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

**Suhani Bashir Bhat**, **Aarif Muhammad Khan**, **Showkeen Muzamil Bashir** and **S.S. Roy**

1Veterinary Public Health and Epidemiology, Apollo College of Veterinary Medicine, Agra Road Jaipur, India.
2Apollo College of Veterinary Medicine, Agra Road Jaipur, India.
3Veterinary Public Health, Shere-kashmir University of Agricultural Sciences & Technology of Kashmir, India.

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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.
(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 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. 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.

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. 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.

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. However, our findings are in contrast with some scientists 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. 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 contamination followed by fins (n=1). None of the isolate was isolated from gills. This is in accordance with various scientists 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 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 be due to heavy rainfall witnessed during this season, thereby reducing the number of microbes

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

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

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

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

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.

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 moderately sensitivity against Ciprofloxacin (55.55%) followed by Norfloxacin, Erythromycin, Gentamicin and Amikacin (22.22% each).
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.

**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.

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**REFERENCES**