African Journal of Microbiology Research Vol. 6(2), pp. 460-464, 16 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1447
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Study of prevalence and antibiotic resistance in *Aeromonas* species isolated from minced meat and chicken samples in Iran

M. M. Soltan Dallal[1,2,3], M. K. Sharifi Yazdi [4,5] and S. Avadisians[1]*

[1]Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
[2]Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[3]Antimicrobial Resistant Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[4]Department of Medical Laboratory Sciences, School of Paramedicine, Tehran University of Medical Sciences, Tehran, Iran.
[5]Zonotic Research Centre. Tehran University of Medical Sciences, Tehran, Iran.

Accepted 27 December, 2011

***Aeromonas* spp. was commonly isolated from ground meat and chicken samples at the retail level. From January to September 2009, 92 samples of chicken and 158 samples of minced meat for sale in retail outlets from regions under supervision of Tehran University of Medical Sciences (TUMS, Iran) were analyzed for the prevalence of *Aeromonas* species. Aeromonads were isolated from 80 (32%) of the samples analyzed, including 53 (57.6%) of chicken and 27 (17%) of minced meat. The isolation rate in chicken was significantly higher than minced meat ($p<0.001$). The highest contamination was found in chicken with *Aeromonas caviae* and minced meat samples contaminated with *Aeromonas hydrophila*. A total of 105 strains were isolated belonging to five species: *A. hydrophila* 43 (41%), *A. caviae* 43 (41%), *Aeromonas sobria* 16 (15.3%), *Aeromonas jandaei* 2 (1.8%) and *Aeromonas veronii* 1 (0.9%). The antibiotic resistance pattern shows that more than 90, 80, 70 and 60% of the strains were resistant to Ampicillin, Cephalothin, Tetracycline and Nalidixic acid, respectively.**

Key words: *Aeromonas*, chicken, meat, antimicrobial resistance, Iran.

INTRODUCTION

Foodborne diseases are an important public health problem in both developed and developing countries. Aeromonads have emerged as foodborne pathogen with worldwide distribution (Merino et al., 1995). There are currently 15 identified and 2 non-identified species that can be categorized into at least 17 DNA hybridization groups (Huys et al., 2005; Tacao et al., 2005). Aeromonads are isolated worldwide from drinking, estuary and wastewater (Ormen and Ostensvik, 2001; Sen and Rodgers, 2004), and also from different kinds of food (Bin Kingombe et al., 2004; Kirov, 1993; Radu et al., 2003; Villari et al., 2000) . In a study, motile *Aeromonas* species were present in all samples including retail lamb meat and offal; so it was concluded that meat products were potentially significant sources of virulent *Aeromonas* species and might play an important role in the etiology of *Aeromonas* gastroenteritis (Majeed et al., 1989). Aeromonads had been regarded as controversial pathogens; however, recent investigations have indicated some strains are entropathogenic (Garibay et al., 2006; Horii et al., 2005; Pokhrel and Thapa, 2004; Sinha et al., 2004; Soltan Dallal and Moezardalan, 2004; Vila et al., 2003). Of the various virulence factors described to

*Corresponding author. E-mail: sovenavedissian@yahoo.com. Tel: + 98-21-88992971. Fax: + 98-21-66462267.

explain the pathogenicity of *Aeromonas* species, beta hemolytic activity seems to be related to enterotoxigenicity (Chopra et al., 1991; Wong et al., 1998). This property is found among clinical samples as well as isolates from environmental samples (Krovacek et al., 1994; Pin et al., 1995; Chopra and Houston, 1999). The antibiotic resistance patterns of such species showed that mostly was resistance to Ampicillin, Amoxicillin, Cotrimoxazole and Cephalothin (Ghenghesh et al., 2008). Moreover, there is good evidence that *Aeromonas* species can express different virulence factors not only at optimal growth temperature, but also at refrigerated temperatures (Merino et al., 1995). This may be of importance to raw food products that are kept at refrigeration and have an extended shelf-life at this temperature. Consequently *Aeromonas* species should be carefully monitored in foodstuffs as they are possible source of food borne infection (Neyts et al., 2000a).

Since meat products are important source of nutrition and could act as a factor in transfer of pathogenic strain and since there is no any published report as to the prevalence and patterns of *Aeromonas* species isolated from raw meat in Iran, we conducted the present study. We investigated *Aeromonas* strains isolated from retail raw chicken samples and minced meat in regions under supervision of TUMS.

MATERIALS AND METHODS

Samples

Between January and September 2009, a total of 250 samples including 71 of fresh chicken, 21 of frozen chicken, 56 of fresh minced meat, 57 of frozen minced meat and 45 of frozen processed minced meat were randomly purchased at the retail level in regions under supervision of TUMS. All samples were transferred to the laboratory and microbiological analyses were carried out immediately.

Isolation and identification

After carrying the samples to the lab, 10 g of the each sample were homogenized with 90 ml of 0.1% (w/v) peptone water (tryptone 1 g/L; NaCl 5 g/L; pH: 7.2) in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 1 min. Then, 1 ml of homogenized solution was inoculated in 9 ml of alkaline peptone water supplemented with cephalotine (CAPW-10) (cephalotine 10 mg/L; pH: 8.4) and was incubated at 28℃ for 18 h. One loo pful of enriched sample was plated onto sheep-blood agar supplemented with ampicillin (ASBA) (Ampicillin 30 mg/L; 5% defibrinated sheep blood) and incubated at 37℃ for 24 h. Presumptive *Aeromonas* colonies were investigated by Gram staining, Oxides and other diagnostic tests: Indole, Motility, Mannitol fermentation, Glucose fermentation, production of gas from glucose and growth on Thiosulfate-Citrate-Bile salts-Sucrose agar (TCBS) medium. In the final stage for separation of *Aeromonas* at the level of species, lactose fermentation, hemolysis, Voges-Proskauer (VP) test, ornithine decarboxylase (ODC), arginine dihydrolase (ADH) and lysine decarboxylase (LDC) tests were used, and for further confirmation, the API-20E kit (bioMerieux, Marcy-l'Etoile, France) was applied.

Antibiotic sensitivity tests

Antibiotic sensitivity was determined by the single disk diffusion method of Bauer et al. (1966) and as recommended by the National Committee for Clinical Laboratory Standards (2004) using Mueller–Hinton agar. Bacteria were cultivated in Luria Bertani broth (tryptone 10 g/L; NaCl 5 g/L; yeast extract 5 g/L) and harvested by centrifugation for 5 min at 8500×g. The cell pellets were resuspended in 0.85% (w/v) saline and were spread onto the surface of the Mueller–Hinton agar plates. Discs containing antibiotics (BBL Sensi-Disc, Becton Dickinson, and Cockeysville, MD, USA) were spotted with a 3 cm interval. The 14 antibiotics and their sensi-disc concentrations were: Ampicillin (10 µg), Carbenicillin (100 µg), Cephalothin (30µg), Chloramphenicol (30 µg), Tetracycline (30 µg), Gentamicin (10 µg), Nalidixic acid (30 µg), kanamycin (30 µg), amikacin (30 µg), neomycin (30 µg), trimethoprim-sulfamethoxazole (25 µg), Ofloxacin (5 µg), Tobramycin (10 µg) and Rifampin (5 µg). The plates were incubated at 37℃ for 24 h. Characterization of strains as sens itive or resistant was based on the size of inhibition zones around each disc according to the manufacturer's instructions.

Hemolysis tests

The strains were tested for hemolytic activity by streaking onto blood agar plates containing 5% defibrinated sheep red blood cells and incubated at 37℃ for 24 h. Hemolytic activity was determined as a zone of hemolysis around the colonies (Brender and Janda, 1987).

Statistics analysis

Statistical analysis of results was performed using SPSS/PC 11.5 software (SPSS Chicago, IL). The chi-square test and Fisher's exact two-tailed test were used for statistical analysis. A P value < 0.05 used for statistical significance.

RESULTS

As seen from Table 1, *Aeromonas* species were isolated from 80 (32%) out of the 250 samples analyzed, including 53 (57.6%) chicken and 27 (17%) minced meat samples. The isolation rate from chicken samples was significantly higher as compared to minced meat ($p<0.001$). More specifically, they were isolated from 70, 14, 21, 9 and 22% of 71 fresh chicken, 21 frozen chicken, 56 fresh minced meat, 57 frozen minced meat and 45 frozen processed minced meat samples, respectively. Fresh and frozen samples were also compared (49 vs. 15%, $p<0.001$) for the whole samples. For chicken samples, 70 vs. 14% ($p<0.001$), while for minced meat 21 vs. 9% ($p=0.06$).

Of the 105 isolates obtained*, Aeromonas hydrophila* (43/105) and *Aeromonas caviae* (43/105) were the most frequent isolated species, followed by *Aeromonas sobria* (16/105), *Aeromonas jandaei* (2/105) and *Aeromonas veronii* (1/105).

Of the present isolates, more than 70% were positive for beta hemolytic activities (Table 2). The antibiotic resistance patterns of the *Aeromonas* species isolated

Table 1. Prevalence of *Aeromonas* species in the various samples examined.

Samples	No. of samples	No. of positive samples	No. of isolates	*A. hydrophila*	*A. caviae*	*A. sobria*	*A. jandaei*	*A. veronii*
Fresh chicken	71	50 (70%)	72	23 (32%)	36 (50%)	11 (15.2%)	1 (1.4%)	1 (1.4%)
Frozen chicken	21	3 (14%)	4	3 (75%)	1 (25%)	-	-	-
Fresh minced meat	56	12 (21%)	12	4 (33.3%)	2 (16.7%)	5 (41.6%)	1 (8.4%)	-
Frozen minced meat	57	5 (9%)	6	4 (66.7%)	2 (33.3%)	-	-	-
Frozen processed minced meat	45	10 (22%)	11	9 (81.8%)	2 (18.2%)	-	-	-
Total	250	80 (32%)	105	43 (41%)	43 (41%)	16 (15.3%)	2 (1.8%)	1 (0.9%)

from the various meat samples are shown in Table 3. More than 90% of the strains were resistant to Ampicillin.

DISCUSSION

The prevalence of *Aeromonas* species observed in this study was 32%. The possibility of the isolation of *Aeromonas* from a variety of sources, such as water and foodstuffs, hinders the assignment of the gastroenteritis secondary to the pathogenic agent to a given source. As the result, dozens of studies performed on the subject yield different findings.

In a study with a total of 563 samples of various foodstuffs, 287 were found to contain *Aeromonas* species. The type of samples most frequently contaminated were poultry (79.3%) and offal (84.3%) (Fricker and Tompesett, 1989). While, another investigation done in Australian lamb meat and offal samples found all of the samples to be contaminated by *Aeromonas* species (Majeed et al., 1989). Finally, having examined 68 samples, Neyts et al. (2000b) isolated *Aeromonas* species from 26% of vegetables, 70% of the meat and poultry samples and 72% of the fish and shrimps.

In the present study, out of 92 samples of chicken and 158 of minced meat, 53 (57.6%) and 27 (17%) respectively, were found to contain *Aeromonas* species. Accordingly, a research done with stool, food and environmental specimens aimed to determining the source and rout of infection, reached the result that 11.1% of diarrheal stools, 2.2% of normal stools were contaminated by the pathogen. Foodstuffs examination showed the unexpectedly high frequency of the pathogen. The authors, thus, suggested that infection might be food borne rather than waterborne (Nishikawa and Kishi, 1988). The idea is still a matter of conjecture and is not established as yet; while a series of studies implicate the waterborne as the main route (Ghenghesh et al., 2001; Huddleston et al., 2006; Koksal et al., 2007; Pokhrel and Thapa, 2004; Rahman et al., 2007; September et al., 2007), the other show the contrary idea to be the case (Bin Kingombe et al., 2004; Neyts et al., 2000 b; Villari et al., 2000).

A biological advantage of Aeromonads is their survival, growth and exotoxin production at low temperatures (Majeed, 1996). In order to show the effect of freezing on the contamination rate, the fresh and frozen (-20°C) samples were compared; according to the results, the effect was statistically significant for chicken samples ($p<0.001$), while for minced meat, despite its reducing effect it did not reach a statistically significant level ($p=0.06$). It is notable that freezing cannot completely prevent from contamination, thus it cannot be suggested as a unique factor to be controlled so as to minimize the contamination.

Concerning the predominant species, results of different studies just slightly differ. Most studies indicate *A. hydrophila* to be more frequently found in meat products (Gobat and Jemmi, 1993; Hanninen, 1993; Kumar, 2000) as well as other specimen (Ghenghesh et al., 2001; Hatha et al., 2005; Koksal et al., 2007; Villari et al., 2000). Interestingly, *A. hydrophila* is the predominant strain in clinical samples, which can prove the role it can play in pathogenesis. Therefore, it deserves even more attention (Garibay et al., 2006; Horii et al., 2005; Pokhrel and Thapa, 2004). In this study, the predominant species in minced meat was *A. hydrophila* (59%) followed by *A. caviae* (20.6%), *A. sobria* (17%), *A. jandaei* (3.4%). In chicken, the predominant species was *A. caviae* (49%) followed by *A. hydrophila* (34%), *A. sobria* (14.4%), *A. jandaei* (1.3%) and *A. veronii* (1.3%). *A. jandaei* and *A. veronii* have been less frequently reported in other studies.

More than 70% of isolates were positive for hemolytic activities (Table 2). The main hemolytic species were *A. sobria* (100%) and *A. veronii* (100%) followed by *A. hydrophila* (93%). *A. caviae*

Table 2. Hemolysin production on SBA plates.

Species	No. isolates	No. positive	Percentage positive
A. hyrophila	43	40	93
A. caviae	43	18	42
A. sobria	16	16	100
A. jandaei	2	0	0
A. veronii	1	1	100
Total	105	75	71

Table 3. Susceptibility of *Aeromonas* species to various types of antibiotics.

Antibiotics (µg)	Resistant (%)	Intermediate (%)	Sensitive (%)
Ampicillin (10)	91	7.5	1.5
Cephalothin (30)	82.5	16	1.5
Tetracycline (30)	69	5	26
Nalidixic acid (30)	61	-	39
Rifampin (5)	4	82	14
Kanamycin (30)	25	44	31
Trimethoprim-sulfamethoxazole (25)	14	4	82
Neomycin (30)	4	12	84
Chloramphenicol (30)	5	5	90
Ofloxacin (5)	-	5	95
Tobramycin (10)	1.5	2.5	96
Amikacin (30)	1.5	1.5	97
Gentamicin (10)	1.5	1.5	97
Carbenicillin (100)	-	2.5	97.5

and *A. jandaei* showed only 42 and 0% hemolytic activity, respectively. In a study with isolates from farm raised fresh water fish, hemolytic activity was detected mostly in *A. hydrophila,* while only half of the *A. sobria* and *A. caviae* showed this activity (Hatha et al., 2005; Ashiru et al., 2011). Thus far, much attention has been paid to the hemolysin of motile *Aeromonas* species for its pathogenicity potential (Namdari and Bottone, 1990). As defined in Wong et al. (1996), all *Aeromonas* isolates with hemolysin-positive genotype were virulent in the suckling mouse assay model.

Most of the strains isolated in this work were resistant to Ampicillin (91%), Cephalothin (82.5%), Tetracycline (69%) and Nalidixic acid (61%). Their resistance was intermediate for Rifampin (82%) and Kanamycin (44%). Sensitivities higher than 80% were observed in the isolates for Trimethoprim-Sulfamethoxazole, Neomycin, Chloramphenicol, Ofloxacin, Tobramycin, Amikacin, Gentamicin and Carbenicillin. Those showed the begging of antibiotic resistance in environmental samples (Ghenghesh et al., 2008). In another study that we performed on diarrheal stools of children, antibiotic resistance pattern was as follows: total resistance to Ampicillin (100%), intermediate resistance to Tetracycline (57%) and Kanamycin (43%) and 100% sensitivity to Gentamicin and Chloramphenicol and finally 92% sensitivity to nalidixic acid (Soltan and Moezardalan, 2004). A comparison of the antibiotic resistance pattern with that of the present study, revealed much similarity. Of note is the altered antibiotic resistant pattern of the bacteria toward resistance to tetracycline and nalidixic acid. This could have been the repercussion of ignorant use of common antibiotics, without testing the diarrhea stool samples of the patient further leading to development of resistance in the said bacterium in the test population. These results confirmed the statement of Munro and Hasting (1993) that using antimicrobial agents would raise the problem of drug-resistance development in the pertinent strains.

In conclusion, it seems completely necessary to pay even more attention to Aeromonads in the field of food microbiology for their abilities to produce toxin, survive under low temperatures and live in a broad spectrum of environments. As the bacteria are able to produce gastroenteritis especially in children, the elderly and immunocompromised patients, more focus must be placed on checking the diarrhoeal stools for Aeromonads as to detect, to identify and to administer proper

antibiotics.

REFERENCES

Ashiru AW, Uaboi-Egbeni PO, Oguntowo JE, Idika CN (2011). Isolation and antibiotic profile of *Aeromonas* species from Tilapia fish (*Tilapia nilotica*) and Catfish (*Clarias betrachus*). Pakistan J. Nutr. 10(10): 982-986.

Bauer AW, Kirby WMM, Sherris JC, Turck M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bin Kingombe CI, Huys G, Howald D, Luthi E, Swings J, Jemmi T (2004). The usefulness of molecular techniques to assess the presence of A*eromona*s spp. harboring virulence markers in foods. Int. J. Food Microbiol., 94, 113-121.

Brender R, Janda JM (1987). Detection, quantification and stability of the beta-haemolysin of *Aeromonas* spp. J. Med. Microbiol., 24: 247-251.

Chopra AK, Houston CW, Kurosky A (1991). Genetic variation in related cytolytic toxins produced by different species of *Aeromonas*. FEMS Microbiol Lett., 62, 231-237.

Chopra AK, Houston CW (1999). Enterotoxins in *Aeromonas*-associated gastroenteritis. Microbes Infect., 1: 1129-1137.

Fricker CR, Tompesett S (1989). *Aeromonas* spp. In foods: a significant cause of food poisoning? Int. J. Food Microbiol., 9: 17-23.

Garibay RI, Aguilera-Arreola MG, Ocañia AN, Cerezo SG, Mendoza MS, López JM, Campos CE, Cravioto A, Castro-Escarpulli G (2006). Serogroups, K1 antigen, and antimicrobial resistance patterns of *Aeromonas* spp. strains isolated from different sources in Mexico. Mem. Inst. Oswaldo Cruz, 101: 157-161.

Ghenghesh KS, El-Ghodban A, Dkakni R, Abeid S, Altomi A, Abdussalam T, Marialigeti, K (2001). Prevalence, species differentiation, haemolytic activity and antibiotic susceptibility of *Aeromonads* in Untreated Well Water. Mem. Inst. Oswaldo Cruz, 96: 169-173.

Ghenghesh KS, Ahmed SF, Abdel El-Khalek R, Al-Gendy A, Klena J (2008). A*eromonas*- associated infections in developing countries. J. Infect. Dev. Ctries, 2(2): 81-98.

Gobat PF, Jemmi T (1993). Distribution of mesophilic *Aeromonas* species in raw and ready-to-eat fish and meat products in Switzerland. Int. J. Food Microbiol., 20: 117-20.

Hanninen ML (1993). Occurrence of *Aeromonas* spp. in samples of ground meat and chichen. Int. J. Food Microbiol ., 18: 339-342.

Hatha M, Vivekanandhan AA, Joice GJ, Christol (2005). Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish. Int. J. Food Microbiol., 98: 131-134.

Horii T, Morita M, Muramatsu H, Monji A, Miyagishima D, Kanno T, Maekawa M (2005). Antibiotic resistance in *Aeromonas hydrophila* and *Vibrio alginolyticus* isolated from a wound infection: A case report. J. Trauma. 58: 196-200.

Huddleston JR, Zak JC, Jeter RM (2006). Antimicrobial susceptibilities of *Aeromonas* spp. isolated from environmental sources. Appl. Environ. Microbiol .,72: 7036-7042.

Huys G, Cnockaert M, Swings J (2005). *Aeromonas culicicola* Pidiyar et al. 2002 is a later subjective synonym of *Aeromonas veronii* Hickman-Brenner et al. (1987). Syst. Appl. Microbiol., 28: 604-609.

Kirov SM (1993). The public health significance of *Aeromonas* spp. in foods. Int. J. Food Microbiol., 20: 179-198.

Koksal F, Oguzkurt N, Samasti M, Altas K (2007). Prevalence and antimicrobial resistance patterns of *Aeromonas* strains isolated from drinking water samples in Istanbul, Turkey. Chemotherapy, 53: 30-35.

Krovacek K, Pasquale V, Baloda SB, Soprano V, Conte M, Dumontet S (1994). Comparison of putative virulence factors in *Aeromonas hydrophila* strains isolated from the marine environment and human diarrheal cases in southern Italy. Appl. Environ. Microbiol., 60: 1379-1382.

Kumar A, Bachhil VN, Bhilegaonakar KN, Agarwal RK (2000). Occurrence of enterotoxigenic *Aeromonas* species in foods. J. Commun. Dis., 32: 169-74.

Majeed K, Egan A, MacRae IC (1989). Enterotoxigenic *Aeromonads* on retail lamb and offal. J. Appl. Bacteriol., 67: 165-170.

Majeed KN (1996). Growth and exotoxin production at low temperatures by *Aeromonas* strains in meat slurries. Microbiol., 85: 105-115.

Merino S, Rubires X, Knochel S, Tomas JM (1995). Emerging pathogens: *Aeromonas* spp. Int. J. Food Microbiol., 28: 157-168.

Munro ALS, Hasting TS (1993). Furunculosis. In: Inglis, V., J. R. Roberts, and N. R.Bromage (eds), Bactreial Disease of Fish, Blackwell, Oxford, pp. 122-142.

Namdari H, Bottone EJ (1990). Microbiologic evidence supported the role of *Aeromonas caviae* as a pediatric enteric pathogen. J. Clin. Microbiol., 28: 837-840.

National Committee for Clinical Laboratory Standards (2004). Performance Standards for Antimicrobial Susceptibility Testing, eighth ed., Fourteenth Informational Supplement Document M2-A8, Wayne, Pennsylvania.

Neyts K, Notebaert E, Uyttendaele M, Debevere J (2000a). Modification of the bile salts-Irgasan-brilliant green agar for enumeration of *Aeromonas* species from food. Int. J. Food Microbiol., 57: 211-218.

Neyts K, Huys G, Uyttendaele M, Swings J, Debevere J (2000b). Incidence and identification of *Aeromona*s spp. from retail foods. Lett. Appl. Microbiol., 31: 359-363.

Nishikawa Y, Kishi T (1988). Isolation and characterization of motile *Aeromonas* from human, food and environmental specimens. Epidemiol. Infect., 101: 213-23.

Ormen O, Ostensvik O (2001). The occurrence of aerolysin-positive *Aeromonas* spp. and their cytotoxicity in Norwegian water sources. J. Appl. Microbiol., 90: 797-802.

Pin C, Marin ML, Selgas D, Garcia ML, Tormo J, Casas C (1995). Differences in production of several extracellular virulence factors in clinical and food *Aeromona*s spp. Strains. J. Appl. Bacteriol., 78: 175-179.

Pokhrel BM, Thapa N (2004). Prevalence of *Aeromonas* in different clinical and water samples with special reference to gastroenteritis. Nepal. Med. Coll J., 6: 139-143.

Radu S, Ahmad N, Ling FH, Reezal A (2003). Prevalence and resistance to antibiotics for *Aeromonas* species from retail fish in Malaysia. Int. J. Food Microbiol., 81: 261-266.

Rahman M, Huys G, Rahman M, Albert MJ, Kühn I, Möllby R (2007). Persistence, Transmission, and Virulence Characteristics of *Aeromonas* Strains in a Duckweed Aquaculture-Based Hospital Sewage Water Recycling Plant in Bangladesh. Appl. Environ. Microbiol., 73: 1444-1451.

Sen K, Rodgers M (2004). Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. J. Appl. Microbiol., 97: 1077-1086.

September SM, Els FA, Venter SN, Brözel VS (2007). Prevalence of bacterial pathogens in biofilms of drinking water distribution systems. J. Water Health., 5: 219-227.

Sinha S, Shimada T, Ramamurthy T, Bhattacharya SK, Yamasaki S, Takeda Y, Nair GB (2004). Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. J. Med. Microbiol., 53: 527-534.

Soltan Dallal MM, Moezardalan K (2004). *Aeromonas* spp associated with children's diarrhoea in Tehran: a case-control study. Ann. Trop. Paediatr., 24: 45-51.

Tacão M, Moura A, Alves A, Henriques I, Saavedra MJ, Correia A (2005). Evaluation of 16S rDNA- and *gyrB*-DGGE for typing members of the genus *Aeromonas*. FEMS Microbiol Lett., 246: 11-18.

Vila J, Rui ZJ, Gallardo F, Vargas M, Soler L, Figueras MJ, Gascon J (2003). *Aeromonas* spp. and traveler's diarrhea: Clinical features and antimicrobial resistance. Emerg. Infect. Dis., 9: 552-555.

Villari P, Crispino M, Montuori P, Stanzione S (2000). Prevalence and molecular characterization of *Aeromonas* spp. in ready-to-eat foods in Italy. J. Food Prot., 63: 1754-1757.

Wong CY, Mayrhofer G, Heuzenroeder MW, Atkinson HM, Quinn DM, Flower RL (1996). Measurement of virulence of Aeromonads using a suckling mouse model of infection. FEMS Immunol. Med. Microbiol., 15: 233-241.

Wong CYF, Heuzenroeder MW, Flower RLP (1998). Inactivation of two haemolytic toxin genes in *Aeromonas hydrophila* attenuates virulence in a suckling mouse model. Microbiol. 144: 291-298.