



Prevalence of *Escherichia coli* Serotypes in Water and the fish *Schizothorax niger* in Dal Lake

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 25-6-2014

Accepted: 23-8-2014

Key Words:

Dal lake

Escherichia coli

Serotypes

Schizothorax niger

ABSTRACT

Escherichia coli serotypes in water and fish (*Schizothorax niger*) samples of Dal Lake in autumn (2010), winter (2010-11) and spring (2011) seasons were analysed. A total of 108 isolates from water and fish were recovered. The predominant serotype in water was O8 (16.66%), followed by O25 (14.81%) and O140 (10.37%) and of fish, serotype O68 (18.87%), followed by O84 (13.20%). Serotypes O78, O86, O153, O84, O68 and O25, known for their association with human and animal diseases, were isolated from both fish and water samples.

INTRODUCTION

Anthropogenic and other environmental pressures on water resources is a global concern. Continuous flow of microorganisms in exceeding numbers in sewage and urban run-off not only pose a risk to human life (Fleisher et al. 1998) but also threaten the biotic ecosystem of the water bodies. Protection of water bodies is therefore, essential for healthy fish production besides limiting the risk of human diseases.

Dal Lake is well adored worldwide due to its proximity with the capital city of Srinagar and also because of its emergence as one of the most beautiful spots in the world. This urban lake is the second largest freshwater lake in the state of Jammu & Kashmir and covers an area of 11.4 sq.km. With increased human settlements around, the physico-chemical and microbial quality of Dal Lake has deteriorated in the recent past due to continuous flow of human and animal wastes from its surroundings. These anthropogenic pressures have dented the pristine glory of the lake resulting in tremendous ecological changes. Various pollutants have increased the organic load of the Dal water, making it a medium for the prolific growth of aquatic vegetation. The aquatic life of the Dal has also changed significantly in past two decades with several species of flora and fauna going under the threat of extinction. The shift in vegetation has further tilted the ecology in favour of the survival of some hardy aquatic species like common carp (*Cyprinus carpio*), thereby affecting the diversity and balance of species richness in the lake leading to the decline in the population of the native fish, the *Schizothoracids*.

Schizothorax niger, is one of the most valued and preferred fish species of Kashmir and is mostly harvested from the Dal Lake, which is one of the major sources of drinking water supply for Srinagar city. Recently, an alarming increase in hospitalized cases of gastroenteritis was reported in those consuming the water directly from the lake. Furthermore, gastrointestinal disorders due to consumption of drinking water from the community water supply in Srinagar are also on record (Mirani 2009).

Serotyping of *Escherichia coli* has been used to identify the incriminated strains and also locate the source of contamination of water. Over 700 serotypes have been identified so far depicting its adaptability to the changing environmental conditions. Most of the serotypes are considered nonpathogenic, but some pathogenic strains cause a number of serious illnesses in man and animals. The present work was undertaken with an objective to identify the serotypes of *Escherichia coli* in lake water and *Schizothorax niger* in different seasons and to trace their possible transmission to humans.

MATERIALS AND METHODS

Sampling: For collection of water samples from the Dal Lake, three sampling sites were selected; the input site (Telbal Nalah, opening into the Dal), the main body (Hazratbal area) and the outlet site (Dalgate area). In all, 54 water and 45 fish samples were collected in an extended period of three seasons, i.e., autumn, winter and spring from August, 2010 to April, 2011, following the standard proto-

col of APHA (1992). Fish were caught with fish nets and brought to the laboratory in sterile polythene bags. All the samples were processed for isolation and identification of *Escherichia coli* within 1-2 hr of collection.

Isolation and Identification of *Escherichia coli*: One mL from each water sample was inoculated in a test tube containing 10 mL of MacConkey's broth. Pieces of fins, gills and the abdominal muscles (after evisceration) were triturated separately in sterile pestle and mortars and incubated each at 37°C for 24 hr in 10 mL sterile MacConkey's broth. Sub-culturing was done on MacConkey's agar plates for cultural identification of the isolates. Lactose fermenting, small, nucleated pink colonies suspected as *Escherichia coli*, were further subjected to IMViC and other biochemical tests. The isolates, identified as *Escherichia coli* were transferred to Eosin Methylene Blue agar (EMB) plates for demonstration of characteristic metallic sheen following incubation at 37°C for 24 hr. Smears from the colonies were stained by Gram's method and observed under oil immersion for purity. Isolates conforming to the characteristics of *Escherichia coli* on the basis of cultural, morphological and biochemical reactions were transferred to nutrient agar slants in duplicate for further studies. One set of the isolates was sent to National *Salmonella* and *Escherichia coli* Center, Kasauli, H.P. for sero-typing.

RESULTS AND DISCUSSION

A total of 214 isolates of *Escherichia coli* were recovered during the course of the study. The distribution of the isolates was almost similar with 108 (50.47%) belonging to water and 106 (49.53%) to the fish. Using somatic O antigen, only 97 isolates from water were typeable in 15 serotypes and of 86 from fish, in 11 serotypes. The results are presented in Table 1. While most of the serotypes demonstrated seasonal variability, serotypes O8, O25, O140, O120, O78 and O157 were isolated from water in all the three seasons depicting their adaptability to climatic conditions of Srinagar as the ambient atmospheric temperature falls below -4°C in the winter season. All these serotypes have a strong history of involvement in human and animal diseases (Nishikawa et al. 2002, Yang et al. 2004, Cookson et al. 2006) and their recovery from Dal water indicates contamination with human and animal wastes.

In the present study, serotype O84 was recovered from fish in all the three seasons and accounted for 13.20% of the total fish isolates. The serotype also contaminated Dal-water in winter and spring seasons to an extent of 6.45% and 3.12%, respectively. There are no reports on its transmission to humans through fish, but its involvement in poultry diseases (Yang et al. 2004) and cases of bovine and hu-

man diarrhoea (Cookson et al. 2006) is well documented.

The repeatability of the serotypes in water samples was highest with serotype O8 accounting for 16.66% of the isolates followed by serotypes O25 (14.81%) and O140 (10.37%). In spite of their higher prevalence in water, serotypes O8 and O140 could not be isolated from any of the fish samples which explain their failure to adapt to the new host species as no previous reports of their association with fish diseases are available. Interestingly, serotype O25 accounted for 5.66% of the isolates from fish suggesting the role of contaminated water in transmission of this serotype to fish.

Serotypes O84 was present in 13.20% of the fish isolates but the predominant serotype was the serotype O68 which accounted for 18.87% of the isolates. Both the serotypes were also isolated from water accounting for 2.78% and 3.7% of the isolates, respectively. Serotypes O78, O86, O153, O84, O68 and O25 were isolated from both fish and water samples of the Dal Lake and are known for their association with the human and animal diseases (Rahman et al. 1997, Nishikawa et al. 2002, Yang et al. 2004, Cookson et al. 2006).

Parts of the fish also varied in the recovery of *Escherichia coli* isolates and the highest percentage was obtained from gills (Table 2) which accounted for 49.05% of the isolates followed by fins (35.84%) and the muscles (15.09%). Serotypes O68 (42.10%), O84 (19.23%) and O25 (25.00%) were the major serotypes, respectively, in fins, gills and muscles. Their higher number in gills may be possibly due to the anatomical disposition of the gills owing to utilization of dissolved oxygen and high vascularity thus supporting the concentration, growth and multiplication of *Escherichia coli*. The adaptability of serotype O84 to *Schizothorax niger* can be argued by the fact that this serotype was isolated from all the parts of fish and in two of the three seasons from water questioning its role in the higher incidence of human cases of gastroenteritis.

While most of the identified serotypes of *Escherichia coli* are known for their role in human and animal diseases, recovery of serotype O157 from Dal water is of a serious concern. The organism is responsible for a number of deadly human diseases like haemorrhagic enteritis, haemolytic uremic syndrome, ulcerative colitis etc. (McCarthy et al. 2001) and has also been detected in drinking water sources of Srinagar (Willayat et al. 2005). However, in the present study, serotype O157 could not be isolated from any of the samples of *Schizothorax niger* but its detection in the exotic common carp (*Cyprinus carpio*) of the Dal Lake is well documented (Suhani 2011).

Table 1: Distribution of *Escherichia coli* serotypes in water and *Schizothorax niger* of Dal Lake in different seasons.

Sr. No.	Serotype	Autumn				Winter				Spring			
		Water, N = 45		Fish, N = 27		Water, N = 31		Fish N = 22		Water N = 32		Fish N = 57	
		No. Isolated	%	No. Isolated	%	No. Isolated	%	No. Isolated	%	No. Isolated	%	No. Isolated	%
1.	O8	10	22.22	-	-	5	16.13	-	-	3	9.37	-	-
2.	O25	09	20.00	-	-	4	12.90	3	13.64	3	9.37	3	5.26
3.	O120	03	6.67	-	-	2	6.45	-	-	3	9.37	-	-
4.	O86	02	4.44	1	3.70	-	-	-	-	-	-	4	7.02
5.	O88	04	8.88	-	-	-	-	-	-	-	-	-	-
6.	O78	02	4.44	6	22.24	3	9.67	-	-	2	6.25	-	-
7.	O20	01	2.22	-	-	1	3.22	-	-	-	-	-	-
8.	O153	01	2.22	-	-	3	9.67	-	-	-	-	6	10.52
9.	O109	02	4.44	-	-	-	-	-	-	2	6.25	-	-
10.	O157	02	4.44	-	-	2	6.45	-	-	2	6.25	-	-
11.	O140	03	6.67	-	-	4	12.90	-	-	4	12.25	-	-
12.	O2	-	-	2	2.74	-	-	1	4.54	-	-	-	-
13.	O84	-	-	2	7.40	2	6.45	6	27.27	1	3.12	6	10.52
14.	O68	-	-	7	25.93	-	-	-	-	4	12.50	13	22.81
15.	O114	-	-	1	3.70	-	-	-	-	-	-	3	5.26
16.	O22	-	-	4	14.81	-	-	-	-	-	-	5	8.77
17.	O164	-	-	-	-	2	6.45	-	-	2	6.25	-	-
18.	O10	-	-	-	-	2	6.45	-	-	-	-	-	-
19.	O11	-	-	-	-	-	-	4	18.18	-	-	2	3.51
20.	O56	-	-	-	-	-	-	3	13.64	-	-	4	7.02
21.	Rough	2	4.44	3	11.12	-	-	3	13.64	3	9.37	5	8.77
22.	Untyped	2	4.44	1	3.70	1	3.22	2	9.09	3	9.37	6	10.52

Table 2: Distribution of *Escherichia coli* serotypes in fins, gills and muscles of *Schizothorax niger* of Dal Lake.

Sr. No.	Serotype	Fins (N = 38)		Sr. No.	Serotype	Gills (N = 52)		Sr. No.	Serotype	Fish Muscle (N = 16)	
		No. recovered	Percentage			No. recovered	Percentage			No. recovered	Percentage
1.	O22	04	10.54	1.	O2	03	05.76	1.	O153	03	18.75
2.	O78	06	15.79	2.	O22	05	09.62	2.	O84	02	12.50
3.	O153	03	07.89	3.	O84	10	19.23	3.	O25	04	25.00
4.	O84	02	05.26	4.	O68	04	07.70	4.	O86	02	12.50
5.	O68	16	42.10	5.	O11	06	11.53	5.	UT	05	31.25
6.	O56	01	02.63	6.	O56	06	11.53				
7.	Rough	06	15.79	7.	O25	02	03.85				
				8.	O114	04	07.70				
				9.	O86	03	05.76				
				10.	Rough	05	09.62				
				11.	UT	04	07.70				

CONCLUSION

The present work was undertaken to identify the *Escherichia coli* serotypes in water and fish (*Schizothorax niger*) samples of Dal Lake in autumn, winter and spring seasons. A total of 108 isolates from 54 samples of water and 106 from 45 samples of fish were recovered. Being a ubiquitous organism, *Escherichia coli* is a normal inhabitant of gastrointestinal tract of man and animals and as such its recovery from Dal Lake and *Schizothorax niger* further confirms contamination of the water bodies with human and

animal excreta/wastes. Using somatic O antigen; only 97 isolates from water in 15 serotypes and of 86 from fish, in 11 serotypes could be typed. The predominant serotype in water was O8 (16.66%), followed by O25 (14.81%) and O140 (10.37%) and of fish, serotype O68 (18.87%), followed by O84 (13.20%). Serotypes O78, O86, O153, O84, O68 and O25, known for their association with human and animal diseases, were isolated from both fish and water samples. Serotype O157 was detected in 5.5% of the water samples but could not be recovered from fish. Serotype O84 was isolated from fish in all the three seasons and from water

in winter and spring seasons depicting its adaptability to the host species and warranting further investigation on its role in human diarrheal diseases.

ACKNOWLEDGEMENT

The authors are highly thankful to Director, Research and Director, Resident instructions cum Dean, Post Graduate studies of SKUAST-Kashmir for providing necessary facilities for the research work.

REFERENCES

- APHA 1992. Standard Methods for the Examination of Water and Wastewater. 18th Ed., American Public Health Association, Washington, D.C.
- Cookson, A.L., Croucher, D., Pope, C., Bennett, J., Carter, F.T. and Graeme, G.T. 2006. Isolation, characterization and epidemiological assessment of shiga toxin-producing *Escherichia coli* O84 isolates from New Zealand. *Journal of Clin. Microbiol.*, 44(5): 1863-1866.
- Fleisher, J.M., Kay, D., Wyer, D. and Godfree, A.F. 1998. Estimates of the severity of illness associated with bathing in marine recreational waters contaminated with domestic sewage. *International Journal of Epidemiology*, 27: 722-726.
- McCarthy, T.A., Barrett, N.L., Hadler, J.L., Salsbury, B., Howard, R.T., Dingman, D.W., Brinkman, C.D., Bibb, W.F. and Carter, M.L. 2001. Hemolytic-uremic syndrome and *Escherichia coli* O121 at a lake in Connecticut, 1999. *Pediatrics*, 108: 59-59.
- Mirani, H. 2009. Kashmir's lake Dal gently weeps. *Online Asia Times*. http://www.atimes.com/atimes/South_Asia/KC26Df04.html.
- Nishikawa, Y., Hase, A., Ogasawara, J., Kitase, T., Abe, N., Nakamura, H., Wada, T., Ishii, E., Haruki, K. and the Surveillance Team 2002. Diarrheagenic *Escherichia coli* isolated from stools of sporadic cases of diarrheal illness in Osaka city, Japan between 1997 and 2000: Prevalence of Enterotoxigenic *E. coli* heat stable enterotoxin 1 gene-positive *E. coli*. *Jpn. J. Infect. Dis.*, 55: 182-190.
- Rahman, M.M., Mahalanabis, D., Ali, M., Mazumder, R.N., Wahed, M.A. and Fuchs, G.J. 1997. Absorption of macronutrients and nitrogen balance in children with dysentery fed an amylase-treated energy-dense porridge. *Acta Paediatr.*, 86(12): 1312-1316.
- Suhani, B.B. 2011. A Study on the Microflora of Common Carp of Kashmir with Particular Reference to Family Enterobacteriaceae. MVSc. Thesis submitted to Division of Veterinary Public Health, SKUAST-K.
- Willayat, M.M., Hussain, S.A. and Nabi, A. 2005. Bacteriological analysis of stream, tube well and community supply water in and around Srinagar. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 26: 66-67.
- Yang, H., Sheng Chen, David G., Shaohua, Z., McDermott, P., Walker, R. and Meng, J. 2004. Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. *J. Clin. Microbiol.*, 42(8): 3483-3489.