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VIRULENCE AND ANTIBIOTIC SUSCEPTIBILITY OF *Aeromonas* spp. ISOLATED FROM NILE TILAPIA FISH, FISH PONDS AND RIVER WATER IN SHARKIA GOVERNORATE, EGYPT

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ABSTRACT

Aeromonas isolates from Nile tilapia fish, fish ponds and River water were investigated for their ability to produce different potential virulence factors *i.e.* hemolysins, proteases, lipases, Gelatinases. In addition, the susceptibility to antibiotics of *Aeromonas* isolates was examined. A total of 376 *Aeromonas* spp. were identified on the basis of biochemical tests and six of them were confirmed by sequencing of 16S rDNA gene as (*A. caviae*, *A. encheleia*, *A. molluscorum*, *A. salmonicida*, *A. veronii* and *A. veronii bv. veronii*). The antibiotic resistance testing revealed that all of the *Aeromonas* strains were resistant to β–lactam (amoxicillin/clavulanic acid) antibiotics. However, the resistance to other antibiotics was variable. All *Aeromonas* strains were found to be resistant to ampicillin, cephalexin, cephradine, rifampin as well as to cephalothin. The majority of the *Aeromonas* strains either isolated from fish or water were capable to produce hemolysins (92.8%), lipases (88.4%), proteases (92.5%) and gelatinases (90.6%) as the virulence factors.

Key words: *Aeromonas* spp., antibiotics, virulence, fish, water.

INTRODUCTION

Genus *Aeromonas* comprises non-motile psychrophilic, and motile mesophilic Gram negative bacteria and include 15 species, being distributed ubiquitously in aquatic environments and are of increasing importance as seafood and waterborne pathogens. Seven species cause gastroenteritis in adults, in children and septicaemia (Dwivedi *et al*., 2008; Khajanchi *et al*., 2012 ; Praveen *et al*., 2016). Also, *Aeromonas* species were etiological agents of fish diseases like furunculosis, septicaemia and skin ulcers (Reith *et al*., 2008; Figueras *et al*., 2009; Sarkar *et al*., 2013 ; Albarral *et al*., 2015). Let alone, its potential as spoilage agent in food (Bezirtzoglou *et al*., 2000). Consequently, *Aeromonas* is a genus of growing interest due to its pathogencity to aquatic organisms as well as high prevalence of multiple antibiotic resistant, hemolysis and proteases producing.

There are numerous reports on isolation of *Aeromonas* spp. from fish and water samples (Rathore *et al*., 2005; Sharma *et al*., 2005; Bagyalakshmi *et al*., 2009; Furmanek-Blaszk, 2014 ; Dahdouh *et al*., 2016). Recognition and monitoring of the potential reservoirs of *Aeromonas* spp. and their drug resistance profile were essential in epidemiological and environmental studies to avoid possible health risks (El-Sayyad *et al*., 2010).

Disease was a primary constraint in aquaculture and can severely impact economic and socioeconomic development in Egypt. The aim of this study was to determine the possible differences among *Aeromonas* spp. isolated from tilapia fish, fish ponds and River Nile water based on their ability to produce different potential virulence factors such as hemolysins, proteases, lipases, gelatinases in addition to their susceptibility to antibiotics.

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MATERIALS AND METHODS

Materials

Nile tilapia fish (*Oreochromis niloticus*)

During the period between June 2014 and May 2015**,** fish samples were purchased from local fish markets at Zagazig City, Sharkia Governorate, Egypt. Fish samples were brought in sterilized plastic bags to the Laboratory of Agricultural Microbiology Department, Faculty of Agriculture, Zagazig University, Egypt, for bacteriological analyses.

Water samples

Fish ponds

During the same period water samples were collected from fish ponds at Abassa, Abo-Hammad Districts, Sharkia Governorate, Egypt for microbiological analyses. The area of each pond was seven faddans, water depth ranged between 60 and 65 cm and stocked with tilapia fish. Water samples were taken from a depth of (20 cm) in a brown glass bottles below the surface of water.

River water and irrigation canal

During the period between June 2014 and May 2015, water samples were collected from three different sites (*i.e.,* Abo-Hammad, Kafr Saker irrigation canals, and Mowees River at Zagazig City), Sharkia Governorate. Water samples were collected according to the standard methods (APHA, 2005). From irrigation canals and Mowees River, the sterilized brown glass bottles (300 ml) were opened below water surface (about 20 cm) where the bottles mouth were directed towards the water current and filled up to two-thirds of each bottle in order to facilitate mixing by shaking before examination. The bottles were closed by its stoppered.

Methods

Microbiological analyses

Fish samples: the different tissues including muscles, skins, gills and intestinal tissues from different fish were homogenized using a stomacher blender in alkaline peptone water (APW).

Water samples: Water samples collected from river water and ponds were diluted in alkaline peptone water. Appropriate dilutions prepared from each of water and fish samples were used for inoculating different nutrient and selective media. The bacteriological examinations of water and fish samples included total bacterial counts (TBC) onto plate count agar and *Aeromonas* spp. counts onto selective *Aeromonas* agar base (Yadav *et al*., 2014). *Aeromonas* spp. were represented by presumptive green with darker (mostly black) centered colonies having a surrounding clear zone and yellow to honey color.

Aeromonas spp. Identification

Aeromonas spp. isolates were isolated from the Nile tilapia fish, fish ponds and river water at Sharkia Governorate, Egypt. The isolates were identified using different biochemical methods according to Martin-Carnahan and Joseph (2005) in the Bergey's Manual of Systematic Bacteriology 2nd edition, volume two, (The Proteobacteria) Part B, "The Gamma Proteobacteria". Bacterial colonies were grown on Luria Bretani (LB) agar plates and subjected to the Gram stain, catalase test, urease test, hydrogen sulfide production test , indole test and Voges-Proskaüer test. Pure bacterial isolates were grown in peptone water supplemented with 0.5% of the tested sugar *i.e.,* glucose, fructose, mannitol, sucrose, sorbitol, trehalose, raffinose, ribose, cellobiose, lactose, mannose, maltose, a rabinose and rhamnose after being sterilized by filtiration. Bromothymol blue and small inverted Durham's tube were included in each tube. Acid production and gas production were detected according to Samelis *et al.* (1994). The ability of *Aeromnas* isolates to grow in the presence of 0% and 3% NaCl was determined in LB broth after inoculated with one ml of 24 hr., old broth culture of each LAB isolate (10^5 CFU/ml) and incubated at 37ºC for 48 hrs. Growth was assayed by assessment of turbidity. To confirm the results, two isolates were identified in Zagazig University Hospitals Clinical Pathology Department. Also, six isolates were identified in El Giza Egypt Sigma Scientific Services Company using 16S rDNA. The DNA of bacteria was isolated according to the protocol by Maniatis *et al*. (1989), and the GeneJet genomic DNA purification Kit (Thermo

K0721). Molecular sequencing of the DNA fragment containing the 16S intergenic spacer corresponding to the conserved region of 16S rDNA (Martinez-Murcia *et al.*, 1992) allowed for an unambiguous classification of the *Aeromonas* isolates. The molecular identification, as primers designs to amplify 16S rDNA gene (forward primer 5′-AGAGTTTGATCATGGCTCAG-3 and reverse primer 5′-GGTTACCTTGTTACGACTT-3′), were performed according to Borrell *et al.* (1997). For genus-specific analysis, DNA was extracted from each bacterial sample (including reference strains) by boiling for 10 min. PCRs were carried out on a thermal cycler Primus 96 Plus (MWGAG-Biotech, Ebersberg, Germany). A 20 ml containing a final concentration of 50 m M KCl, the strains were maintained on LB slant agar at 4℃.

Incidence of some virulence activities in *Aeromonas* spp.

Aeromonas spp. were tested for hemolytic activity by streaking them onto trypticase soy agar (TSA) plates containing 5% sheep blood for 48 hr., at 37°C. Beta hemolytic zones of 2 mm or more around the colonies were regarded as the sign of positive hemolytic activity (Erdem *et al*., 2010). Hemolytic activity of the *Aeromonas* spp. strains was categorized as alpha, beta, or gamma (Brender and Janda, 1987).

Protease activity was determined on the surface of skimmed milk agar, in which skimmed milk was added just before pouring the medium into the petri plates. The plates were incubated at 37°C for 4 days. After incubation, the clear zones of hydrolysis were measured and recorded. The presence of a transparent zone around the colonies indicated proteases activity (Gudmundsdottir, 1996; Pang *et al.*, 2015).

Lipase activity was determined by streaking the culture onto plate's containing 0.5% tributyrin emulsified with 0.2%Triton X- 100 and incubated at 37˚C for 24 hr. (Anguita *et al*., 1993 ; Sarkar *et al*., 2013). The presence of transparent zones around the colonies indicated lipase activity.

Gelatinase activity was tested by using gelatin agar plates. The cultures were streaked onto the plates and incubated at 37˚C for 24 hr., then the plates were immersed with mercuric chloride $HgCl_2$ solution "15% in 20% (*V/V*) concentrated HCl solution" (Kannan 2002). The presence of transparent zone around the colonies indicated gelatinase activity (Bagyalakshmi *et al*., 2009).

Antimicrobial susceptibility test

The antibiotic susceptibility test was performed by the standard disc diffusion method (NCCLS 2003 and 2004). The following commercial discs antimicrobial substances used were: erythromycin (15 µg), ofloxacin (5 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), ampicillin (10 µg), amikacin (30 µg), trimethoprim/ sulphamethoxazole (1.25/23.75 µg), kanamycin (30 µg), amoxicillin (25 µg), cefoxitin, cephalothin (30 µg), neomycin (30 µg), nalidixic acid (30 µg), norfloxacin (10 µg), rifampin (5 µg), tetracycline (30 µg), amoxicillin/clavulanic acid (30 µg), aztreonam (10 µg), cephradine (30 µg), cephalexin (30 µg) and doxycycline (30 µg). Pure cultures of *Aeromonas* were enriched in LB broth media at 37^{o}C for 24 hr. Using a strile glass spreader, 100µl (~ $2x10^{8}$ CFU/ml) from each bacterial culture were spread onto LB agar plates (Costa *et al*., 1998). The antibiotic discs were dispensed with a sufficiently separated from each other so as to avoid overlapping of inhibition zones. After 30 min, the plates were inverted and incubated at 37^{o}C for 18–24 hr. Results were recorded by measuring the diameter of the inhibition zones (mm) and compared with standards for antimicrobial disk susceptibility tests, supplied by the LB media Laboratories, and were classified as resistant, intermediate and sensitive (Costa and Cyrino, 2006; Furmanek-Blaszk, 2014).

RESULTS AND DISCUSSION

Microbial Loads in Fish and Water Samples

Total counts of bacterial loads and *Aeromonas* spp. expressed as Log CFU/ml in water samples collected from various locations and fish samples in Sharkia Governorate can be shown in Tables 1 and 2. The highest total bacterial counts and *Aeromonas* counts were obtained in intestine,

Table 1. Total counts of bacterial and *Aeromonas* species load (Log CFU/g) in different fish organ samples collected during one year period (2014–2015) from Sharkia Governorate markets

Season	Month	Fish organ							
		Intestine		Skin		Gills		Muscles	
		TC	AC	TC	AC	TC	AC	TC	AC
Summer	**June**	6	4.1	6.7	4.2	6.7	3.9	5	3.1
	July	6.8	4.9	6.9	5.3	6.5	4.8	4.9	2.8
	August	7.1	5	7.8	5.3	7.6	5.1	4.1	2.7
	Mean	6.6	4.7	7.1	5	7	4.6	4.7	2.9
Autumn	**September**	8	4.4	8.1	6.3	8	6.2	7.1	3
	October	8.1	6.2	8.1	6.3	8.1	5.2	4.1	3.3
	November	7.8	6.1	7.9	6	7	5	4.2	3.1
	Mean	8	5.6	8	6.2	7.7	5.5	5.1	3.1
Winter	**December**	7.5	4.1	7	4.3	6.7	4.2	5.1	3.1
	January	8	5.3	8	5.9	6.9	5.2	5.2	3.3
	February	7.8	5.2	7.9	6	7.7	5.9	7.2	4.2
	Mean	7.8	4.9	7.6	5.4	7.1	5.1	5.8	3.5
Spring	**March**	7.3	5	7.7	5.4	6.9	5.3	6.7	5
	April	6.9	4.3	6.7	5.1	6.6	5	4.9	3.3
	May	6.6	3.2	7.3	4.1	7.1	4.2	5.7	2.5
	Mean	7	4.2	7.2	4.9	6.9	4.8	5.8	3.6
	Over total	7.3 (26.6%*)	4.8 (25.9%**)	7.5 (27.4%*)	5.4 (29.2%**)	7.2 (26.3%*)	5 (27%**)	5.4 (19.7%*)	3.3 (17.8%**)
	AC/TC percent	65.8%		72%		69.4%		61.1%	

TC : Total counts., * : TC in one organ / TC in four organs percent.
AC : *Aeromonas* counts. , ** : AC in one organ / AC in four organs percent.

Table 2. Total counts of bacterial and *Aeromonas* species load (Log CFU/ml) in different water samples collected during one year period (2014 – 2015) from Sharkia Governorate

Season	Abo- Hammad irrigation canal		Kafr Sakr irrigation canal		Mowees river		Abassa ponds	
	TC	AC	TC	AC	TC	AC	TC	AC
Summer	5.9	3.5	4.9	3.4	5.3	4.3	8.5	6.7
Autumn	3.1	2.1	3.9	2.7	4.1	3.1	7.2	4.9
Winter	4	3.1	4.9	2.7	3.9	2.9	5.2	3.7
Spring	5.1	3.5	4	3.1	5.1	4.1	8.1	5.8
Over total	4.5	3.1	4.4	3	4.6	3.6	7.3	5.3
AC/TC percent	68.9%		68.2%		78.3%		72.6%	

TC : Total counts.
AC : *Aeromonas* counts.

skin and gills of fish samples (~ 6-8 Log cfu/g) during September and October 2014. On the other hand, the lowest counts were also obtained during June 2014 and May 2015 with level (~ 3-7 Log cfu/g) in both total counts and *Aeromonas* counts. However, the total bacterial load and *Aeromonas* counts in muscles ranged between 4.1-7.2 and 2.5 -5 Log cfu/g, respectively. The highest total bacterial counts and *Aeromonas* counts were recorded in muscles during February and March 2015 and the lowest counts were recorded during August 2014 and May 2015 (Table 1). Also, the incidence of *Aeromonas* spp. varied depending on fish samples (gills, skin, intestine and muscles) examined. The percentage of *Aeromonas* incidence over the total bacteria was higher in the skin (29.2%) rather than in muscles (17.8%). Similar trend was observed in the total counts of bacteria since the highest percentage was found on the skin giving (27.4%) whereas the lowest percentage was also recorded in the muscles (19.7%). These results in general indicated that the highest incidence of *Aeromonas* spp. was observed in the skin (72%) of fish samples. These results were comparable very well with those obtained by (Erdem *et al*., 2010). Regarding the water samples, the highest total bacterial counts and *Aeromonas* counts were detected in Abassa ponds during the Summer and Spring (*i.e.,* 8.5 and 8.1 Log CFU/ml) and (6.7- 5.8 Log CFU/ml), respectively. On the other hand, the lowest counts of (TBC) and *Aeromonas* counts were obtained in Abo-Hammad irrigation canal during Autumn 2014 (*i.e.,* 3.1 and 2.1 Log CFU/ml), respectively (Table 2).

Identification of *Aeromonas* spp.

The results for the identification of the aforementioned isolates based on the biochemical tests reported by (Martin-Carnahan and Joseph, 2005) are presented in Table 3. The isolates were identified as *Aeromonas caviae*, *Aeromonas enecheleia*, *Aeromonas hydrophila*, *Aeromonas molluscorum*, *Aeromonas salmonicida*, *Aeromonas sobria*, *Aeromonas veronii*, *Aeromonas veronii bv. Veronii*. Generally, the criteria to identify species were primarily based on biochemical tests then the sequencing of the 16S rDNA gene has proven to be valuable in the identification of *Aeromonas* spp. (Demarta *et al*., 1999). The overall sequence similarity between *Aeromonas* spp. was very high, but there was sufficient variability to discriminate different species. PCR-RFLP analysis of 16S rRNA gene was considered to be a rapid and powerful method for identifying isolates of *Aeromonas* to the species level (Borrell *et al*., 1997; Figueras *et al*., 2000; Ghatak *et al*., 2006).

The amplified 16S rDNA gene products of representative isolates (n=6) from each identified group in 16S rDNA RFLP were sequenced using primer F:-AGA GTT TGA TCC TGG CTC AG, R:-GGT TAC CTT GTT ACG ACT T., from a commercial sequencing facility (Sigma Labs). The sequences were aligned independently and phylogenetically analysed using GATC Company by use ABI 3730xl DNA sequencer [Gene JET™] (Saitou and Nei, 1987). The sequencing of the 16S rDNA gene showed that the closest strain relatedness of AFg, AWz, AFm, AWh, AFs_2 and AFi strains with that of *A. caviae* (98%), *A. enecheleia* (98%), *A. molluscorum* (90%), *A. salmonicida* (97%), *A. veronii* (98%) and *A. veronii bv. veronii* (97%), respectively. These strains were isolated from gills, Mowess river, Abassa ponds, muscles, Abou-Hammad irrigation canal, skin and intestine samples, respectively (Table 4).

Incidence of Some Virulence Factors in *Aeromonas* Strains

Both the quantitative and qualitative production of proteases, lipases and gelatinases are important factors in the spoilage of foods, and the presence of proteases, lipases and gelatinases were used as indicators of potential pathogenicity (Majeed and MacRae, 1993; Santos *et al*., 1999; McMahon, 2000). Hemolysins, proteases, lipases and gelatinases were thought to contribute to the virulence of aeromonads for fish and other hosts. However, their contribution to human pathogenicity still needs to be determined (Erdem *et al*., 2010). Data in Table 5 show that the majority of the *Aeromonas* strains either isolated from fish or water were active in producing hemolysins, lipases, proteases and gelatinases since (92.8, 88.4, 92.5 and 90.6%) of the *Aeromonas* strains, respectively were active. These results are in harmony with those obtained by (Pemberton *et al*., 1997) who stated that the virulence factors by *Aeromonas* spp. has

Table 3. Biochemical properties of some *Aeromonas* spp. isolated from fish and water

Characteristics	Bacterial isolates							
	AFg	AWz	AWa	AFm	AWh	AFs_1	AFs_2	AFi
Motility	+	+	+	+	+	-	+	+
Indole production	+	+	+	+	+	+	+	+
Voges – proskauer	-	-	+	-	+	-	-	+
Urea hydrolysis	-	-	-	-	-	-	-	-
H_2S production	-	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	-	+	+
Oxidase	+	+	+	+	+	+	+	+
Acid glucose	+	+	+	+	+	+	+	+
Gas glucose	-	+	+	+	+	+	+	+
Growth in 0% NaCl	+	+	+	+	+	+	+	+
Growth in 3% NaCl	+	+	+	+	+	-	+	+
Acid mannitol	+	+	+	+	+	+	+	+
Rabinose	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+
Raffinose	-	-	-	-	-	-	-	-
Rhamnose	-	+	+	-	-	-	-	-
Mannose	+	-	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Lactose	+	-	+	+	+	-	+	+
Sucrose	+	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	+	-	-	-
Trehalose	+	+	+	+	+	+	+	+
Cellobiose	+	-	-	+	+	+	+	+

AFg: isolated from gills. , AWz: isolated from mowees river., AWa: isolated from Abassa ponds., AFm: isolated from muscles. , AWh: isolated from Abou-Hammad irrigation canal. , AFs_1 and AFs_2: were isolated from skin., AFi : isolated from intestine.

Table 4. Identification of six *Aeromonas* species

Isolate	Identification By biochemical and 16S rRNA	Identity	International *Aeromonas* strains
AFg	*Aeromonas caviae*	98%	*Aeromonas caviae* strain CECT 4221 16S ribosomal RNA gene, partial sequence
AWz	*Aeromonas encheleia*	98%	*Aeromonas encheleia* strain CECT4342 16S ribosomal RNA gene, partial sequence
AFm	*Aeromonas molluscorum*	90%	*Aeromonas molluscorum* strain LMG 22214 16S ribosomal RNA gene, complete sequence
AWh	*Aeromonas salmonicida*	97%	*Aeromonas salmonicida* strain ATCC 33658 16S ribosomal RNA gene, complete sequence
AFs_2	*Aeromonas veronii*	98%	*Aeromonas veronii* B565 strain B565 16S ribosomal RNA, complete sequence
AFi	*Aeromonas veronii bv.veronii*	97%	*Aeromonas veronii bv.veronii* strain ATCC 35624 16S ribosomal RNA gene, complete sequence

Table 5. Incidence of some virulence factors in *Aeromonas* spp. isolated from fish and water samples

Specie	No. of Strains	Hemolytic activity Types		Proteases	Lipases	Gelatinase
Aeromonas caviae	54	α-Hemolytic	43 (80%)	38 (70%)	38 (70%)	54 (100%)
Aeromonas encheleia	34	β-Hemolytic	33 (97%)	32 (94%)	33 (97%)	31 (91%)
Aeromonas hydrophila	27	β-Hemolytic	27(100%)	27 (100%)	25 (93%)	17 (63%)
Aeromonas molluscorum	24	α-Hemolytic	18 (75%)	23 (96%)	23 (96%)	21 (88%)
Aeromonas salmonicida	65	β-Hemolytic	65 (100%)	65 (100%)	54 (83%)	55 (85%)
Aeromonas sobria	33	β-Hemolytic	31 (94%)	29 (88%)	29 (88%)	33 (100%)
Aeromonas veronii	50	β-Hemolytic	50 (100%)	50 (100%)	50 (100%)	48 (96%)
Aeromonas veronii bv. veronii	32	α-Hemolytic	29 (91%)	31 (97%)	30 (94%)	30 (94%)
All the strains	319	92.8%		92.5%	88.4%	90.6%

been identified by production of exoenzymes, although the importance and exact mechanism of each factor associated to the virulence has not been well established. *Aeromonas* strains were active in hemolysin production and could be divided into beta (β–heamolysin) and alpha (α–heamolysin) on sheep blood agar plates. Our results contradict those obtained by (Erdem *et al*., 2010) who found that no type of hymolysis, except for β-hemolysis was found in *Aeromonas* isolates. The highest percentage of β-hemolytic isolates (100%) was recorded in *A. hydrophila* , *A. salmonicida* and *A. veronii* followed by *A. encheleia* (97%) and the least one was *A. sobria* which recorded 94%. Beta hemolysin has been reported as a virulence factor in motile aeromonads (Majeed and MacRae, 1993). Also (Erdem *et al*., 2010) found that *A. hydrophila* and *A. veronii* bv. *sobria* strains exhibited beta-hemolytic activity to different extents, but not *A. caviae* strains. Concerning α–heamolysin, data in the previous table show also that *Aeromonas veronii bv. veronii* recorded the highest percentage of heamolytic activity (91%) followed by *Aeromonas caviae* and *Aeromonas molluscor*um which were recorded 80% *and* 75%, respectively. Proteolytic activity of *Aeromonas* strains was 100% of each of *A. hydrophila*, *A. salmonicida* and *A. veronii* followed by 97% of *A. veronii bv.veronii*, 96% of *A. molluscor*um, 94% of *A. encheleia*, 88% of *A. sobria* and 70% of *A. caviae*. These results are comparable with those obtained by (Erdem *et al*., 2010) who found that 100% of *A. hydrophila* and *A. veronii bv.sobria* were producer of proteases followed by 81.8 % of *A. caviae*. The lipases are important not only for bacterial nutrition but also are involved in *Aeromonas* virulence character (Pemberton *et al*., 1997). Data in Table 5 show also the lipases activity percent of different *Aeromonas* strains from fish and water. While *A. veronii* recorded the highest lipases activity (100%), *A. caviae* showed the lowest percent (70%). The other *Aeromonas* strains showed (97%) *A. encheleia* (96%) *A. molluscor*um (94%) *A. veronii bv.veronii* (93%) *A. hydrophila* (88%) *A. sobria* and (83%) *A. salmonicida*. These results were in accordance with those obtained by (Bagyalakshmi *et al*., 2009) who found that *A. caviae* and *A.sobria* showed 70% and 88% of the lipases activity, respectively.

The highest gelatinases activity (100%) in *Aeromonas* strains was recorded in *A. caviae* and *A. sobria* but the lowest activity was recorded in *A. hydrophila* (63%). On the other hand, the other *Aeromonas* strains tested gave an average range of 85–96% (Table 5). Gelatinases activity in *A. sobria*, *A. caviae* and *A. salmonicida* was comparable very well with those obtained by (Bagyalakshmi *et al*., 2009) who reported that gelatinases activity for these three species were 100, 100 and 83%, respectively.

Anti-bacterial Activity of Different Antibiotics Against *Aeromonas* spp.

The resistance patterns of eight *Aeromonas* strains against 22 antibiotics are shown in Table 6. Based on the average inhibition zone for each antibiotic with 8 tested *Aeromonas* strains, there was an obvious variation in the sensitivity of the bacterial strains studied. Data in the previous table show that all *Aeromonas* strains were resistant to ampicillin, cephalexin, cephradine, amoxicillin/clavulanic acid, rifampin as well as to cephalothin. These results agree very well with those obtained by Jagoda *et al*. (2014) who observed that *in vitro* antimicrobial susceptibility testing showed highest resistances towards tetracycline (58.5%) and erythromycin (54.7%). Also (Hatha *et al*., 2005) stated that antibiotic resistance patterns of the strains revealed that they had acquired a relatively higher resistance to oxytetracycline, amoxycillin, ampicillin, novobiocin and polymixin-B, implicating possible use of these antibiotics in the aquaculture systems.

On the other hand, *A. hydrophila* was resistant to all antibiotic tested in this study except doxycycline and kanamycin. In contrast, chloramphenicol was the most active antibiotic against 5 *Aeromonas* strains used in this study compared to the others since this antibiotic resulted in 17.8 mm as an average of the zone of inhibition followed by ciprofloxacin which resulted in 16.3 mm (Table 6). These results are in harmony with those of Costa and Cyrino, (2006), who observed that the *A. hydrophila* type strain presented resistance to the same antimicrobial substances and also against rifampicin. The bacterial isolate from pacu was the only resistant strain to tetracyclin. However, Laith and Najiah (2013) stated that the majority of *Aeromonas* spp. isolated strains from diseased

Table 6. The susceptibility or resistance of *Aeromonas* strains to 22 antibiotics, based on inhibition zone diameter (mm)

Antibiotic / *Aeromonas* strain	AK (30 µg)		AM (10 µg)		AMC (30 µg)		ATM (10 µg)		AX (25 µg)		C (30 µg)		CE (30 µg)		CIP (5 µg)		CL (30 µg)		CN (10 µg)		DO (30µg)	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
A.caviae	22	S	8	R	0	R	9	R	18	I	23	S	0	R	21	R	0	R	17	I	14	I
A.encheleia	13	R	0	R	0	R	0	R	0	R	16	I	8	R	23	I	0	R	18	S	20	S
A.hydrophila	0	R	8	R	7	R	9	R	0	R	0	R	0	R	0	R	0	R	13	R	17	S
A. molluscorum	13	R	0	R	0	R	21	I	11	R	22	S	0	R	26	S	7	R	13	R	18	S
A.salmonicida	16	I	12	R	0	R	0	R	16	I	16	I	0	R	16	R	0	R	16	I	18	S
A.sobria	13	R	7	R	0	R	14	R	7	R	23	S	0	R	22	I	0	R	18	S	0	R
A.veronii	16	I	8	R	0	R	0	R	0	R	21	S	0	R	0	R	0	R	15	R	0	R
A.veronii.bv. veronii	15	I	13	R	7	R	21	I	12	R	21	S	9	R	22	I	8	R	17	I	10	R
Average	13.5		7		1.8		9.3		8		17.8		2.1		16.3		1.9		15.9		12.1	

AK: Amikacin, AM: Ampicilin, AMC: Amoxicillin/clavulanic acid, ATM: Aztreonam, AX: Amoxicillin, C: chloramphenicol, CE : Cephradine, CIP: Ciprofloxacin, CL: Cephalexin, CN: Gentamicin, DO: Doxycycline.

(1): Inhibition zone (mm), (2): S/R/I: S: sensitive, R: resistant, I: intermediate.

Table 6. Cont.

Antibiotic / *Aeromonas* strain	E (15 µg)		FOX (30 µg)		K (30 µg)		KF (30 µg)		N (30 µg)		NA (30 µg)		NOR (10 µg)		OFX (5 µg)		RA (5 µg)		SXT (1.25/ 23.75µg)		TE (30 µg)	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
A.caviae	7	R	0	R	17	S	0	R	16	I	21	S	18	S	17	R	0	R	7	R	0	R
A.encheleia	7	R	15	I	16	I	0	R	11	R	0	R	17	S	23	I	0	R	11	I	17	I
A.hydrophila	0	R	13	R	17	S	7	R	11	R	0	R	9	R	0	R	0	R	0	R	0	R
A. molluscorum	0	R	23	S	12	R	0	R	22	S	0	R	30	S	18	R	10	R	15	I	0	R
A.salmonicida	12	R	11	R	17	S	0	R	12	R	0	R	0	R	15	R	0	R	18	S	0	R
A. sobria	5	R	13	R	16	I	7	R	12	R	0	R	15	I	15	R	0	R	8	R	0	R
A.veronii	0	R	12	R	13	R	0	R	0	R	0	R	0	R	17	R	0	R	11	I	0	R
A.veronii.bv. veronii	14	I	13	R	16	I	0	R	20	S	23	S	25	S	17	R	0	R	13	I	7	R
Average	5.6		12.5		15.5		1.8		13		5.5		14.3		15.3		1.3		10.4		3	

E: Erythromycin, FOX: Cefoxitin, K: Kanamycin, KF: Cephalothin, N: Neomycin, NA: Nalidixic acid, NOR: Norfloxacin, OFX: Ofloxacin, RA: Rifampin, SXT: Trimethoprim/sulphamethoxazole, TE: Tetracycline.

(1): Inhibition zone (mm), (2): S/R/I: S: sensitive, R: resistant, I: intermediate.

fish were *A. hydrophila*. All isolates of *A. hydrophila* were resistant to ampicillin and susceptible to tetracycline. Multiple drug resistance index (MAR) for all isolates ranged between 0.10 to 0.50. Therefore, routine monitoring of drug susceptibility pattern over time is necessary. Samal *et al*. (2014) found that *A. hydrophila* isolated from diseased fish were sensitive to oxytetracycline, ofloxacin, azithromycin, doxycycline, nitrofurazone, streptomycin, chlorotetracycline and norfloxacin.

None of *Aeromonas* strains were resistant to amoxicillin antibiotic while two strains only gave intermediate reaction toward this antibiotic namely *A. caviae* and *A. salmonicida.* However, none of *Aeromonas* strains were resistant to ofloxacin antibiotic, while one strain only gave intermediate reaction toward this antibiotic namely *Aeromonas encheleia*. These results are comparable with those obtained by Omojowo and Omojasola (2013) who found that testing by the disc diffusion method was conducted using ofloxacin, amoxicillin, tetracycline, ampicillin, erythromycin, gentamicin, nalidixic acid and chloramphenicol. All the isolated organisms were 100% sensitive to ofloxacin. Multiple antibiotic resistant was observed in *Aeromonas* strains in our study since *A. hydrophila*, *A. veronii* and *A. sobria* were resistant to 20, 19 and 17 antibiotics, respectively. Also *A. salmonicida, A. molluscorum* and *A. caviae* were resistant to 15, 14 and 13 different antibiotics, respectively. While *A. encheleia* was resistant to 12 antibiotics and *A.veronii.bv. veronii* was resistant to 11 antibiotics. These results are in harmony with those reported by Pettibone *et al*., (1996); Son *et al*., (1997) and Vivekanandhan *et al*., (2002) who stated that multiple antibiotic resistance (MAR) has been registered for *A. hydrophila* isolated from freshwater fish farms in association with a variety of drugs, commonly used as feed additives. Also, Costa and Cyrino (2006) found that *A. hydrophila* presented resistance to amoxicillin, ampicillin, lincomycin, novobiocin, oxacillin, penicillin trimetoprim + sulfametoxazole and rifampicin. In this connection Dias *et al*. (2012) found that all the tested strains presented multi-resistance to the tested antibiotics, and the antibiotic susceptibility profile to tetracycline, ticarcillin, carbenicillin, ampicillin and erythromycin revealed resistance levels of more than 80%.

In conclusion, the predominance of *Aeromonas* strains in most the total fish body and water samples was observed. These strains belongs to *A. caviae*, *A. encheleia*, *A. hydrophila*, *A. molluscorum*, *A. salmonicida*, *A. sobria*, *A. veronii* and *A. veronii bv. veronii* and may pose risk to public health. Since the resistance to antibiotics shown is already high, special attention should be paid to indiscriminate use of antibiotics. Most of these environmental *Aeromonas* strains produced many virulence factors involved haemolysins, lipases, proteases, gelatinases.

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ضراوة بكتيريا الإيروموناس المعزولة من السمك البلطي أو الأحواض السمكية ومياه النهر في محافظة الشرقية – مصر وحساسيتها للمضادات الحيوية

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تم دراسة عزلات الإيروموناس المعزولة من سمك البلطي والأحواض السمكية ومياه النهر لقدرتها على إنتاج عوامل الضراوة المختلفة مثل الهيموليسينز، البروتييزس، الليبيزس والجيلاتينيزس، بالإضافة إلى الحساسية للمضادات الحيوية. وتم تعريف عدد كلي ٣٧٦ إيروموناس على أساس الإختبارات البيوكيميائية وتم التحقق منها في ست عزلات بدراسة تتابعات جين ١٦ إس لـ DNA الريبوسومي لبكتيريا (إيروموناس كافي، إيروموناس مولوسكورم، إيروموناس سالمونيسيدا، إيروموناس سوبيريا، إيروموناس فيرونياي، إيروموناس فيرونياي بيوفار فيرونياي)، وبدراسة المقاومة للمضادات الحيوية وجد أن سلالات الإيروموناس كانت مقاومة لمضادات البيتا لاكتام (أموكسيسيلين/حامض كلافيولانيك) بينما كانت متباينة المقاومة للمضادات الأُخَر، ووجد أن كل السلالات كانت مقاومة لكلا من المضادات الحيوية الآتية: أمبسيللين، سيفاليكسين، سيفرادين، أموكسيسيلين/ حامض كلافيولانيك، ريفامبين وسيفالوثين، وأظهرت الغالبية من سلالات الإيروموناس سواء كانت معزولة من السمك أو المياه نشاطا في إنتاج الهيموليسينز، البروتييزس، الليبيزس والجيلاتينيزس.