Diversity and Antibiotic Resistance of Aeromonas in Inflow and Outflow Water in Carp Ponds

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Abstract

The diversity and antibiotic resistance of the genus *Aeromonas* were studied in the inflow region and outflow region in three carp ponds of a commercial fish farm. The collected data indicated that the abundance of *Aeromonas* differed among the research water basins, regions and seasons. The results of the present study showed that *Aeromonas* inhabiting the water of the studied ponds strongly differed in the resistance level to tested antibiotics. This genus of bacteria was the most resistant to amoxicillin, ampicillin, clindamycin and penicillin and susceptible to streptomycin. The *Aeromonas* bacteria inhabiting the inflow were more resistant to tested antibiotics than those inhabiting the outflow of the studied ponds. Results of this study showed that multiple antibiotic resistance was observed among *Aeromonas* isolated from studied water basins which indicates the pollution of this environment with antibiotic substances. *Aeromonas* inhabiting the inflow and outflow regions of studied three carp ponds show different level resistence to antibiotics belonging to the different classes.

1. Introduction

Gram-negative bacteria, autochthonous and ubiquitous in aquatic environments which are members of the genus *Aeromonas* are considered to be one of the most important bacteria among ecological agents of bacterial fish diseases around the world (Dahanayake et al. 2019; Silva et al. 2019; Gołaś et al. 2019). Motile *Aeromonas* species including many species are well recognized as characteristic opportunistic bacterial pathogens that are responsible for diseases and mortality of numerous fish species (Yu et al. 2015; Chenia, 2016; Mulyani et al. 2018). Gross symptoms in fish infected with *Aeromonas* sp. include dermal ulceration, fin rot, red sores, lethargy, pop-eyes, ocular ulceration, rotting of tails, haemorrhagic septicaemia, loss of appetite, histopathological changes in intestine, liver and kidney, moreover, due to infection up to 80–100% of fish die within 1–2 weeks (Yang et al. 2017; Mulyani et al. 2018; Dahanayake et al. 2019). The hypervirulent pathotype *A. salmonicida* responsible for fish furunculosis can cause host death within hours (Skwor et al. 2014; Menanteau-Ledouble et al. 2016). *Aeromonas* sp. have been used to explain the pathogenesis of infections as they synthesize different toxins including haemolysins, enterotoxins, endotoxins, aerolysins, cytotoxins, adhesions and exoenzymes including lipase, elastase, DNAse, and protease which together contribute to overall disease progress and degrade the host cells (Odeyemi and Ahmed, 2014; Hossain et al. 2018; Syrova et al. 2018). Naturally occurring plasmids encoding these virulence factors have been identified in the genus *Aeromonas* (Furmanek-Blaszk 2014). Changes in environmental conditions, such as stress, which decreases effectiveness of fish immune system, overcrowding, a sudden temperature change, hypoxia, high concentration of nitrite, unhygienic handling, poor feed quality, organic pollution, non-bacterial pathogenic infections and rough weather conditions are the most common predisposing factors associated with disease among fish (Rajpakshe et al. 2012; Dar et al. 2016, Hossain et al. 2018, Zdanowicz et al. 2020). Diseases in fish farming systems, which are currently one the most relevant food sources in several countries are recognized as an important limiting factor to production and cause the great economic loss in the fish farming industry (Yang et al. 2017; Silva et al. 2019).

In aquaculture, in order to control and limit spread of bacterial fish infections and diseases different antibiotics are used (Saidi et al. 2013; Mulyani et al. 2018). Consequently, the widespread abuse of these pharmaceuticals by most farmers for therapeutic and prophylactic reason in aquaculture exerts selective pressures on environmental microbes and may accelerate evolution and dissemination of antibiotic resistance by bacteria (Piotrowska and Popowska 2014; Patil et al. 2016, Zdanowicz et al. 2020). The mechanisms of antibiotic resistance among aquatic bacteria are diverse, e.g., (i) reduction of membrane permeability, (ii) drug inactivation, (iii) rapid efflux of antibiotic and (iv) mutation of cellular target (Saidi et al. 2013; Kim et al. 2015). According to Zhang et al. (2009) bacterial resistance to certain antibiotic may be based on more than one of aforementioned mechanisms. The dissemination of antibiotic resistance determinants among the bacterial community is mainly attributed to rapid horizontal gene transfer of genetic elements, such as integrons, plasmids and transposons (Yang et al. 2017; Dahanayake et al. 2019). The genetic plasticity of the microbial community enables resistance genes to move quickly throughout different environmental bacterial populations and communities. Aquaculture systems and farms have been designated as “genetic reactors” or “hotspots for antibiotic resistance genes” (Watts et al. 2017).

Recently, *Aeromonas* genus was proposed by several authors (Patil et al. 2016; Varela et al. 2016; Baron et al. 2017) as a good potential indicator for monitoring the spread of antibiotic resistance in the aquatic environment. Also as suggested in the Terrestrial Animal Health Code (2005) *Aeromonas* could be a potential candidate as a model indicator organism in order to study the spread of antibiotic resistance in fish farming. Therefore, the aim of the present work was (i) to determine the abundance of *Aeromonas* inhabiting water of the inflow and outflow regions of three carp (*Carpinus carpio)* ponds of a commercial fish farm (ii) to investigate

the seasonal dynamics in the number of these bacteria (iii) to study level of antibiotic resistance of *Aeromonas* bacteria inhabited investigate water basins. This study is a continuation of the research conducted on these ponds in 2013, the results of which were published in Zdanowicz et al. (2020).

2. Material And Methods

2.1. Study and sampling area

This study was carried out in three carp ponds, fry (FRY), fingerling (FING) and marketing (MAR) situated in Wiklino (Poland) (Fig. 1). All these ponds are located in the depression surrounded by arable land. Basic data on morphological, physicochemical and bacteriological characteristics of the studied ponds are presented in Table 1. Another detailed description of characteristics these water basins is given by Zdanowicz et al. (2020).

Table 1
Values of selected morphometry, some physicochemical and bacteriological parameters of studied ponds (*Kamińska 2015)

Parameters			Fry pond	Fingerling pond	Marketing pond
Pond type			artificial	artificial	natural
Area			0.5 ha	3 ha	3 ha
Average depth			0.7 m	0.8 m	0.9 m
Temperature	sp	inflow	12	13	15
		outflow	14	14	14
	s	inflow	21	23	25
		outflow	24	24	25.5
	a	inflow	12	14	14
		outflow	13.5	14	14.5
pH	sp	inflow	8.3	7.7	7.6
		outflow	8.4	7.8	7.7
	s	inflow	7.9	8.0	7.6
		outflow	8.1	7.9	7.6
	a	inflow	8.0	7.7	7.6
		outflow	8.1	7.6	7.6
*Total Bacterial Number ($10^9 \cdot dm^{-3}$)			1.35	1.40	1.79
*Psychrophilic bacteria number (10^3 CFU$\cdot cm^{-3}$)			51.14	28.39	21.56
*Mesophilic bacteria number (10^3 CFU$\cdot cm^{-3}$)			4.75	11.53	8.39

Water samples were collected from the studied ponds in two regions: at inflow (If) of water from the Brodniczka River and at outflow (Of) of water at the outlet monk sluice (Fig. 1) in the spring(sp), summer(s) and autumn(a) seasons in 2014.

Similary as presented in publication Zdanowicz et al. (2020) water samples were collected from the depth of about 15 cm below the water surface directly into sterile glass bottles. Collected water samples were stored in an ice-box, transported to the laboratory and were subjected to microbiological analysis.

2.2 Determination of Aeromonas number

For detection of *Aeromonas* bacteria in the collected samples of water, these samples according to Zdanowicz et al. (2020) were diluted with sterile phosphate-buffered saline (PBS) to reach final concentration ranging from 10^{-1} to 10^{-3}. Diluted water samples were filtered using a 0.45-µm pore size, 47 mm - diameter membrane filters (Whatman ME 25/31 ST). The filters were then aseptically transferred into Petri dish with Aeromonas Isolation Agar (Biocorp). Dark green colonies (sulfise production and no acid fermentation) was considered as presumptive *Aeromonas*. The number of these bacteria was counted and results were calculated as colony forming units (CFU) per 1 cm^3 of water. All presumptive *Aeromonas* isolates were than subjected to a series of morphological and conventional biochemical tests according to Jeeva et al. (2013), Rashid et al. (2013) and Zdanowicz et al. (2020) which eventually led to their identification. *Aeromonas* strains were kept on nutrient agar slants at room temperature and maintained by subculturing every month and then used to determine their antibiotic resistance.

2.3 Antibiotic resistance profiling of Aeromonas isolates

Aeromonas strains isolated from collected water samples were subjected to resistance testing using the agar disk diffusion method as described detailed by Mudryk et al. (2015) and Zdanowicz et al. (2020). The following diffrent 12 clinical and aquaculture used antibiotics (their codes and concentrations [µg disc] were given in parentheses) grouped into seven classes depending on their mechanisms of action and their chemical structure: aminoglycosides (AMG) (gentamycin - GN, 10µg, neomycin - N 30µg, streptomycin - S 300µg), β-lactams (LA) (amoxicillin - AX 25µg, ampicillin - AM 10µg, penicillin - P 10µg), chloramphenicols (CHL) (chloramphenicol - C 30µg), lincosamides (LIN) (clindamycin - CA 2µg), macrolides (MAC) (erythromycin - E 15µg), fluoroquinolones (FLU) (ciprofloxacin - CIP 5µg) and tetracyclines (TET) (tetracycline - TE 30µg, oxytetracycline - OT 30µg) (Reinthaler et al., 2003; Li et al., 2017). The results were used to calculate the Antibiotic Resistance Index (ARI) (Webster et al. 2004).

According to EUCAST the strains showing resistant or intermediate behaviour were subsumed under the category resistant. All others strains were classified as sensitive.

2.4 Statistic analyses

Standard deviation (SD), coefficient of variation (CV), and coefficient of dispersion (CD) were calculated according to Velji and Albright (1986). The relationships among the parameters within the whole data set were examined with the Spearman's rank correlation coefficient. The significance of the differences in *Aeromonas* abundance between the ponds and seasons was assessed using 2-way ANOVA. The analyses were performed using the STATISTICA 12 software.

3. Results

In Table 2 are presented data on number planktonic *Aeromonas* bacteria inhabited three studied water basins. The present study has shown that those bacteria in fingerling pond (mean: $5.35 \cdot 10^2$ CFU · cm^{-3}) was two-fold higher than in fry and marketing ponds (mean: $1.91–2.61 \cdot 10^2$ CFU · cm^{-3}). In all three studied ponds *Aeromonas* abundance was higher in the outflow region ($2.09–5.9 \cdot 10^2$ CFU · cm^{-3}) than in the inflow region ($1.73–2.19 \cdot 10^2$ CFU · cm^{-3}). Studied taxonomic group bacteria isolated in the inflow and outflow regions of the analyzed water basins characterized seasonal variation (Fig. 2). The maximum abundance of *Aeromonas* in If region in all studied ponds was recorded in summer. The lowest number of the studied bacteria in this region was noted in fry and marketing ponds in spring, while in fingerling pond in autumn. In Of region the higher abundance of *Aeromonas* was noted in fry and marketing ponds in summer, while in fingerling pond in autumn. In all studied ponds in the outflow region the minimum number of the studied bacteria was recorded in spring.

Table 2
Occurrence and abundance of *Aeromonas* in water samples collected from studied

Pond	Site	CFU (ml)	Range	SD	CV(%)	CD
Fry	inflow	173	20–440	38.8	43.9	33.4
	outflow	209	60–400	59.9	112.0	262.0
	mean	191				
Fingerling	inflow	480	260–720	102.6	97.3	454.3
	outflow	590	40–1380	183.1	55.8	183.5
	mean	535				
Marketing	inflow	239	50–600	77.2	73.6	129.6
	outflow	283	68–540	49.5	95.1	255.7
	mean	261				

The genus *Aeromonas* inhabited water of the studied water basins was show differences in the level of resistance to antibiotics (Table 3). In the studied ponds all isolated bacteria the genus *Aeromonas* were resistant to AM, AX, CA, and P. These studies show that 90% of bacterial strains were resistant to erythromycin and only 10–30% to tetracycline and oxytetracycline. In the present study, all isolates inhabiting the studied water bodies were susceptible to streptomycin. *Aeromonas* inhabiting the inflow region of the studied ponds were more resistant (ARI = 0.50) to tested antibiotics than those inhabiting the outflow region (ARI = 0.37–0.38).

Table 3
Percentage of antibiotic resistance among *Aeromonas* isolated from surface water of

Pond	Sampling site	Numbers of studies strains	Antibiotics												
			C	OT	TE	S	P	E	N	AM	AX	CIP	GN	CA	ARI
Fry	inflow	44	9.1	18.2	22.7	0.0	100	90.9	4.5	100	100	0.0	0.0	100	0.5
	outflow	45	0.0	15.5	26.6	0.0	100	87.5	1.9	100	100	0.0	0.0	100	0.37
Fingerling	inflow	47	4.3	21.3	21.3	0.0	100	87.2	12.8	100	100	2.1	2.1	100	0.5
	outflow	41	0.0	30.6	26.4	0.0	100	81.8	2	100	100	0.0	2.1	100	0.38
Marketing	inflow	36	2.8	16.7	22.2	0.0	100	91.7	8.3	100	100	2.8	2.8	100	0.5
	outflow	34	0.0	11.9	23.8	0.0	100	92.9	11.1	100	100	0.0	0.0	100	0.37

Aeromonas bacteria isolated from the inflow and outflow regions of the studied ponds were analysed for multiple antibiotic resistance (Fig. 3). The results showed that 40–60% of *Aeromonas* inhabited both studied regions of the water basins were resistant to 5 antibiotics and about 20% to 4, 6 and 7 of the 12 antibiotics tested. No strains of the studied bacteria genus inhabiting If and Of regions were resistant to 1–3 and 10–12 antibiotics.

Data on bacterial strains resistance to different classes of the studied antibiotics are given in Fig. 4. All *Aeromonas* bacteria inhabited in studied ponds in both regions were resistant to β-lactams (LA) and lincosamides (LIN). About 80–90% of bacteria isolated from If and Of regions of the carp ponds were resistant to macrolides (MAC). Bacteria isolated from both studied regions showed the lowest (0-9.1%) resistance level to aminoglycosides (AMG), fluoroquinolones (FLU) and chloramphenicols (CHL).

The relationship between the abundance of *Aeromonas* in the inflow and outflow regions of the studied carp ponds are presented as the correlation matrix in Table 4. In the inflow region positive correlation ($r = 0.84$, $p < 0.05$) was noted between fingerling and fry pond and also between fry and marketing pond ($r = 0.74$, $p < 0.01$). In the inflow region positive correlation in the abundance of *Aeromonas* between all studied seasons was documented. In the outflow region positive correlation ($r = 0.92$, $p < 0.05$) in the abundance of *Aeromonas* between marketing and fingerling pond and marketing and fry pond ($r = 0.70$, $p < 0.01$) was recorded. In the outflow region of the studied ponds there was no correlation in the abundance of the studied taxonomic bacterial group between the seasons.

Table 4 Correlation matrix coefficient numbers of *Aeromonas* in the water fish ponds

inflow

Correlation coefficients				
		fry	fingerling	marketing
	fry			
ponds	fingerling	0.84**		
	marketing	0.35	0.74*	
		sp	s	a
	sp			
seasons	s	0.85**		
	a	0.61*	0.87**	

outflow

Correlation coefficients				
		fry	fingerling	marketing
	fry			
ponds	fingerling	0.56		
	marketing	0.70*	0.92**	
		sp	s	a
	sp			
seasons	s	0.57		
	a	0.31	0.55	

Significance (p) is indicated by asterisks: * $p < 0.05$ ** $p < 0.01$

By grouping the results by the ponds and seasons 2-way ANOVA was applied to detect significant differences between the abundance of *Aeromonas* strains in the inflow and outflow regions (Table 5). There were significant differences in the number of *Aeromonas* between ponds and seasons in both studied regions.

Table 5
Analyses of 2-way-ANOVA in the numbers of *Aeromonas* in the water due to fish ponds and seasons. Significance (p) is indicated by asterisks: * p < 0.05, ** p < 0.01, *** p < 0.001

Correlation coefficients				
		fry	fingerling	marketing
	fry			
ponds	fingerling	0.56		
	marketing	0.70*	0.92**	
		sp	s	a
	sp			
seasons	s	0.57		
	a	0.31	0.55	
	inflow		**outflow**	
Source of variation	F	p	F	p
ponds	7.73	**	4.07	*
seasons	6.42	**	5.42	*
ponds × seasons	11.10	***	9.54	***
Explanations:				
F – Fisher test				
p – significance level				

4. Discussion

Aeromonas are ubiquitous bacteria primarily recovered in all kinds of aquatic environments and are commonly isolated from different fish farms (Jha et al. 2008; Harnisz and Tucholski 2010; Yu et al., 2015 Piotrowska et al. 2017; Silva et al. 2019). The abundance of *Aeromonas* strains in the studied fish ponds oscillated from 1.73 to 5.90 · 10^2 CFU · cm^{-3}. These data corresponded with the results (1.56·10^2 – 4.22·10^2 CFU · cm^{-3}) previous research we conducted on these water bodies (Zdanowicz et al., 2020), which indicates that the size of this taxonomic group in the studied water bodies maintained a constant level of many years of research cycle. Similar results were also reported by Jha et al. (2008) from the fish farm in India (3.63 · 10^2 CFU · cm^{-3}) and those noted by Skwor et al. (2014) in Lake Erie (0.47 · 10^2 – 3.6 · 10^3 CFU · cm^{-3}) but they were lower than the number of these organisms (4.0 · 10^2 – 2.0 · 10^5 CFU · cm^{-3}) in riverine freshwater of Marrakech (Morocco) and the number (2.1–2.6 · 10^6 CFU · cm^{-3}) of *Aeromonas* recorded in river of Lotcha (West Bengal, India) (Roy et al., 2013).

In all studied ponds, the number of *Aeromonas* was higher in the outflow region than near its inflow. Also Gołaś et al. (2019) when studying recirculating aquatic ecosystem noted that *Aeromonas* abundance in water samples at the outflow was several hundred times higher than at the inflow.

Imzilin (2001) and Maalej et al. (2003) demonstrated that abundance of bacteria in water basin shows clear seasonal variation. According to Naviner et al. (2006) and Huddleston et al. (2006) the highest abundance of bacteria in water basins was usually noted in summer. The results of our study also showed similarly to the results of research in these water reservoirs obtained by Zdanowicz et al. (2020) in 2013 that the the highest abundance of *Aeromonas* in the studied water basins was mostly observed in the summer season, when phytoplankton blooms are often noted. For bacteria, extracellular algal excretions constitute a very good source of easily accessible nutrients, stimulating increased bacterial abundance (Romani Sabater 2000). High temperature in these season is able stimulated high metabolism and generation of these organisms (Maalej et al. 2003; Huddleston et al. 2006). Temperature next to

the availability of nutrients is a key abiotic parameter influencing significantly the seasonal variation in the number of bacteria in water basins (Cottrell and Kirchman 2000).

In recent decades the abundance of antibiotic-resistant bacteria in fish farms has increased drastically as a consequence of the widespread pollution and uncontrolled use of antibiotics, prophylactically and therapeutically some on a daily basis against diseases and also as growth stimulator for fish (Rajpakshe et al. 2012; Piotrowska and Popowska 2014; Chung et al. 2017). Consequently resistance of bacteria to antibiotics has been widely spread in aquaculture environments (Shah et al. 2012; Piotrowska and Popowska 2014). The rapid increase in the number of antibiotic resistant aquatic bacteria and antibiotic resistance genes is due, in part, to the ability of these organisms to transfer antibiotic resistance agents among bacterial populations by cell to cell contact (Zhang et al. 2009; Oliveria et al. 2010).

The results of the present study showed that the genus *Aeromonas* isolated from the inflow and outflow regions of the studied ponds strongly differed in the resistance level to tested antibiotics. All strains were resistant to β-lactam antibiotics, like amoxicillin, ampicillin and penicillin which indicates that the β-lactamase gene, which inactivates these antibiotics might be present in the gene pool of *Aeromonas* inhabiting water of the studied ponds (Lin et al. 2004). High percentage (70–100%) of *Aeromonas* bacteria resistant to these three β-lactam antibiotics was also noted in these ponds in 2013 (Zdanowicz et al. 2020) and also by Vaseeharn et al. (2005) in the ponds in India, Penders and Stobberinh (2008) in the indoor catfish and eel farm in the Netherlands, Harnisz and Tucholski (2010) in carp ponds in Poland and Laith and Najah (2013) in fish farm on Marang River, Terenggangu (Malyasia).

All *Aeromonas* strains isolated from water of three studied water basins were also resistant to clindamycin. These results corespondend with the data obtained by Stratev et al. (2013) who observed that all *Aeromonas* bacteria isolated from the rainbow trout were resistant to clindamycin, moreover 98% of these bacteria inhabiting inland ponds located in Bangkok city were resistant to this antibiotic (Yano et al. 2015).

On the other hand we observed that all isolated *Aeromonas* bacteria were susceptible to streptomycin. The sensitivity of this taxonomic group of bacteria to streptomycin is coresponding with the data reported from many fish farms, for example, by Radu et al. (2003) from fish farms of Merisian province (Turkey), Belèm-Costa and Cyrio (2006) from tilapia and pacu farms and Kanchan et al. (2016), from fish collected from farm in Kosumpisi District Maha Sarakham Province (Thailand).

According to the data from our study *Aeromonas* bacteria inhabited IF and OF regions of three studied fish water basins differed in the level of antibiotic resistance. Those inhabiting the inflow region were resistant to nearly all tested antibiotics compared to bacteria inhabiting the outflow region of the studied water bodies. Also Mudryk et al. (2014) and Cisar et al. (2014) in their research documented that bacteria that differed in antibiotic resistance inhabited different parts of the water basins.

Uncontrolled and extensive use of antibiotics in aquaculture leads to the selection of genes encoding resistance and may cause the frequent occurrence of multiple antibiotic resistance (Piotrowska et al. 2017; Hossain et al. 2018; Silva et al. 2019). The development of multiple antibiotic resistance in recent decades has become a serious global threat in aquaculture (Orozova et al. 2010; Igbinosa et al. 2012; Islam et al. 2015). According to Dar et al. (2016) multiple antibiotic resistance may be coded on plasmids, mutational events or on smaller and mobile genetic elements called transposons, which are able to move between plasmids and bacterial chromosomes. The present study similarly to the research conducted on these ponds in 2013 (Zdanowicz et al. 2020) also documented multiple antibiotic resistance of planktonic *Aeromonas* bacteria. The higest percentage of bacteria isolated from the studied ponds were resistant to 5 antibiotics of the 12 used in this study. This means that *Aeromonas* are able to detoxicate those pharmaceutical organic compounds and according to Radu et al. (2003) the high level of multiple resistance may arise from selective pressure due to the indiscriminate use of antibiotics. Adaptive responses of bacterial communities to several antibiotics noted in the present study may presumably reflect the history of antibiotics application in the studied ponds (Mudryk et al. 2014). Most classes of antibiotics that are approved for human and animal use are introduced into water basins (Ecconomou and Gouisa 2015; Kim et al. 2015). Therefore resistance to multiple classes of antibiotics is not uncommon in bacteria isolated from aquaculture; this is also confirmed by the results of the present study. All planktonic *Aeromonas* isolated from three studied ponds were most resistant to β-lactam antibiotics, which inhibit biosynthesis process of cell wall. Presented results correspond with data obtained during previous studies of these reservoirs published by Zdanowicz et al. (2020). Also these results confirm that *Aeromonas* has a consistent pattern of continuous resistance to this group of antibiotics. The high level of *Aeromonas* resistance to β-lactam antibiotics has also been documented in the previous studies by Roy et al. (2013), Mudryk et al. (2015) and Dahanayake et al. (2019). The resistance of *Aeromonas* to β-lactam antibiotics is due to their ability to synthesise three extracellular enzymes: β-lactamase, acylase and

penicillinase, which hydrolyse the amide bond of the β-lactam ring of β-lactam antibiotics (Jalal et al. 2010). These enzymes limit the permeability of the cytoplasmic membrane to these antibiotics and transform these compounds into antibiotically inactive penicilloic acid (Saaverda et al. 2004). According to Schwartz et al. (2003) and Aminov (2009) the number of bacterial strains producing an extended spectrum of β-lactamases capable of hydrolysing β-lactam antibiotics is rapidly increasing all over the world.

Beside β-lactam antibiotics, all *Aeromonas* bacteria inhabited studied water basins were resistant to lincosamide antibiotics. The high resistance level of *Aeromonas* strains to lincosamide antibiotics has also been documented in the previous studies (Calamari et al. 2003; Andreozzi et al. 2006). Lincosamides inhibit bacterial protein biosynthesis by binding reversibly to the subunit 50S of the bacterial ribosome (O`Dowd et al., 2008). This binding was reported to block peptide bond formation and/or peptidyl - tRNA translocation from the A to the P site of the ribosome (Menninger and Coleman 1993).

Many *Aeromonas* strains isolated from the water of the studied ponds were susceptible to aminoglycoside antibiotics which is also confirmed by the data obtained during the research of these water bodies in 2013 (Zdanowicz et. al. 2020). This means that the studied genus of bacteria is not capable of actively detoxifying those antibiotics and according to Yu et al. (2015) may be recommended to treat fish infected with *Aeromonas*. Moreover this supports previous findings of Orazova et al. (2010) and Kim et al. (2015) who studied *Aeromonas* resistance to aminoglycoside antibiotics. Aminoglycosides are a large and diverse class of antibiotics, which have bactericidal activity against some gram-positive and many gram-negative bacteria (Ryu and Rando 2008). Bacterial resistance to aminoglycosides is due to one of four mechanisms: reduced uptake, mutational modification of 16S rRNA, enzymatic modification of 16S rRNA, and modification and inactivation of antibiotics by three extracellular enzymes: phosphotransferase, nucleotidyltransferase and acetyltransferase (Wright 2003).

Aeromonas strains isolated from inflow and outflow water of the studied ponds were susceptible to fluoroquinolones. This class of antibiotics has been used extensively in aquaculture to control bacterial fish diseases and to promote fish growth (Naviner et al. 2011; Rico et al. 2012; Ruiz et al. 2012). Fluoroquinolones were the first choice in treating of *Aeromonas* infections (Ozarova et al. 2010). Plasmid–mediated quinolone resistance in bacteria associated with fish farms was detected in several countries (Buschmann et al. 2012; Jiang et al. 2012; Miranda et al. 2013). The extensive administration of quinolones in fish farming has been linked to increased mutations in DNA gyrase and topoisomerase IV in quinolone-resistant fish pathogens (Shah et al. 2012).

Beside aminoglycoside and fluoroquinolone antibiotics many *Aeromonas* bacteria inhabited studied three water bodies were susceptible to chloramphenicols. These data support the results of the previous studies on bacterial resistance to chloramphenicols in aquaculture as well as the results of the studies on clinical isolates (Igbinosa et al. 2012; Stratev et al. 2013; Silva et al. 2014; Yano et al. 2015). Chloramphenicols are used mainly prophylactically in many aquacultures (Rajpakshe et al. 2012).

Conclusion

In conclusion, we showed that the genus *Aeromonas* inhabiting the studied aquaculture may play an important role as a reservoir of antibiotic resistance thereby posing not only a potential threat to fish health but also to public health by the transmission of resistance genes to human populations through fish carriers consumption. Therefore, antibiotic resistance to antibiotics of *Aeromonas* bacteria inhabiting this habitat still require further studies, particularly when long-term widespread pollution and abuse of these pharmaceuticals in aquaculture is observed all over the world.

Declarations

Ethical Approval - Not applicable

Consent to Participate - Not applicable

Consent to Publish - Not applicable

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by P Perliński, ZJ Mudryk M Zdanowicz. The first draft of the manuscript was written by P Perliński and ZJ Mudryk and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing Interests - The authors declare that they have no competing interests.

Availability of data and materials - The data sets generated during and/or analysed dur-ing the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Map studied ponds where the sampling sites inflow and outflow regions are located. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

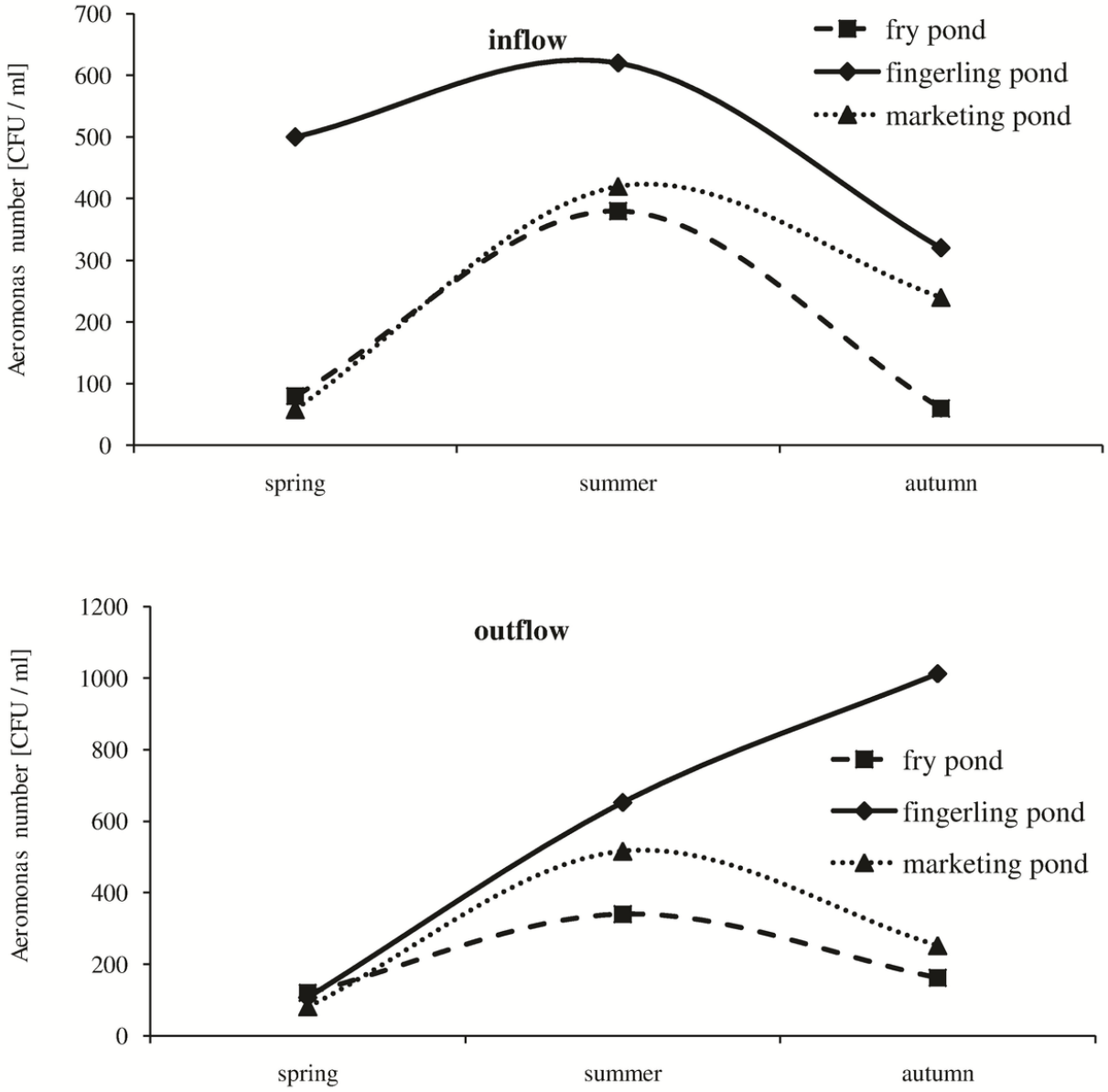

Figure 2

Seasonal dynamics change of Aeromonas bacteria number in water studied ponds (average from the pooled data of all sites and seasons)

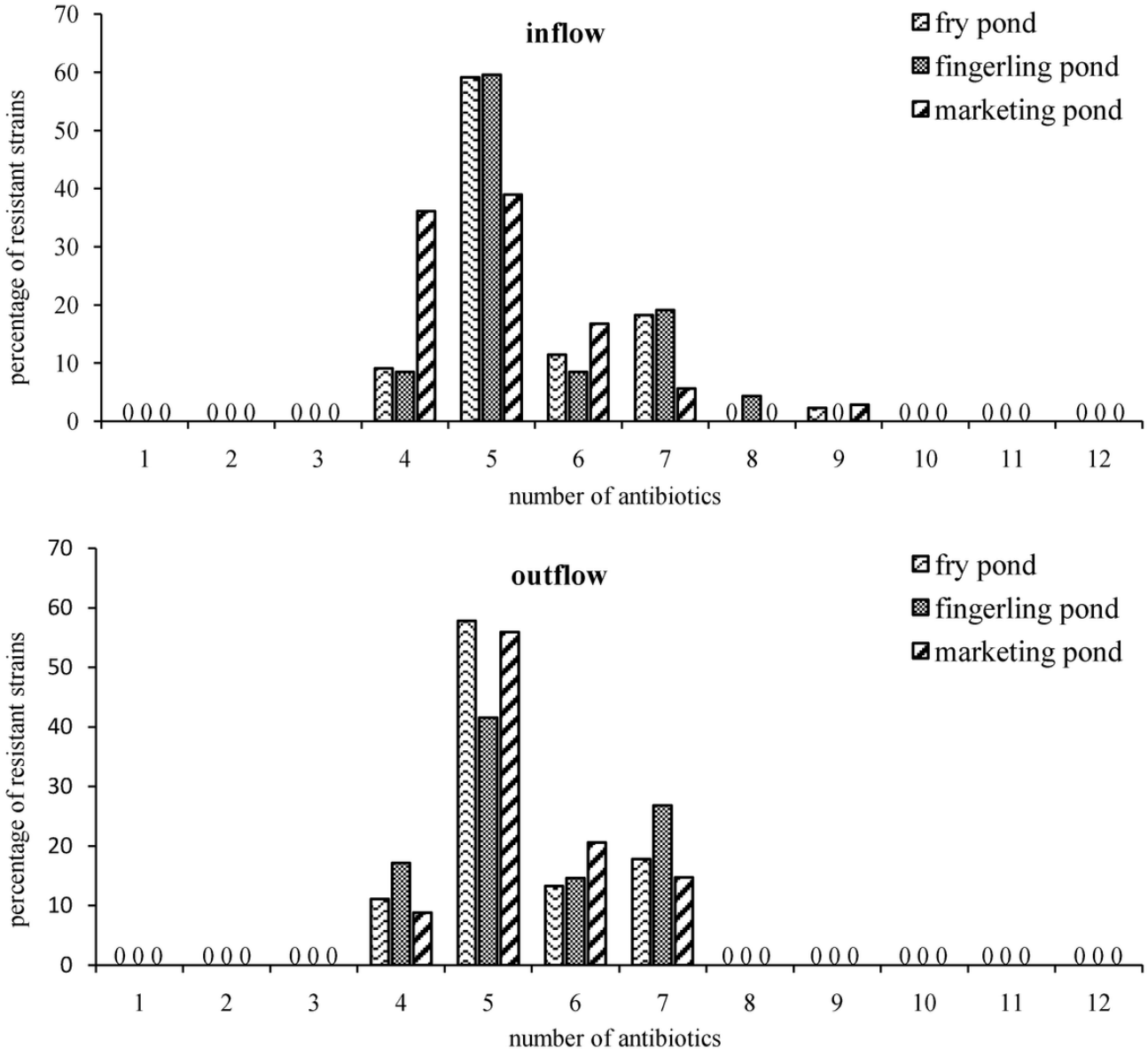

Figure 3

Percentage of resistance studied antibiotics of the Aeromonas strains isolated from studies water basins in inflow and outflow region (data derived from the pooled data of all sites during the study period)

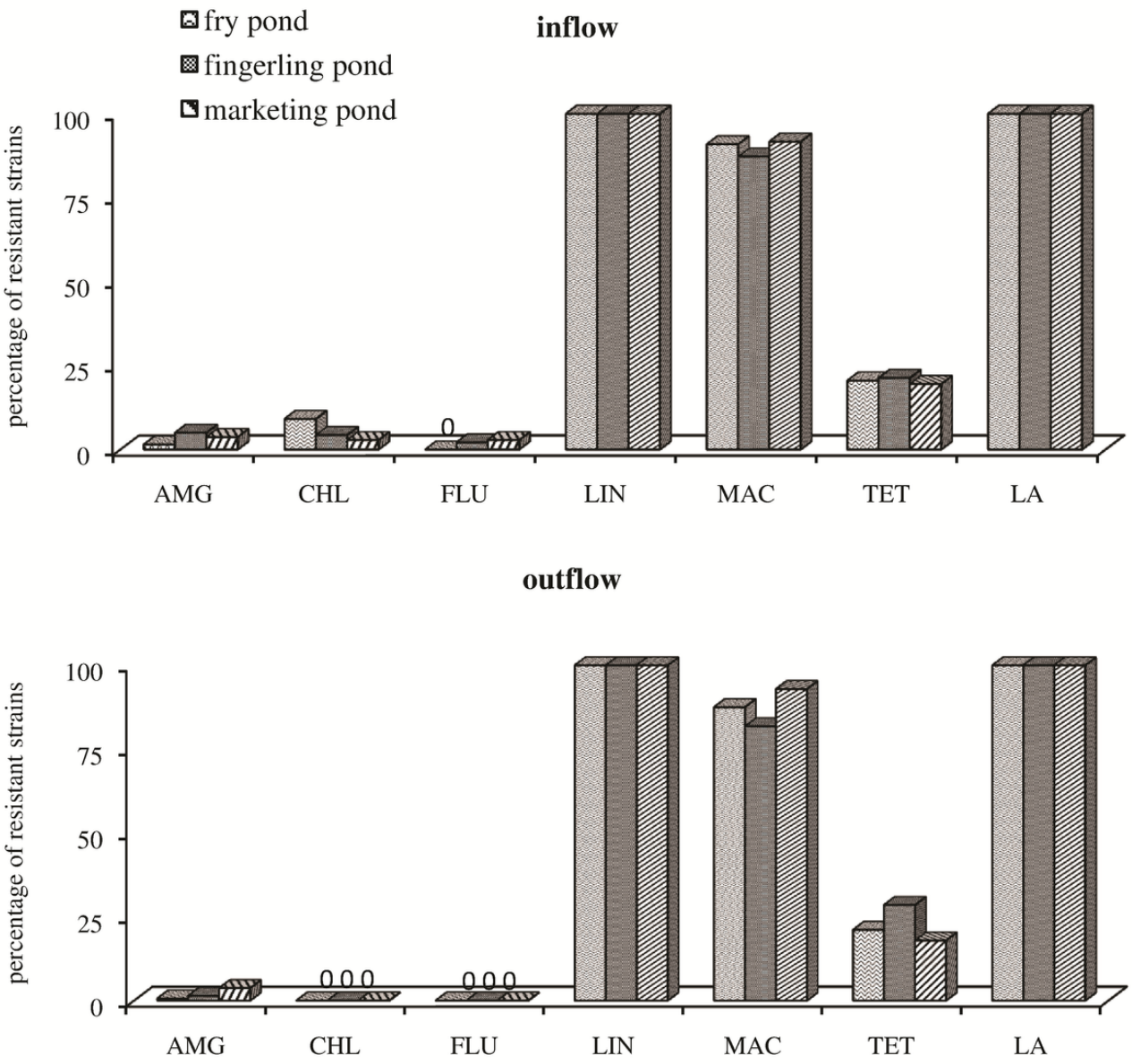

Figure 4

The resistance of studied bacteria with respect to antibiotics chemical structure (in percentage, percentages derived from the pooled data of all sites and seasons)