EMERGENCE AND SPREAD OF ANTIMICROBIAL RESISTANCE IN MOTILE AEROMONADS OF THE AQUACULTURE ENVIRONMENT

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The global rise in antimicrobial resistance (AMR) among bacteria causing infectious diseases and the associated risks on human health are well known. The uproar of AMR has provided with the most severe health threat of the century. The research on the AMR of the organisms present in the environment is, however, meagre. The bacterial species of the genus *Aeromonas* is widely distributed in the environment that comprises both motile and non-motile variants. Several of them are autochthonous to the aquatic environment. The motile aeromonads, in particular, are notorious and contribute to severe health hazards to the humans and aquatic animals. In a variety of fish, they cause motile *Aeromonas* septicemia (MAS). Immunosuppressive events like nutritional, environmental and handling stress may trigger MAS. Different groups of antibiotics are used in the treatment of human and animal diseases and the indiscriminate use of which leads to disturbing consequences of AMR. Much of the susceptibility information available on the genus *Aeromonas* are based solely upon three major species associated with human diseases. It is not entirely clear whether those patterns can be extrapolated to other less frequently encountered taxa causing illness. In recent years, the emergence of AMR among the motile aeromonads of fish demonstrated the abuse of antibiotics in aquaculture. In this review, the AMR among motile aeromonads of the aquaculture environment and their mitigation are discussed. The available data indicated that the opportunistic pathogens like motile aeromonads might serve as important vectors of AMR in the aquatic environment.

Key words: Antibiotic-resistant genes, Antimicrobial-resistance, Aquaculture, Motile aeromonads, Motile *Aeromonas* septicemia

Aeromonads are ubiquitous and autochthonous to the aquatic environment. The oldest and first report of an organism resembling *Aeromonas* was made by Zimmerman (1890) at Chemnitz (Germany),

who coined the name *Bacillus punctatus* due to its nature of colonies on gelatin plates. The genus *Aeromonas* formally accepted and published in the 7th edition of Bergey's Manual of Determinative Bacteriology, have

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a complex taxonomy and is divided into two major groups namely motile group comprised of over 30 species and a non-motile group comprising a singular species *A. salmonicida* (Graf, 2015). The motile representatives of the genus *Aeromonas* have been in a state of constant flux through the continuous introduction of new phenotypic and genotypic data. As a consequence, the taxonomic history of motile aeromonads has been one of confusion and controversy, that led to several reclassifications (Janda and Abbott, 2010; Beaz-Hidalgo *et al.*, 2013; Graf, 2015).

Motile aeromonads are widely distributed in the aquatic environment throughout the world and their autochthonous presence among freshwater, marine and brackishwater (estuarine) environments suggested their popularity as the indigenous microflora of fish (Janda and Abbott, 2010). As large numbers of species are being identified and classified among the motile aeromonads group, the advent of modern taxonomic methods may question the reliability of earlier identifications (Graf, 2015). It is of noteworthy that some names that have been proposed are still not universally accepted (Rasmussen-Ivey *et al.*, 2016; Králová *et al.*, 2016). Multiple research studies have been done employing 16S rRNA gene sequence analysis of the genus *Aeromonas*, which elucidated that the members of this genus form a distinct line within Gamma-Proteobacteria. This genus is comprised of mesophiles and psychrophiles, which can cause a number of diseases to warm and cold-blooded animals (Graf, 2015). *Aeromonas* can cause extra-intestinal diseases in humans, especially in

immunocompromised individuals, including septicemia, wound infections, urinary tract infections, hepatobiliary tract infections and necrotizing fasciitis (Janda and Abbott, 2010). This report discusses the role of motile aeromonads as a pathogen of fish in aquaculture, the emergence of AMR in the aquaculture environment and their mitigation.

Motile *Aeromonas* septicaemia (MAS) in aquaculture

The MAS has been known by several names like *Aeromonas* infection, motile aeromonad infection (MAI), haemorrhagic septicemia, red sore and infectious abdominal dropsy (Öztürk and Altinok, 2014). The first report of MAS was by Schäperclaus (1930) in carps and subsequently, the works of several authors on motile aeromonads have been amply reviewed (Graf, 2015; Austin and Austin, 2016; Plumb, 2018). Also, the epizootiology of MAS has been studied vastly and reviewed by Janda and Abbott (2010). These species can associate themselves with algae, biofilms and zooplankton. They are indigenous to the gut microflora of many vertebrates and invertebrates and have the potential to harbour and spread motile aeromonads (Janda and Abbott, 2010). The uptake of these pathogenic motile aeromonads is mainly by dermal and oral routes, with the former occurring after disruption of the fishes' first line of mucosal defences. Asymptomatic carriers are more likely to deposit the bacterium in their faeces, which accumulate among the benthic flora (Stratev *et al.*, 2015). Diseased individuals may shed these bacteria from open lesions and are preyed upon by other fish (Janda and Abbott, 2010).

The MAS can be correlated to many predisposing factors such as injury, infection with different aetiological agents, or immunosuppressive events like nutritional, environmental and handling stress. Besides, the MAS is often a complicating factor in columnaris disease, saprolegniosis and several cases of viral aetiology (Austin and Austin, 2016). *Aeromonas hydrophila*, *A. sobria*, *A. caviae*, *A. schubertii* (Öztürk and Altinok, 2014) and possibly other motile aeromonads are capable of producing diseases in fish (Hanson *et al.*, 2014; Plumb, 2018). Typical MAS outbreaks are most prevalent in spring and fall when fluctuating temperatures compromise the immune system (Hanson *et al.*, 2014). The external symptoms of MAS range from abscesses and ulcers to accumulation of fluid in scale

pockets (lepidorthosis) and abdomen. Internal symptoms include blood in ascites and swollen friable kidney (Stratev and Odeyemi, 2017). Control measures for an *Aeromonas* epidemic must, therefore, address both environmental and bacteria-related constituents. Reducing stress is both challenging and vital to *Aeromonas* control (Austin and Austin, 2016; Plumb, 2018). The rapid increase in water temperature and avoidance of pathogens, diseased fish introduction and contaminated water are suggested (Stratev and Odeyemi, 2017; Plumb, 2018). Prophylactic antibiotics have been recommended following known stress, but this approach can increase the incidence of antibiotic resistance, and the cost may be prohibitive (Plumb, 2018).

Table 1. The dose and dosage of antibiotics used against MAS in fish

Antibiotic/Class	Dose and dosage	References
Oxytetracycline	2.5 - 3.75 g /100 pound fish/ day for 10 days	USFWS, 2015
Furanace (Nifupirinol)*	Immersion treatment with 1–2 ppm for 5–10 min in water	Austin and Austin, 2016
Ormethoprim and sulfadimethoxine	50 mg/kg fish/day for 5 days	USFWS, 2015

*: Currently unapproved for aquaculture use

Table 2. The resistance and susceptibility of motile aeromonads' towards various antibiotics

Susceptible (90–100%)	Aminoglycosides, carbapenems, cephalosporins (extended-spectrum), cephalosporins ("fourth generation"), macrolide (azithromycin), monobactams, nitrofurans, penicillin (extended-spectrum), phenicols, quinolones, tetracyclines
Intermediate (70–90%)	Aminoglycosides, antifolates, cephalosporins (expanded spectrum)
Resistant (<70%)	Antifolates, cephalosporins (narrow spectrum), penicillins (extended spectrum), macrolides, penicillins (narrow spectrum)

(Source: Janda and Abbott, 2010)

Antibiotic usage against motile *Aeromonas* septicemia

Disease control in aquaculture relies on an amalgamation of good management practices, the use of few approved and commercially available drugs and vaccines, and prevention of infection (Austin and Austin, 2016). In aquaculture, the use of antimicrobial compounds essentially started with the work of Gutsell (1946), who recognized the potential of sulphonamides for combating furunculosis in trout. A range of antimicrobial compounds in aquaculture rapidly expanded following the introduction of sulphonamides (Austin and Austin, 2016). The detailed descriptions of the antibiotics that are used against MAS in fish are presented in Table 1. Drug choices for the treatment of common infectious diseases have become progressively restricted. To date, only three antimicrobials are approved by the United States Food and Drug Administration (USFDA) as feed additives for the treatment of bacterial disease in fish, which has been recognized worldwide as food additives used for the treatment of bacterial infection in fish (USFWS, 2015). It includes (i) oxytetracycline (Terramycin® 200), (ii) florfenicol (Aquaflor®) and (iii) ormethoprim and sulfadimethoxine (Romet-30). The use of unapproved drugs or misuse of approved drugs in aquacultured fish poses a potential human health hazard. These substances may be toxic, allergenic, or carcinogenic, and/or may cause antibiotic-resistance in pathogens that affect humans (Leonard *et al.*, 2015).

Antimicrobial resistance in motile aeromonads

The general view of aquacultured motile aeromonads' resistance and susceptibility towards various antibiotics as classified by Janda and Abbott (2010) are presented in

Table 2. *Aeromonas* spp. are intrinsically resistant to ampicillin and cefazolin due to their beta-lactamase production (Dias *et al.*, 2012). Most aeromonads continue to be susceptible to tetracyclines, aminoglycosides, quinolones, trimethoprim-sulfamethoxazole and third-generation cephalosporins (Janda and Abbott, 2010). In recent years, increasing resistance references to tetracyclines, trimethoprim-sulfamethoxazole, some extended-spectrum cephalosporins (ceftriaxone, cefotaxime and cefixime) are emerging (Piotrowska and Popowska, 2014, 2015; Deng *et al.*, 2016; Zhou *et al.*, 2019). In clinical biology, the overall susceptibility profile for the genus *Aeromonas* does not appear to have changed appreciably from what was recorded in studies conducted between the mid-1980s and mid-1990s (Stratev and Odeyemi, 2016). Fluoroquinolone resistance among *Aeromonas* isolates remained uncommon, and occur in <10% of the motile aeromonads (Aravena-Roman *et al.*, 2012). An increasing ciprofloxacin-resistance rate was, however, noted in *Aeromonas* isolates associated with intra-abdominal infections in the Asia-Pacific region, from 7.8% between 2003 and 2006 to 16.6% between 2007 and 2010 (Li *et al.*, 2012). Aminoglycoside resistance remains rare worldwide (Kimura *et al.*, 2013). Overall, in addition to well-known chromosomally encoded beta-lactamases, aeromonads would acquire plasmids or integron-mediated resistance genes, expressing resistance to one or multiple classes of antimicrobial agents (Chen *et al.*, 2012).

Antimicrobial resistance in motile aeromonads of aquaculture environment

The intensive use of antimicrobials in aquaculture to combat diseases provides a selective pressure creating reservoirs

of drug-resistant bacteria and transferable resistance genes in fish pathogens and other bacteria in the aquatic environment (Romero *et al.*, 2012). Various authors have emphasized the putative negative effects of using antimicrobial agents in fish farms (Romero *et al.*, 2012; Watts *et al.*, 2017; Aich *et al.*, 2018). Motile aeromonads remain the most dominant group isolated from aquatic environments due to its omnipresence. The major studies dealing with the general susceptibility of motile aeromonads of fish to various classes and combinations of antimicrobial agents are very limited. Susceptibility patterns of *Aeromonas* spp. from aquatic environments to various antimicrobial agents are well documented (Romero *et al.*, 2012). *Aeromonas hydrophila* strains resistant to third and fourth generations cephalosporins, aminoglycosides, quinolone and fluoroquinolones and other commonly used antibiotics, and sensitive to cephalosporins (cefuroxime, ceftriaxone, cefpodoxime, cefalotin, ceftazidime, cephalexin, cefoxitin, cefotaxime), chloramphenicol, tetracycline, imipenem, quinolones, gentamicin, kanamycin, nitrofurantoin, norfloxacin, streptomycin, sulphamethoxazole, ciprofloxacin, oxytetracycline, doxycycline, amikacin, aminoglycosides, amoxicillin, ticarcillin, ofloxacin, pefloxacin, neomycin, oxacillin, gatifloxacin and levofloxacin have been isolated from different diseased fish in different parts of world (Kaskhedikar and Chhabra, 2010; Ashiru *et al.*, 2011; Aravena-Román *et al.*, 2012; Romero *et al.*, 2012; Afizi *et al.*, 2013; Samal *et al.*, 2014; Wamala *et al.*, 2018). Few studies reported the susceptibility data for *Aeromonas* species other than *A. hydrophila*, *A. caviae*, and *A. veronii* bv. *sobria* (Zhou *et al.*, 2019). Several other

studies have shown a high prevalence of drug-resistant *Aeromonas* spp. from fish (Čížek *et al.*, 2010; Bardhan, 2019). Antibiotic-resistance profile of *A. veronii*, *A. media*, *A. jandaei*, *A. hydrophila*, *A. caviae*, *A. culicicola* and *A. aquariorum* isolated from the skin of ornamental fish imported to Portugal revealed a resistance level of >80% to tetracycline, ticarcillin, carbenicillin, ampicillin and erythromycin. Ciprofloxacin and norfloxacin resistance was 43% and 34%, respectively among *A. hydrophila* (Dias *et al.*, 2012). *Aeromonas schubertii* isolated from diseased snakeheads in China was susceptible to cefoxitin and cefoperazone and resistant to polycyclin, cefotamine, penicillin-G, clindamycin and oxacillin (Chen *et al.*, 2012).

Paul *et al.* (2015) documented antibiotic-resistant motile aeromonads induced septicemia in catfish. *Aeromonas* spp. of diseased catfish were resistant to broad-spectrum co-trimoxazole, chloramphenicol, ciprofloxacin, gatifloxacin, gentamicin, sulphafurazole and oxytetracycline. The results of Bardhan (2019) demonstrated that motile *Aeromonas* spp. of the carp aquaculture systems of West Bengal were highly resistant to amoxyclav (77.69%), cefalexin (76.86%) and nitrofurantoin (67.88%). Considerably lower resistance to ciprofloxacin and sulfafurazole was noted. The multiple antibiotic-resistance (MAR) index of these motile aeromonad strains varied between 0.33 and 1.0 (Bardhan, 2019). The uprise in AMR among these motile aeromonads suggested a heavy uproar and concern. These observations are of great concern because a sample with MAR index above 0.2 is considered to be exceedingly manifested with antibiotics and having a high-risk factor (Saka *et al.*, 2017). The results of several earlier studies also

Table 3. Antibiotic resistance genes of motile aeromonads of different environments

<i>Aeromonas</i> species	Resistance gene	Resistance phenotype	Environmental source
<i>Aeromonas media</i> , <i>A. allosaccharophila</i>	accA4-cr	Quinolones	WWTP (TWW, RWW), WTP, NW (L)
<i>A. media</i> , <i>A. allosaccharophila</i>	qnrS2	Quinolones	WWTP (TWW, RWW),
<i>A. allosaccharophila</i> , <i>A. aquariorum</i>	gyrA	Quinolones	WWTP (TWW, RWW), WTP,
<i>A. hydrophila</i> , <i>A. media</i> , <i>A. caviae</i> , <i>A. popoffii</i>			NW (L, R), F
<i>A. allosaccharophila</i> , <i>A. aquariorum</i> ,	parC	Quinolones	WWTP (TWW, RWW),
<i>A. hydrophila</i> , <i>A. media</i> , <i>A. veronii</i> ,			WTP, NW (R, F)
<i>A. caviae</i> , <i>A. taiwanensis</i>			
<i>Aeromonas</i> sp., <i>A. caviae</i> , <i>A. media</i> , <i>A. hydrophila</i>	aadA1	Aminoglycosides	WWTP (TWW, EW), NW (M, F), HC, AQ
<i>Aeromonas</i> sp., <i>A. caviae</i> ,	aadA2	Aminoglycosides	WWTP (TWW), NW
<i>A. hydrophila</i> , <i>A. eucrenophila</i> , <i>A. media</i> , <i>A. veronii</i>			(M, R, F), HC, AQ (S, F)
<i>Aeromonas</i> sp.	sat2	Aminoglycosides	WWTP (EW)
<i>A. hydrophila</i> , <i>A. sobria</i> , <i>A. veronii</i> , <i>A. isthiosoma</i> , <i>A. encheleia</i> ,	aadA1a	Aminoglycosides	AQ (F, S)
<i>A. bestiarum</i>			
<i>A. caviae</i> , <i>A. hydrophila</i>	accA4	Aminoglycosides	AQ (F)
<i>A. caviae</i> , <i>A. bestiarum</i>	strA-strB	Aminoglycosides	AQ, (R, S, F)
<i>A. hydrophila</i> , <i>A. isthiosoma</i> , <i>A. sobria</i> , <i>A. encheleia</i>	aacA	Aminoglycosides	AQ
<i>A. hydrophila</i> , <i>A. veronii</i> , <i>A. jandei</i>	cphA	β -lactams	WWTP (TWW), WTP,
<i>A. allosaccharophila</i> , <i>A. aquariorum</i> , <i>A. media</i> , <i>A. bestiarum</i> ,			NW (M), HC
<i>A. eucrenophila</i>			
<i>Aeromonas</i> spp.	bla _{XPC-2}	β -lactams	WWTP, SW
<i>A. caviae</i> , <i>A. allosaccharophila</i>	bla _{VEB-1a}	β -lactams	NW (R)
<i>A. veronii</i> , <i>A. media</i>			
<i>A. allosaccharophila</i> , <i>A. veronii</i>	bla _{SHV-12}	β -lactams	NW (R, M)
<i>A. media</i> , <i>A. caviae</i> , <i>A. hydrophila</i>			
<i>A. allosaccharophila</i>	bla _{PER(1,6)}	β -lactams	NW (R, M)
<i>A. veronii</i> , <i>A. media</i> , <i>A. caviae</i>			
<i>A. veronii</i>	bla _{GES-7}	β -lactams	NW (R)
<i>A. allosaccharophila</i>	bla _{TLA-2}	β -lactams	NW (R)
<i>A. media</i> , <i>A. caviae</i> , <i>A. sobria</i> , <i>A. caviae</i>	bla _{OXA(2,7)}	β -lactams	NW (M, F), AQ
<i>Aeromonas</i> sp., <i>A. media</i> , <i>A. veronii</i> , <i>A. hydrophila</i> , <i>A. caviae</i>	bla _{TEM}	β -lactams	WWTP (RWW), NW (M, R, L, F), HC
<i>A. hydrophila</i> , <i>A. caviae</i>			NW (R, M)
<i>Aeromonas</i> sp., <i>A. isthiosoma</i>		β -lactams	WWTP, AQ

Cont. Table 3.

Aeromonas species	Resistance gene	Resistance phenotype	Environmental source
<i>Aeromonas</i> sp., <i>A. caviae</i>	bla _{SE1}	β-lactams	NW (R, M)
<i>Aeromonas</i> sp., <i>A. media</i> , <i>A. sobria</i> , <i>A. hydrophila</i>	dfra1/7	Trimethoprim	WWTP (TWW, EW), HC, AQ, NW (F)
<i>A. hydrophila</i> , <i>A. media</i> , <i>A. veronii</i> , <i>A. eucrenophila</i> , <i>A. caviae</i>	dfra12	Trimethoprim	NW (estuarine, F), HC, AQ
<i>A. hydrophila</i>	dfra13	Trimethoprim	AQ (F)
<i>A. hydrophila</i>	dhfr2a	Trimethoprim	AQ, S
<i>A. hydrophila</i>	dhfr1	Trimethoprim	F, AQ, S
<i>A. hydrophila</i> , <i>A. sobria</i> , <i>A. veronii</i> , <i>A. bestiarum</i>	sul1	Sulphonamides	AQ (S, F)
<i>A. veronii</i> , <i>A. caviae</i> , <i>A. hydrophila</i>	sul2	Sulphonamides	AQ, NW (R, S)
<i>A. bestiarum</i>	estX	Streptothricin	WWTP (EW)
<i>Aeromonas</i> sp.	Cat	Chloramphenicol	NW (M)
<i>Aeromonas</i> sp.	catB2	Chloramphenicol	F, AQ (S)
<i>A. hydrophila</i> , <i>A. sobria</i> , <i>A. veronii</i> , <i>A. bestiarum</i>	catB3	Chloramphenicol	NW (L) AQ
<i>A. allasaccharophila</i> , <i>A. hydrophila</i>	catB8	Chloramphenicol	NW (M), HC, AQ (F)
<i>A. hydrophila</i> , <i>A. caviae</i>	flo _R	Chloramphenicol	AQ (F), NW (R, S)
<i>A. hydrophila</i>	tetA	Tetracycline	WWTP, HC, AQ (F),
<i>A. hydrophila</i> , <i>A. allosaccharophila</i>	tetA	Tetracycline	F, S, NW (R)
<i>A. veronii</i> , <i>A. sobria</i> , <i>A. encheleia</i> , <i>A. media</i> , <i>A. caviae</i>	tetB	Tetracycline	AQ
<i>A. hydrophila</i>	tetC	Tetracycline	HC, AQ, S, F
<i>A. hydrophila</i>	tetD	Tetracycline	HC, AQ, S, F (AQ), WWTP, HC,
<i>Aeromonas</i> sp., <i>A. hydrophila</i> , <i>A. encheleia</i> , <i>A. veronii</i> , <i>A. sobria</i> , <i>A. caviae</i> , <i>A. eucrenophila</i> , <i>A. hydrophila</i> , <i>A. ichthiosomia</i> , <i>A. sobria</i> , <i>A. encheleia</i> , <i>A. veronii</i> , <i>A. caviae</i>	tetE	Tetracycline	AQ, S, F,
<i>Aeromonas</i> sp., <i>A. hydrophila</i> , <i>A. encheleia</i> , <i>A. veronii</i> , <i>A. sobria</i> , <i>A. caviae</i>	tetG	Tetracycline	AQ (F)
<i>A. hydrophila</i> , <i>A. encheleia</i>	tetH	Tetracycline	AQ
<i>A. hydrophila</i> , <i>A. sobria</i> , <i>A. hydrophila</i>	tetM	Tetracycline	AQ (F)
<i>A. bestiarum</i>	tetY	Tetracycline	NW
<i>A. caviae</i>	vatE	Streptogramin	AQ (F)
<i>Aeromonas</i> sp., <i>A. hydrophila</i> , <i>A. encheleia</i> , <i>A. veronii</i> , <i>A. sobria</i> , <i>A. caviae</i> , <i>A. eucrenophila</i> , <i>A. hydrophila</i> , <i>A. ichthiosomia</i> , <i>A. sobria</i> , <i>A. encheleia</i> , <i>A. veronii</i> , <i>A. caviae</i>	mefA, ermC, ermE, ermX, ermC	Macrolides	AQ (F)

WWTP- wastewater treatment plant; RWW- raw wastewater; TW- treated wastewater; WTP- water treatment plant; EW- effluent water; SW- special wastewater from hospital; NW- natural water; HC- human consumption; AQ- aquaculture; F - fish; S- sediment; L- lake, R-river; M-marine waters, estuaries (Source: Piotrowska and Popowska, 2014; Watts *et al.*, 2017)

suggested the role of aeromonads as reservoirs or vectors of antimicrobial resistance determinants through horizontal gene transfer between aeromonads and coexistent bacteria in aquatic or gut microenvironments (Sreedharan *et al.*, 2012; Bharathkumar and Abraham, 2013). The MAR *Aeromonas* spp. found in different cultured freshwater fish implied that difficulties might arise in the prophylaxis and therapy of *Aeromonas* infections (Romero *et al.*, 2012; Bharathkumar and Abraham, 2013; Bardhan, 2019). The recent trends of AMR among motile aeromonads and other bacteria isolated from fish demonstrated the abuse of antibiotics (Romero *et al.*, 2012; Watts *et al.*, 2017; Bardhan, 2019). Such trends of AMR among the most dominant bacterial flora of the aquatic environment indicated a danger to humans and animals as well (Watts *et al.*, 2017).

Antibiotic-resistant genes (ARGs) in motile aeromonads

Antimicrobial resistance in bacterial populations can occur from the clonal selection under antimicrobial selective pressure or through horizontal gene transfer. Mobile genetic elements viz., plasmids, integrons and transposons contribute to wider dissemination of genetic resistance determinants among bacterial populations (Piotrowska and Popowska, 2015). The Tn3 family of transposons play a central role in disseminating resistance to antibiotics in Gram-negative bacteria such as *Aeromonas* (Carnelli *et al.*, 2017). They are especially associated with MAR plasmids of *Aeromonas* (del Castillo *et al.*, 2013). Genetic determinants such as integrons, transposons and resistance plasmids,

involved in the spread of antibiotic resistance, were often detected in *Aeromonas* strains and may be a crucial genotypic determinant for AMR among motile aeromonads (Carnelli *et al.*, 2017). *Aeromonas* spp. are the part of the bacterial population with several chromosomal β -lactamase genes, including *cphA*, *cepH* and *ampH*, which encode class B, C and D β -lactamases, respectively (Balsalobre *et al.*, 2010). Integrons-inserted gene cassettes have been documented in widespread *Aeromonas* species isolated from diseased fish (Piotrowska and Popowska, 2014). The most often found resistance gene cassettes contained several aminoglycoside resistance genes *aadA1*, *aadA2*, and the trimethoprim resistance gene *dfrA1* (Piotrowska and Popowska, 2015). *Aeromonas* spp. isolated from natural waters frequently demonstrated a tetracycline-resistance phenotype (Tação *et al.*, 2012). The resistance to oxytetracycline was most often coded by the *tet(E)* gene or by a combination of the *tet(E)* gene with *tet(A)* and *tet(D)* genes in motile aeromonads from common carp and koi carp (Čížek *et al.*, 2010). A wide range of antibiotic-resistance genes (ARGs) have been documented in motile aeromonads from different environments and the details of the same are presented in Table 3. The prevalence of several of these genes suggested that motile *Aeromonas* spp. can be unique vectors in spreading the ARGs in the aquatic environment.

Mitigation

The last decade of the 20th century and the first decade of the 21st century have witnessed the emergence and spread of

antibiotic resistance in pathogenic bacteria around the World, and the consequent failure of antibiotic therapy (Palmer and Kishony, 2013). *Aeromonas hydrophila* was reportedly caused lung colonization with cystic fibrosis, believed to have originated from the tropical fish tank (Cremonesini and Thomson, 2008) and zoonotic via spine puncture or open wounds (Haenen *et al.*, 2013). Although no cases of resistant motile aeromonad infections that has been transferred from fish to humans are available, it has been speculated that transmission to humans may occur when cultured fish containing AMR bacteria and ARGs are consumed. Therefore, to address the problem of antibiotic-resistance, effective strategies are required. As global production of aquatic species (fish, shellfish, shrimp and molluscs) has been growing rapidly in the last decade, the use of antibiotics in aquaculture also increased (Watts *et al.*, 2017). To prevent the emergence and transfer of antibiotic resistance in aquatic food animals, new methods to manage infectious diseases in aquaculture are required. For example, optimal use of existing vaccines can be a viable alternative (Assefa and Abunna, 2018). Improving hygiene (Austin and Austin, 2016), using enzymes, probiotics, prebiotics and acids to improve health (Sayes *et al.*, 2018; Hoseinifar *et al.*, 2018), and utilizing bacteriocins, antimicrobial peptides and bacteriophages as substitutes for antibiotics might be good methods to promote fish growth and decrease infectious diseases in them (Rao and Lalitha, 2015).

The Study for Monitoring Antimicrobial Resistance Trends (SMART) is the premier

global surveillance system on antimicrobial resistance of microbes since 2002. It is a global surveillance program designed to longitudinally monitor the epidemiological trends and *in vitro* antimicrobial susceptibilities of 12 antimicrobial agents against a variety of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections (Hsueh, 2012). Data from SMART studies showed that the level of antimicrobial resistance differs by geographic region and is highest in Asia-Pacific countries (Sumpradit *et al.*, 2017). Likewise, it is worthwhile to formulate internationally acceptable standard protocols about the use of antibiotics in aquaculture and about surveillance programs to monitor the global emergence of MAR bacteria. Reducing antibiotic use in aquaculture is also important. Policies to change the use of antibiotics in aquaculture can include ban or restriction on using medically important antibiotics, promising financial incentives for developing aquatic animal-specific antibiotics, making drug licensing rules more stringent and imposing penalties on defaulters. Continuous efforts to educate people about antibiotic resistance are a very important strategy. Improvement of sanitation and hygiene in aquaculture is also meaningful (Romero *et al.*, 2012; Stratev and Odeyemi, 2017). As the major suitable targets of antibiotics have already been identified, to discover new classes of antibiotics, novel strategies for rational design and screening-based approaches are required. Unlike conventional antimicrobial drugs, new strategies are also presented for the treatment of microbial diseases, such as host defence peptides, bacteriophages,

vaccines, immunoglobulins, and probiotics (Lloyd, 2012). Future prospects for MAS control include the development of a modified-live vaccine as well as a vaccine against the various enzymes and toxins produced by *Aeromonas* spp. (Stratev and Odeyemi, 2017).

Conclusion

The tenacity and propagation of AMR in the environment represent a global health crisis. According to FAO/OIE/WHO (2019), if AMR is not controlled or monitored, we will be moving to a post-antibiotic era, an era where no new antibiotics replacement will be available and common, simple, treatable diseases would lead to a new epidemic. Therefore, it is critical that we understand environmental hotspots for genetic exchange of AMR genes such as aquaculture systems and determine how they might transfer to clinically equivalent strains. In general, infections caused by *Aeromonas* spp. do not directly pose a substantial threat to public health. However, the transfer of ARGs causes ineffectiveness of antibiotic treatment

of fish diseases and leads to significant economic losses in aquaculture. Given the role of *Aeromonas* spp. as ARG vectors between environmental bacteria and clinical human pathogens, as well as the existence of MAR *Aeromonas* strains, and the location of ARG on mobile genetic elements, the presence of resistant *Aeromonas* strains in the environment may pose a serious threat to human health. The alternative disease management strategies combined with a better understanding of the effects on the microbiome of the farmed fish may provide alternative solutions to improve aquaculture health and function while reducing the potential for the spread of antimicrobial resistance.

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