JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2013. Vol. **7**(2), p. 1165-1169

Isolation and Antibiogram Profile of *Aeromonas* species from Common Carp (Fish) of Kashmir Valley

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(Received: 30 September 2012; accepted: 12 November 2012)

The present study was undertaken for the isolation, identification and antibiogram of *Aeromonas* spp. isolated from Common Carp of Lakes and River of Kashmir valley during May 2009-April 2010. A total of 144 fish samples were examined. The organisms were identified by cultural, morphological and biochemical tests for *Aeromonas* spp. The overall percentage prevalence of *Aeromonas* spp. in common carp was 6.25%. Wular lake recorded highest presence of *Aeromonas* spp. (3.47%) followed by Dal lake (2.08%). Maximum recovery was recorded in intestines (n=8 out of 9 isolates), followed by fins (n=1). Season wise prevalence showed increased reports in autumn (4.86%). All the isolates showed sensitivity to Oxytetracycline (77.78%) and resistant to Amoxicillin (88.89%).

Key words: Common Carp, *Aeromonas* spp., Antibiotic sensitivity tests and Kashmir.

Fish form an important part of a nutritious and healthy diet. Minor components of fish muscle include minerals, vitamins, sugars, and low molecular weight non-protein nitrogenous compounds. India has 2.25 million hectors of land under ponds and tanks, 1.30 million hectors under lakes, 2.09 million hectors as reservoirs and 1.23 million hectors of brackish water areas. The state of Jammu and Kashmir is bestowed by various water reservoirs, which mainly includes lakes, rivers, streams and ponds. Among these Wular (Asia's largest lake) and Dal Lakes (world famous) have major contribution in the fish production of our state with an average annual fish yield of 16.5kg ha-^{1} and 21 kg ha-^{1} for Wular and Dal Lake, respectively[1]. The two major fish species found in these water reservoirs include *Cyprinus carpio* (common carp), an exotic fish species introduced in Kashmir in 1959 and *Schizothorax niger*, which is native to this region[2]. Fish has also been implicated in transmission of various pathogens to man e.g. trematodes, nematodes, bacteria and viruses. The flesh of live and healthy fish is normally sterile, but microorganisms start growing after death and when only lightly preserved. Bacteria are mostly present on the outer surfaces

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(skin, gills) and in the intestinal tract, the total counts of which varies with the temperature and environmental factors. There are two broad groups of bacteria of public health significance that can contaminate products of aquaculture. The first group includes those that are indigenous to fish and its environment e.g. *Aeromonas hydrophila*, *Clostridium botulinum*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus*, *Listeria monocytogenes.* The second group includes those introduced through contamination by human/ animal excreta wastes[3] i.e. members of family *Enterobacteriaceae* such as *Salmonella* spp., *Shigella* spp., *Escherichia coli* etc. Most of these bacterial species are pathogenic for both fish and man. Owing to the importance of fish as an excellent and safe source of animal protein, the present study was undertaken for the isolation, identification and antibiogram of *Aeromonas* spp. isolated from Common Carp of Kashmir Valley.

MATERIALS AND METHODS

The samples of Common Carp were collected from various input sources of Kashmir (Jammu & Kashmir, India) viz- Dal, Aanchar, Nigeen Lake and River Jhelum in Srinagar, Manasbal Lake in Ganderbal and Wular Lake in Bandipora districts of Valley. A total of 144 samples were collected from these input sources as depicted in Table 1.

A total of 144 fresh raw fish samples were collected in UV sterilized polyethylene zip lock sachets. They were transported to the laboratory on ice packs and processed immediately for the isolation of various bacteria. From each fish a representative samples from gills, fins and intestines were taken. The samples (gills and fins) were collected aseptically with the help of sterile scissors and paired forceps. However, the intestinal samples were taken by cutting the part of intestines using sterile scalpel and removing the contents.

For isolation of *Aeromonas* spp. the samples viz. gills, fins and intestines were enriched in alkaline peptone water containing Novobiocin and incubated at 37°C for 24 h. A loopful of broth culture was streaked on Rimler-Shotts agar media and incubated at 37°C for 24 hr. All the isolates were collected on nutrient agar slants as pure culture and stored at refrigeration temperature for further characterization.

Identification of the microorganisms was carried by Gram's staining, colony characteristics, and biochemical profile as per the standard methods[4]. The presumptive isolates were assigned to genus *Aeromonas* on the basis of colony characteristics (yellowish colonies surrounded by haloes), oxidase test, catalase test, motility and production of acid from arabinose and sucrose and gas from glucose[5].

There is a global concern over the use and abuse of antimicrobials in human medicine, animal husbandry, aquaculture practice and parallel rise in emergence of drug resistant pathogens with increasing frequency[6]. It was therefore of immense importance to determine the antibiotic sensitivity of the *Aeromonas* isolates during the present study.

RESULTS AND DISCUSSION

Out of total 144 samples from each of gills, fins and intestines, 9 were positive for *Aeromonas* spp. (6.25%). The isolates [Fig. 1-5] were confirmed on the basis of colonial, morphological, cultural and biochemical characteristics as per standard methodology[4].

In Dal Lake, bacterial isolates recovered from fish showed per cent prevalence for *Aeromonas* spp. as 2.08. Isolates recovered from fish samples of Wular Lake showed *Aeromonas* spp. as (3.47%), Manasbal Lake (0.69%). From Aanchar and Nigeen lakes as well as in river Jhelum no reports of the species were registered. Our findings are in total agreement with various workers[7-10]. However, our findings are in contrast with some scientists[11] who reported 40% fish samples positive for *Aeromonas* spp.

Heavy recovery of *Aeromonas* spp. recorded in the fish samples of Wular Lake as compared to others could due to an increase in the illegal human settlements around the lake during last two decades leading to accumulation of human and animal wastes which finally found their way directly into the lake. Fish from Dal also presented a higher percentage of contamination in comparison to Manasbal lakes. In Dal Lake the higher percentage (2.08%) of contamination could be due to deterioration in the quality of water as a result of untreated sewage and solid wastes dropped directly into the lake by peripheral human

settlements. The lake have invariably heavy tourist rush than Manasbal lake which is of less tourist attraction. Encroachments of water channels and consequent clogging have also led to diminished circulation and inflows into the lakes, causing an increase in the concentration of phosphates and nitrogen which in turn resulted in extensive microbial and weed growth. These findings correlate with earlier workers[12]. Fish from the Aanchar and Nigeen lakes and river Jhelum showed no reports of *Aeromonas* spp., which could be due to free flowing nature of the river and also due to stringent steps taken by the state Government in making the banks of the river more eco-friendly and by minimizing the flow of wastes from human settlements directly into the river.

Out of 9 *Aeromonas* isolates, intestines (n=8) recorded highest extend of contimation followed by fins (n=1). None of the isolate was isolated from gills. This is in accordance with various scientists[13-15] who also recovered highest percent of *Aeromonas* spp. from intestines of fish than gills and fins. However, our findings are in contrast with some workers[16-17] who reported isolation of *Aeromonas* spp. from the liver and kidney of marine fish.

The highest prevalence (4.86%) of *Aeromonas* spp. was recorded in autumn season followed by summer (0.69%) and winter (0.69%), while no reports were found in spring season. Higher percentage in the autumn season could be due to environmental factors like higher ambient temperature supporting the growth and multiplication of microbes, low precipitation, dry and windy weather conditions increasing the inflow of microbes into the lakes. The season receives the lowest rainfall which causes reduction in the water levels of the lakes leading to higher concentration of bacterial species in a given volume. Absence of the isolates in spring season could also heavy rainfall witnessed during this season, thereby reducing the number of microbes

Table 1. Season wise sample collection of Common Carp for microbial isolation

S. No.	Input source	Summer (May-Aug)	Autumn (Sept-Nov)	Winter (Dec-Feb)	Spring (Mar-Apr)	Total No. of samples to be collected
1.	Dal Lake	6	6	6	6	24
2.	Wular Lake	6	6	6	6	24
3.	Manasbal Lake	6	6	6	6	24
4.	Aanchar Lake	6	6	6	6	24
5.	Nigeen Lake	6	6	6	6	24
6.	Jhelum River	6	6	6	6	24
	Total	36	36	36	36	144

Table 2. Antibiotic Sensitivity for *Aeromonas* spp. (9)

S. No.	Drugs	Highly Sensitive	Moderately Sensitive	Resistant
1.	Norfloxacin (10 mcg)	5 (55.56)	2 (22.22)	2 (22.22)
2.	Ciprofloxacin (30 mcg)	4 (44.44)	5 (55.56)	0
3.	Erythromycin (15 mcg)	4 (44.44)	2 (22.22)	3 (33.33)
4.	Gentamicin (10 mcg)	6 (66.67)	2 (22.22)	1 (11.11)
5.	Amikacin (10 mcg)	6 (66.67)	2 (22.22)	1 (11.11)
6.	Amoxycillin/Clavulonic acid (30 mcg)	0	1 (11.11)	8 (88.89)
7.	Oxytetracycline (30 mcg)	7 (77.78)	0	2 (22.22)
8.	Chloramphenicol (30mcg)	6 (66.67)	0	3 (33.33)
9.	Sulphamethoxazole-trimethoprim (30mcg)	6 (66.67)	1 (11.11)	2 (22.22)

The figures in parenthesis indicate the percentage

in a given volume in the lakes which further reduced the chances of contamination of fish. Human infections due to consumption of contaminated fish have been related to seasonal variations. It had been reported that human infections caused by organisms transmitted from fish or the aquatic environment were quite common depending on the season, contact with the fish and related environment, dietary habits and the immune status of the exposed individual[18].

Out of 9 *Aeromonas* [Fig. 6 & Table 2] isolates highest sensitivity was shown against Oxytetracycline (77.77%) followed by Gentamicin, Amikacin, Chloramphenicol and Sulphonamides (66.66% each) and Erythromycin and Ciprofloxacin (44.44% each). The isolates recorded moderatively sensitivity against Ciprofloxacin (55.55%) followed by Norfloxacin, Erythromycin, Gentamicin and Amikacin (22.22% each).

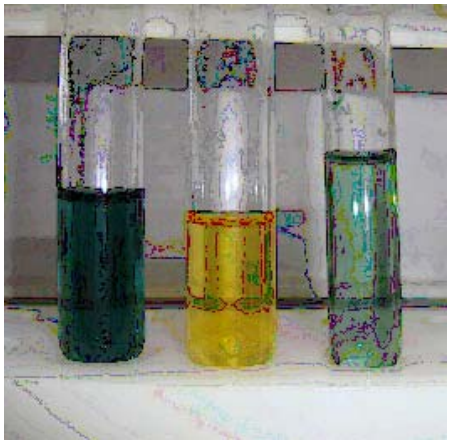

Fig. 3. Acid from Arabinose test for *Aeromonas* spp. recovered from fish

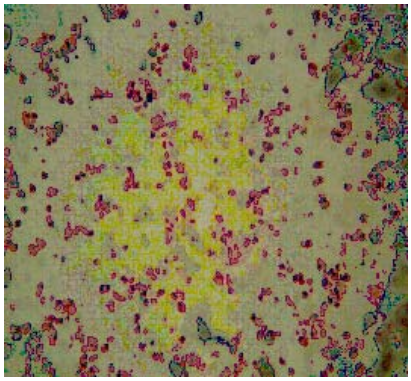

Fig. 1. Microscopic view of Gram Negative cocco-bacilli *Aeromonas* spp isolated from fish (100X)

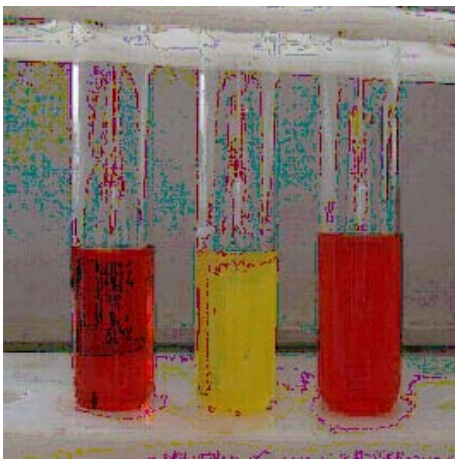

Fig. 4. Acid from Sucrose test for *Aeromonas* spp. recovered from fish

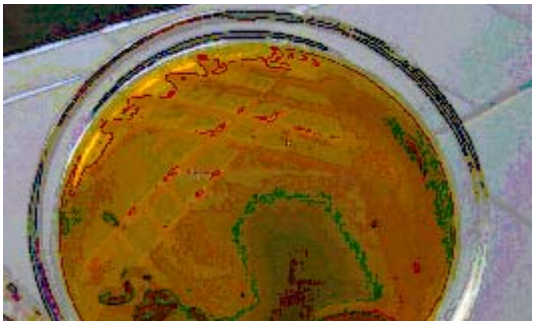

Fig. 2.Yellow colonies of *Aeromonas* spp. on Rimler-Shotts Agar plate

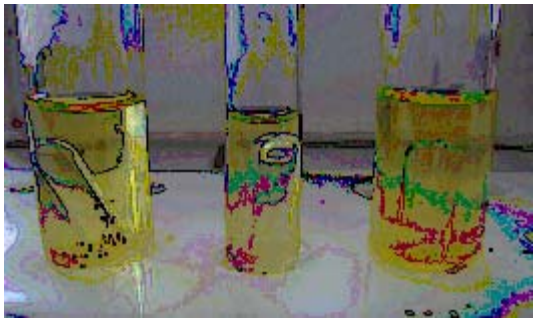

Fig. 5. Gas from Glucose test for *Aeromonas* spp. recovered from fish

Highest resistance by *Aeromonas* isolates was recorded against Amoxycillin (88.88%), Erythromycin and Chloramphenicol (33.33% each) followed by Norfloxacin, Oxytetracycline and Sulphonamides (22.22% each). These findings are well supported[19].

CONCLUSION

The results revealed that overall contamination of *Aeromonas* spp. as 6.25% in the fish samples of Kashmir valley. Wular lake recorded highest prevalence followed by Dal lake. Maximum isolates were recovered from intestines. Among seasons, autumn recorded highest number of the species. The *Aeromonas* spp. was highly sensitive and resistant to Oxtetracycline and Amoxicillin respectively.

ACKNOWLEDGEMENTS

Author is thankful to Dean, Faculty of Veterinary Sciences and Animal Husbandry, Shuhama, Kashmir for providing the necessary facility to carry out the research. Thanks are due to my Major Advisor whose immense help saved my time and energy. I am highly thankful to Head, Department of Veterinary Public Health and Epidemiology, Apollo College of Veterinary Medicine.

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