JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2009. Vol. **3**(2), p. 517-526

Isolation and Identification of Mesophillic Aeromonas Bacteria from Meat and Fish Foods of North East India

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(Received: 18 July 2009; accepted: 16 August 2009)

Screening of 332 samples of animal food origin comprising 104 poultry, 137 fish, 51 pork and 40 goat meats (chevon) was carried out for the presence of Aeromonas infection. The isolation and identification of different Aeromonas species was done on different media and on the basis of morophological, cultural characters and biochemical tests.respectively. Out of 332 samples 38 samples were found positive with Aeromonas species. *Aeromonas hydrophila, A. caviae* and *A. sobria* were isolated from different food samples. *A. hydrophila* was commonly isolated from different meat and fish foods samples. *A.sobria* and *A. caviae* were isolated from fish and chicken meat samples repectively. The incidence of *A. sobria* infection was very low. Chevon harboured no infection of Aeromonas.

Key words: Aeromonas, Isolation, Identification, Meat and Fish foods.

Man has always relished meat and has incorporated it as one of the most important and favorite part of his diet ever since pre-historic times as it has endowed him with high satiety value, excellent source of high quality proteins, fats, carbohydrate, vitamins and minerals (Gracey, 1985).

In the recent times, there have been reports of many cases of diseases caused by pathogenic zoonotic bacteria in India, especially due to *Aeromonas, Listeria, Vibrio, Plesiomonas*, Salmonella and Staphylococcus. There has also been a wide array of microorganisms like bacteria, viruses, rickettsia, helminthes and fungi that may be transmitted through meat. Micro flora primarily incorporating the fresh meat are *Escherichia coli, Salmonella, Acrobacter, Aeromonas, Listeria, Micrococcus, Proteus, Staphyllococcus, Clostridium, Pseudomonas, Klebsiella*, etc. which may be transmitted through meat to human beings. Fresh raw meat while in distribution may be a source of zoonotic infection, which is insidious and go unnoticed (Bachhil and Ahullwalia 1973).As trends in food borne diseases continue to rise, the effective identification and control of pathogens becomes more important for

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the food industry. Bacterial zoonoses have been of great significance, not only affecting the public health but also causing a great economic loss both to the population and the nation. The major factor contributing to the appearance of new zoonotic pathogens in human populations is increased contact between humans and wildlife. Sudden epidemic outbreaks of food-borne zoonotic diseases lead to drastic public health hazard and among the recently encountered is the *Aeromonas.* In view of the potential losses to the commercial and sport fishing industries and the serious implications for human health, definitive isolation and identification of *Aeromonas* spp. are necessary to develop therapeutic strategies. By definition, the detection of a pathogen requires rapid and specific methods for isolation, identification, and enumeration. Such procedures may assist in the control of potentially pathogenic microorganisms from environmental and food samples, which are mostly regarded as the main transport vectors to human populations. A complete review of methodologies for the isolation, identification, and enumeration of *Aeromonas* spp. from clinical, environmental and food samples has been done by Joseph *et al*., (1987). Since then, other isolation methods have been evaluated and compared for the ability to isolate or detect *Aeromonas* spp. in food and environmental items and this indicates the need for a reliable, universal, and standard method for the detection of these pathogens in clinical, environmental and food samples.

Aeromonas spp. is also common contaminants and readily isolated from a wide range of food animals (Palumbo *et al*., 1985; Majeed *et al*., 1989; Palumbo *et al*., 1989). However, the first occurrence of *Aeromonas* spp. was reported from food (milk), but the full potential of food in the etiology of *Aeromonas* infections still needs to explored, more so in Indian context where the reports are scanty.It is in this context that the present investigations was envisaged with a view to isolate and characterize the *Aeromonas* spp. in foods of animal origin.Since no study is available on Aeromonas infection in animal origin food, the present study aims to isolate and identify the mesophilic Aeromonads infection in meat and fish food of North East India.

MATERIAL AND METHODS

Collection of Meat and Fish Food Samples

The materials for the present study was collected from food of animal origin that is pig, goat, poultry and fish which are commonly consumed by the people of North Eastern Region.The samples were procured from retail shops of Meghalaya and Assam states.A total of 332 samples(chicken 104,fish 137,pork 51,goat meat(chevon) 40) were collected in the early morning hours immediately after slaughter of animals.The period of collection varied from month of March 2007 to December 2007.The samples were brought to the laboratory aseptically in chilled state and processed immediately for their bacteriological profile of public health significance.

Enrichment

100 g of sample collected from different parts of slaughtered carcass (intestine, gills and meat) of the aforesaid food samples were inoculated in alkaline peptone water (APW) with pH 8.4 were incubated at 37°C for 18 to 24 hrs.

Isolation of Bacteria

A loopful of inoculum from the enrichment broth was streaked on Ampicillin Dextrin Agar (ADA) plates. The inoculated plates were incubated at 37°C for 18-24 hours for the growth of distinguished colonies of *Aeromonas* species. Subcultures were made from single colony to obtain pure culture of the organism. The growth on nutrient agar slants and stabs were stored at 4°C and the cultures were maintained by sub-culturing the slants every month and the stabs every two months.

Identification of Bacteria

The identification of the bacteria was made according to the scheme of Bergeys's manual of Systemic Bacteriology (Vol. I-1984, Vol. II-1986, Vol. III-1989), Cruickshank *et al.* (1975). The isolated bacteria were identified on the basis of morphological characters, cultural characters and biochemical tests.

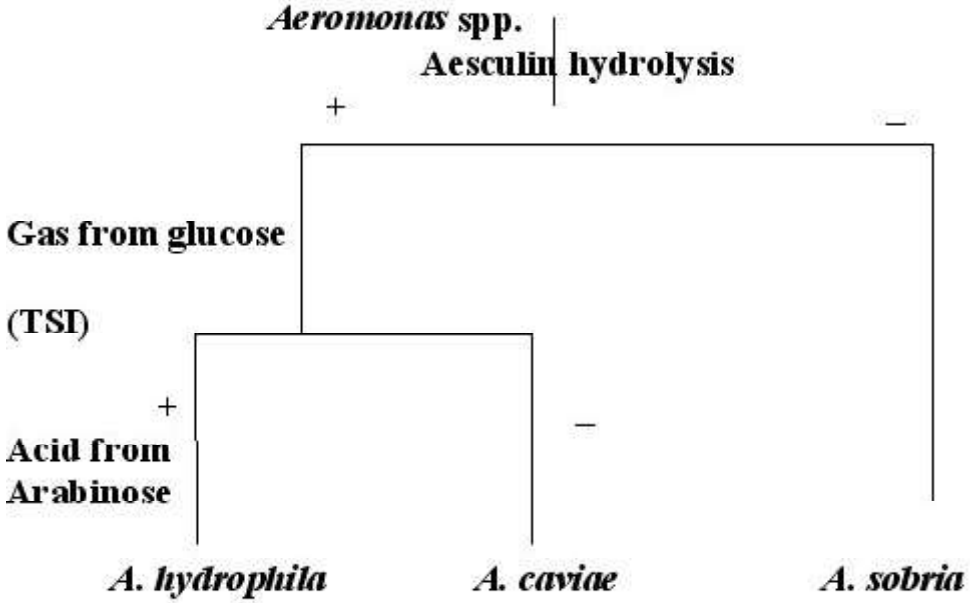
Morphological Characters

The isolates were stained with Gram's staining method on the lines advocated by Cruickshank *et al*. (1975). Observations were particularly made on shape, size, axis, sides, ends, arrangements and motility. The compositions of

the staining solutions used are given in the appendix.

Cultural Characters

Cultural characteristics were observed on Ampicillin Dextrin Agar plates and nutrient agar slants. On solid medium the colonies were examined for shape, size, surface, elevation, edge, colour, consistency, opacity, and emulsifiability. Colour of the isolates varied with specificity of bacterial to the media used. Finally biochemical tests were employed to categorize the bacteria in its respective genera and species by the Aerokey (Modified) methods as per the flow chart given below.



***In vitro* Antiobiotic sensitivity test**

The disk diffusion test for sensitivity to different antibiotics was conducted as per the lines advocated by Cruickshank *et al*. (1975).

Preperation of the Inoculum

The test organisms were picked up from a 24 hours young culture plate and inoculated into BHI broth. The broth cultures were incubated for 18-24 hours at 37°C.

Sensitivity Test Procedure

About 100-200 µl of the inoculum was poured on MHA plates and spread uniformly. The plates were kept undisturbed for about 5 mins. The excess inoculum was sucked out from the plate with the help of a sterile Pasteur pipette and the antimicrobial discs were placed gently on the agar surface maintaining adequate distance among them. Each disc was gently pressed down with a pair of sterile forceps to ensure complete contact with the medium. The plates were then incubated aerobically at 37°C for 18-24 hours in inverted position.

Positive test was indicated by zones of inhibition which were measured by using the zone size interpretative tables provided by the manufacturer of the discs.

The antibiotic disks used were of HI-MEDIA Laboratories, Mumbai, India, consisting of the following:

Ampicillin (10µg), Kanamycin (10µg), Sulphafurazole (300µg), Amikacin (30µg), Carbenicillin (100µg), Streptomycin (25µg), Chlortetracycline (30µg), Trimethoprim (30µg), Ciprofloxacin (5µg), Tetracycline (10µg), Gentamicin (30µg), Co-trimoxazole (25µg), Chloramphenicol (30µg), Cephotaxime (30µg), Cephalothin (30µg), Cefuroxime (30 µg) and Nalidixic acid (30µg).

RESULTS

Result of screening of a total of 332 samples of animal food origin comprising 104 poultry (chicken), 137 fish, 51 pork and 40 goat meat (chevon) for the presence of aeromonads, species identification, pathogenecity tests and antimicrobial sensitivity are presented.

The samples were enriched in APW, streaked on to selective agar (ADA) and incubated at 37°C for 24 hrs. Of 332 samples processed for isolation of *Aeromonas* spp. only 38 isolates tested positive for *Aeromonas*. The details of the isolates isolated have been summarized (Table 1).

The *Aeromonas* colonies appeared yellow-coloured, small, smooth and honey drop-like. The results indicated that ADA yielded higher number of isolates, thus proving to be the best selective medium and differentiate recovery from mixed cultures as recommended by Sahu *et al*. (1996).Comparatively higher incidences of aeromonads were observed in fish followed by poultry, pig and goat samples (Table 1).

The results of *in-vitro* antibiotic tests of 38 *Aeromonas* isolates isolated in the present study are presented in Table (2). and Fig.(2).

An overall analysis of drug sensitivity test revealed that out of 38 *Aeromonas* isolates from chicken, fish, pork and chevon, which were highly sensitive to antibiotic disks were recorded as Ciprofloxacin (97.36%), Streptomycin (86.84%), Amikacin (84.21%), Nalidixic acid (78.94%), Cephotaxime (73.68%), Gentamicin (63.15%), Cefuroxime (55.26%), Co-trimoxazole (18.42%), Chloramphenicol (13.15%), Sulphafurazole (13.15%), Carbenicillin (10.52%), Trimethoprim (7.89%), Tetracycline (5.26%), Chlortetracycline (5.26%), Ampicillin (2.63%),

Fig. 1. TSI test showing positive hydrogen sulphide gas and glucose fermentation

Fig. 2. Different sugar fermentation test is showin by Aeromonas isolates

Table 1. Incidence of *Aeromonas* spp. in foods of animal origin

S. No.	Source	No. of samples tested	No. of positive samples	Percentage
1.	Chicken	104	12	11.5
2.	Fish	137	18	13.13
3.	Pork	51	7	22.5
4.	Chevon	40	1	10.00
	Total	332	38	36.88

Table 2 . Drug Sensitivity of *Aeromonas* Isolates from Chicken, Fish, Pork and Chevon

S. No.	Drugs	Attributes	Aeromonas isolates				Total
			Chicken	Fish	Pork	Chevon	
1.	Streptomycin	No. of	10	16	6	1	33
		isolates	83.33	88.88	85.71	100	86.84
		Percentage N	12	18	7	1	38
2.	Kanamycin	No. of	0	1	0	0	1
		isolates	0	5.55	0	0	2.63
		Percentage N	12	18	7	1	38
3.	Sulphafurazole	No. of	1	3	1	0	5
		isolates	8.33	16.66	14.28	0	13.15
		Percentage N	12	18	7	1	38
4.	Amikacin	No. of	9	17	5	1	32
		isolates	75.00	94.44	71.42	100	84.21
		Percentage N	12	18	7	1	38
5.	Ampicillin	No. of	0	0	0	0	0
		isolates	0	0	0	0	0
		Percentage N	12	18	7	1	38
6.	Carbenicillin	No. of	1	3	0	0	4
		isolates	8.33	16.66	0	0	10.52
		Percentage N	12	18	7	1	38
7.	Chlor-Tetracycline	No. of	0	2	0	0	2
		isolates	0	11.11	0	0	5.26
		Percentage N	12	18	7	1	38
8.	Trimethoprim	No. of	1	2	0	0	3
		isolates	8.33	11.11	0	0	7.89
		Percentage N	12	18	7	1	38
9.	Cephotaxime	No. of	9	14	5	0	28
		isolates	75.00	77.77	71.42	0	73.68
		Percentage N	12	18	7	1	38
10.	Ciprofloxacin	No. of	11	18	7	1	37
		isolates	91.66	100	100	100	97.36
		Percentage N	12	18	7	1	38
11.	Tetracycline	No. of	0	1	1	0	2
		isolates	0	5.55	14.28	0	5.26
		Percentage N	12	18	7	1	38
12.	Cephalothin	No. of	0	1	0	0	1
		isolates	0	5.55	0	0	2.63
		Percentage N	12	18	7	1	38
13.	Gentamicin	No. of	7	12	4	1	24
		isolates	58.33	66.66	57.14	100	63.15
		Percentage N	12	18	7	1	38
14.	Co-trimoxazole	No. of	2	4	1	0	7
		isolates	16.66	22.22	14.28	0	18.42
		Percentage N	12	18	7	1	38
15.	Chloram-Phenicol	No. of	3	2	0	0	5
		isolates	25.00	11.11	0	0	13.15
		Percentage N	12	18	7	1	38
16.	Cefuroxime	No. of	7	10	4	0	21
		isolates	58.33	55.55	57.14	0	55.26
		Percentage N	12	18	7	1	38
17.	Nalidixic Acid	No. of	9	15	5	1	30
		isolates	75.00	83.33	71.42	100	78.94
		Percentage N	12	18	7	1	38

Cephalothin (2.63%), and Kanamycin (2.63%). All the 38 *Aeromonas* isolates from chicken, fish, pork and chevon exhibited their highest sensitivity towards Ciprofloxacin, Streptomycin and Amikacin; sensitive towards Nalidixic acid, Cephotaxime, Gentamicin, and Cefuroxime. Other drugs which showed significant resistance are recorded as Co-trimoxazole, Chloramphenicol, Sulphafurazole, Carbenicillin, Trimethoprim, Tetracycline, Chlortetracycline, Ampicillin, Cephalothin, and Kanamycin. Similar results were also recorded by Chang and Bolton (1987). Differences in the frequency of resistance towards Ampicillin, Chloramphenicol, Tetracycline and Trimethoprim was observed and such differences may well be related to the source and the frequency and type of antimicrobial agents prescribed for treating *Aeromonas* infections in different geographical areas. *Aeromonas* species may easily develop resistance in presence of low concentrations of antibiotics due to the indiscriminate use of these antibiotics (Chang and Bolton, 1987).

Pathogenecity Test

Paw oedema test

The result of Paw oedema test is depicted in Table (3).Of the 38 isolates examined, CFF of 21 (55.26 %) isolates produced significant oedema (Table 3). The relative thickness (%) for enterotoxicity was observed to be higher than 121.0 ± 38 that is 283.4.

Rabbit ligated ileal loop test (RLIL)

Twenty µg to 30 µg of CFF was injected into the intestinal loop of the rabbits. Positive results in RLIL exhibited fluid accumulation at the rate of around 0.2 to 0.6 ml/cm, respectively. The accumulated fluid was found to be thick and dark in nature.

Table 3. Mouse Paw Oedema Test

S. No	Source	Species	Number of isolatesexamined	Number of isolates Positive (%) (CFF)
1.	Fish	*A. hydrophila*	16	10 (62.5%)
		A. sobria	2	1 (50%)
2.	Chicken	*A. hydrophila*	11	6 (54.54%)
		A. caviae	1	0 (0%)
3.	Pig	*A. hydrophila*	7	4 (57.14%)
4.	Goat	*A. hydrophila*	1	0 (0%)
		Total	38	21 (55.26%)

DISCUSSION

Mesophillic *Aeromonas* spp. have emerged as important human and animal pathogen, with worldwide distribution (Merino *et al*., 1995). Voluminous literature bears the testimony that aeromonads are involved in the etiology of gastrointestinal diseases in human beings (Burke *et al*., 1983; Gracey *et al*., 1982) with illness ranging from mild to dysentery-like diarrhoea (Champsaur *et al*., 1982). Acute self limiting gastroenteritis seems to be more common in children, particularly those below 2 years and adults above 50 years (Burke *et al*., 1983; Agger, 1986). *Aeromonas* spp. also causes extra intestinal infections that may become fatal especially among immunocomprised patients.

Aeromonas spp. are considered to be regular flora of poikilotherm and homotherm animals implicated in the etiology of a variety of systemic and localized diseases in different animals, reptiles and fish (Boulanger *et al*., 1977). Prevalence of virulent *Aeromonas* spp. has also been recorded from farm animals (Gray *et al*., 1990). In India, its occurrence has been reported from buffalo with the history of abortions (Das Paranjape, 1990) and ulcerative condition of fish (Pal and Pradhan,1990).

The possible sources of infection are water, food and feacal contamination. Water is frequently shown to be contaminated with *Aeromonas* spp., being found in domestic water supply (Burke *et al*., 1984) including chlorinated water. In India, it has been recorded from river

and sewage water (Garg *et al*., 1994).

The selection of a reliable medium for the primary isolation of the organism is an essential requirement for the proper understanding of the clinical and epidemiological significance. It is more so with *Aeromonas* spp. because of its unique nature (Kay *et al*., 1985). One of the main problems in the isolation of mesophilic *Aeromonas* spp. is that no universal selective medium is available. Different workers have recommended different medium that was found to be much superior according to them. Markwardt *et al*., (1989) found Coomassie Brilliant Blue agar to be very valuable, Jenkins and Taylor reported Rimler-Shotts and Starch-Glutamate-ampicillin-penicillin-based medium proved to be better choice and Gobat and Jemmi revealed Bile-salts-irgasan-brilliant green agar to be very selective.

In this study, 332 samples were tested on ADA medium and the results indicated that ADA was the best medium for recovery of *Aeromonas* isolates. The application of this medium in assessing the incidence of the bacterium in foods of animal origin obtained from retail markets permitted rapid recovery in presence of very large numbers of competing micro flora (Palumbo *et al*., 1985 and Kersters *et al*., 1996). Also this medium had a greater confirmation rate along with its high specificity and no false negative colonies were encountered (Havelaar *et al*., 1987).

All the samples procured from the retail shops were enriched in APW broth without addition of ampicillin. On inoculation on ADA medium, maximum growth of the *Aeromonas* spp. was observed the following day indicating that APW was the best enrichment broth for recovery of aeromonads from the samples. The results observed in this present study are in conformity with that of VonGraevenitz and Bucher (1993).

All the isolates showed visible growth at 37°C after 24 hrs of incubation which became abundant after 72 hrs. Maximum growth temperature was observed from 37ºC to 42ºC (Merino *et al*., 1995). However, it has been reported that most clinical strains grow well at 42ºC (Palumbo *et al*., 1985), where as only few isolates from vegetables stored at 5ºC are capable of growing at this temperature. It has been reported in another study that 40 strains from India also grew well at 41ºC, but all of them were from clinical cases (Schubert, 1987).

The isolation studies indicated the overall incidence of aeromonads in food to be 36.88%. Highest share of these isolates (Table. 1, Fig.2) was achieved from fish, followed by poultry, pig and goat. However, the isolation rates were much lower than what has been reported from most of the western countries. In a comparable study from Romania, 51.40% from food of animal origin were found to be positive, but lesser isolation rates (20-24%) were also reported by other workers (Majeed *et al*., 1989; Gobat and Jemmi, 1993). The results recorded in the present study are in agreement with few reports which are in our country, which has reported isolation rates to be about 4-10% (Yadav *et al*., 1995). Lower isolation rates recorded in this study could be due to geographical variations and the type of samples processed (Khardori and Fainstein, 1998).

One hundred thirty seven Fish samples examined in this study yielded 18 *Aeromonas* isolates (13.13%). Incidently, fish isolates had the highest share in total no. of isolates.

In the present study, *A. hydrophila* (92.10%) was the predominant species from fish, followed by *A. sobria* (5.26%) and *A. caviae* (2.63%). This similar pattern has been observed in earlier studies (Hudson and deLacy, 1991).

Isolation of *Aeromonas* at a higher rate is highly significant, as fish is considered to be the reservoir of *Aeromonas* spp. in this sub-continent (Rahim *et al*., 1984). It assumes great importance as *Aeromonas* spp. may cause morbidity and mortality in fishes and also pose public health threat.

One hundred and four Chichen samples examined in this study yielded 12 (11.5%) *Aeromonas* isolates. *A. hydrophila* was the predominant species. This was because the birds are regularly fed fish meal in the farms, which might be the source of infection. In a comprehensive study, Glunder and Siegmann (1990) observed that, the rate of isolation of *Aeromonas* was higher in wild birds and the infection was dependent on the habitats and dietary habits which influenced the occurrence of aeromonads in them.

Birds may be an important reservoir and may play an important role in disseminating the *Aeromonas* spp. because of their flying capacity. Fifty-one Pork samples were examined in the present investigation that yielded 7 *Aeromonas* isolates. The predominant species observed was *A. hydrophila*.

Among all the Goat samples examined, the lowest incidence was observed in goat meat and the isolate was identified as *A. hydrophila*. It appears that meat is not the primary source of infection in India (Amadi *et al*. (2005) had also reported presence of *A. hydrophila* and *A. sobria* in meat and offals but these were unlikely to pose any public health problems in Nigeria as meat usually undergoes prolonged cooking procedures. This is contrary to the reports from other western countries, where meat is considered to be reservoir for *Aeromonas* spp.

From the studies it appears that the occurrence of *Aeromonas* spp. varies from country to country in regard to differences in food and geographical conditions.

The frequencies of bacterial strains resistant to antimicrobial agents have increased dramatically in the environment as a consequence of the wide spread use of drugs (Kruse and Sorum, 1994). A significant public health concern and the possibility of transfer of resistant genes between bacteria in the natural habitats have attracted attention.

In the present study, antimicrobial susceptibility pattern against 17 antibiotics was studied for 38 *Aeromonas* isolates. All the 38 isolates were recovered from a media (ADA) containing ampicillin. The results were interpreted according to the diameter of the zone of inhibition as per the manufacturer's (HI-MEDIA Laboratories, Mumbai) instructions.

Rabbit ileal loop assay is commonly used to detect the enterotoxigenic potential of many entero pathogens including *Aeromonas* spp. (Asao *et al*., 1984). In the present study, CFF of 12 isolates were tested by this method. A dilation index (DI) of 0.4 and above was considered positive. Singh and Sanyal (1997) reported that all the three species of *Aeromonas* were shown to be potentially enterotoxigenic regardless of the source and strains that showed little or no fluid accumulation in initial experiments but became enterotoxin producers after one to three passages through rabbit ileal loops. Asao *et al*. (1984) had also reported positive RIL test with purified haemolysin.

CONCLUSION

The information on aeromonads infection in food and water is lacking from North East India.The diseases caused by aromonas are not known from contaminated foods.The main diet of people of this region comprises meat and fish products.Isoolation and identification of aeromonads from meat and fish food can be used for knowing the cause of traveler's diahoerrea and other food born diseases.This will be helpful in reducing the aeromonad infection in food.Among the different species of aeromonas hydrophila was found commonly associated with all kinds of foods of animal origin.However the infection level differed in different types of meat and fish food samples.

ACKNOWLEDGMENTS

The first author is thankful to the university authorities for providing financial assistance in the form of scholarship.

REFERENCES

1. Gracey, J.F. Thornton's meat hygiene. 7th Edn. (E.L.B.S.). Beccles and London.William Clowes. Ltd.1985
2. Bachill, V.N. Ahluwalia, S.S. Occurrence of coliform in raw meat. *J. Microbiol.,* 1973; **13:**165-167.
3. Joseph, S.W.; Colwell, R.R.McDonell, M.T. Taxonomy, ecology, isolation and identification: *Aeromonas* taxonomy. *Experrientia*. 1987; **43:** 349-550.
4. Palumbo, A.S.; Maxino, F.; Williams, C.A.; Buchannan, L.R.Thayer, W.D. Starch-Ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. *Appl. Environ. Microbiol*. 1985; **50(4):** 1027-1030.
5. Majeed, K.N.; Egan, A. MacRae, I.C. Enterotoxigenic aeromonads on retail retail lamb meat and offal. *J. Appl. Bacteriol.* 1989; **67(2):** 165-170.

6. Palumbo, A.S.; Bencivengo, M.M.; Corral, D.F.; Williams, C.A. Buchanan, L.R. . Characterization of the *Aeromonas hydrophila* group isolated from retail foods of animal origin. *J. Clin. Microbiol.,* 1989;**27(5):** 854-859.
7. Cruickshank, R.; Duguid, J.P.; Marmion, B.P. Swain, R.H.A. Medical Microbiology. 12th Edn. 2. Edinburg, London and New York. Churchill Livingstone 1975.
8. Sahu, K.R.; Palumbo, A.D. Galvani, D.K. Selective media for *Aeromonas* spp. *J., Bacteriol*. 1996;**23:** 456-460.
9. Chang, J.B. and Bolton, M.S. Plasmids and resistance to antimicrobial agents in *Aeromonas sobria* and *Aeromonas hydrophila* clinical isolates. *Antimicrob. Agents. Chemother*. 1986; **31(8):** 1281-1282.
10. Merino, S.; Rubires, X.; Knochel, S. Thomas, J.M. Emerging Pathogens *Aeromonas* spp., *Int. J. Food Microbiol.,* 1995;**28:** 157-168.
11. Gracey, M.; Burke, V. Robinson, J. Short reports- *Aeromonas* spp. in traveller's diarrhoea. *British Med. J*. 1982; 289:658.
12. Burke, V.; Robinson, J.; Beaman, J.; Gracey, M.; Lesmana, M.; Rockhill, R.; Echeverria, P. Janda, J.M. Correlation of enterotoxicity with biotype in *Aeromonas* spp. *J. Clin. Microbiol*. 1983;**18(5):** 1196-1200.
13. Champsaur, H.; Andermont, A.; Matheiu, D.; Rottman, E. Auzepy, P. Cholera-like illness due to *Aeromonas sobria*. *J. Infec. Dis.* 1982; **145:** 361-366.
14. Agger, W.A. Diarrhoea associated with *Aeromonas hydrophila*. *Pediatr. Infect. Dis.,* 1986; **5** (Suppl.)**:** 105-108.
15. Boulanger, Y.; Lallier, R. Cousineau, G. Isolation of enterotoxigenic *Aeromonas* from fish. *Can. J. Microbiol*. 1977;**23(9):** 1161-1164.
16. Gray, S.J.; Stickler, D.J. Bryant, T.N. The incidence of virulence factors in mesophilic *Aeromonas* species isolated from farm animals and their environment. *Epidemiol. Infect*., 1990;**105:** 277-294.
17. Das, A.M. Paranjape, V.L. *Aeromonas sobria* in babaline (water buffalo) abortion : growth requirements, biochemical characters, antibiotic susceptibility, experimental pathogenicity and serology. *Ind. J. Exp. Biol.,* 1990; **28:** 341-345.
18. Pal, J. Pradhan, K. Bacterial involvement in ulcerative condition of air breathing fish from India. *J. Fish. Biol*., 1990; **36:** 833-839.
19. Burke, V.; Robinson, J.; Cooper, M.; Beaman, J.; Partridge K.; Peterson, D. Gracey, M. Biotyping and virulence factors in clinical and environmental isolates of *Aeromonas* species. *Appl. Environ. Microbiol*. 1984; **47(5):** 1146-1149.
20. Garg, S.R.; Jindal, N.Kumar, A. Characterization of motile Aeromonas – isolated from sewage. *Indian J. Comp. Microbiol. Immunol. Infect. Dis*., 1994;**15:** 48-50.
21. Kay, B.A.; Gnerreo, C.E. Sack, B. Media for the isolation of *Aeromonas hydrophila*. *J. Clin. Microbiol.* 1985; **22:** 888-890.
22. Markwardt, M.N.; Gocha, M.Y. Klontz, W.G. A new application for Coomassie Brilliant Blue agar: Detection of *Aeromonas salmonicida* in clinical samples. *Dis. Aquat. Org*. 1989; **6:** 231-233.
23. Kersters, I.; Smeyers, M. Verstreate, W. Comparison of different media for the enumeration of *Aeromonas* spp. in fresh waters. *J. Appl. Bacteriol*. 1996; **1:** 257-261.
24. Havelaar, A.H.; During, M. Versteegh, J.F.Ampicillin dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filteration. *J. Appl. Bacteriol.* 1987; **62(3):** 279-287.
25. Von Graevenitz, A. Bucher, C. Evaluation of different selective media for isolation of *Aeromonas* and *Plesiomonas* spp. from human feces. *J. Clin. Microbiol.*, 1993; **17:** 16-21.
26. Schubert, R.H.W. Ecology of Aeromonads and isolation from environmental samples. *Experientia,* 1987;**43:** 351-354.
27. Gobat, P.-F. Jemmi, T. Comparison of seven selective media for the isolation of mesophilic *Aeromonas* species in fish and meat. *Int. J. Food. Microbiol.* 1995; **24(3):** 375-384.
28. Yadav, A.S.; Singh, B.R.; Kapoor, K.N.; Rathore, R.S.Verma, S.S. Occurrence of enterotoxigenic *Aeromonas hydrophila* in fish. Compendium, second Annual Conference of IAAVR, Hisar, 1995; pp. 33.
29. Khardori, N. Fainstein, V. *Aeromonas* and *Plesiomonas* as etiological agents. *Ann. Rev. Microbiol*. 1998; **42:** 395-419.
30. Hudson, J.A. Delacy, K.M. Incidence of motile aeromonads in New Zealand retail foods. *J. Food Protect.* 1991; **54:** 696-699.
31. Rahim, Z.; Sanyal, S.C.; Aziz, S.M.K.; Huq, I.M. and Chowdhury, A.A. (1984). Isolation of enterotoxigenic, haemolytic and antibiotic resistant *Aeromonas hydrophila* strains from infected fish in Bangladesh. *Appl. Environ. Microbiol.* **48(4):** 865-867.
32. Glunder, G. Siegmann, O. Occurrence of

Aeromonas hydrophila in wild birds. *Avian Pathol.*, 1990; **18:** 685-695.

33. Amadi, E.N.; Obunwenre, B. and Akani, N.P. (2005). Occurrence of hemolysin-producing aeromonads in meat and offal sold in Port Harcourt, *Nigeria. J. Food Sfy.* **25(3):** 183-192.
34. Kruse, H. sorum, H. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Appl. Environ. Microbiol.* 1994; **60(11):** 4015-4021.
35. Asao, T.; Kinoshita, Y.; Kozaki, S.; Uemura, T. Sakaguchi, G. Purification and some properties of *Aeromonas hydrophila* haemolysin. *Infect. Immun.* 1984; **46(1):** 122-127.
36. Singh, D.V. Sanyal, S.C. Enterotoxicity, haemolytic activity and antibiotic susceptibility if *Aeromonas eucrenophila* strains isolated from water and infected fish. *Indian. J. Exp. Biol.* 1997; **35**(2): 144-147.