*Recent Research in Science and Technology 2009, 1(1): 014–019*
*ISSN: 2076-5061*
*www.recent-science.com*

MICROBIOLOGY

# CHARACTERIZATION, HEMOLYSIS AND MULTIDRUG RESISTANCE AMONG *AEROMONAS* SPP. ISOLATED FROM BHAVANI RIVER, ERODE, SOUTH INDIA

**B. Bagyalakshmi[1*], D. Sridhar[2], P. Ponmurugan[1], A.J. Smitha[2], K. Arti[1], T. Vidyashri[1], P. Vinothini[1], R. Sowmya[1]**
[1]*Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode-637 215, Tamil Nadu, India*
[2]*Department of Microbiology, Maharaja College for Women, Perundurai-638 052 Tamil Nadu, India*

## Abstract

A total of 87 strains of *Aeromonas* spp. were identified biochemically. The strains were isolated from 50 samples of water from Bhavani river Erode, Tamil Nadu, India. In the present study among 87 *Aeromonas* spp. the prevalence strain was identified as *A. hydrophila* (60.9%), while the other strains belonged to the species *A. sobria* (20.7%), *A. caviae* (11.5%) and *A.salmonicida* (6.9%). The virulence factors like hemolysin, lipase, and serine protease were present in 96%, 93% and 94% of the strains respectively. Antibiotic susceptibility of *Aeromonas* spp. was determined by disc diffusion method. All *Aeromonas* spp. were examined for resistance against 16 antibiotics. All strains showed 100% of resistance to Ampicillin, Carbenicillin and Cephalothin. The highest resistances encountered were 91.9% to streptomycin, 90.8% to polymyxin-B, 85% to rifampicin while the rest were under 50%. In contrast all the strains were sensitive to cefotaxime. The present work highlights the important incidence of *Aeromonas* spp., with virulence potential and antimicrobial resistance, isolated from river Bhavani.

**Key Words**: *Aeromonas*; Water; Hemolysis; Antibiotic resistance.

## Introduction

*Aeromonas* spp. are widely distributed in nature and were found in soil, drinking water and chlorinated tap water (1, 2). Members of the genus *Aeromonas* have been considered to be opportunistic pathogens they are involved in a number of diarrhoeal and extra intestinal infections including septicemia, wound infections burn associated sepsis, respiratory tract infections and peadiatric gastroenteritis (3, 4 and 5). The presence of *A. hydrophila* and *A. sobria*, in drinking water is considered a relevant factor, since it is associated with digestive tract disorders (6). Food of animal origin, seafood and vegetables also act as an important vehicle for *Aeromonas* spp. infections (7, 8). *Aeromonas* spp. infections normally associated with immuno suppressed patients have been reported (9).It also causes infections in fishes (4). Antibiotic resistant *Aeromonas* spp. have been reported by many scientists (10, 11). A number of virulence factors like hemolysin, protease, lipase and DNase produced by *Aeromonas* spp. play an important role in the development of disease in human and in fish (12, 13).

Although the number of water borne outbreaks caused by A*eromonas* spp. has been quite limited, the presence of *Aeromonas* spp. in river system should not be ignored. Despite the number of surveys on the incidence of other kind of organisms like *Escherichia coli* and *Salmonella* spp. in water bodies, no report is available in Bhavani river regarding *Aeromonas* spp. All water samples were collected from areas largely used by mankind for drinking, cooking, bathing and other utilities.

The present study therefore carried out to document the prevalence of *Aeromonas* spp. The production of virulence factors and their antimicrobial susceptibility patterns of the strains isolated were also studied.

* *Corresponding Author*

## Materials and Methods

### Collection of water samples

A total of 50 samples were examined during this study .Two hundred milliliters of water was collected from Bhavani river by using pre strerilized screw capped glass bottles. All samples were processed within 3 h of collection.

### Examination of water samples

The water samples were enriched in and a loopful of enriched culture was streaked onto Starch Ampicillin agar (14) medium (SAA) (Hi-Media, Mumbai,India).The plates were incubated at 35˚C for 24 h. Yellow to honey coloured colonies surrounded by a clear zone of amylolytic activity after flooding the plates with iodine solution were scored as presumptive *Aeromonas* spp. These colonies were picked, twice streaked to purify on trypticase soy agar plates, and transferred to trypticase soy agar slants for further characterization.

### Characterization and identification of *Aeromonas* strains

A total of 87 isolates were examined by biochemically to species level by referring description of work (15). Gram stain, motility, oxidase and catalase production, fermentation of glucose, and resistance to vibriostatic agent O/129 (12).All isolated *Aeromonas* spp. further tested for growth at 37˚C; indole production; gas from glucose, Voges-Proskauer; lysine and ornithine decarboxylase; aesculine hydolysis; $H_2S$ from cysteine; susceptibility to 30ug of cephaotin; methyl red test; L-arginine hydrolysis; fermentation of L- arabinose, D-mannitol, salicin sucrose, and D-sorbitol (3) casein hydrolysis, gelatinase, and DNase cactivity (16) and β-hemolysis on sheep blood agar plates (12).

### Hemolytic activity

The *Aeromonas* strains were tested for β-hemolytic activity on blood agar base (Hi-Media, Mumbai, India) supplemented with 5% sheep blood. The culture is streaked on to the plates and incubated at 37˚C for 24 h. The presence of clear colorless zone surrounding the colonies considered as β-hemolytic activity (12).

### Proteolytic activity

Casein hydrolysis was tested on Mueller- Hinton agar containing 10 % (w/v) skimmed milk by streaking onto the plates and incubating at 37˚C for 24 h. The presence of a transparent zone around the colonies indicated caseinase activity.

### Gelatinase activity

Gelatinase activity was tested by using gelatin agar plate, the cultures were streaked onto the plates and incubated at 22˚C for 24 h. Then the plates were immersed with mercuric chloride solution. The presence of transparent zone around the colonies indicated gelatinase activity.

### Lipolytic activity

Lipase activity was determined by streaking the culture on to the plate's containig0.5% tributyrin emulsified with 0.2%Triton X- 100 and incubated at 37˚C for 24 h. (17).The presence of transparent zone around the colonies indicated lipase activity.

### Nuclease activity

DNases activity were determined on DNase agar plates with 0.005% methyl green .The culture is streaked on to the plates and incubated at 37˚C for 24 h. A pink halo around the colonies indicated nuclease activity.

### Antimicrobial susceptibility test

The antimicrobial resistance of all strains to various antibiotic was determined by the disc diffusion method (18). The antibiotics and concentration ranges of the discs (Hi-media)tested were as follows: amikacin (30µg), ampicillin (10µg), Carbenicillin (100µg), cephalothin (30µg), cefotaxime (30µg), ciprofloxacin (5µg), chloramphenicol (30µg), erythromycin (15µg), streptomycin (10µg), gentamycin (10µg), kanamycin (30µg), neomycin (30µg), polymyxin-B (300U), rifampicin (5µg), tetracycline (30µg), piperacillin (100µg). After enrichment in tryptic soy broth (Himedia) for 6-8 h at 37˚C, the cultures were streaked on Mueller- Hinton agar (Himedia) plates using a sterile cotton swab. The antibiotic discs were placed on the agar surfaces sufficiently separated from each other so as to prevent over-lapping of the inhibition zones. After 30 minutes, the plates were inverted and incubated at 37˚C for 24h. Results were recorded by measuring the inhibition zones, comparing with interpretive chart of the Kirby-Bauer sensitivity test method.

## Results

A total of 50 numbers of water samples were collected from river Bhavani during the period of April 2008 –May 2009. Only 86% of water samples showed the presence of *Aeromonas* spp. (Table 1). A total of 87 *Aeromonas* spp. were isolated from water samples.Yellow to honey coloured colonies surrounded by clear halo zone of amylolytic activity were selected for

further analysis. The most commonly found *Aeromonas* spp. were *Aeromonas hydrophila* (60.9%). *Aeromonas sobria* (20.7%), *Aeromonas caviae* (11.5%) and *Aeromonas salmonicida* (6.9%) were also recorded (Table 2).

Table 1: Percent incidence of *Aeromonas* spp in water from Bhavani river

| Source | No. of water samples | Positive for *Aeromonas* spp. | % |
|---|---|---|---|
| Water | 50 | 43 | 86 |

Table 2: Percentage incidence of *Aeromonas* strains isolated from Bhavani river water samples

| *Aeromonas* strains | Number of isolates | Percentage of Occurrence (%) |
|---|---|---|
| *Aeromonas hydrophila* | 53 | 60.90 |
| *Aeromonas sobria* | 18 | 20.70 |
| *Aeromonas caviae* | 10 | 11.50 |
| *Aeromonas salmonicida* | 6 | 06.90 |

All *Aeromonas* spp.(100%) showed DNase activity. The virulence factors like hemolysin, lipase, and serine protease were present in 96%, 93% and 94% of the strains respectively. However, protease activity was different when evaluated with the caseinase test (66%) than with geltinase (94%) (Table 3).

Table 3: Incidence of virulence factors (%) in *Aeromonas* spp. isolated from Bhavani river

| *Aeromonas* strains | β-hemolysis test (37°C) | Lipase test | Serine Protease test | Caseinase test | Gelatinase test | DNase test |
|---|---|---|---|---|---|---|
| *A. hydrophila, n=53* | 100 | 100 | 100 | 51 | 92 | 100 |
| *A. sobria, n=18* | 94 | 88 | 88 | 100 | 100 | 100 |
| *A. caviae, n=10* | 80 | 70 | 70 | 100 | 100 | 100 |
| *A. salmonicida, n=6* | 100 | 83 | 100 | 50 | 83 | 100 |
| All the strains | 96 | 93 | 94 | 66 | 94 | 100 |

The resistance patterns obtained with the 87 *Aeromonas* spp. against 16 antibiotics are shown in table 3. A 100% of resistance was observed in *Aeromonas* spp. against Ampicillin, Carbenicillin and Cephalothin. In addition the highest resistances showed by *Aeromonas* spp. encountered were 91.9% to streptomycin, 90.8% to polymyxin-B, and 85% to rifampicin (Table 4).

Table 4: Percentage antimicrobial resistance of *Aeromonas* spp.isolated from Bhavani River.

| Antibiotics with concentration | *A. hydrophila n=53* | *A. sobria n=18* | *A. caviae n=10* | *A. salmonicida n=6* | Resistant strains |
|---|---|---|---|---|---|
| Amikacin (30µg) | 0 | 38.8 | 40 | 33 | 14.9 |
| Ampicillin (10µg) | 100 | 100 | 100 | 100 | 100 |
| Carbenicillin (100µg) | 100 | 100 | 100 | 100 | 100 |
| Cephalothin (30µg ) | 100 | 100 | 100 | 100 | 100 |
| Cefotaxime (30µg) | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin (5µg ) | 49 | 50 | 50 | 33 | 48.2 |
| Chloramphenicol (30µg) | 0 | 11 | 20 | 16.6 | 5.74 |
| Erythromycin (15µg) | 52.8 | 44.4 | 40 | 50 | 49.4 |
| Streptomycin (10µg) | 100 | 77.7 | 80 | 83.3 | 9.9 |
| Gentamycin (10µg) | 37.7 | 55.5 | 60 | 66.6 | 45.9 |
| Kanamycin (30µg) | 0 | 38.8 | 20 | 33 | 12.6 |
| Neomycin (30µg) | 33.9 | 44.4 | 40 | 50 | 37.9 |
| Polymyxin- B (300 U) | 100 | 77.7 | 80 | 66.6 | 90.80 |
| Rifampicin (5µg) | 100 | 55.5 | 70 | 66.6 | 85.05 |
| Tetracycline (30µg) | 33.9 | 50 | 50 | 50 | 19.54 |
| Piperacillin (100µg) | 0 | 27.7 | 10 | 16.6 | 18.3 |

Least resistance was observed against Chloramphenocol (5.7%), Kanamycin (12.6%), Tetracycline (19.54%), Neomycin (37.9%), Gentamycin (45.9%), Erythromycin (49.4%). *Aeromonas* stains showed 14.9%, 48% resistance to amickacin, ciprofloxacin respectively. In contrast all the strains were sensitive to cefotaxime. Only 18.3 % of the strains showed resistance to Piperacillin.

## Discussion

A total of 87 *Aeromonas* spp. belonging to four different species isolated from samples of Bhavani river water. The prevalence of *Aeromonas* spp. revealed remarkable diversity in this water system, which includes potential pathogens like *A. hydrophila*. In the present study this particular species accounts for 60.9%. The prevalence of *A. hydrophila* from drinking water samples already have been reported (2,19). *Aeromonas sobria* (20.7%), *Aeromonas caviae* (11.5%) and *Aeromonas salmonicida* (6.9%). have reported in our study (Table 2).Occurrence of *A. sobria* in water has been already reported (20). *A. salmonicida* has been considered as a fish pathogen (21,22) but also reported in water (23,1). The interesting observation of *Aeromonas* spp. in river bhavani which suggests the possible release of waste waters into Bhavani river that might enhanced the population of *Aeromonas* spp.

The virulence factors by *Aeromonas* spp. has been identified by production of exo enzymes, although the importance and exact mechanism of each factor associated to the virulence has not been well established (24). Our study showed that *Aeromonas* spp. produced hemolysin, Lipase, Serine protease, gelatinase and caseinase (Table 3). *Aeromonas hydrophila and Aeromonas salmonicida* had a high occurrence of hemolytic activity. The presence of hemolytic activity in *Aeromonas* spp. has been reported. 66% of the *Aeromonas* spp. showed hemolytic activity was noted by many scientists (25,1,26, 27). Hemolytic *A. hydrophila* from water sample was also reported (28). 97% correlation between hemolysis and enterotoxin production was noticed in many reports (29).

The lipases are important for bacterial nutrition (24) but they also involved in virulence character. In the present study *A. caviae* alone showed 70% of the activity. The proteolytic activity is important to induce pathology in living system. In our study, proteolytic activity of the isolates was evaluated by determining caseinase and gelatinase production. This study revealed

(94%) activity showed for serine protease and gelatinase. But only 66% of the strains produced caseinase. 61% of the *Aeromonas* isolates produed protease in this study (30). The DNase activity was detected in 100% of the strains. This may indicate their virulence ability.

The antibiotic resistance testing revealed that all *Aeromonas* spp. were resistant to β- lactum antibiotics (1) (Table 4). However, the resistance to other antibiotics was variable. Only 18.3% of the strains showed resistance to piperacillin. Penicillin resistance *Aeromonas* spp. from food samples have been reported (31). None of the strains were showed resistant to cefoxime antibiotic. Our results are consistent with data reported by (32, 33, 34) found that strains of *A. salmonicida* isolated from environment samples were 100% susceptible to ampicillin, cephalothin, polymyxin, rifampicin. But in our study it showed a different degree of resistance to these antibiotics, ranging from 16% to 100%. (28) reported all *Aeromonas* spp. were found to be susceptible to amikacin and ciprofloxacin in his studies. In the present study 14.9%, 48% strains showed resistance to amickacin, ciprofloxacin respectively. Resistance to streptomycin was high (91.9%). Our results correlated with his findings (35). Least resistance was observed against Chloramphenocol (5.7%), Kanamycin (12.6%), Tetracycline (19.54%), Neomycin (37.9%), Gentamycin (45.9%), Erythromycin (49.4%). Our results are not correlated with the findings of this study (10).

The present study highlights the presence of *Aeromonas* spp in water system indended for human consumption in the city. High prevalence of multiple antibiotic resistant, hemolysis and protease producing *Aeromonas* spp. was noticed. The productions of hemolysis and resistant to various antibiotics are normally associated to enteric infections caused by species of *Aeromonas*.

## Conclusion

The result obtained in the present study indicates that different *Aeromonas* spp. is able to multiply in water distribution systems. The increased occurrence of these strains should not be ignored. These microorganisms could reach the food product, becoming a potential human pathogen. It should be kept in mind that these microorganisms in water system might be a potential risk for public health.

## Acknowledgement

The authors are thankful to the Principal and the Management for providing necessary facilities and constant encouragement to carry out this study.

## Reference

1. Abraha, G., and R.K. Agarwal, 2001. Occurrence of enteropathogenic and multidrug resistance *Aeromonas* spp.in drinking water. Ind. J. of Comparative Microbiol, Immunology and Infectious Diseases. 22: 128-30.
2. Koksal, F., N. Oguzkurt, M. Samast, and K. Altas, 2007. Prevalence and Antimicrobial Resistance Patterns of *Aeromonas* Strains Isolated from Drinking Water Samples in Istanbul, Turkey. Chemotherapy. 53: 30-35.
3. Janda, J.M. and S.L. Abbott, 1998. Evolving concepts regarding the Genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Clin. Infect. Dis. 27:332– 344.
4. Austin, B. and C. Adams, 1996. Fish pathogens. In: Austin, B., Altwegg, M., Gosling, P.J., Joseph, S. (Eds.), The Genus Aeromonas. Wiley, Chichester, 198–243.
5. Subashkumar, R., T. Thayumanavan, G. Vivekanandhan, and P. Lakshmana Perumalsamy, 2006. Occurrence of *Aeromonas hydrophila* in acute gasteroenteritis among children. Indian J. Med. Res.123: 61-66.
6. Delmar, B., and B. Adriano, 2001. Antimicrobial susceptibility, hemolysis, and hemagglutination among *Aeromonas* spp. Isolated from water of a bovine abattoir. Braz. J. Microbiol. 32, 231-234.
7. Mattick, K.L., and T.J. Donovan, 1998. The risk to public health of *Aeromonas* in ready-to-eat salad products. Commun. Dis. Public Health. 1, 263– 266.
8. Kaskhedikar, M., and Chhabra, D., 2009. Multiple drug resistance of *Aeromonas hydrophila* isolates from Chicken samples collected from Mhow and Indore city of Madhyapradesh Veterinary World.2, 31-32.
9. Ko, W.C., H.C. Lee, Y.C. Chuang, C.C. Liu, and J.J .Wu, 2000. Clinical features and therapeutic implications of 104 episodes of monomicrobial *Aeromonas bacteraemia*. J. Infect. 40: 267–273.
10. Vila, J., F.Marco, L. Soler, M.R.Chaco´n, and M.J. Figueras, 2002. In vitro antimicrobial susceptibility of clinical isolates of *Aeromonas caviae*, *Aeromonas*

*hydrophila* and *Aeromonas veronii* biotype sobria. J. Antimicrob. Chemother. 49:525– 529.

11. Thayumanavan, T., R. Subashkumar, G. Vivekanandhan, K. Savithamani, and P. Lakshmana perumalsamy, 2007. Haemolytic and Multidrug Resistant *Aeromonas hydrophila* Cross Contamination in Retail Seafood Outlets of Coimbatore, South India. American J. Food Technol. 2: 87-94.
12. Santos, J.S,A., J. Ce´Sar, A.R. Gonza´Lez, and Mari´A-Luisa, G.L., 1999. Hemolytic activity and siderophore production in different *Aeromonas species* isolated from fish. Appl. Environ. Microbiol. 65: 5612–5614.
13. Soler, L., M.J. Figueras, M.R. Chaco´n, J. Vila, F. Marco, A. Martı´-nez-Murcia, and J. Guarro, 2002. Potential virulence and antimicrobial susceptibility of *Aeromonas popoffii* recovered from freshwater and seawater. FEMS Immunol. Med. Microbiol. 32:243–247.
14. Palumbo,S.A.,F.Mexino,A.C.Williams,Buchanon,R.L. andD.W. Thayer,1985.Starch Ampicillin Agar for the quantitative detection of *Aeromonas hydrophila*. Appl. Environ. Microbio.50:1027-1030.
15. Altwegg, M., 1999. Aeromonas and Plesiomonas. In: Murray, P.R.,Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H. (Eds.), Manual of Clinical Microbiology, 7a ed. American Society for Microbiology, Washington, DC, 507–511.
16. Huys, G., P. Kampfer, M. Altwegg, R. Coopman, P. Janssen, M. Gillis, and K. Kersters, 1997. Inclusion of *Aeromonas* DNA hybridization group11 in *Aeromonas encheleia* and extended descriptions of the species *Aeromonas eucrenophila* and *Aeromonas encheleia*. Int. J. Syst. Bacteriol. 47: 1157-1164.
17. Anguita, J., Rodriguez-Aparicio, L.B., and Naharro, G., 1993. Purification, gene cloning, amino acid sequence analysis, and expression of an extracellular lipase from an *Aeromonas hydrophila* human isolate. Appl. Environ. Microbiol. 59:2411-2417.
18. Bauer, A.W., Kirby, W.M.M., Sherris, J.S., and M.Turck, 1966. Antibiotic susceptibility testing by a standard single disc method. Am.J. Clin. Pathol. 45:493-496.
19. Neyts, K., G. Huys, M. Uyttendaele, J. Swings and J. Debevere, 2000. Incidence and identification of mesophilic *Aeromonas* spp. from retail foods. Belgium Lts. In Appli. Microbiol. 31: 359-363.
20. Rasel,K.O.,T. Eiza, N. Hironori, A. Mohammed, B.Sukalyani, R. Thandavarayan,and O. Keinsuke, 2008.Toxin production by *Aeromonas sobria* in Natural environment: River water vs Sea water. Acta. Med. Okayama. 62: 363-371.
21. Sorum, H., G .Holstad, T. Lunder,and T. Hastein, 2000. Grouping by plasmid profiles of atypical *Aeromonas salmonicida* isolated from fish with special reference to salmonid fish. Dis. Aquat.Org. 41:159– 171.
22. Gardun˜o, R.A., A.R. Moore, G. Oliver, A.L. Lizama, E. Gardun˜o, and W.W.Kay, 2000. Host cell invasion and intracellular residence by *Aeromonas* salmonicida: role of the S-layer. Can. J. Microbiol.46: 660– 668.
23. Marsol, G.U., C.Lio nel, R. Christine, C. Pierre, and Q. Claudine, 2000. Antibacterial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. J. Antibacterial Chemotherapy.46: 297-301.
24. Pemberton, J.M., S.P. Kidd, and R. Schmidt, 1997. Secreted enzymes of *Aeromonas*. FEMS Microbiol. Lett. 152:1– 10.
25. Kingombe, C.I., G .Huys, M. Tonolla, M.J. Albert, J. Swings, R. Peduzzi, and T. Jemmi, 1999. PCR detection, characterization and distribution of virulence genes in *Aeromonas* spp. Appl. Environ. Microbiol. 65: 5293– 5302.
26. Santos J.S.A., C.E. Sar, J., Gonzalez, A.O., Garci´A-o´and M.L. Pez, 2008. Hemolytic Activity and Siderophore Production in Different *Aeromonas* Species Isolated from Fish.African J.of Biotechnol. 7:373-380.
27. Sechi, L.A., A. Deriu, M.P. Falchi, G. Fadda, and S. Zanetti, 2002. Distribution of virulence genes in *Aeromonas* spp. isolated from Sardinian waters and from patients with diarrhea.J.Appl.Microbiol.92:221-227.
28. Gurol, E.,A. Gonul, T. Seda, S. Mehmetsami, Y. Ciliem, O. Haken, and D Riza, 2006. Detection of the frequency, antimicrobial susceptibility, and genotypic discrimination of *Aeromonas* strains isolated from municipally treated tap water samples by cultivation and AP-PCR. Int. J. food microbial.107: 310- 314.
29. Burke, V., J. Robinson, H.M. Atkinson, and M. Gracey, 1982. Biochemical characteristics of enterotoxigenic *Aeromonas* spp. J. Clin. Microbiol.15: 48-52.
30. Escarpulli,G.C., M.J.Figueras, G.Aguilera-Arreola, L. Soler, E. Ferna´ndez- Rendo´n, G. O. Aparicio, J. Guarro, and M.R. Chaco´n, 2003. Characterisation of *Aeromonas* spp. isolated from frozen fish intended for human consumption in Mexico. Int. J. Food Microbiol.84: 41-49.

31. Mohammed, B.A., M. Ahmed, and B.K. Abdul, 2009. Antibiotic susceptibility profile of *Aeromonas* spp. Isolates from food in Abu Dhabi, United Emirates. New Microbiolo. 32: 7-23.
32. Gon˜i-Urriza, M., L.Pineau, M. Capdepuy, C. Roques, P.umette, and C.Quentin, 2000. Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. J. Antimicrob. Chemother. 46:97– 301.
33. Jennifer, R.H., C.Z. John, and R.M. Jeter, 2006. Antimicrobial Susceptibilities of *Aeromonas* spp. isolated from environmental sources. Appl. Environ. Microbiol. 72: 7036–7042.
34. Dalsgaard, I., B. Nielsen, and J.L. Larsen, 1994. Characterization of *Aeromonas Salmonicida* a comparative study of strains of different eographic origin. J. Appl. Bacteriol.77: 21–30.
35. Sinha, S., T. Shimada, T. Ramamurthy, S.K Bhattacharya, S. Yamasaki, Y. Taked, and G. Balakrishna Nair, 2004. Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas species* isolated from hospitalized diarrhoeal cases in Kolkata, India. J. of Medical Microbiol.53: 527–534.