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Prevalence and antibiotic susceptibility of *Aeromonas hydrophila* isolated from freshwater fishes

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Abstract

Aeromonas hydrophila is an opportunistic microorganism. It is a secondary biological agent that contributes to the occurrence of fish diseases and its deterioration. This research was undertaken to determine the prevalence of *A. hydrophila* in some freshwater fishes collected from three different fish markets of Dhaka City and to test their antibiotic susceptibility. Total bacterial count and total aeromonas on different aeromonas selective media were enumerated using serial dilution technique. Bacterial isolates were characterized to identify *A. hydrophila* using biochemical tests and with comparison to reference strain (ATCC 7966). The lowest Aeromonas count was detected to be $2.83\pm0.40\times10^2$ cfu/g in *Anabas testudineus* and the highest was $1.03\pm0.153\times10^3$ cfu/g in *Oreochromis mossambicus*. On market basis highest aeromonas count was found in Anando Bazar ($8.10\pm1.09\times10^2$ cfu/g) and lowest in Hatirpool Bazar ($5.63\pm0.90\times10^2$ cfu/g) with no significant difference. Maximum susceptibility to amikacin and gentamicin was observed whereas all of the isolates were found resistant to a commonly used antibiotic amoxycillin. The obtained results point that antimicrobial susceptibility was more or less similar regardless of the origin of the samples collected. All the fishes investigated in this study contained *A. hydrophila* in their different organs.

Keywords: *Aeromonas hydrophila*; total aeromonas count; biochemical tests; antibiotic susceptibility; motile aeromonas septicemia

INTRODUCTION

Aeromonas hydrophila is the most common bacteria in freshwater habitat throughout the world, and this bacterium frequently causes disease among cultured and feral fishes (Cipriano 2001). In a variety of freshwater species, the existence and pathogenicity of *A. hydrophila* have been reported, comprising *Salmo gairdneri* (Peters *et al.* 1988), *Clarias batrachus* (Angka 1990), *Carassius auratus* (Citarasu *et al.* 2011), *Cyprinus carpio* (Citarasu *et al.* 2011), *Oreochromis niloticus* (Ibrahem *et al.* 2008) and *Channa striata* (Duc *et al.* 2013). A wide variety of primarily freshwater fish species, including carp, tilapia, perch, catfish and salmon were affected by mesophilic *A. hydrophila* and caused Motile Aeromonas Septicemia (MAS) (Joseph *et al.* 1994). Septic arthritis, diarrhea, corneal ulcers, skin and wound infections, meningitis and fulminating septicemia may be caused by *A. hydrophila* in immune-compromised human hosts (von Gravenitz and Mensch 1968). It was reported widely infecting freshwater fish and marine fish species associated with skin lesions, tail and fin rot, hemorrhagic septicemia over the body and tissue destruction, epizootic ulceration and

necrosis in the liver and kidney of fish (Austin and Adams 1996; Doukas *et al.* 1998; Janda and Abbott 2010). In freshwater cultured cyprinid fishes *A. hydrophila* has also been described as the dominant infectious agent of 'fish-bacterial-septicemia' (Qian *et al.* 1997).

Epidemic disease outbreaks in fish caused by *A. hydrophila*, resulting in millions of dollars of lost revenue, have been reported worldwide. It was considered as a significant economic problem, particularly in China and India over the past decade (Citarasu *et al.* 2011). In parts of south-east Asia such as in Bangladesh and India, it was reported that epizootic ulcerative syndrome (EUS) caused by *A. sobria* resulted in great damage to fish farms (Rahman *et al.* 2002). Intensification of fish farming in Bangladesh has increased the number of disease outbreaks in intensive production systems. Most etiological agents were not yet identified and their morbid processes are still not studied. Both *A. hydrophila* and *A. sobria* produce enterotoxins, dermo-necrotic factors and haemolysins (Olivier *et al.* 1981). Production of capsule has been reported for *A. hydrophila* serogroups (Martinez *et al.* 1995), but it is not clear the function of capsule material. Perhaps it is supposed to resist complement activity and enhance adherence (Merino *et al.* 1996).

Conventional methods of identifying the prevalence of aeromonads are based on isolation and biochemical identification (Austin and Austin 1989). Identification of aeromonads is difficult by using biochemical schemes but most clinical microbiology laboratories still routinely rely on easy-to-use phenotypic methods (Sinha *et al.* 2004). In this study, both bacterial isolation and biochemical identification were performed.

The use of antibiotics is the most important factor in amplifying the level of resistance in a given reservoir (Wegener and Frimodt-Moller 2000). 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 (Aoki *et al.* 1971; Pettibone *et al.* 1996; Son *et al.* 1997; Vivekanandhan *et al.* 2002). The main problem involving the use of antibiotics against aeromonas infections is the development of resistance by these bacteria (Aoki *et al.* 1971), generally related to the presence of plasmids (Ansary *et al.* 1992). The MAR among *A. hydrophila* strains has been reported from many parts of the world (Pettibone *et al.* 1996; Son *et al.* 1997). Under these circumstances, it will be worthwhile to find out the prevalence of antibiotic resistance of the aeromonas strains that may be considered as an emerging pathogen and to identify the high-risk source.

In this study, we have reported the prevalence of *A. hydrophila* in some commonly available freshwater fish species of Bangladesh using selective agar and biochemical tests; and also checked antibiotic susceptibility of the isolated strains.

METHODOLOGY

Sample collection: Fish samples [Sarpunti, *Systomus sarana* (Hamilton, 1822); Koi, *Anabas testudineus* (Bloch, 1792); Tatkini, *Cirrhinus reba* (Heckel, 1838); Tilapia, *Oreochromis mossambicus* (Peters, 1852); Meni, *Nandus nandus* (Hamilton, 1822)] were collected aseptically from three different fish markets of Dhaka city *viz.*, Palashi Bazar, Anando Bazar and Hatirpool Bazar on June to November, 2015. Fish samples were collected in sterile plastic bags and were labeled. Identification of sampled fish was done according to Rahman (2005). Then the muscle, gill and gut samples were collected from the fishes aseptically following the method of APHA (1998). Three specimens per species in each fish market considered for present study.

Bacteriological analysis: Nutrient agar (NA) medium was used for the enumeration and isolation of heterotrophic aerobic bacteria present in fish samples, while aeromonas Agar, AH (Hi-media, Bombay, India); AO (Oxoid, Hampshire, UK) and AL (LAB, Lancashire, UK) media were used for the enumeration and isolation of *Aeromonas* sp. present in studied fish samples. Total aeromonas count of three species (*S. sarana*, *A. testudineus* and *N. nandus*) from two markets (Palashi and Hatirpool Bazar) were enumerated to compare three different aeromonas agar media.

Serial dilution technique was used for the enumeration and isolation of the bacteria. In each sample, 10^{-4} dilution for NA medium and 10^{-3} dilution for the plating of Aeromonas Agar (Hi-media, Oxoid and LAB) medium were performed.

One ml of each of the diluted sample was taken in a sterilized petri plate by sterilized pipette. Pour plating in duplicate plates was performed for each diluted sample. After 48 h of incubation at 37°C temperature the plates having well discrete colonies were selected for counting from the respective culture plate. The selected plates were placed on a digital colony counter to count the colonies (DC-8 OSK 100086, Kayagaki, Japan). Discrete bacterial colonies were isolated immediately after counting.

Identification of Aeromonas hydrophila: 15 suspected isolates of *Aeromonas* sp. in selective media were used for the identification to species level by performing important biochemical tests viz. Oxidase test, Catalase

test, KOH solubility test, MR test, Indole production, V.P. test, brown water pigment solution test, hydrolysis of esculin and arginine, carbohydrates fermentation, KIA test, utilization of citrate and motility test (SAB 1957; Eklund and Lankford 1967; Collins and Lyne 1984; Sneath *et al.* 1986; Schaad 1988; Claus 1995; Atlas 1997).

Antibiotic susceptibility assay: Susceptibility of *A. hydrophila* to different antibiotics was measured in vitro according to Kirby-Bauer methods (Bauer *et al.* 1966). Commercially available 14 antibiotics in diffusion disks (Table 9) were used for this test. 15 identified *A. hydrophila* including one reference strain (ATCC 7966) were inoculated in nutrient broth and cultured for 16 hours. The bacterial suspension was then spread onto the surface of the Mueller-Hinton agar (Hi-media, M173-500G, India) using sterile cotton swabs, which were then left to dry for several minutes. The antibiotic discs (Oxoid, Hampshire, UK) were placed on the surface of the agar plate and incubated for 24 h at 37 °C. Finally, the zone of inhibition was measured and compared with the reference data of antibiogram pattern to know the susceptibility of the bacteria.

Statistical analyses: The statistical package for the social sciences (SPSS) v. 20.0 for windows (SPSS, SAS Institute Inc. Cary, USA) was used for statistical analysis. One way ANOVA, Tukey's HSD post hoc for the multiple comparisons were subjected using 5% level of significance to present the data as mean±SEM.

RESULTS AND DISCUSSION

Total bacterial and aeromonas load of the studied fish samples: The bacterial counts of the samples are shown in Table 1. A large number of aerobic heterotrophic bacteria were found to be associated with the samples. The total heterotrophic bacterial count (TBC) ranged in between $1.60\pm0.252\times10^5$ and $5.04\pm0.74\times10^5$ cfu/g.

The highest count of TBC was $5.04\pm0.74\times10^5$ cfu/g in *N. nandus* and lowest TBC count was $1.60\pm0.252\times10^5$ cfu/g in *O. mossambicus*. For the total aeromonas count, it also showed differences in mean among the selected freshwater fishes. The highest aeromonas count was $1.03\pm0.153\times10^3$ cfu/g in *O. mossambicus* and the lowest was $2.83\pm0.40\times10^2$ cfu/g in *A. testudineus*. According to Hatha *et al.* (1998) high microbial abundance might be due to contaminated source of water, poor hygiene and sanitation condition of processing of the fishes. In this study, total bacterial count was found maximum $5.04\pm0.74\times10^5$ cfu/g which are within the acceptable limit (ICMSF 1998). So this study clarifies that the collected freshwater fishes from different fish markets were safe for human consumption from microbial point of view.

Table 1: Bacterial load (cfu/g; mean±SEM) on NA and aeromonas agar (Hi-Media) of studied fish species (N=45, ANOVA, HSD, p<0.05)

Species	*TBC*	*Total Aeromonas count*
Systomus sarana	$3.34\pm0.63\times10^{5ab}$	$9.72\pm1.21\times10^{2bc}$
Oreochromis mossambicus	$1.60\pm0.25\times10^{5b}$	$1.03\pm0.15\times10^{3b}$
Cirrhinus reba	$2.28\pm0.46\times10^{5b}$	$5.73\pm0.82\times10^{2ab}$
Anabas testudineus	$2.69\pm0.42\times10^{5b}$	$2.83\pm0.40\times10^{2a}$
Nandus nandus	$5.04\pm0.74\times10^{5a}$	$4.83\pm0.92\times10^{2a}$

Means within column followed by different superscript small letters indicate significant differences

Table 2 describes the differences in mean according to different organs of fishes. The highest count of TBC was $4.21\pm0.42\times10^5$ cfu/g in gill and lowest TBC count was $5.83\pm0.73\times10^4$ cfu/g in muscle. For the total aeromonas count, it also showed differences in mean among the selected freshwater fish organs. The highest aeromonas count was $1.09\pm0.103\times10^3$ cfu/g in gill and the lowest was $1.76\pm0.24\times10^2$ cfu/g in muscle.

Table 2: Bacterial load (cfu/g; mean±SEM) on NA and aeromonas agar (Hi-media) of three different organs of five fish species (N=45, ANOVA, HSD, p<0.05).

Organs	*TBC*	*Total Aeromonas Count*
Muscle	$5.83\pm0.73\text{x}10^{4a}$	$1.76\pm0.24\text{x}10^{2c}$
Gill	$4.21\pm0.42\text{x}10^{5b}$	$1.09\pm0.10\text{x}10^{3a}$
Gut	$4.18\pm0.46\text{x}10^{5b}$	$7.38\pm0.66\text{x}10^{2b}$

Means within column followed by different superscript small letters indicate significant differences

Figure 1 shows total bacterial count (TBC) (cfu/g, mean± SEM) in *S. sarana*, *O. mossambicus*, *C. reba*, *A. testudineus* and *N. nandus* measured from different organs of fishes. From muscle, *S. sarana* showed maximum TBC ($1.02\pm0.29\times10^5$ cfu/g), from gill and gut, *N. nandus* showed maximum TBC ($7.66\pm0.32\times10^5$ and $6.82\pm1.26\times10^5$ cfu/g). Minimum TBC was counted from the muscle and gut of *O. mossambicus* $3.07\pm0.61\text{x}10^4$ and $1.43\pm0.12\text{x}10^5$ cfu/g, respectively.

Figure 2 shows total aeromonas count (cfu/g, mean± SEM) in all fish samples measured from selected organs. The highest count of total aeromonas was $1.57\pm0.12\times10^3$ cfu/g found from gill of *S. sarana* and the lowest count was $1.56\pm0.97\times10^1$ cfu/g measured from muscle of *A. testudineus*.

It is important to note that when the total bacterial load reaches $1.0\text{x}10^7$ cfu/g or more in food and food products, these foods are considered as spoiled (Shewan 1970).The total bacterial count was $5.83\pm073\text{x}10^4$, $4.21\pm0.42\text{x}10^5$

and 4.18±0.46x10^5 cfu/g in muscle, gill and gut respectively and total aeromonas count 1.76±0.24x10^2, 1.09±0.10x10^3 and 7.38±0.66x10^2 cfu/g which means that the fish samples collected from the local markets were not harmful for consumption. Bacteria associated with fish muscle and their great variation in percentage has been reported by Anwar *et al.* (1988).

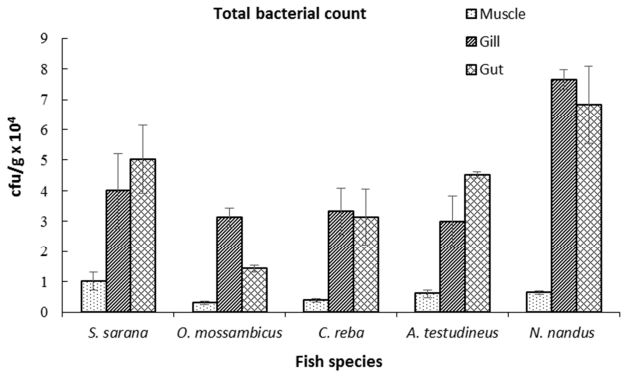

Figure 1: Variation of total bacterial count (cfu/g; mean± SEM) in different organs of studied freshwater fishes.

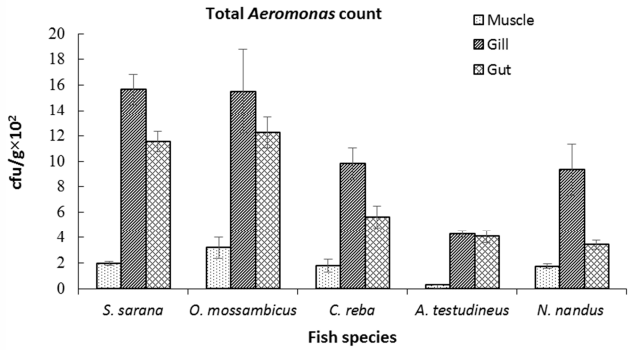

Figure 2: Variation of total *Aeromonas* count (cfu/g; mean± SEM) in different organs of studied freshwater fishes.

Table 3 describes the differences in mean according to various fish markets of Dhaka city. The highest count of TBC was 3.33±0.47×10^5 cfu/g in Palashi and lowest TBC count was 2.35±0.36×10^5 cfu/g in Hatirpool. The highest aeromonas count was 8.10±1.09×10^2 cfu/g in Anando Bazar and the lowest was 5.63±0.90×10^2 cfu/g in Hatirpool Bazar. Figure 3 and 4 show the species-specific variation of TBC and aeromonas load among different markets.

Table 4 shows variation of *Aeromonas* sp. in different aeromonas agar media for some selected fish species. The highest count of total aeromonas was 3.59±1.07×10^3 cfu/g found in *S. sarana* and the lowest count was 2.21±0.26×10^2 cfu/g measured from *N. nandus*. Zaky *et al.* (2011) reported that *Aeromonas* sp. were counted on EndoAgar, and aeromonas selective agar plates which reached the highest counts in water samples collected from Bahr-El-Bakar drain (1.2x10^3 cfu/ml) and in intestine of fishes collected from El-Mataryia area (4.1x10^2 cfu/g).

Table 3: Bacterial load (cfu/g; mean±SEM) on NA and aeromonas agar (Hi-Media) of five selected fish species collected from three different fish market of Dhaka city (N=45, ANOVA, HSD, p>0.05).

Market	*TBC*	*Total Aeromonas Count*
Anando	3.29±0.48×10^5	8.10±1.09×10^2
Palashi	3.33±0.47×10^5	6.33±0.67×10^2
Hatirpool	2.35±0.36×10^5	5.63±0.90×10^2

Means within column with no letters denote no significant differences

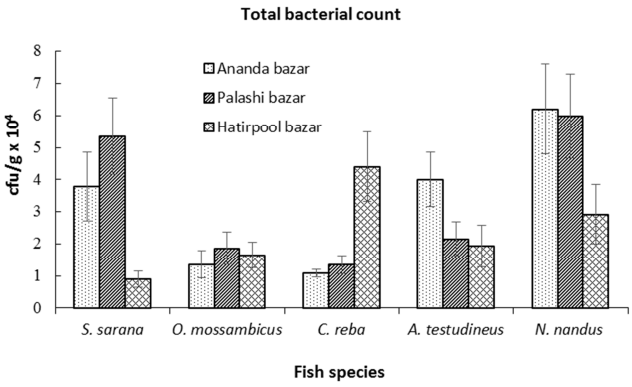

Figure 3: Variation of total bacterial count (cfu/g; mean± SEM) in different fish markets of selected freshwater fishes.

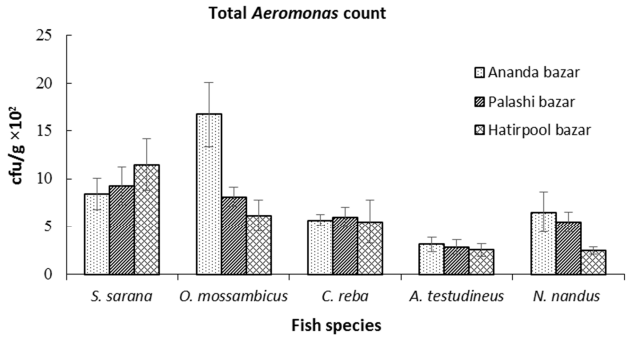

Figure 4: Variation of total *Aeromonas* count (cfu/g; mean± SEM) in different fish markets of selected freshwater fishes.

Table 4: Aeromonas load (cfu/g; mean±SEM) on different agar media of three fish species collected from two different fish markets of Dhaka city (N=18, ANOVA, HSD, p<0.05)

Species	*AH*	*AO*	*AL*
Systomus sarana	1.04±0.16×10^{3a}	2.97±0.65×10^{3a}	3.59±1.07×10^{3a}
Anabas testudineus	2.68±0.49×10^{2b}	7.16±0.77×10^{2b}	2.35±0.24×10^{2a}
Nandus nandus	3.98±0.88×10^{2b}	6.13±1.06×10^{2b}	2.21±0.26×10^{2a}

Means followed by different superscript small letters indicate significant differences

Table 5 shows variation of *Aeromonas sp.* in different aeromonas agar media from muscle, gill and gut of some selected fish species. The highest count of total aeromonas was $2.53\pm0.67\times10^{3}$ cfu/g found from gill and the lowest count was $9.67\pm1.13\times10^{1}$ cfu/g measured from muscle. On the surface and in the intestinal tract of tilapia fish, total plate count of the isolates, *A. caviae* showed a colony forming unit (cfu/ml) of $3.4\text{x}10^{7}$ and $2.2\text{x}10^{7}$, respectively, *A. sobria* had a cfu/ml of $2.8\text{x}10^{7}$ on the surface of *C. batrachus* and *A. hydrophila* had a cfu/ml of $3.8\text{x}10^{7}$ and $4.0\text{x}10^{6}$ in the intestinal tract and on the surface of catfish, respectively (Ashiru *et al.* 2011).

Table 5: Aeromonas load (cfu/g; mean±SEM) on different agar media of three fish organs collected from two different fish markets of Dhaka city (N=18, ANOVA, HSD, p<0.05)

Organs	*AH*	*AO*	*AL*
Muscle	$1.13\pm0.2\times10^{2b}$	$3.15\pm0.17\times10^{2b}$	$9.67\pm1.13\times10^{1b}$
Gill	$9.62\pm1.57\times10^{2a}$	$2.53\pm0.67\times10^{3a}$	$5.36\pm0.79\times10^{2a}$
Gut	$6.27\pm1.02\times10^{2a}$	$1.46\pm0.24\times10^{3ab}$	$1.82\pm0.25\times10^{2b}$

Means followed by different superscript small letters indicate significant differences

Table 6 represents variation of *Aeromonas* sp. in different aeromonas agar media from some selected fish species collected from two fish markets in Dhaka Metropolitan City.

Table 6: Aeromonas load (cfu/g; mean±SEM) on different agar media of fish species collected from two different fish markets of Dhaka city. (N=18, ANOVA, HSD, p>0.05)

Market	*AH*	*AO*	*AL*
Palashi	$5.86\pm0.99\text{x}10^{2}$	$1.58\pm0.48\text{x}10^{3}$	$2.45\pm0.33\text{x}10^{2}$
Hatirpool	$5.49\pm1.23\text{x}10^{2}$	$1.28\pm0.23\text{x}10^{3}$	$2.98\pm0.68\text{x}10^{2}$

Means with no letters denote no significant differences

Biochemical tests: After a preliminary diagnosis of the isolates conducted by observation of colony morphology, some important biochemical tests were performed and their results listed in Table 7. Among the 15 isolates, 11 (68.75%) isolates were fermented all the tested carbohydrates and four couldn't ferment Arabinose, Sucrose and Xylose. For the inositol fermentation, 10 of the isolates with reference *A. hydrophila* (ATCC 7966) couldn't ferment inositol whereas rest 5 isolates could ferment inositol.

All the tested organisms were catalase negative but oxidase and KOH solubility positive. All isolates showed motility in sloppy agar medium. Phenotypically, motile aeromonads are cytochrome oxidase positive, ferment glucose with or without the production of gas (Cipriano 2001) which is comparable to this study.

Table 7: Biochemical characteristics of 15 representative isolated strains from the studied fishes collected from different fish market of Dhaka city with one reference strain (*Aeromonas hydrophila* ATCC 7966)

Sl. No.	*Characteristics*	*Results (%)*	
		Positive	*Negative*
	Fermentation tests-		
1	a. L- Arabinose	15 (93.75)	1 (6.25)
2	b. D- Mannitol	16 (100)	0 (0)
3	c. D- Xylose	13 (81.25)	3 (18.75)
4	d. Inositol	5 (31.25)	11 (68.75)
5	e. Sucrose	15 (93.75)	1 (6.25)
6	f. Glucose	14 (87.5)	2 (12.5)
7	H_2S Production	16 (100)	0 (0)
8	Motility test	16 (100)	0 (0)
9	Citrate	13 (81.25)	3 (18.75)
10	Esculin	14 (87.5)	2 (12.5)
11	Arginine	16 (100)	0 (0)
12	Indole	11 (68.75)	5 (31.25)
13	VP test	10 (62.5)	6 (37.5)
14	Methyl red	8 (50)	8 (50)
15	Gram staining	0 (0)	16 (100)

The results of the biochemical characterization of the isolates were interpreted and found in agreement with those reported by Nieto *et al.* (1984). However, variable results were obtained in voges-proskauer reaction, citrate utilization and arabinose fermentation tests.

Identification of A. hydrophila: Consulting all morphological, biochemical and physiological characters of the isolated organisms, identifications were done with the help of Bergey's manual of systematic bacteriology (Sneath *et al.* 1986). All the selected bacterial isolates belonged to a single species *A. hydrophila* (Table 8).

Antibiotic susceptibility of isolated A. hydrophila: Culture and sensitivity showed all the strain were resistant to amoxycillin whereas all the strain showed sensitivity to amikacin and gentamycin (Table 9). In this study, nine isolates including the reference strain showed sensitivity to tetracycline and for the antibiotic kanamycin no strain showed resistance but two isolates (Tt 1 and Tt 3) showed intermediate resistance. Interestingly, one strain (M 1) showed all similar result for the antibiotic sensitivity test with reference strain (ATCC 7966) but showed opposite result with the antibiotic sulphamethoxazole.

Table 8: Origin (fish species, organs and markets) of the representative fifteen isolates from five freshwater fish species identified as *Aeromonas hydrophila* using biochemical tests

SL No.	Isolate Name	Fish species	Organ	Market
1	Sp1	*S. sarana*	Muscle	Anando Bazar
2	Sp2	*S. sarana*	gill	Palashi Bazar
3	Sp3	*S. sarana*	gut	Hatirpool Bazar
4	Tp 1	*O. mossambicus*	Muscle	Anando Bazar
5	Tp 2	*O. mossambicus*	gill	Palashi Bazar
6	Tp 3	*O. mossambicus*	gut	Hatirpool Bazar
7	Tt 1	*C. reba*	Muscle	Anando Bazar
8	Tt 2	*C. reba*	gill	Palashi Bazar
9	Tt 3	*C. reba*	gut	Hatirpool Bazar
10	K 1	*A. testudineus*	Muscle	Anando Bazar
11	K 2	*A. testudineus*	gill	Palashi Bazar
12	K 3	*A. testudineus*	gut	Hatirpool Bazar
13	M 1	*N. nandus*	Muscle	Anando Bazar
14	M 2	*N. nandus*	gill	Palashi Bazar
15	M 3	*N. nandus*	gut	Hatirpool Bazar

Table 9: Percentage of antibiotic susceptibility for 15 representative isolates with one reference strain (ATCC 7699) against 14 antibiotics.

Name of antibiotics	Isolates		
	R (%)	I (%)	S (%)
Amikacin (30µg) (AK)	0 (0)	0 (0)	16 (100)
Nitrofurantoin (300µg) (F)	6 (37.5)	0 (0)	10 (62.5)
Gentamicin (10µg) (CN)	0 (0)	0 (0)	16 (100)
Erythromycin (15µg) (E)	10 (62.5)	3 (18.75)	3 (18.75)
Tetracycline (30µg) (TE)	6 (37.5)	0 (0)	10 (62.5)
Ampicillin (10 µg) (AMP)	13 (81.25)	0 (0)	3 (18.75)
Polymixin B (300 unit) (PB)	9 (56.25)	6 (37.5)	1 (6.25)
Chloramphenicol (30 µg) (C)	2 (12.5)	1 (6.25)	13 (81.25)
Sulphamethoxazole (25 µg) (RL)	11 (68.75)	1 (6.25)	4 (25)
Streptomycin (10 µg) (S)	4 (25)	3 (18.75)	9 (56.25)
Amoxycillin (10µg) (AML)	15 (93.75)	0 (0)	1 (6.25)
Kanamycin (30µg) (K)	0 (0)	2 (12.5)	14 (87.5)
Ciprofloxacin (5µg) (CIP)	1 (6.25)	0 (0)	15 (93.75)
Nalidixic acid (30µg) (NA)	6 (37.5)	0 (0)	10 (62.5)

R, Resistant; I, Intermediate; S, Sensitive

In some previous studies, *A. hydrophila* was reported to be sensitive to chloramphenicol, erythromycin, kanamycin, neomycin (Boonyaratpalin 1989) and resistant to amoxycillin and clindamycin (Belem-Costa and Cyrino 2006; Adanir and Turutoglu 2007). The results from the present study were similar to them, but different from the results reported by Son *et al.* (1997) and Vivekanandhan *et al.* (2002) who found that *A. hydrophila* was resistant to chloramphenicol, erythromycin, kanamycin and tetracycline. More than 50% of the *A. hydrophila* strains was resistant to tetracycline and occurrence of tetracycline resistant strains of *A. hydrophila* from different sources was reported previously (Ansary *et al.* 1992; Pettibone *et al.* 1996; Son *et al.* 1997). In the present study, one strain (M2) from Anando Bazar was resistant to tetracycline whereas two strains (K 2, M 3) isolated from Palashi Bazar and three strains (Tp 1, Tt 2 and K 3) isolated from Hatirpool Bazar showed resistance to tetracycline (Figure 5). Among the strains tested, most of the strains were resistant to erythromycin (Table 10).

Table 10: Susceptibility of 15 representative isolates of *Aeromonas hydrophila* with one reference strain (ATCC 7966) to tested antibiotics

Isolates /Strains	Antibiotics		
	Sensitive	Intermediate	Resistant
Sp1	AK, AML, AMP, C, CIP, E, GN, K, NA, TE, F	RL	S, PB
Sp2	AK, C, CIP, GN, K, F	S, PB	AML, AMP, E, NA, RL, TE
Sp3	AK, C, CIP, GN, K, NA, S, RL, TE, PB	E	AML, AMP, F
Tp1	AK, C, CIP, GN, K, F		AML, AMP, E, NA, S, RL, TE, PB
Tp2	AK, C, CIP, GN, K, NA, RL, TE	S	AML, AMP, E, F, PB
Tp3	AK, AMP, C, CIP, E, GN, K, NA, S, RL, TE, F	PB	AML
Tt1	AK, C, CIP, GN, S, TE	K	AML, AMP, E, NA, RL, F, PB
Tt2	AK, GN, K, S		AML, AMP, C, CIP, E, NA, RL, TE, F, PB
Tt3	AK, CIP, GN, TE, F	C, K, PB	AML, AMP, E, NA, S, RL
K1	AK, CIP, GN, K, TE	PB	AML, AMP, C, E, NA, S, RL, F
K2	AK, C, CIP, GN, K, S, RL, F		AML, AMP, E, NA, TE, PB
K3	AK, C, CIP, GN, K, NA, S, F		AML, AMP, E, RL, TE, PB
M1	AK, AMP, C, CIP, E, GN, K, NA, TE, F	S	AML, RL, PB
M2	AK, C, CIP, GN, K, S, F		AML, AMP, E, NA, RL, TE, PB
M3	AK, C, CIP, GN, K, S, TE	E, PB	AML, AMP, NA, RL, F
Refere-nce	AK, C, CIP, GN, K, NA, S, TE, F	E, PB	AML, AMP, RL

This is partially supported with previous study by Ansary *et al.* (1992) and Son *et al.* (1997). However, Pettibone *et al.* (1996) have not reported any erythromycin resistant *A. hydrophila* strains. The variation in the drug resistance may be related to the source of the *A. hydrophila* isolates and the frequency and type of antimicrobial agents prescribed for treating aeromonas infections, e.g. in cultured fish of different geographical areas (Son *et al.* 1997). The chloramphenicol resistant strains were few. Except the strain isolated from Tatkini gill none of the strains isolated from the selected fishes was chloramphenicol resistant. Similar findings have been recorded from Malaysian and American aeromonas isolates from fish (Ansary *et al.* 1992; Pettibone *et al.* 1996). Resistance towards chloramphenicol, erythormycin, kanamycin, nalidixic acid, streptomycin, sulphamethoxazole and tetracycline has been observed among *A. hydrophila* isolates from *Tilapia mossambica* (Son *et al.* 1997). In this study, all the strains isolated from tilapia showed sensitivity to Chloramphenicol and kanamycin whereas resistant to erythromycin, nalidixic acid, streptomycin, sulphamethoxazole and tetracycline was seen. So it partially supports the study reported by Son *et al.* (1997).

Results of this study showed that the strains in all samples were exposed to antibacterial drugs and developed resistance. This means that antibiotics are used inappropriately and a further development of the resistance in microorganisms may be expected. In the antibacterial susceptibility test, *A. hydrophila* strains were found to be resistant to most used drugs. So the number of effective antibacterial drugs is diminishing. Since this is a microorganism that may threaten human health in course of time, transmission of the reduced susceptibility may have negative consequences for humans.

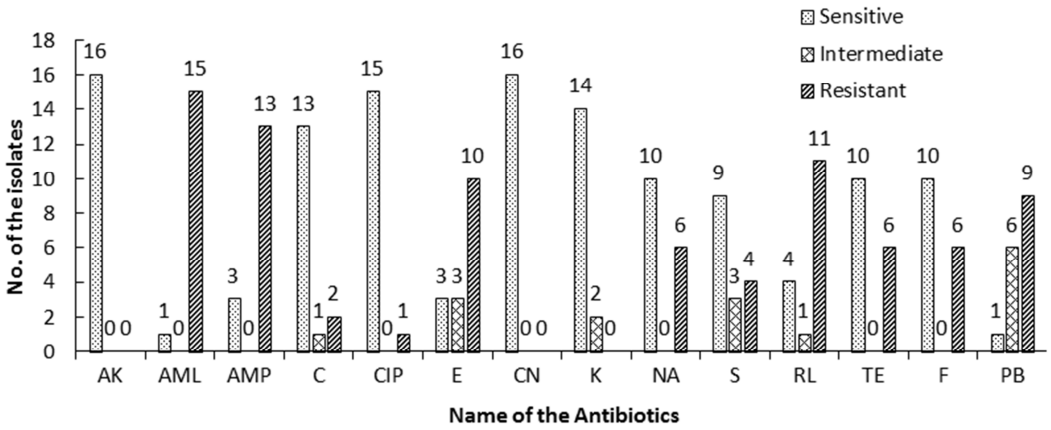

Figure 5: Susceptibility pattern of 15 isolates with a reference strain (*Aeromonas hydrophila* ATCC 7966) in tested antibiotics (AK, Amikacin; AML, Amoxycillin; AMP, Ampicillin; C, Chloramphenicol; CIP, Ciprofloxacin; E, Erythromycin; CN, Gentamicin; K, Kanamycin; NA, Nalidixic acid; S, Streptomycin; RL, Sulphamethoxazole; TE, Tetracycline; F, Nitrofurantoin; PB, Polymixin B; No., Number)

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