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Antimicrobial resistance of *Aeromonas spp.* isolated from the carp farm following Streptomycin treatment

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Abstract
Seven fresh bacterial isolates were isolated from disease affected fish species such as *Labeo rohita*, Cyprinus carpio, *H. molitrix*, and *Puntius sarana* from different carp farms and market sources. Bacterial isolates were collected from different tissues or organ such as skin, gill, spot lesions on the skin, abdomen, kidney etc. Several tests were conducted for characterization of bacteria. Susceptibility of Aeromonas spp. bacteria to Streptomycin (10 µg/200 mL) antibiotic were 150 colony and in control 320 colony in R-1. Where in R-2, 142 colony and in control 296 colony and finally in R-3, 145 colony and in control 311 colony were found in T-4. Where resistance of *Aeromonas* spp. bacteria to Streptomycin (30 µg/200 mL) antibiotic were 1 colony and in control 318 colony in R-1. Where in R-2, 0 colony and in control 309 colony and finally in R-3 also 0 colony and in control 314 colony were found in T-4. All the treatments were performed through the serial dilution method at 310×10^{-4} bacterial dilution factor.
Keywords: Antimicrobial resistance, *Aeromonas*, carp, Streptomycin and treatment

1. Introduction
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) among *Aeromonas hydrophila* strains has been reported from many parts of the world (Pettibone *et al*., 1996; Son *et al*., 1997; Ko*et al*., 1998; RajeswariShome and Shome, 1999). 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. Antimicrobial resistances of bacterial pathogens are a major problem for the treatment of fishes with bacterial diseases. Determination of minimum inhibitory concentration (MIC) of antibiotics for bacteria plays a crucial role for the determination of antibiotic resistance of bacteria (Islam, M. A. *et al*. 2008). The present research work was undertaken for determining resistance of *Aeromonas spp.* against most commonly used antibiotics Streptomycin. For the last few years a series of antimicrobial resistance of *Aeromonas spp.* following various types of antibiotic treatment have been carrying out. Magali Naviner *et al*. 2011, Orozova, P. *et al*. 2008 and 2010, Vivekanandhan, G. *et al*. 2002, Islam, M. A. *et al*. 2008, Rahman M. M. and M. N. Hossain 2010 detected antimicrobial resistance of *Aeromonas spp.* following various types of antibiotic treatment named Ampicillin, Amoxicillin, Tetracycline, Oxytetracycline, Renamycin, Erythromycin, Ciprofloxacin, etc. on specific kind of fish, but they could not launch experimental on the growth pond in a carp farm by Streptomycin antibiotic. So, it becomes very important to know the fact.
Considering the above facts, the present study was designed to attain the following major objectives:

1. To determine the antimicrobial resistance of *Aeromonas spp.* bacteria against Streptomycin antibiotic.

2. To determine susceptibility of *Aeromonas spp.* bacteria to Streptomycin antibiotic.

2. Materials and methods

2.1. Collection of bacterial isolates

A total of 7 fresh bacterial isolates (Table 1.) were isolated from disease affected fish species Fig. 1 of different farms and market sources.

Fresh isolates	Host species	Tissue/organs	Locations/area
N-1	*Labeo rohita*	Skin	Fish market (Borobazar)
N-2	*Cyprinus carpio*	Gill	Fish market (Bablatola)
N-3	*Cyprinus carpio*	spot lesions on the skin	Fish farm
N-4	*H. molitrix*	Lesion	Fish farm
N-5	*Puntius sarana*	Abdomen	Fish farm
N-6	*Puntius sarana*	Lesion	Fish farm
N-7	*P. ticto*	Kidney	Fish farm

N: Number

Fig 1: The appearance of lesions on the skins of the sarputi, (*Puntius sarana*) from which *Aeromonas spp.* was isolated.

2.2. Characterization of *Aeromonas* isolates

At first lesion from skin, gill and scale were collected by inoculating loop from live fish and were aseptically streaked onto previously prepared BHI with nutrient agar media inside the laminar flow. Then the live fish were killed with a sharp scalpel by cutting below the head. After then bacterial samples were collected from the abdomen and kidney by sterilized inoculating loop by the same way, and incubated at 37 ^{0}C for 24 hours.

2.2.1. Primary characterization

Before characterization pure stocks were sub-cultured onto BHI with nutrient agar plates to obtain a fresh 24 hour culture. Morphological characteristics bacterial colonies (shape, size, and color) were recorded. The shape of individual bacterium was determined by Gram's staining method using a fresh 24-hour old culture. Motility test was performed by preparing a dilute suspension of fresh bacterial culture on a clean glass slide with a coverslip and observing on a monitor attached to a binocular microscope.

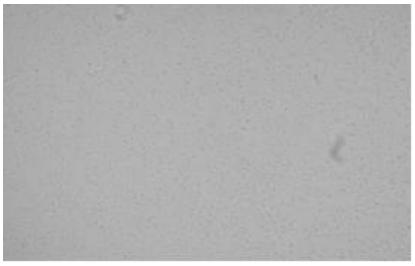

Fig 2: Motility test of bacteria was performed by Hanging drop examination under 40X magnification binocular microscope.

Color and shape: Color and shape was observed under photography microscope (AxioCamERc 5s with Axio Vision driver Carl Zeiss Germany).

Gram's staining: Gram's staining method according to Hans Christian Gram(1884) result show bacteria get decolorized and take the light pink color after counterstaining with safranin, natural red or dilute carbolfuchsin are called Gram negative bacteria.

Table 2: Primary characterization of bacterial isolates.

Test	Result
Colony Color	Yellowish
Shape	Small rod
Motility	Motile
Gram's staining	-

– Negatively responded

2.3. Bacteria Culture and Antibiotic Susceptibility and Resistant Test

For 200 ml distill water, 2.44 g BHI and 1 g nutrient agar are taken in a flask and homogeneously mixed by magnetic stirrer. Then media was autoclaved at 121 ^{0}C. After completing autoclave media were allowed to cool inside the laminar flow until reaches 50 ^{0}C. After then streptomycin antibiotic at different doses (3 µg-10 µg) in case of susceptibility test and (15 µg-30 µg) in case of resistance test as in (Table 3.) are given inside the flask and mixed thoroughly by a magnetic stirrer about 500 rpm. And finally antibiotic-containing media was poured immediately on the petridishes carefully.

2.3.1. Inoculum preparation

Inocula were obtained from an overnight agar culture of the test organism. Inoculum was prepared by taking a single well-isolated colony of the same morphology from an agar plate culture. The top of each colony was touched with a sterile loop and the growth was transferred into mother test-tube containing 10 ml of distill water. Then perform serial dilution.

2.3.2. Spreading and incubation

And then 1000 µl/1 ml bacterial dilution from each test-tube were taken by micropipette and given to an antibiotic containing petri dish and at the same time control petri dish at the same concentration. After then spread the bacterial solution onto the agar surface of the petridishes by well sterile spreader thoroughly. After completing all the petridishes are kept in an incubator at 37 ^{0}C for 24 hours.

3. Results and Discussion

3.1. Antimicrobial susceptibility test

Antimicrobial susceptibility represents the doses of antibiotic at which start inhibition of growth are referred to as Susceptible (S). And the doses of antibiotic at which there is complete inhibition of growth are referred to as Resistant (R) and in relation to the doses of antibiotic were tested as per (Table-03).

Table 3: Antimicrobial susceptibility test at different doses of streptomycin antibiotic.

Name of antibiotic	value (µg/200 ml)	
	Susceptible (S)	Resistant (R)
Streptomycin(T-1)	3	15
Streptomycin(T-2)	5	20
Streptomycin(T-3)	8	25
Streptomycin(T-4)	≤10*	≥30**

≤ Less or equal; ≥ Greater or equal

* indicates less than 10 µg or at 10 µg *Aeromonas spp.* bacteria were sensitive and start inhibition of growth.
** indicates that greater than 30 µg or at 30 µg *Aeromonas spp.* bacteria were complete inhibition of growth.

3.2. Discussion

Continuous practice of streptomycin antibiotic at the dose of 30 µg for longer period may improve resistance power in fish body. So, treatment of streptomycin antibiotic at the dose of 30 µg will not work properly. In that case the dose of streptomycin antibiotic should increase slightly.

Orozova, P. *et al*. (2010) checked antibiotic susceptibility by means of disk-diffusion method (Bauer *et al*., 1966) according to the recommendations of CLSI (Clinical Laboratory Standards Institute). The disks were positioned upon Muller-Hinton agar, inoculated in advance with 100 µL of bacterial suspensions of the isolated strains cultivated in meat-peptone broth for 24 h at 28 ^{0}C (Costa *et al*., 1998). After additional incubation for 24 h at 28 ^{0}C, the inhibition zone has been measured and showed results that *Aeromonas spp.* were susceptible to 10 µg and resistant to 30 µg streptomycin. The result of the present study was related with the results of other scientists.

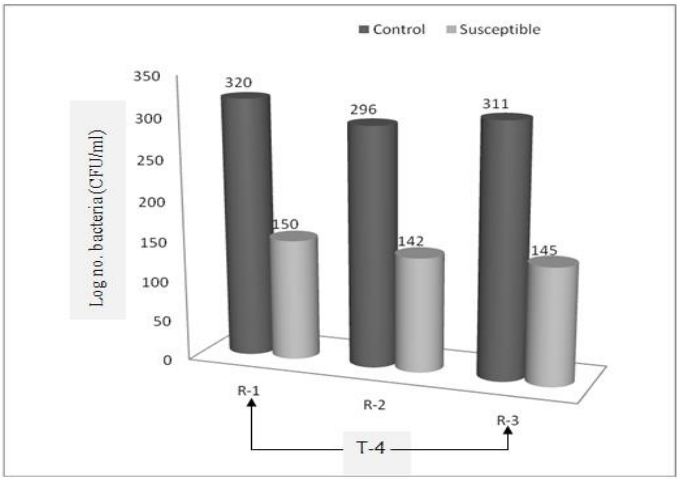

Fig 3: Determination of susceptibility of *Aeromonas spp.* bacteria to Streptomycin (10 µg/200 mL) antibiotic

All the treatments were performed through the serial dilution method at 310×10^{-4} bacterial dilution factor. Susceptibility of *Aeromonas spp.* bacteria to Streptomycin (10 µg/200 mL) antibiotic were 150 colony and in control 320 colony in R-1. Where in R-2, 142 colony and in control 296 colony and finally in R-3, 145 colony and in control 311 colony were found in T-4.

All the treatments were performed through the serial dilution method at 313×10^{-4} bacterial dilution factor. Resistance of *Aeromonas spp.* bacteria to Streptomycin (30 µg/200mL) antibiotic were 1 colony and in control 318 colony in R-1. Where in R-2, 0 colony and in control 309 colony and finally in R-3 also 0 colony and in control 314 colony were found in T-4.

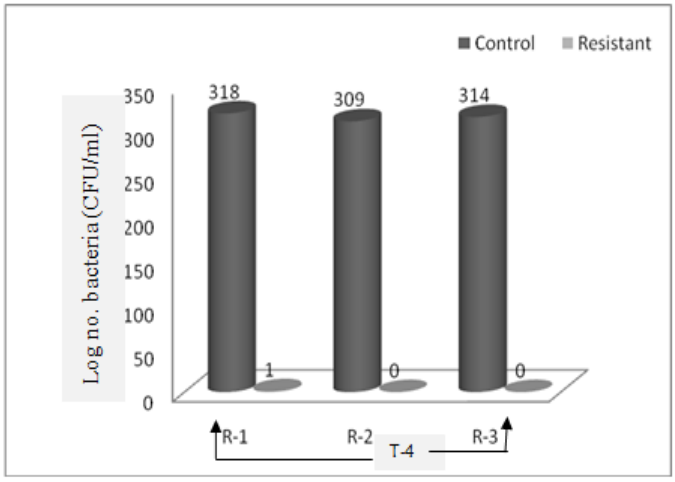

Fig 4: Determination of resistance of *Aeromonas spp.* bacteria to Streptomycin (30 µg/200 mL) antibiotic.

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