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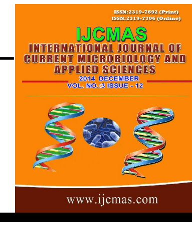

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Original Research Article

Isolation, biochemical characterization, antibiotic susceptibility study of *Aeromonas hydrophila* isolated from freshwater fish

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A B S T R A C T

Keywords

Aeromonas hydrophila, Isolation, Biochemical characters, Antibiotic sensitivity study, Fish, Rimler Shott's medium

The isolation, phenotypic and biochemical characterization, antibiotic susceptibility test of *Aeromonas hydrophila* isolated from diseased fish as rohu, catla, mrigal, cat fish, gold fish and *Channa* spp., were done in the present experiment. *A. hydrophila* strains along with reference strain MTCC 646 produced small, round, smooth, convex and translucent, yellow colonies on Rimler Shott's medium, Gram negative short rod, motile by polar flagella, fermentative, showed positive reaction towards oxidase, catalase, produced gas from glucose, utilize citrate for growth, produced indole, reduced nitrate, oxidate gluconate, produced amylase enzyme and decompose starch. Strain differentiation of *A. hydrophila* was done on the basis of 34 different sugar fermentation tests used in the study. *A. hydrophila* strains sensitive to oxytetracycline, ofloxacin, azithromycin, doxycycline, nitrofurazone, streptomycin, chlorotetracycline and norfloxacin; resistance to amoxicillin, ampicillin, bacitracin, cefuroxime, cloxacillin, cephalexin, co-trimoxazole, flumequine and erythromycin.

Introduction

Aquaculture has demonstrated significant growth during the past two decades to meet the protein requirement of the increasing global population. Aquaculture has become one of the major economic activities in several nations (Balc´azar *et al.*, 2006). With the intensification of aquaculture practices, the favorable environmental required for pathogenic bacterial growth gets generated in the culture systems (Aguirre-Guzman and Filipe, 2000).

Aeromonas spp. has been known to be pathogenic and is responsible for a multiple of infections to humans, reptiles, fish and other cold-blooded animals. *A. hydrophila* causes haemorrhagic and ulcerative diseases and septicaemia in Indian major carps (Das and Mukherjee, 1997, 1998). *A. hydrophila* has been reported to be associated with epizootic ulcerative syndrome and other infections in fishes in South East Asian countries, Malayasia, Sri Lanka, Japan and

India (Das and Mukherjee, 1997, 1998; Miyazaki *et al.,* 2001; Shome *et al.,* 2005). Several studies have incriminated these organisms in gastroenteritis ranging from mild diarrhea to life-threatening cholera-like illness in children and adults, wound infections, meningitis, broncho-pulmonary infections and osteomyelitis (Figueras *et al.,* 2007). The antibiotic sensitivity test of *Aeromonas hydrophila* against different antibiotics isolated from different sources of diseased fish, clinical and environmental samples from India and other parts of world was studied by several authors (Du *et al.,* 2011; Ali Alzainy, 2011; Dias *et al.,* 2012; Khairul Afizi *et al.,* 2013).

Materials and Methods

Isolation of Bacteria

Isolation of *Aeromonas hydrophila* bacteria was done from diseased fish viz. catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), gold fish (*Carassius auratus*), Anabas (*Anabas testidenus*) and clarias (*Clarias betrachus*), cat fish and Channa species (*Channa punctatus* & *Channa marulius*) showing gross pathological lesions as ulcerated skin, tail and fin rot, from different experimental and culture ponds of Central Institute of Freshwater Aquaculture (CIFA), commercial farms of Odisha and Andhra Pradesh. Samples were collected aseptically from different organs like skin, gill, kidney, liver and intestine etc. and grown on brain heart infusion (BHI) broth and turbid growth cultures from BHI broth streaked onto Rimler Shott's medium and Aeromonas isolation agar medium for isolation of *Aeromonas* spp.

Biochemical characterization of *Aeromonas hydrophila*

The biochemical tests were conducted to determine the physiological characters, carbohydrate metabolism and production of nitrogenous compounds. The characters such as capsule formation, motility and formation of flagella were recorded under morphological studies. The physiological characters included catalase production, growth on KCN medium, methyl red (MR) and voges-proskauer (VP) medium; different enzyme hydrolysis and amino acid decarboxylase tests, maximum temperature for growth in nutrient broth and growth in peptone water in the presence or absence of NaCl (Holt and Krieg, 1984). Observations on carbohydrate metabolism reaction included production of acid and gas in glucose and glycerol, production of acid in L-arabinose, D-xylose, L-rhamnose, D-mannose, sorbose, D-lactose, D-maltose, D-sucrose, raffinose, D-mannitol, D-dulcitol, D-sorbitol, salicin, esculin hydrolysis and production of β-galactosidase (West and Colwell, 1984).

Antibiotics sensitivity test

The antibiotic susceptibility test was done using standard antibiotic discs by disc diffusion techniques (Cruikshank *et al.,* 1975). A total of 34 types of antibiotics disc (Hi-media) were used for antibiotic sensitivities study and the different discs with their disc potency were summarized in Table 3. Different *A. hydrophila* cultures were grown in brain heart infusion (BHI) broth for 24 hours at 37^0C. The suspension was reinoculated on diagnostic sensitivity (DST, Hi Media, India) medium plates by swabbing. The antibiotic discs were then kept on the Diagnostic sensitivity medium (DSM) plates by help of a sterile forceps. The antibiotic discs were then kept on the DSM plates and the plates were incubated at 37^0C for 24 hours. Cultures were judged as resistant or sensitive on the basis of size of the zone of bacteria growth inhibition according to the guidelines of the CLSI, (2010).

Results and Discussion

Isolation of *Aeromonas hydrophila* isolates

Total of 197 samples were collected from skin lesion, liver, kidney, intestine, gill and spleen of different diseased fish as *Channa* spp., mrigal, catfish, goldfish, rohu, catla, etc. of Pathology laboratory, catfish unit, wet laboratory of Central Institute of Freshwater Aquaculture (CIFA); Commercial farm of Puri, Cuttack and Khordha districts and selected commercial farms of Andhra Pradesh. Out of 197 isolates, 59 isolates grown in Rimler-Shott's (RS) and Aeromonas isolation agar (AIA) agar medium and produced yellow, round, small to medium, convex, elevated and transparent colonies. After Gram staining, a series of biochemical tests and sugar fermentation tests; only 12 isolates confirms as *Aeromonas hydrophila* and their source of isolation were; skin lesion of mrigal of CIFA pond, skin lesion, liver, kidney of *Channa* spp. of commercial fish farm of Puri district, CIFA wet laboratory and Andhra Pradesh. Various authors used different mediums for isolation of *Aeromonas* spp. from aquatic environments, aquatic animals, seafoods, raw milk, animal products, fish and humans as Rimler-Shotts medium, starch ampicillin agar, SA, Rimler's-Shotts (RS) and starch-glutamate-ampicillin-penicillin based medium, Pseudomonas aeromonas selective agar base (GSP) and Starch ampicillin agar (SAA) in Eastern Canada (Shotts and Rimler, 1973; Jenkins and Taylor, 1995; Singh, 1997).

Biochemical characterization of *A. hydrophila* isolates

In our study, we found that *A. hydrophila* isolates (Ah1–Ah12) produced small, round, smooth, convex, translucent, yellow colonies on Rimler Shott's (RS) medium and Aeromonas isolation agar (AIA) medium. Microscopically *A. hydrophila* was a Gram negative short rod, motile by polar flagella with swarming movement and they were 0.5–1.2 µm in diameter and 1.4–15.0 µm in length. All the 12 isolates were compared with the reference strain *A. hydrophila* MTCC 646 (Ah13), (Microbial Institute, Chandigarh, India). *A. hydrophila* isolates including reference strain (MTCC 646) were fermentative, motile by polar flagella, vibriostatic compound (O/129, 150 µg), and novobiocin- resistant, showed positive reaction towards oxidase, catalase, produced gas and acids from glucose, utilize citrate for growth and produced acetoin, produced indole, reduced nitrate, oxidate gluconate, produced amylase enzyme and decompose starch, showed positive reaction towards lipase test, gelatinase test, caseinase test, DNase test, oxidation of ONPG test, lysine and arginine decarboxylase test; esculin hydrolysis test, methyl red (MR) test, alkyl sulfatase test, acetate utilization test; showed negative reaction towards ornithine decarboxylase test, Voges proskauer (VP) test, urease test, malonate utilization test and DL-lactate utilization test. *A. hydrophila* isolates utilize glucose, dextrose, lactose, D-mannitol, sucrose, D-maltose, trehalose, starch, D-galactose, D-ribose, glycerol, salicin, sorbitol, rhamnose, D-mannose, amygdalin, L-arginine and arabinose; do not ferment D-sorbose, L-rhamnose, D-melibiose, m-inositol, raffinose, adonitol, D-cellobiose, inulin, L-arabinose, xylose and dulcitol; do not reduced ferrous sulfate to ferric sulfate and not produced H_2S gas (Table 1). *A. hydrophila* strains grow in nutrient broth and other basal medium with 0–2% NaCl and do not grow in presence of 4–8% NaCl. Similar results with minor variations in biochemical properties of *A. hydrophila* isolates was reported by various authors as negative for lysine decarboxylase, ornithine decarboxylase positive, Voges proskauer positive (Jayavignesh *et al.,* 2011), H_2S positive, negative towards DNase test, nitrate positive, potassium cyanide positive, isolated from rainbow trouts in Korea (Lee *et al.*, 2000), from fish (*Etroplus suratensis)* and the waters of traditional brackishwater farms in Cochin, India (Thomas *et al.*, 2009), from *Labeo rohita* and from various organs of freshwater fish (Sahu *et al.,* 2011), from skin ulcerated fish (*Schizothorax prenanti*) in Yaan city, China (Du *et al.*, 2011), isolated from diseased fish (Sarkar *et al.,* 2012).

In our study, we reported that *A. hydrophila* isolates showed variation in few biochemical reactions such as lysine decarboxylase test (Ah7 & Ah11), arginine decarboxylase test (Ah4 & Ah9), citrate utilization test (Ah8 & Ah12), MR test (Ah5 & Ah10), VP test (Ah3 & Ah9), gluconate oxidation test (Ah10), acetate utilizetion test (Ah6), alkyl sulfatase test (Ah12) and oxidation of ONPG test (Ah1). We observed that the ratio of caseinase, gelatinase, amylase and DNase activity varied in the range of 1.56–2.36, 2.06–2.75, 1.57–1.89 and 1.56–2.09, respectively (Table 2). The ratio of caseinase activity of Ah4 & Ah5 was same (2.07), the ratio of gelatinase activity of Ah6 & Ah12 was same (2.18), the ratio of amylase activity of Ah1 & Ah6 was same (1.87), Ah4, Ah9, Ah11 were same (1.69), Ah3 & Ah10 was same (1.75) and the ratio of DNase activity of Ah6 & Ah12 was same (1.87).

Antibiotic sensitivity study of *A. hydrophila* isolates

Aeromonas hydrophila isolates were 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, cephotaxime), chloramphenicol, tetracycline, imipenem, quinolones, gentamicin, kanamycin, nitrofurantoin, norfloxacin, streptomycin, sulphamethoxazole, ciprofloxacin, oxytetracycline, doxycycline, amikacin, aminoglycosides, amoxicillin, ticarcillin, ofloxacin, pefloxacin, neomycin, oxacillin, gatifloxacin and levofloxacin isolated from different diseased fish, environmental and clinical samples in different parts of world as Venezuela, South East Brazil, Vietnam, Asia, Abu Dhabi, UAE, Mhow and Indore city of Madhyapradesh, Meghalaya and Assam states, China, Lagos metropolis in Nigeria, Malaysia and Egypt (Ottaviani *et al.,* 2006; Ghenghesh *et al.,* 2008; Kaskhedikar and Chhabra, 2009, 2010; Ashiru *et al.,* 2011; Odeyemi *et al.,* 2012; Khairul Afiziul *et al.*, 2013). Like antibiotics, various extracts as hexane, ethyl acetate, acetone, ethanolic, methanolic and aqueous extracts of *Mangifera indica* (mango) kernel have potentialities in using against different aquatic bacterial pathogens including *A. hydrophila* (Sahu *et al.*, 2013). In our present experiment, all the isolates of *A. hydrophila* isolates including reference strain MTCC 646 (Ah1-Ah13) were 100% sensitive to oxytetracycline, ofloxacin, azithromycin, doxycycline, 92.3% sensitive to nitrofurazone, streptomycin, 84.6% to chlorotetracycline, cephalothin, chloramphenicol, norfloxacin, furazolidone, 76.9% to amikacin, trimethoprim, neomycin, oxacillin, 69.2% to ciprofloxacin, gentamycin, flomequine, 61.5% to tetracycline and resistance to amoxicillin, ampicillin, bacitracin, cefuroxime, cloxacillin, cephalexin, co-trimoxazole, flumequine, erythromycin, penicillin-G, novobiocin, nalidixic acid, vibriostatic compound (O/129), polymixin-B and cephotaxime (Table 3).

Table.1 Phenotypic and biochemical characterization of different strains of *A. hydrophila* (Ah1-Ah 12) and reference strain (MTCC 646). (K/Ag- Alkaline butt and alkaline slant; K/A- Acid butt and alkaline slant)

Biochemical tests		Ah1	Ah2	Ah3	Ah4	Ah5	Ah6	Ah7	Ah8	Ah9	Ah10	Ah11	Ah12	MTCC 646
Gram's reaction		-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase test		+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidative/Fermentative test		F	F	F	F	F	F	F	F	F	F	F	F	F
Motility test		+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test		+	+	+	+	+	+	+	+	+	+	+	+	+
Gas from glucose		+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase test		+	+	+	+	+	+	-	+	+	+	-	+	+
Arginine decarboxylase test		+	+	+	-	+	+	+	+	-	+	+	+	+
Ornithine decarboxylase test		-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate utilization test		+	+	+	+	+	+	+	-	+	+	+	-	+
Triple sugar iron agar test		K/Ag	K/A	K/Ag	K/Ag	K/Ag	K/Ag	K/Ag	K/A	K/Ag	K/Ag	K/A	K/A	K/Ag
Indole production test		+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red (MR) test		+	+	+	+	-	+	+	+	+	-	+	+	+
Voges proskauer (VP) test		-	-	+	-	-	-	-	-	+	-	-	-	-
Nitrate reduction test		+	+	+	+	+	+	+	+	+	+	+	+	+
Urease test		-	-	-	-	-	-	-	-	-	-	-	-	-
Esculin hydrolysis test		+	+	+	+	+	+	+	+	+	+	+	+	+
Gluconate oxidation		+	+	+	+	+	+	+	+	+	-	+	+	+
Malonate utilization test		-	-	-	-	-	-	-	-	-	-	-	-	-
Acetate utilization test		+	+	+	+	+	-	+	+	+	+	+	+	+
DL-lactate utilization test		-	-	-	-	-	-	-	-	-	-	-	-	-
Alkyl sulfatase test		+	+	+	+	+	+	+	+	+	+	+	-	+
Amylase production test		+	+	+	+	+	+	+	+	+	+	+	+	+
Lipase production test		+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatinase test		+	+	+	+	+	+	+	+	+	+	+	+	+
Caseinase test		+	+	+	+	+	+	+	+	+	+	+	+	+
DNase test		+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidation of ONPG		-	+	+	+	+	+	+	+	+	+	+	+	+
Acid production from:														
	Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+
	D-fructose	+	+	+	+	+	+	+	+	+	+	+	+	+
	D-mannose	+	+	+	-	+	+	+	+	+	-	+	+	+
	D-mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+
	D-maltose	+	+	+	+	+	+	+	+	+	+	+	+	+
	Trehalose	+	-	+	+	+	+	+	+	+	+	+	-	+
	Dextrin	+	+	+	+	-	+	+	+	+	-	+	+	+
	Starch	+	+	+	+	+	+	+	+	+	+	+	+	+
	D-galactose	-	+	+	+	+	+	+	+	+	+	+	+	+
	D-ribose	+	+	+	+	+	+	+	-	+	+	+	+	+
	Glycerol	+	+	+	-	+	+	+	+	+	+	+	+	+
	Salicin	+	+	+	+	+	+	-	+	+	+	+	+	+
	D-sorbose	-	-	-	-	-	-	-	-	-	-	-	-	-
	L-rhamnose	-	-	+	-	-	-	-	-	-	-	-	-	-
	D-melibiose	-	-	-	-	-	-	-	+	-	-	-	-	-
	m-inositol	-	-	-	-	-	-	-	-	-	-	-	-	-
	Raffinose	-	-	-	-	-	-	-	-	-	-	+	-	-
	Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-
	D-cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-
	Inulin	-	-	-	-	-	-	-	-	-	-	-	-	-
	L-arabinose	-	-	-	-	-	+	-	-	-	-	-	-	-
	Xylose	+	-	+	-	+	+	-	+	+	-	-	-	+
	Dulcitol	-	-	-	-	+	-	-	-	-	-	-	-	+
Growth in NaCl:														
	0%	+	+	+	+	+	+	+	+	+	+	+	+	+
	2%	+	+	+	+	+	+	+	+	+	+	+	+	+
	4%	-	-	-	-	-	-	-	-	-	-	-	-	-
	6%	-	-	-	-	-	-	-	-	-	-	-	-	-
	8%	-	-	-	-	-	-	-	-	-	-	-	-	-

Table.2 Zone diameter (cm) of β-haemolysis, caseinase, gelatinase, amylase and DNase activities produced by 12 *A. hydrophila* (Ah1-Ah12) strains and *A. hydrophila* reference strain MTCC 646 (Ah13)

		β-haemolysis			**Caseinase activity**			**Gelatinase activity**			**Amylase activity**			**DNase activity**		
Sl No.	Strain	Colony Diameter in cm	Zone Diameter in cm	Ratio	Colony Diameter in cm	Zone Diameter in cm	Ratio	Colony Diameter in cm	Zone Diameter in cm	Ratio	Colony Diameter in cm	Zone Diameter in cm	Ratio	Colony Diameter in cm	Zone Diameter in cm	Ratio
1	Ah1	1.7	3.2	1.88	1.2	2.1	1.75	0.8	1.9	2.37	0.8	1.5	1.87	0.9	1.6	1.78
2	Ah2	1.8	3.4	1.89	1.1	1.9	1.73	1.2	2.6	2.17	0.9	1.7	1.89	1.0	1.9	1.90
3	Ah3	2.2	3.7	1.68	1.2	2.7	2.25	1.3	2.9	2.23	1.2	2.1	1.75	1.2	2.1	1.75
4	Ah4	2.1	3.6	1.71	1.4	2.9	2.07	1.4	3.1	2.21	1.3	2.2	1.69	1.3	2.2	1.69
5	Ah5	2.3	3.8	1.65	1.5	3.1	2.07	1.6	3.3	2.06	1.4	2.3	1.64	1.1	2.3	2.09
6	Ah6	1.4	2.9	2.07	0.9	1.5	1.67	1.1	2.4	2.18	0.8	1.5	1.87	0.8	1.5	1.87
7	Ah7	1.6	3.0	1.87	0.8	1.7	2.13	0.9	2.1	2.34	0.7	1.3	1.86	0.9	1.4	1.56
8	Ah8	1.5	3.1	2.07	0.9	1.4	1.56	1.0	2.2	2.20	1.1	1.9	1.73	0.7	1.3	1.86
9	Ah9	2.2	3.6	1.64	1.4	3.2	2.28	1.4	3.4	2.43	1.3	2.2	1.69	1.4	2.3	1.64
10	Ah10	2.4	3.9	1.63	1.3	2.8	2.15	1.3	3.1	2.38	1.2	2.1	1.75	1.3	2.4	1.85
11	Ah11	2.3	3.6	1.57	1.5	2.7	1.80	1.2	3.3	2.75	1.1	1.8	1.69	1.2	2.2	1.84
12	Ah12	1.6	3.1	1.94	1.0	1.7	1.70	1.1	2.4	2.18	1.0	1.8	1.80	0.8	1.5	1.87
13	Ah13 MTCC 646	1.9	3.7	1.95	1.1	2.6	2.36	1.3	3.2	2.46	1.4	2.2	1.57	1.1	1.9	1.73

Table.3 Antibiogram test of different strains of *A. hydrophila* (Ah1-Ah 12) and reference strain (MTCC 646). S= sensitive, R= Resistant

Antibiotics with symbols	Disc potency (mcg)	Isolates													Zone of inhibition (mm)		
		Ah1	Ah2	**Ah3**	**Ah4**	**Ah5**	**Ah6**	**Ah7**	**Ah8**	**Ah9**	**Ah 10**	**Ah 11**	Ah 12	**MTCC 646**	R	S	%S
Amikacin, Ak	30	S	S	S	**R**	S	S	S	**R**	S	S	S	S	**R**	14	17	76.9
Amoxycillin, Am	30	R	R	R	R	R	R	R	R	R	R	R	R	R	13	18	0
Ampicillin, A	10	R	R	R	R	R	R	R	R	R	R	R	R	R	14	17	0
Bacitracin, B	10 U	R	R	R	R	R	R	R	R	R	R	R	R	R	08	13	0
Cefuroxime, Cf	30	R	R	R	R	R	R	R	R	R	R	R	R	R	14	18	0
Chlorotetracycline, Cl	30	S	S	S	S	S	S	**R**	S	S	**R**	S	S	S	14	19	84.6
Cloxacillin , Cx	10	R	R	R	R	R	R	R	R	R	R	R	R	R	19	20	0
Cephalexin,Cp	30	R	R	R	R	R	R	R	R	R	R	R	R	R	11	21	0
Cephalothin, Ch	30	S	S	S	S	S	**R**	S	S	S	S	S	S	**R**	14	18	84.6
Ciprofloxacin, Cf	5	S	**R**	S	S	**R**	S	S	**R**	S	S	**R**	S	S	10	16	69.2
Chloramphenicol, C	10	S	S	**R**	S	S	S	S	S	S	S	S	S	**R**	12	18	84.6
Co-trimoxazole, Co	25	R	R	R	R	R	R	R	R	R	R	R	R	R	10	16	0
Flumequine, F	5	R	R	R	R	R	R	R	R	R	R	R	R	R	15	21	0
Gentamycin, G	10	**R**	S	S	**R**	S	**R**	S	S	**R**	S	S	S	S	12	15	69.2
Erythromycin, E	15	R	R	R	R	R	R	R	R	R	R	R	R	R	13	23	0
Penicillin-G, P	2 U	R	R	R	R	R	R	R	R	R	R	R	R	R	14	15	0
Novobiocin, No	5	R	R	R	R	R	R	R	R	R	R	R	R	R	17	22	0
Trimethoprim, Tr	5	S	**R**	S	S	S	S	S	**R**	S	S	S	**R**	S	10	16	76.9
Nalidixic acid, Na	30	R	R	R	R	R	R	R	R	R	R	R	R	R	13	19	0
Oxytetracycline, Ot	30	S	S	S	S	S	S	S	S	S	S	S	S	S	14	19	100
Tetracycline, T	30	S	**R**	S	**R**	S	S	**R**	S	**R**	S	S	S	**R**	14	19	61.5
Ofloxacin, Of	2	S	S	S	S	S	S	S	S	S	S	S	S	S	12	16	100
Neomycin, N	30	S	S	**R**	S	S	S	**R**	S	S	**R**	S	S	S	12	17	76.9
Nitrofurazone, Nf	100	S	S	S	S	S	S	S	**R**	S	S	S	S	S	19	26	92.3
Norfloxacin, Nx	10	S	S	**R**	S	S	S	S	S	S	**R**	S	S	S	12	17	84.6
Vibriostatic (O/129)	15	R	R	R	R	R	R	R	R	R	R	R	R	R	13	16	0
Polymixin B, Pb	30	R	R	R	R	R	R	R	R	R	R	R	R	R	8	12	0
Cephotaxime, Ce	15	R	R	R	R	R	R	R	R	R	R	R	R	R	10	16	0
Furazolidone, Fr	30	S	S	**R**	S	S	S	S	S	**R**	S	S	S	S	12	17	84.6
Oxacillin, O	30	S	**R**	S	S	**R**	S	S	S	S	**R**	S	S	S	14	19	76.9
Flomequine, Fm	10	S	S	**R**	S	S	S	**R**	S	S	S	**R**	S	**R**	14	17	69.2
Streptomycin, S	50	S	S	S	S	S	S	S	**R**	S	S	S	S	S	11	15	92.3
Azithromycin, Az	30	S	S	S	S	S	S	S	S	S	S	S	S	S	14	20	100
Doxycycline, Do	30	S	S	S	S	S	S	S	S	S	S	S	S	S	12	16	100

In conclusion, the findings of the present study indicate the involvement of *A. hydrophila* from the disease affected fish as a major pathogen. The results of phenotypic, biochemical characterization and antibiogram studies to a wide no. of antibiotics could be used for diagnosis of aeromoniasis, EUS in different outbreak and epidemiological condition.

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