation (NRI) determined cut-off values (COWT). Information derived from the AMU and AMR survey was entered into an MS Excel file which was linked with another one that consisted of laboratory test results and this combined data were used for descriptive analysis.

The results of AMU and AMR surveys in tilapia and traditional freshwater fish under the project FAO/FMM/RAS/298 as follows:

Production of tilapia and traditional freshwater fish: Descriptive analyses indicated that of the 60 investigated household owners, 78.3 % were male and 27.7 % were female (Table 2). As of November 2017, these owners had an average of 13.6 years of experience in the production of tilapia and traditional freshwater fish, with the longest having 29 years of experience and the youngest having only one year of experience. A majority (78.3 %) of the owners responded that they participated in one or more training courses on disease control, while 21.7 % said that they had never participated in any training courses. Most of the surveyed households (76.7 %) said that they cultured more than two species of tilapia and traditional freshwater fish in their production areas, while 23.2 % said that they cultured only one species (Table 2).

Knowledge about antibiotics: Although 95 % of the surveyed household owners said that they could detect aquatic animals with signs of disease, only 30 % of them had asked for technical advice on treatment. The majority (75 %) replied that they used antibiotics, although they could not differentiate well

Table 2. Baseline information regarding 60 households interviewed on the use of antibiotics and antibiotic resistant in aquaculture.

No.	Category	Number of households n = 60	Proportion of total number of households
1.	Gender		
	Male	47	78.3
	Female	13	21.7
2.	Year started fish culture	60	
	Oldest	1988	
	Newest	2016	
3.	Participated in training courses on disease control	60	
	Yes	47	78.3
	No	13	21.7
4.	Number of fish species cultured	60	
	Single	14	23.3
	Multiple	46	76.7

between antibiotics and other supplements such as vitamins and general mineral materials. Importantly, 65 % of the households said that they used a larger volume of antibiotics than that indicated in the instructions for use. This overuse could result in antibiotic resistance or imply that the quality of the antibiotics was not good enough. About 13.3 % of the households believed that antibiotics could be used to treat viral diseases, while 65 % said that they could be used to treat bacterial diseases. At least 16.7 % said that they used antibiotics to promote better growth.

Practices on using antibiotics: A majority (61.7 %) of the surveyed households said that they used antibiotics in compliance with manufacturer's instructions, and 78.3 % of them replied that they procured antibiotics from veterinary shops, compared with 11.7 % who said that sometimes they purchased antibiotics from both medical drug and veterinary drug shops. The reasons cited for the use of human antibiotics were cheaper cost and increased effectiveness. This is important information, as using antibiotics intended for human medicine likely results in AMR. Of the surveyed households, 38.8 % said that they received an introduction for the use of antibiotics from drug sellers, compared with 15 % who said that they did not have any information on usage. While 91.7 % said that they mixed antibiotics with food to feed fish, and 98.3 % of the surveyed households said that they considered various criteria (e.g. coverage, source, purpose of the antibiotic, expiry date, etc.) when buying antibiotics for their fish.

Bacterial isolation and AMR analysis: Among the 177 samples isolated for targeted pathogens, only two were positive for *Streptococcus* sp. and six were positive for *A. hydrophila*. Of these, two samples were collected from Nam Sach District and six were collected from Tu Ky District. The sampled fish did not show any clinical signs of disease. The isolated strains

were identified as *A. hydrophila* and *S. agalactiae* based on their biochemical characteristics (Fig. 3).



Fig 3. (A) *Aeromonas hydrophila* was isolated from tilapia (*Oreochromis niloticus*) collected in Hai Duong province in 2017; (B) *Streptococcus agalactiae* was isolated from climbing perch (*Anabas testudineus*) collected in Hai Duong province in 2017.

A total of 20 strains of *A. hydrophila* (including six strains isolated from this survey and 14 other strains isolated from tilapia and traditional freshwater fish in previous years) were tested for AMR (Table 3). The results showed that 45 % of the tested strains of *A. hydrophila* were non-wild type (NWT) for ciprofloxacin, 35 % were NWT for trimethoprim/sulfamethoxazole, 20 % were NWT for tetracycline and chloramphenicol, 15 % were NWT for erythromycin and doxycycline, 10 % were NWT for florphenicol and 5 % were NWT for rifampicin. Disc diffusions-based COWT were identified for WT of *A. hydrophila* strains as follows: ≥ 11 mm for florphenicol, ≥ 12 mm for erythromycin, ≥ 14 mm for rifampicin and neomycin, ≥ 18 mm for doxycycline, ≥ 19 mm for tetracycline and trimethoprim/sulfamethoxazole, ≥ 25 mm for chloramphenicol, and ≥ 34 mm for ciprofloxacin (Fig. 4).

Table 3. *Aeromonas hydrophila* strains used for antibiotic resistance testing.

No.	Bacterial strain	Host source	Year of isolation	Location (Province)
1.	<i>A. hydrophila</i> HDPT15.6	Tilapia (<i>Oreochromis niloticus</i> , Linnaeus, 1758)	2015	Phu Tho
2.	<i>A. hydrophila</i> CEDMA16.19	Tilapia (<i>O. niloticus</i>)	2016	Vinh Phuc
3.	<i>A. hydrophila</i> CEDMA16.20	Tilapia (<i>O. niloticus</i>)	2016	Vinh Phuc
4.	<i>A. hydrophila</i> CEDMA16.34	Tilapia (<i>O. niloticus</i>)	2016	Bac Ninh
5.	<i>A. hydrophila</i> CEDMA16.42	Tilapia (<i>O. niloticus</i>)	2016	Bac Ninh
6.	<i>A. hydrophila</i> HBTT16.01	Channel catfish (<i>Ictalurus punctatus</i> , Rafinesque, 1818)	2016	Hoa Binh
7.	<i>A. hydrophila</i> CEDMA17.001	Spotted catfish (<i>Hemichromis guttatus</i> , Gunther, 1862)	2017	Ha Nam
8.	<i>A. hydrophila</i> CEDMA17.002	Spotted catfish (<i>H. guttatus</i>)	2017	Ha Nam
9.	<i>A. hydrophila</i> CEDMA17.008	Tilapia (<i>O. niloticus</i>)	2017	Hoa Binh
10.	<i>A. hydrophila</i> CEDMA17.009	Tilapia (<i>O. niloticus</i>)	2017	Hoa Binh
11.	<i>A. hydrophila</i> CEDMA17.019	Channel catfish (<i>I. punctatus</i>)	2017	Hai Duong
12.	<i>A. hydrophila</i> CEDMA17.020	Tilapia (<i>O. niloticus</i>)	2017	Hai Duong
13.	<i>A. hydrophila</i> CEDMA17.021	Tilapia (<i>O. niloticus</i>)	2017	Hai Duong
14.	<i>A. hydrophila</i> CEDMA17.022	Grass carp (<i>Ctenopharyngodon idella</i> , Valenciennes, 1844)	2017	Bac Ninh
15.	<i>A. hydrophila</i> CEDMA17.044	Common carp (<i>Cyprinus carpio</i> , Linnaeus, 1758)	2017	Hai Duong
16.	<i>A. hydrophila</i> CEDMA17.045	Grass carp (<i>C. idella</i>)	2017	Hai Duong
17.	<i>A. hydrophila</i> CEDMA17.046	Grass carp (<i>C. idella</i>)	2017	Hai Duong
18.	<i>A. hydrophila</i> CEDMA17.047	Tilapia (<i>O. niloticus</i>)	2017	Hai Duong
19.	<i>A. hydrophila</i> CEDMA17.048	Grass carp (<i>C. idella</i>)	2017	Hai Duong
20.	<i>A. hydrophila</i> CEDMA17.049	Common carp (<i>C. carpio</i>)	2017	Hai Duong

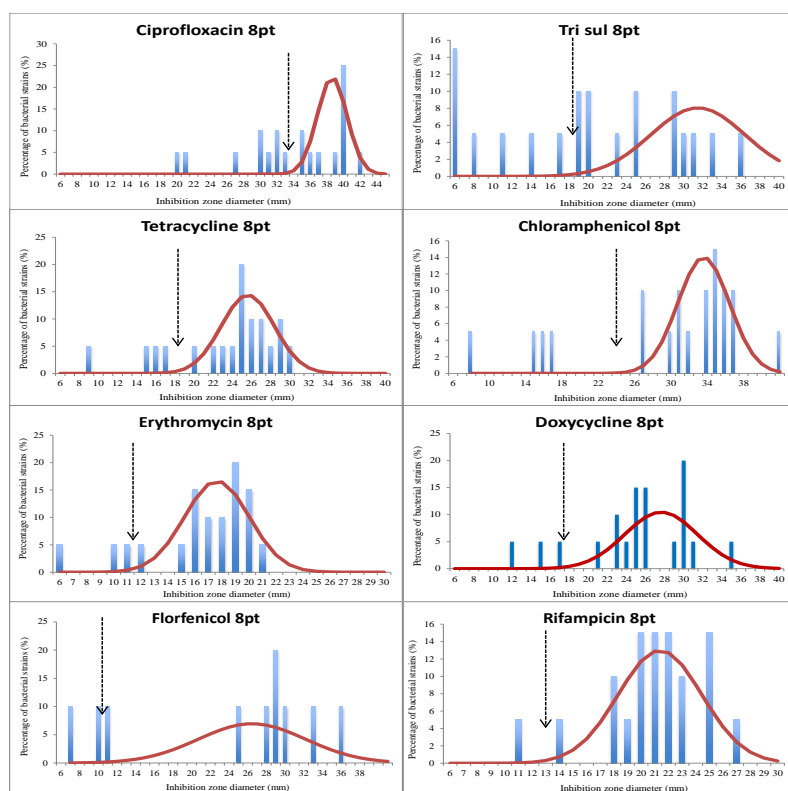


Fig. 4. AMR analysis of *Aeromonas hydrophila* strain isolated from tilapia *Oreochromis niloticus* and traditional freshwater fish common carp *Cyprinus carpio*, grass carp *Ctenopharyngodon idella*, silver carp *Hypophthalmichthys molitrix*, and climbing perch *Anabas testudineus*.

Best Practice

In terms of best practices, some national programmes for disease prevention and control have been established for main aquaculture species such as shrimp *P. monodon* and *P. vannamei* and catfish *P. hypophthalmus*, including national programmes for: (1) prevention and control of disease in *Pangasius* catfish, conducted between 2015 and 2020 (Decision 4995/QĐ-BNN-TY dated 20/11/2014) (MARD, 2014); (2) the establishment of disease-free compartments and zones for shrimp production (Decision 4088/QĐ-BNN-TY dated 01/10/2016) (MARD, 2016); and (3) for the active surveillance of diseases in shrimp and *Pangasius* catfish to be exported between 2017 and 2020 (Decision 1038/QĐ-BNN-TY dated 29/3/2017) (MARD, 2017b).

Annually, both national and local authorities issue national and local action plans for aquatic animal disease control and organise training activities on the principles of good antibiotic use in aquaculture. Best practices in shrimp, *Pangasius* catfish, tilapia, and other species have also been improved through the application of aquaculture technologies including VietGAP and GlobalGAP, and are encouraged through research and evaluation of alternative treatment measures to AMU (e.g. probiotic products, herbal/plant extract products).

Conclusion

Viet Nam has increased its capacity for the better management of AMU and AMR by using the One Health Approach. Several key activities have been implemented for the aquaculture component, such as awareness communication, training and education, legislation development, surveys to obtain basic information about AMU and AMR, applying best practices, and closely collaborating with international organisations such as WHO, OIE, FAO and NACA to implement activities on AMU and AMR. Because aquaculture is one of the most important sectors for economic growth in Viet Nam, the Vietnamese Government needs to continue implementing activities related to AMU and AMR in aquaculture in order to minimise the risk of AMR in the future.

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Guidance in Development of Aquaculture Component of a National Action Plan on Antimicrobial Resistance

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Abstract

Since the adoption, in May 2015, of the Global Plan of Action (GAP) on Antimicrobial Resistance (AMR), during the 68th World Health Assembly of the World Health Organization (WHO) and the subsequent adoption by the delegates of the World Organisation for Animal Health (OIE) of the OIE AMR Strategy and the adoption of Resolution 4/2015 by the 39th Conference of the Food and Agriculture Organization of the United Nations (FAO), a political declaration was made during a high-level meeting on AMR at the 71st United Nations General Assembly (UNGA, September 2016). The UNGA called upon the Tripartite (i.e. WHO, OIE, and FAO) and other intergovernmental organisations to support the development and implementation of National Action Plans (NAPs) and AMR activities at the national, regional, and global levels under the One Health platform. This paper provides preliminary guidance in the development of the aquaculture component of NAP on AMR under the auspices of the FAO project FMM/RAS/298/MUL: Strengthening capacities, policies, and national action plans on prudent and responsible use of antimicrobials in fisheries. Such guidance, consisting of two levels (i.e. generic and aquaculture-specific), are important first steps in the process. Having such a plan will now allow the responsible Competent Authority to raise the profile of aquaculture in national discourses on AMR, generate an understanding of the sector and its importance by other relevant sectors engaged in the One Health platform, stimulate investment and support towards further development and implementation of the action plan. The aquaculture component needs to be fully integrated in a country's NAP on AMR.

Keywords: NAP, AMR, One Health

Introduction

Antimicrobial resistance (AMR) is a global concern and is now recognised as one of the greatest threats to public health worldwide. In order to address the crisis brought about by systematic misuse and overuse of antimicrobial drugs that contributed to the emergence and spread of antimicrobial-resistant organisms and that threatens the sustainability of an effective, global public health response to risks of infectious diseases, in May 2015, the Global Plan of Action on AMR (GAP) was adopted during the 68th World Health Assembly of the World Health Organization (WHO). The World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) contributed to the GAP. The OIE delegates, in May 2015, adopted the OIE AMR strategy (OIE, 2016), and

the 39th FAO Conference (June 2015) adopted Resolution 4/2015 to support the GAP (FAO, 2016). A political declaration was made during a high-level meeting on AMR at the 71st United Nations General Assembly (UNGA, September 2016) (WHO, 2015) which called upon the Tripartite (i.e. FAO as the global leader for food and agriculture, the OIE as the global leader for animal health and welfare, and WHO as the global leader for human health) and other intergovernmental organisations to support the development and implementation of National Action Plans (NAPs) and AMR activities at the national, regional and global levels under the One Health platform.

In 2017, the FAO implemented a project FMM/RAS/298/MUL: Strengthening capacities, policies, and national action plans on the prudent and

responsible use of antimicrobials in fisheries. The objectives of this project were to develop and/or enhance the knowledge, skills, and capacity of the participating Competent Authorities (CA) on fisheries and aquaculture, as well as to assist the CAs in the development and implementation of policies and national action plans (NAPs) on the prudent and responsible use of antimicrobials (FAO, 2017; Bondad-Reantaso et al., 2020).

This paper provides preliminary guidance in the development of the aquaculture component of NAP on AMR under the auspices of the above mentioned FAO project FMM/RAS/298/MUL. Two levels of guidance were provided: (i) generic guidance and (ii) specific guidance in aquaculture.

Guidance in the Development of the Aquaculture Component of National Actions Plans (NAPs) on Antimicrobial Resistance (AMR)

The guidance involves two levels, i.e., generic and aquaculture-specific.

1. **Generic guidance.** The first generic step is to review the WHO Global Plan of Action and the subsequent plans of actions of OIE and the FAO (Table 1). These action plans can guide countries in the development of their national action plans and determine what objectives or pillars of a country action plan may be appropriate. A country's AMR NAP is usually led and coordinated by the relevant health ministry.

2. **Aquaculture-specific guidance.** The next step is to understand the aquaculture sector and examine different aspects and how they may relate to the emergence of AMR in aquaculture. The following actions are recommended:

- a) Review/prioritisation of the most important cultured species based on production statistics;
- b) Review/prioritisation of the most important bacterial diseases (Table 1) affecting the most important cultured species based on agreed criteria;
- c) Review Codex Alimentarius maximum residue limit (MRL);
- d) Collect information on actions to deal with bacterial diseases (prevention, good aquaculture/biosecurity practices, treatment with antibiotics, alternative treatment, and other measures);
- e) Provide guidance on the mechanisms for collection of information on AMU and AMR surveillance; and
- f) Identify requirements for AMU and AMR surveillance (such as personnel, field/laboratory procedures, skills, facilities, policies/legislation, reporting/record keeping, monitoring).

For 2a, most aquaculture-producing countries have national aquaculture production statistics and aquaculture action plans and/or aquaculture policy documents which may provide information on priority aquaculture species; these can be a very useful source of data.

Table 1. Objectives of the action plans on AMR of the World Health Organization, the World Organisation for Animal Health, and the Food and Agriculture Organization of the United Nations.

World Health Organization (WHO)	World Organisation for Animal Health (OIE)	Food and Agriculture Organization of the United Nations (FAO)
<p>The WHO global action plan sets out five strategic objectives:</p> <ul style="list-style-type: none"> • To improve awareness and understanding of antimicrobial resistance; • To strengthen knowledge through surveillance and research; • To reduce the incidence of infection; • To optimise the use of antimicrobial agents; and • To develop the economic case for sustainable investment that takes account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines, and other interventions. 	<p>The OIE Strategy supports the objectives established in the Global Action Plan and reflects the mandate of the OIE as described in its Basic Texts and Strategic Plans. It has four objectives:</p> <ul style="list-style-type: none"> • Improve awareness and understanding • Strengthen knowledge through surveillance and research • Support good governance and capacity building • Encourage implementation of international standards 	<p>To support the implementation of Resolution 4/2015, the FAO Action Plan on AMR (2016-2020) addresses four major Focus Areas:</p> <ul style="list-style-type: none"> • Improve awareness on AMR and related threats; • Develop a capacity for surveillance and monitoring of AMR and AMU (antimicrobial use) in food and agriculture; • Strengthen governance related to AMU and AMR in food and agriculture; • Promote good practices in food and agricultural systems and the prudent use of antimicrobials.

For 2b, in terms of bacterial pathogens, potential data sources at the country level may include the following: (1) a country's National Pathogen List, if this exists as part of a National Strategy on Aquatic Animal Health or Aquaculture Biosecurity; (2) disease information from diagnostic laboratories;

(3) quarantine records; (4) disease information from primary producers; (5) disease information published in the scientific and grey literature; and (6) national residue testing programme, if it exists. A compilation of a list of important bacterial pathogens in aquaculture production can be found in Table 2 below.

Table 2. List of important bacterial pathogens in aquaculture production (Bondad-Reantaso et al., 2020).

According to	Pathogen	Reference
Responsible management of bacterial diseases in aquaculture (in preparation)		FAO/HQ Aquaculture Biosecurity team (Regional consultation on AMR Monitoring and Surveillance Guidelines Volume 3: "Monitoring and surveillance of AMR in bacteria from aquaculture"
Gram negative	Vibriosis, Aeromonas, Edwardsiellosis, Pseudomonas, Flavobacteriosis, Infection with intracellular bacteria	
Gram positive	Mycobacteriosis, Streptococcosis, Renibacteriosis, Infection with anaerobic bacteria	
Best practice guidelines for the performance of bacterial susceptibility tests		Smith, 2019
Non-fastidious Gram-negative bacteria	<i>Aeromonas caviae</i> , <i>Aeromonas hydrophila</i> , <i>Aeromonas jandaei</i> , <i>Aeromonas salmonicida</i> , <i>Aeromonas sobria</i> , <i>Aeromonas veronii</i> , <i>Acinetobacter</i> spp., <i>Citrobacter freundii</i> , <i>Edwardsiella anguillarum</i> , <i>Edwardsiella ictaluri</i> , <i>Edwardsiella piscicida</i> , <i>Edwardsiella tarda</i> , <i>Pseudomonas anguilliseptica</i> , <i>Pseudomonas fluorescens</i> , <i>Yersinia ruckeri</i>	
Halophilic Gram-negative bacteria (facultative and obligate halophiles)	<i>Aliivibrio salmonicida</i> , <i>Photobacterium damsela</i> , <i>Vibrio alginolyticus</i> , <i>Vibrio anguillarum</i> , <i>Vibrio harveyi</i> , <i>Vibrio parahaemolyticus</i> , <i>Vibrio vulnificus</i>	
Flavobacteria and related species	<i>Flavobacterium branchiophilum</i> , <i>Flavobacterium columnare</i> , <i>Flavobacterium psychrophilum</i> , <i>Tenacibaculum maritimum</i>	
Gram-positive cocci	Mesophilic species (<i>Aerococcus viridans</i> , <i>Lactococcus garvieae</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus dysgalactiae</i> , <i>Streptococcus iniae</i> , <i>Streptococcus phocae</i> , <i>Weissella</i> spp., <i>Psychrophilic</i> spp., <i>Lactococcus piscium</i> , <i>Vagococcus salmoninarum</i>)	
Mycobacteria and related species	<i>Mycobacterium fortuitum</i> , <i>Mycobacterium marinum</i> , <i>Nocardia asteroides</i> , <i>Nocardia crassostreae</i> , <i>Nocardia seriolae</i>	
NACA/FAO/OIE List	Mollusc (<i>Xenohaliotis californiensis</i>) Crustacean (<i>Vibrio parahaemolyticus</i> , <i>Hepatobacter penaei</i>) Finfish (<i>Edwardsiella ictaluri</i>) Crustacean (<i>Spiroplasma eriocheiris</i>)	OIE, 2020 NACA/FAO/OIE Quarterly Aquatic Animal Disease Report
Review papers	<i>Vibrio parahaemolyticus</i> , <i>Vibrio harveyi</i> , <i>Vibrio alginolyticus</i> , <i>Vibrio campbellii</i> , <i>Spiroplasma penaei</i> <i>Aeromonas</i> spp., <i>Edwardsiella</i> spp., <i>Flavobacterium</i> spp., <i>Streptococcus</i> spp., <i>Lactococcus</i> spp., <i>Vibrio</i> spp., <i>Yersinia</i> spp.	Morales-Covarrubias, 2018 Reverter et al., 2020
Country information		
China	Finfish (<i>Aeromonas caviae</i> , <i>Aeromonas hydrophila</i> , <i>Aeromonas sobria</i> , <i>Edwardsiella tarda</i> , <i>Flavobacterium columnare</i> , <i>Pseudomonas alcaligenes</i> , <i>Pseudomonas putida</i> , <i>Streptococcus</i> spp., <i>Vibrio anguillarum</i> , <i>Yersinia ruckeri</i>) Crustaceans (<i>Vibrio anguillarum</i> , <i>Vibrio harveyi</i> , <i>Vibrio parahaemolyticus</i>) Mollusc (<i>Aeromonas hydrophila</i> , <i>Vibrio alginolyticus</i> , <i>Vibrio fluvialis</i> II)	2019 National Surveillance - National Pathogen List
Malaysia	<i>Campylobacter</i> , <i>Enterococcus faecalis</i> , <i>Escherichia coli</i> , <i>Salmonella</i> , <i>Staphylococcus aureus</i> <i>Aeromonas salmonicida</i> , <i>Streptococcus</i> <i>Vibrio parahaemolyticus</i> , <i>Escherichia coli</i>	NAP on AMR National Pathogen List NAP on AMR
Viet Nam	<i>Streptococcus</i> spp., <i>Aeromonas hydrophila</i>	

This list was compiled by Bondad-Reantaso et al. (2020) from several sources including the following:

- Responsible management of bacterial diseases in aquaculture, a publication in preparation, contains six groups of Gram-negative bacteria (Vibriosis, Aeromonosis, Edwardsiellosis, Pseudomonosis, Flavobacteriosis, Infection with intracellular bacteria) and four groups of Gram-positive bacteria (Mycobacteriosis, Streptococcosis, Renibacteriosis, Infection with anaerobic bacteria).
- Best practice guidelines for the performance of bacterial susceptibility tests: this publication (Smith 2019) contains a list of bacteria isolated from aquatic animals or aquatic environment categorised as: Non-fastidious Gram-negative bacteria; Halophilic Gram-negative bacteria (facultative and obligate halophiles); Flavobacteria and related species; Gram-positive cocci; and Mycobacteria and related species.
- NACA/FAO/OIE lists: bacterial pathogens affecting finfish, molluscs, and crustaceans. There are only three pathogens in the OIE list and five in the NACA/FAO/OIE regional list of diseases that is part of the Quarterly Aquatic Animal Disease reporting system.
- Review papers: two review papers: (1) bacterial diseases of farmed shrimp in Latin American countries (Morales-Covarrubias, 2018) and (2) major bacterial pathogens in aquaculture (Reverter et al., 2020).
- Country information: bacterial pathogens of concern can be part of a surveillance programme, national pathogen list, or NAP on AMR.

For 2c, there are existing guidance documents on maximum residue limit (MRL) for fish products. The MRL is the maximum concentration of residue legally tolerated in a food product obtained from an animal that has received a veterinary medicine (FAO, 2018). Information on 2c is needed for 2d and 2e.

After identification of the farmed species and bacterial pathogens of concern, the next step is to understand how these disease problems are being addressed which may include any or a combination of the following measures, such as prevention, good aquaculture/biosecurity practices, treatment with antibiotics, alternative treatment.

For 2d, information can be generated from a country's Competent Authority responsible for the management and control of diseases in the aquaculture sector, as well as from the industry and academic and research stakeholders.

Items 2d and 2e are interrelated in the sense that if bacterial diseases are being addressed through the use of antibiotics, then the next step will be to understand AMU especially antibiotics. The specific guidance in the collection of information on AMU and AMR can be found in Bondad-Reantaso et al. (2020). The guidance consists of nine sections, namely: Section 1 (Contact person profile); Section 2 (Farm information); Section 3 (Types of antimicrobial agents used in cultured species in terms of treatment, prevention, disinfection, or other uses); Section 4 (Doses of antimicrobial agents used in cultured species, expressed in mg); Section 5 (Duration of antimicrobial agents used in cultured species, expressed in days); Section 6 (Effectiveness of antimicrobial agents used in cultured species, expressed in percent); Section 7 (In your experience, what is the availability of these agents, in terms of: freely available in the market, through prescription, no information?); Section 8 (Drug sales); and Section 9 (Drug sales by routes of administration, e.g. medicated feed; bath treatment; directly to the pond; parenteral delivery). In addition to the suggested forms to be filled up, guidance notes were also provided on the following aspects: collection of data; logistics/operational aspects, stakeholders, surveillance objectives, sampling design, target microorganisms, and laboratory methodology.

Item 2f is also an essential step. Surveillance is an economic activity and thus it is essential to identify requirements for AMU and AMR surveillance (personnel, field/laboratory procedures, skills, facilities, policies/legislation, reporting/record keeping, monitoring, etc.).

Other essential and relevant guidance that can be used include the following:

- OIE International Standards on Responsible and Prudent Use of Antimicrobials: Use of antimicrobial agents in aquatic animals (2020a).

The relevant chapters are:

- a) Chapter 6.1: Introduction to the recommendations for controlling AMR
- b) Chapter 6.2 Principles for responsible and prudent use of antimicrobial agents in aquatic animals
- c) Chapter 6.3 Monitoring the quantities and usage patterns of antimicrobial agents used in aquatic animals
- d) Chapter 6.4 Development and harmonization of national AMR surveillance and monitoring programmes for aquatic animals
- e) Chapter 6.5 Risk analysis for AMR arising from the use of antimicrobial agents in aquatic animals.

- Antimicrobial susceptibility testing programmes. This technical paper (Smith, 2019) addresses best practice guidelines for the performance of antimicrobial susceptibility testing of bacteria isolated from aquatic animals as part of a monitoring or surveillance programme or to provide guidance for clinical treatments of diseased animals. It contains six sections, namely: Section 1: Relevance of the document to the Action Plan; Section 2: Principles of antimicrobial susceptibility testing; Section 3: Standard protocols recommended for use in antimicrobial susceptibility testing of bacteria isolated from aquatic animals; Section 4: Design of programmes aimed at monitoring or surveillance of AMR associated with the use of antimicrobial agents in the rearing of aquatic animals; Section 5: Conclusions; and Section 6: References.
- FAO Code of Conduct for Responsible Fisheries: Aquaculture development. 8. Recommendations for prudent and responsible use of veterinary medicines in aquaculture. These Technical Guidelines (FAO, 2019) are developed to support Section 9 – Aquaculture Development of FAO’s CCRF (No. 5, Suppl. 8) and The FAO Action Plan on Antimicrobial Resistance 2016–2020. They also support the international aquatic animal health standards of the OIE, food safety standards of the FAO/WHO Codex Alimentarius, and the One Health platform under the FAO/OIE/WHO Tripartite Collaboration on AMR. Their objective is to assist countries in encouraging the prudent and responsible use of veterinary medicines (antimicrobial agents and other chemotherapeutants) in aquaculture production through appropriate government regulation and the promotion and encouragement of awareness and responsible use by the concerned government agencies, private-sector aquaculture producers, and aquatic animal health professionals. They emphasise, among the guiding principles, that responsible use of veterinary medicines in aquaculture requires collaboration among all stakeholders and a strong commitment to governance, awareness, best practices, surveillance, and research, including monitoring of AMR, tracking of antimicrobial usage (AMU), assessing risk in different settings and evaluating strategies to reduce AMR and maintain the efficacy of antimicrobial agents.

Once the specific guidance in understanding the aquaculture sector are put in place, the next step will be to look at the other relevant objectives, in terms of awareness, knowledge generation, and capacity development as in Table 1 that can be a useful reference. The aquaculture component will need to

be integrated in the country's overall AMR NAP and within the One Health approach/framework.

Conclusion

The important role played by aquaculture in providing high-quality nutrition, improving livelihoods, stimulating and creating decent work and economic growth, and alleviating poverty, particularly in low-income food-deficit countries and the need to address biosecurity, one of the most important sustainability challenges, can be important drivers for supporting AMR stewardship.

The preliminary guidance (generic and specific) in the development of the aquaculture component of a country's NAP on AMR are important first steps in the process. Having such a plan will now allow the responsible Competent Authority to raise the profile of aquaculture in national discourses on AMR, generate an understanding of the sector and its importance by other relevant sectors engaged in the One Health platform, stimulate investment and support towards further development and implementation of the action plan. The aquaculture component needs to be fully integrated in a country's NAP on AMR.

These guidelines were used, to a certain extent, by participating countries (e.g. China, Malaysia, the Philippines and Viet Nam) in the mentioned FAO project FMM/RAS/298/MUL. The example set by these countries shows that the guidance can be a straightforward method from which national strategies on AMR can build upon.

There is an urgent need for aquaculture countries, especially those with substantial aquaculture production and food security objectives through aquaculture, to pay high attention to the emergence of antimicrobial-resistant organisms that can result from antimicrobial imprudent and irresponsible use in the aquaculture sector.

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