Indian Journal of Animal Sciences **82** (8): 785–793, August 2012

Molecular detection of food-borne *Aeromonas* species

S GHATAK[1], R K AGARWAL[2], K N BHILEGAONKAR[3] and J P S GILL[4]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 5 October 2010; Accepted: 21 March 2012

ABSTRACT

The members of the genus *Aeromonas* have emerged as an important food-borne pathogen that pose many challenges to the scientific community. Difficulties regarding aeromonads involve its debated taxonomy, true role as pathogen and detection, particularly from foods. Various conventional techniques involving culture media and broths have been devised for identification form foods and immunological techniques *viz.* ELISA protocols have been standardized. Due to shortcomings of these methodologies molecular techniques were explored. These included nucleic acid hybridization probes, various formats of PCR, restriction fragment length polymorphism, amplified fragment length polymorphism, pulsed field gel electrophoresis, real time PCR, sequence analysis, DNA microarray technique, etc. Many of these modern assays have been promising, indicating that future detection paradigm for detection of *Aeromonas* will predominantly be based on molecular techniques. In this communication we have attempted to review these available molecular tools for detection of aeromonads from foods.

Key words: *Aeromonas*, Detection, Food, Molecular

Once considered an opportunistic pathogen, the organisms of the genus *Aeromonas* has emerged as an important food-borne pathogen for humans (Merino *et al.* 1995, Isonhood and Drake 2002, Pund and Theegarten 2008). Despite the discovery of these organisms dating more than a hundred years ago this collection of Gram negative, coccobacillary, oxidase and catalase positive organisms kept microbiologists busy debating its taxonomy, food-borne nature and clinical relevance (Janda and Abott 1998) However, years of insightful research findings world wide have led to a better understanding of the aeromonal biology and recognition of this unique organism as an emerging food-borne pathogen.

Emergence of Aeromonas species as food-borne pathogen: Aeromonads are autochthonous inhabitants of aquatic environment (Schubert 1991). In addition various foods also act as important reservoir for these organisms. The foods include seafood, chicken and red meat, vegetables, raw milk and milk products (Isonhood and Drake 2002).

Present address: [1] Senior Scientist (ghataksnd@rediffmail.com), Division of Animal Health, ICAR Research Complex for NEH Region, Umiam, Meghalaya 793103 India.

[2]Principal Scientist and Head (rajeshbly@ivri.res.in), Division of Bacteriology and Mycology; [3]Principal Scientist (kiranvph@rediffmail.com), Division of Veterinary Public Health.

[4]Professor and Head (gilljps@yahoo.com), Department of Veterinary Public Health, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab 141 004 India.

Among sea foods, aeromonads are reported in fish and fish-eggs, shrimp (Hanninen *et al.* 1997), prawn (Borch *et al.* 1996), finfish, shell fish (Palumbo *et al.* 1985, Tsai and Chen 1996). Among shellfishes, oyster had the highest incidence (Tsai and Chen 1996).

Aeromonas spp. has also been found in many types of meats including ground beef, chicken thigh meat, pork sausage, ground and unseasoned pork (Okrend *et al.* 1987). They are also found in high numbers in pig carcass processing equipment such as dehairing equipment, dehairing water, loin trimming belt and mesh gloves (Gill and Jones 1995). Keeping a view of endemicity of aeromonads in food processing lines in mind, Borch *et al*. (1996) proposed that *Aeromonas* spp. could be useful as an indicator of processing hygiene.

Reports on the prevalence of *Aeromonas* spp. in foods in India are far more limited. However, the organism has been isolated from variety of foods including fish, meat, milk, egg, tortoise and snail (Singh *et al.* 1997, Agarwal *et al.* 2000). Doke *et al*. (1994) isolated *Aeromonas* spp. from Indian mackerel. In 1997, a study by Khurana and Kumar in Haryana, showed distribution pattern of aeromonads to be 37.5% in sheep meat, 32.6% in edible organ of poultry, 28% in poultry meat, 24.2% in edible organs of sheep and 0.9% in poultry eggs. Agarwal *et al*. (2000) after investigating a wide range of foods found aeromonads in 22.22% of fish, 5% of tortoise, 6.25% of snail, 4.08% of other aquatic foods,

8.88% of goat meat, 2.85% of buffalo milk, 18% of quail eggs, whereas Kumar (1998) isolated aeromonads from fish (28.57%), poultry meat (16.67%), poultry eggs (12.50%), goat meat (12%), buffalo meat (7.69%) and cow milk (5.56%). *Aeromonas* spp. have also been isolated from about 12.5% of ice cream samples in Chennai (Shankar *et al.* 1994).

Besides foods, aeromonads have also been implicated in a number of food-borne outbreaks in various parts of the world including France, Japan, Norway and Sweden (Granum *et al.* 1998, Kingombe *et al.* 2004). Usually the food-borne aeromoniasis are now considered as emerging human pathogens as well as enterotoxigenic "neoenteropathogen" (Ljungh and Wadstrom 1985, Merino *et al.* 1995). Up to 13% of reported gastroenteritis cases in United States might be caused by *Aeromonas* spp. (Buchanan 1984). Gastroenteritis caused by *Aeromonas* spp. may assume either secretory (Gracey *et al.* 1982, Burke *et al.* 1983), dysenteric (Rahman and Willoughby 1980), chronic (for >10 days) (Gracey *et al.* 1982), choleraic (Gurwith *et al.* 1977, Champsaur *et al.* 1982, Swale *et al.* 1986) or traveller's diarrhoea form (Echeverria *et al.* 1984, Gracey *et al.* 1984, Vila *et al.* 2003). Several outbreaks of gastroenteritis caused by *Aeromonas* spp. have also been documented (Bloom and Bottone 1990, de La Morena *et al.* 1993). In addition to these, a unique outbreak of toxi-infection caused by preformed toxin of *Aeromonas hydrophila* has been reported by Cantoni *et al*. (2002).

Common housefly (*Mucsa* sp.) is often termed the 'porter of infection' and is usually related to gastroenteritis in humans. Reports of isolation of *Aeromonas* spp. from housefly indicated the possible role of flies in spreading aeromonal gastroenteritis (Nayduch *et al.* 2001, 2002).

Aeromonads are able to grow in raw, cooked and processed foods that are stored under refrigeration or packaged under modified atmosphere (Kirov 1993, Merino *et al.* 1995). Under these conditions, aeromonads are also able to elaborate toxins, posing a serious public health threat to the consumers of refrigerated and / or packaged ready to eat foods (Callister and Agger 1987, Kirov 1993).

Troubled taxonomy: Following initial placement of these organisms clubbed within genus *Aeromonas* under the family *Vibrionaceae*, the taxonomic position for aeromonads were reviewed. Finally, the genus was positioned under family *Aeromonadaceae* under order *Aeromonadales* within the class gamma-proteobacteria (Garitty *et al.* 2001). One of the most important consideration behind forming a new order was the phylogenetic depth and diversity displayed by these organisms. This is evident from the discovery of new species within the genus as recent as in the year 2008 (Alperi *et al.* 2008). Now 19 species comprise the genus *Aeromonas* (Table 1).

Detection dilemma: Despite convincing scientific evidences in favour of *Aeromonas* spp. as an important and emerging food-borne pathogen, till date there are relatively few attempts to detect aeromonads routinely from suspected foods. This may perhaps be explained due to the inability of the conventional isolation methods (Sachan and Agarwal 2000), more demanding biochemical tests for speciation of the organisms (Abott *et al.* 2003) and inadequacy of the current biochemical tests to differentiate *Aeromonas* spp. (Ormen *et al.* 2005).

Table 1. Members of the genus *Aeromonas*

A. allosaccharophila	*A. molluscorum*
A. aquariorum	*A. popoffi*
A. bestiarum	*A. salmonicida*
A. bivalvium	*A. schubertii*
A. caviae	*A. simiae*
A. encheleia	*A. sobria*
A. eucrenophila	*A. tecta*
A. hydrophila	*A. trota*
A. jandaei	*A. veronii* *
A. media	

* *A. veronii* has two distinct biogroups (bg.) – *A. veronii* bg. veronii and *A. veronii* bg. sobria

The resultant scenario therefore is presence of a number of enrichment media (viz. alkaline peptone water, cephalothin alkaline peptone water, etc.), selective plating media (viz. rimler-shotts agar, ampicillin blood agar, ampicillin dextrin agar, UNISC agar etc.) and commercial identification systems with none winning universal acceptance across researchers and laboratories (Khan and Cerniglia 1997, Soler *et al.* 2003a, Kingombe *et al.* 2004, da Rocha *et al.* 2008).

In a bid to overcome detection limitations of conventional methods, immunological techniques were explored by various researchers. One of the prime targets for the development of immunoassays for aeromonads was the outer membrane proteins (OMPs) of the organism. OMPs were characterized and looked into for presence of homogeneity (Ghatak *et al.* 2001) and then the common fractions were used for standardization ELISA for detection of *Aeromonas* from foods (Merino *et al.* 1993, Sendra *et al.* 1997, Sachan and Agarwal 2002, Ghatak *et al.* 2003). However, the application of such immunoassay protocols remained somewhat limited.

Molecular tools - a way forward: The advent of molecular techniques in food microbiology has given a fillip for the detection of *Aeromonas* spp. from foods. These techniques not only circumvented the limitations faced by conventional as well as immunological techniques, they proved to be rapid, able to identify hitherto unsuspected aeromonads from foods and were robust in their applications (Kingombe *et al.* 2004).

Polymerase chain reaction (PCR): Two years after the first description of polymerase chain reaction by Saiki *et al.* (1988), Pollard *et al.*(1990) for the first time published a PCR methodology for *A. hydrophila*, targeting a well known virulence gene called aerolysin (*aer*). Inspired by the initial success the same target was employed for PCR detection of *Aeromonas* from foods, fishes and other sources (Baloda *et*

al. 1995). These early developments were followed by a number of PCR protocols optimized for detection of aeromonads involving various targets namely, haemolysin gene, enterotoxin gene, cytotoxin gene (Hirono *et al.* 1992, Chopra *et al.* 1993, Wang *et al.* 1996, Santos *et al.* 1999, Ormen and Ostenvik 2001, Sechi *et al.* 2002, Abdullah *et al.* 2003, Agarwal *et al.* 2003) (Table 2).

The common feature in these assays was that the targets for PCR amplification were various virulence genes, based on virulence assays. Once optimized, many of these assays found successful application in determining distribution of virulence genes among collections of *Aeromonas* isolates (Gonzalez-Serrano *et al.* 2002, Sechi *et al.* 2002, Abdullah *et al.* 2003, Chacon *et al.* 2003, Seethalakshmi *et al.* 2008).

A significant advancement of virulence based assays were reported which were able to standardize a multiplex-PCR protocol for simultaneous detection of one cytotoxic (*Act*), and two cytotonic (*Alt*, *Ast*) enterotoxin gene for detection of *Aeromonas* spp. from food samples. The assay was applied to a relatively large number of suspected food poisoning samples and was reportedly rapid and specific (Chang *et al.* 2008).

Another class of PCR assays was also developed in parallel targeting housekeeping genes of aeromonads e.g. 16S rRNA, gyr B, rpo D, etc. These assays were employed for two main purposes, one for phylogenetic studies (Yanez *et al.* 2003) and the others for detection of aeromonads from various sources especially from food items like milk, chicken and fish samples (Porteen *et al.* 2006, 2007).

A valuable extension of application of PCR technology on housekeeping genes (16S rRNA and gyr B) of *Aeromonas* spp. was optimization of typing scheme for aeromonads based on PCR-denaturing gradient gel electrophoresis (PCR-DGGE). *gyr* B gene based PCR-DGGE was reportedly a powerful detection tool for aeromonads in complex samples (Tacao *et al.* 2005b). This technology is relatively new to food microbiology and promising for culture-independent food quality assessment (Ercolini 2004). Therefore it is expected and would be interesting to see the application of PCR-DGGE for monitoring and diversity evaluation of aeromonads in food.

Table 2. Common targets for PCR detection of *Aeromonas* spp.*

Virulence genes	Housekeeping genes
Aerolysin	16S rRNA
Cytotonic enterotoxin	*rpo D*
Cytolytic enterotoxin	*gyr B*
Haemolysin	Type III secretion systems
Lipase	¨ *asc V*
Protease	¨ *aex T*
Histone like protein	¨ *asc F*
Elastase	¨ *asc G*
AHCYTOEN	

*This list is indicative only.

As PCR technology kept evolving and found many new applications *Aeromonas* researchers experimented with newer technologies and reported many different PCR formats for diverse objectives. Among them notable are RAPD, ERIC and BOX-PCR techniques.

Randomly amplified polymorphic DNA (RAPD) – PCR: RAPD-PCR methodology comprises use of arbitrary primers to enable random priming at various locations of the target genome which is followed by low stringency amplification generating PCR products of varying sizes that are indicative of the nucleotide polymorphisms in the genomes under study. Since the technique is poised for delineating polymorphisms, it has been employed for strain differentiation and source discrimination among isolates of *Aeromonas* spp. (Oakey *et al.* 1996, Davin Regli *et al.* 1998, Talon *et al.* 1998, Alavandi *et al.* 2001, Aguilera-Arreola *et al.* 2005, Korbsrisate *et al.* 2007).

Enterobacterial repetitive intergenic consensus (ERIC) – PCR: Despite aeromonads not being members of *Enterobacteriaceae* family, ERIC-PCR technique has been employed by many researchers to gain insight into the differences among strains and to achieve other typing goals (Davin Regli *et al.* 1998, Soler *et al.* 2003, Aguilera-Arreola *et al.* 2005). ERIC elements comprise repetitive sequences in bacterial genomes not restricted only to enterobacterial genomes, which are targeted to produce unique fingerprint for target bacteria based on the relative abundance of the repeats within the bacterial genome. The discriminatory power of ERIC-PCR have been claimed to be at par or more than the RAPD-PCR for *Aeromonas* spp. (Soler *et al.* 2003b, Aguilera-Arreola *et al.* 2005).

BOX-PCR: Like ERIC elements, BOX elements too are repetitive sequences of 154 bp length found within the bacterial genome. PCR amplifying these repeats can be useful for fingerprinting and typing of bacteria. Based on this principle, Tacao *et al.* (2005a) successfully optimized a BOX-PCR protocol yielding fingerprints applicable for typing and epidemiological studies of *Aeromonas* spp.

Nucleic acid probes: Probes are usually short stretches (15 – 30 bp) of nucleic acid that are labeled with radio tracer of fluorogenic tracer. Probe assays utilize the binding ability of denatured nucleic acid strands between a target organisms' DNA/RNA and a complementary sequence bearing labeled probe. In case of binding or hybridization target DNA/RNA will emit signal which can be detected by suitable sensor.

As a downstream study to characterize RAPD band by cloning and sequencing, Okay *et al.* (1999) developed a digoxigenin (DIG) labeled probe for *A. hydrophila*. However, this probe was specific for only hybridization group 1 (HG1) of *Aeromonas* spp. and have not been reported to be applicable for detection of *Aeromonas* from foods. While, a labeled DNA probe based on the highly conserved outer membrane protein of *Aeromonas* spp. specifically detected

aeromonads in dot-blot assay, and was claimed to be suitable as rapid diagnostic tool for *Aeromonas* from foods (Khusiramani *et al.* 2009).

Restriction fragment length polymorphisms (RFLP): RFLP techniques involving endonuclease digestion of DNA segment offer valuable tool for detection, confirmation and speciation of food-borne pathogens. For aeromonads, RFLP assays developed, have however mostly been directed towards non-food applications including detection of clinical isolates (Borrell *et al.* 1997, Ghatak *et al.* 2006), for taxonomic inference (Figuears *et al.* 2000, Martinez-Murcia *et al.* 2005), for species identification (Iganowska and Kaznowski 2004, Kaznowski and Konecka 2005). These studies involved PCR amplification of a target piece of DNA within *Aeromonas* genome and then looking for enzyme specific restriction sites by digesting the amplicon with a selected enzyme(s). Polymorphisms were revealed by the differing banding patterns among isolates or strains or species when digested amplicon were analyzed by gel electrophoresis. Most of these above mentioned studies looked for polymorphism within 16S rRNA gene or in the 16S – 23S rRNA intergenic spacer region of *Aeromonas* spp. and, Kingombe *et al.* (2004) successfully employed RFLP technique on PCR amplicons for detection and speciation of aeromonads from foods. Moreover, this technique has also been utilized to detect and characterize *Aeromonas* isolates from chicken carcasses and in fish farm (Lee *et al.* 2002, Abdullah *et al.* 2003). One limitation that most of these studies suffered from is apparent similarity among RFLP fingerprints of various strains and isolates. Only close examination could reveal the true identity of the isolates. To circumvent this limitation Ghatak *et al.* (2006) devised a stepwise RFLP protocol that was based on either presence or absence of fragmentation of the 16S rDNA amplicon or highly discernible banding profile among more important of the *Aeromonas* species. The protocol was developed in such a fashion that once the identity of an isolate was established, there was no need to go through the further steps thus saving valuable time and costly chemicals.

Amplified fragment length polymorphism (AFLP): AFLP technique is similar to RFLP with difference being in the use of specific adapter molecules to detect and amplify polymorphisms within a set of DNA molecules. This unique technique has been widely used for the detection and species identification of *Aeromonas* spp. (Huys *et al.* 1996, Lund *et al.* 2002) and also for molecular epidemiological studies of aeromonads (Kuhn *et al.* 1997, Rahman *et al.* 2007). Moreover, AFLP has also been employed for the detection of *Aeromonas* spp. from foods (Kingombe *et al.* 2004).

Pulsed field gel electrophoresis (PFGE): When it comes to discriminatory ability of typing technique there are only a few tests that may equal PFGE. The technique essentially involves restriction enzyme digestion of carefully extracted intact genomic DNA of bacteria followed by electrophoresis on agarose gel under the influence of an alternating field of electric current, so that the large DNA fragments can wriggle through the pores of the gel without being stuck up (Jay *et al.* 2005). Early experimentation on aeromonads started with application of this technique to construct physical and genetic map of *A. hydrophila* (Dodd and Pemberton 1998). Subsequently, PFGE was employed to study and characterize genetic diversity of *Aeromonas* isolates from diverse sources including food isolates (Hanninen and Hirvela-Koski 1999, Villari *et al.* 2000, Bonadonna *et al.* 2002).

Real time PCR: Extension of PCR, especially the quantitative (q-PCR) technology to combine with advances in fluorescence optics yielded the intelligently conceived technology of real time PCR. This technique is regarded truly revolutionary in that it has catapulted traditional analytical goal of end point analysis to dynamic detection ability during exponential amplification phase of PCR in real time (hence the name is) (Espy *et al.* 2006).

One of the first reports of application of real time PCR for aeromonads was by Fukushima *et al.* (2003) who reported standardization of a duplex real time SYBR green PCR assay, wherein the researchers were able to detect *Aeromonas* spp. along with 16 other food and waterborne pathogens. Since one of the principal advantages of real time PCR has been the ability to measure the target (bacteria), a number of researchers reported real time assays for detection and quantification of aeromonads (Balcazar *et al.* 2007, Ahmed *et al.* 2008, Yu *et al.* 2008, Khan *et al.* 2009, Trakhna *et al.* 2009). These assays were reportedly rapid (Fukushima *et al.* 2003, Khan *et al.* 2009), sensitive (Balcazar *et al.* 2007) and were culture independent (Khan *et al.* 2009). However, the assays suffered the weakness in detecting aeromonads chiefly from water. As the sensitivity of real time PCR is of many orders higher than conventional one (Espy *et al.* 2006), it is expected that many assays will come up for *Aeromonas* spp. for detection from foods.

DNA microarray: DNA microarrays are systematic collection of discretely immobilized oilgonucleotides on a solid matrix that can produce a signal pattern upon hybridization with labeled target DNA (Rasooly and Herold 2008). DNA microarrays have found wide range of application including gene expression studies, genotyping and detection of pathogens important in food safety, medicine, and others (Kostrzynska and Bachand 2006). This novel technology is particularly capable in parallel identification of multiple targets (much higher than conventional multiplex PCR) (Rasooly and Herold 2008). Several researchers, therefore, attempted development of assays for multiple food-borne pathogens that included *Aeromonas* spp. also. (Kostic *et al.* 2007, Lee *et al.* 2008). As microarray technology enjoys diversified applications, many workers employed this technology for non-food studies on aeromonads including elucidation of toxin induced gene expression (Galindo *et al.* 2003, 2005) comparative genomic

profiling (Nash *et al.* 2006), comparison between virulence assay models (Hayes *et al.* 2006), detection of fish pathogens (Gonzalez *et al.* 2004). The array technology is especially suitable for food-borne pathogens when foods normally harbour numbers of harmless and innocuous organisms. Moreover, sensitivity and rapidity of this technology have been reported to be comparable or more than the conventional detection protocols (Hong *et al.* 2004, Wang *et al.* 2007)

Sequence analysis: Sequence analysis is one of the most convincing tools in modern molecular biology for the detection and confirmation of identity of food-borne pathogens. The technique involves chemical determination of nucleotide sequence of a desired segment of genome of the suspected pathogen and then comparing the same with available sequences for that particular segment of the same organism from computerized database(s). Sequence analyses for aeromonads have chiefly been utilized for phylogenetic and taxonomic studies (Yanez *et al.* 2003). Nevertheless, amplicon sequence analysis (ASA) of PCR amplified genome segments has been found to be particularly useful for detection and species determination of *Aeromonas* spp. from foods (Kingombe *et al.* 2004).

Conclusion and future trends: Detection of *Aeromonas* spp. from foods poses many challenges. For decades, researchers all over the world had to rely on conventional detection methodologies despite their documented limitations. These bottlenecks ironically prompted next-in-the-line researchers to explore and apply the benefits of molecular techniques for *Aeromonas* research in general detection in food in particular. Result is a smorgasbord of molecular techniques that were innovatively experimented upon, painstakingly optimized, rigorously evaluated and ready to use.

However, the true test of a technique lies with its ability to cope with changing demands of researchers, industry and law enforcers over time. Amongst molecular tools, PCR appear to possess this rare quality, that for last two decades its applications for detection of aeromonads have been steady. Although traditional culture based assays are considered natural reference test to compare molecular assays, it has been convincingly documented that many a times rapid molecular assays outperform the former detecting additional 'truly' positive cases (Abubakar *et al.* 2007).

Another concern that haunts molecular techniques is the inability to differentiate live and dead cells (Ghatak and Gill 2009). However, newer protocols have been reported that incorporated azide dyes in the PCR cycles to effectively rule out amplification of DNA from dead cells (Soejima *et al.* 2007, Brescia *et al.* 2009). These newer PCR protocols are yet to be reported for *Aeromonas* spp., but may be expected ahead in time.

The future holds many promises for *Aeromonas* researchers particularly engaged in detection from foods and perhaps we will see many new assays, innovative modification of existing ones, especially, of PCR, real time PCR and microarray technology.

REFERENCES

Abdullah A I, Hart C A and Winstanley C. 2003. Molecular characterization and distribution of virulence-associated genes amongst *Aeromonas* isolates from Libya. *Journal of Applied Microbiology* **95**:1001–07.

Abott S L, Cheung W K W and Janda J M. 2003. The genus *Aeromonas:* Biochemical characteristics atypical reactions and phenotypic identification schemes. *Journal of Clinical Microbiology* **41**: 2348–57.

Abubakar I, Irvine L, Aldus C F, Wyatt G M, Fordham R, Schelenz S, Shepstone L, Howe A, Peck M and Hunter P R. 2007. A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. *Health Technology Assessment* **11**:1–216.

Agarwal R K, Bhilegaonkar K N, Noubade R and Purshottam. 2003. Detection of hemolytic *Aeromonas hydrophila* from foods by polymerase chain reaction. *Journal of Veterinary Public Health* **1**: 97–101.

Agarwal R K, Kapoor K N, Ashok Kumar and Bhilegaonkar K N. 2000. Aeromonads in foods of animal origin. *Indian Journal of Animal Sciences* **70** (9): 942–43.

Aguilera-Arreola M G, Hernández-Rodríguez C, Zúñiga G, Figueras M J, Castro-Escarpulli G. 2005. *Aeromonas hydrophila* clinical and environmental ecotypes as revealed by genetic diversity and virulence genes. *FEMS Microbiology Letters* **242**:231–40.

Ahmed W, Huygens F, Goonetilleke A and Gardner T. 2008. Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland Australia. *Applied Environmental Microbiology* **74**: 5490–96.

Alavandi S V, Ananthan S and Pramod N P. 2001. Typing of *Aeromonas* isolates from children with diarrhoea and water samples by randomly amplified polymorphic DNA polymerase chain reaction and whole cell protein fingerprinting. *Indian Journal of Medical Research* **113**: 85–97.

Alperi A, Figueras M J, Inza I, Martinez-Murcia A J. 2008. Analysis of 16S rRNA gene mutations in a subset of *Aeromonas* strains and their impact in species delineation. *International Microbiology* **11**: 185–94.

Balcázar J L, Vendrell D, de Blas I, Ruiz-Zarzuela I, Gironés O and Múzquiz J L. 2007. Quantitative detection of *Aeromonas* salmonicida in fish tissue by real-time PCR using self-quenched, fluorogenic primers. *Journal of Medical Microbiology* **56**: 323–28.

Baloda S B, Krovacek K, Eriksson L, Linne T and Mansson T. 1995. Detection of aerolysin gene in *Aeromonas* strains isolated from drinking water, fish and foods by the polymerase chain reaction. *Comparative Immunology Microbiology and Infectious Diseases* **18**: 17–26.

Bloom H G and Bottone E J. 1990. *Aeromonas hydrophila* diarrhoea in a long-term care setting. *Journal of American Geriatric Society* **38**: 804–806.

Bonadonna L, Briancesco R, Filetici E, Manuppella A, Pourshaban M and Semproni M. 2002. Genomic heterogeneity of environmental and clinical aeromonads. *New Microbiology* **25**: 21–29.

Borch E, Nesbakken T and Christensen H. 1996. Hazard identification in swine slaughter with respect to food-borne bacteria. *International Journal of Food Microbiology* **30**: 9–25.

Borrell N, Acinas S G, Figueras M J and Murcia A J M. 1997. Identification of *Aeromonas* clinical isolates by restriction fragment length polymorphism of PCR-amplified 16S rRNA genes. *Journal of Clinical Microbiology* **35**: 1671–74.

Brescia C C, Griffin S M, Ware M W, Varughese E A, Egorov A I and Villegas E N. 2009. Cryptosporidium propidium monoazide-PCR, a molecular biology-based technique for genotyping of viable cryptosporidium oocysts. *Applied Environmental Microbiology* **75**: 6856–63.

Buchanan R L. 1984. The new pathogens. An update of selected examples. *Association of Food Drug Officials Quarterly Bulletin* **48**: 142–55.

Burke V, Gracey M, Robinson J, Peck D, Beaman J and Bundell C. 1983. The microbiology of childhood gastroenteritis: *Aeromonas* species and other infective agents. *Journal of Infectious Diseases* **148**: 68–74.

Callister S M and Agger W A. 1987. Enumeration and characterization of *Aeromonas hydrophila* and *Aeromonas caviae* isolated from grocery store produce. *Applied Environmental Microbiology* **53**: 249–54.

Cantoni C, Stella S and Amendola F. 2002. An outbreak of toxi-infection caused by high quantity of *Aeromonas hydrophila*. *Industrie Alimentarie* **41**: 798–801.

Chacón M R, Figueras M J, Castro-Escarpulli G, Soler L and Guarro J. 2003. Distribution of virulence genes in clinical and environmental isolates of *Aeromonas* spp. *Antonie Van Leeuwenhoek.* **84**: 269–78.

Champsaur H, Ademont A, Matthieu D, Rottman E and Anzepy P. 1982. Cholera-like illness due to *Aeromonas sobria*. *Journal of Infectious Diseases* **145**: 248–54.

Chang Y C, Wang J Y, Selvam A, Kao S C, Yang S S. and Shih D Y. 2008. Multiplex PCR detection of enterotoxin genes in *Aeromonas* spp. from suspect food samples in northern Taiwan. *Journal of Food Protection* **71**: 2094–99.

Chopra A K, Houston C W, Peterson J W and Jin G F. 1993. Cloning expression and sequence analysis of a cytolytic enterotoxin gene from *Aeromonas hydrophila. Canadian Journal of Microbiology* **39**: 513–23.

da Rocha M P, Scheid L A and Alves S H. 2008. New selective indicator medium for detection of *Aeromonas* and Plesiomonas: UNISC agar. *Revista da Sociedade Brasileira de Medicina Tropical* **41**: 431–33.

Davin-Regli A, Bollet C, Chamorey E, Colonna D'istria V and Cremieux A. 1998. A cluster of cases of infections due to *Aeromonas hydrophila* revealed by combined RAPD and ERIC-PCR. *Journal of Medical Microbiology* **47**: 499–504.

de La Morena M L, Van R, Singh K, Brian M, Murray B E and Pickering L K. 1993. Diarrhoea associated with *Aeromonas* species in children day care centres. *Journal of Infectious Diseases* **168**: 215–18.

Dodd H N and Pemberton J M. 1998. Construction of a physical and preliminary genetic map of *Aeromonas hydrophila* JMP636. *Microbiology* **144**: 3087–96.

Doke S N, Warrier S B, Alur M D, Venugopal V and Nair P M. 1994. Protease secretion by resting cells of *Aeromonas hydrophila* as a possible mode of survival in flesh foods. *Journal of Food Science and Technology India* **31**: 476–79.

Echeverria P, Sack R B, Blacklow N R, Bodhidatta P, Rowe B and McFarland A. 1984. Prophylactic doxycychine for travellers' diarrhoea in Thailand. *Amarican Journal of Epidemiology* **120**: 912–21.

Ercolini D. 2004. PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. *Journal of Microbiological Methods* **56**: 297–314.

Espy M J, Uhl J R, Sloan L M, Buckwalter S P, Jones E A, Vetter E A, Yao J D C, Wengenack N L, Rosenblatt J E, Cockerill F R and Smith T F. 2006 Real-time PCR in clinical microbiology: Applications for routine laboratory testing. *Clinical Microbiology Review* **19**: 165–256.

Figueras M J, Soler L, Chacon M R, Guarro J and Murica A J M. 2000. Extended method for discrimination of *Aeromonas* spp. by 16S rDNA RFLP analysis. *International Journal of Systematic and Evolutionary Microbiology* **50**: 2069–73.

Fukushima H, Tsunomori Y and Seki R. 2003. Duplex real-time SYBR green PCR assays for detection of 17 species of food- or water-borne pathogens in stools. *Journal of Clinical Microbiology* **41**: 5134–46.

Galindo C L, Fadl A A, Sha J, Pillai L, Gutierrez C Jr. and Chopra A K. 2005. Microarray and proteomics analyses of human intestinal epithelial cells treated with the *Aeromonas hydrophila* cytotoxic enterotoxin. *Infection and Immunity* **73**: 2628–43.

Galindo C L, Sha J, Ribardo D A, Fadl A A, Pillai L and Chopra A K. 2003. Identification of *Aeromonas hydrophila* cytotoxic enterotoxin-induced genes in macrophages using microarrays. *Journal of Biological Chemistry* **278**: 40198–212.

Garrity G M, Winters M and Searles D B. 2001. Taxonomic outline of the prokaryotic genera. *Bergey's Manual of Systematic Bacteriology*. 2nd edn. pp. 13 Bergey's Manual Trust.

Ghatak S and Gill J P S. 2009. Molecular Techniques for Detection and Typing of Food-borne Pathogens from Livestock Products. *Winter School on Recent developments in post harvest processing and value addition to livestock products produce.* Pp 52–57. Central Institute of Post Harvest Engineering and Technology, Ludhiana, Punjab.

Ghatak S, Agarwal R K and Banerjee R. 2001. Characterization of outer membrane protein of *Aeromonas hydrophila* and A. sobria of diverse serotypes. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases* **22**: 47– 50.

Ghatak S, Agarwal R K and Bhilegaonkar K N. 2003. Detection of *Aeromonas* spp. from milk and meat by enzyme-linked immunosorbent assay. *Journal of Veterinary Public Health* 1: 17–24.

Ghatak S, Agarwal R K and Bhilegaonkar K N. 2006. Species identification of clinically important *Aeromonas* spp. by restriction fragment length polymorphism of 16S rDNA. *Letters in Applied Microbiology* **44**: 550–54.

Gill C O and Jones T. 1995. The presence of *Aeromonas, Listeria* and *Yersinia* in carcass processing equipment at two slaughtering plants. *Food Microbiology* **12**: 135–41.

González S F, Krug M J, Nielsen M E, Santos Y and Call D R. 2004. Simultaneous detection of marine fish pathogens by using multiplex PCR and a DNA microarray. *Journal of Clinical Microbiology* **42**:1414–19.

González-Serrano C J, Santos J A, García-López M L, Otero A. 2002. Virulence markers in *Aeromonas hydrophila* and *Aeromonas* veronii biovar sobria isolates from freshwater fish

and from a diarrhoea case. *Journal of Applied Microbiology* **93**: 414–19.

Gracey M, Burke V and Robinson J.1982. *Aeromonas*–associated gastroenteritis. *Lancet*. **2**: 1304–06.

Gracey M, Burke V, Robinson J, Masters P L, Stewart J and Pearman J. 1984. *Aeromonas* spp. in traveller's diarrhoea. *British Medical Journal* **289**: 658.

Granum P E, O'Sullivan K, Tomas J M and Ormen O. 1998. Possible virulence factors of *Aeromonas* spp. from food and water. *FEMS Immunology and Medical Microbiology* **21**: 131–37.

Gurwith M, Bourque C, Cameron E, Forrest G and Green M. 1977. Cholera-like diarrhoea in Canada. Report of a case associated with enterotoxigenic *Escherichia* coli and a toxin-producing *Aeromonas hydrophila*. *Archives of Internal Medicine* **137**: 1461–64.

Hänninen M L and Hirvelä-Koski V. 1999. Genetic diversity of atypical *Aeromonas* salmonicida studied by pulsed-field gel electrophoresis. *Epidemiology and Infection* **123**: 299–307.

Hanninen M L, Oivanen P and Hirvela-Koski V. 1997. *Aeromonas* species in fish, fish-eggs, shrimp and freshwater. *International Journal of Food Microbiology* **34**: 17–26.

Hayes S L, Lye B R, Lye D J, Rodgers M R, Stelma G, Malard J M, Vandewalle A and Vesper S J. 2006. Identification by microarray of a common pattern of gene expression in intact intestine and cultured intestinal cells exposed to virulent *Aeromonas hydrophila* isolates. *Journal of Water and Health* **4**: 381–88.

Hirono I, Aoki T, Asao T and Kozaki S. 1992. Nucleotide sequences and characterization of haemolysin genes from *Aeromonas hydrophila* and *Aeromonas sobria. Microbial Pathogenesis* **13**: 433–66.

Hong B X, Jiang L F, Hu Y S, Fang D Y and Guo H Y. 2004. Application of oligonucleotide array technology for the rapid detection of pathogenic bacteria of food-borne infections. *Journal of Microbiological Methods* **58**: 403–11.

Huys G, Coopman R, Janssen P and Kersters K. 1996. High-resolution genotypic analysis of the genus *Aeromonas* by AFLP fingerprinting. *International Journal of Systematic Bacteriology* **46**: 572–80.

Iaganowska M and Kaznowski A. 2004. Restriction fragment length polymorphism of 16S–23S rDNA intergenic spacer of *Aeromonas* spp. *Systematic and Applied Microbiology* **27**: 549–57.

Isonhood J H and Drake M. 2002. *Aeromonas* species in foods. *Journal of Food Protection* **65**: 575–82.

Janda J M and Abbott S L. 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding Panorama of species, disease presentations, and unanswered questions. *Clinical Infectious Disease* **27**: 332–44.

Janda J M and Duffey P S. 1988. Mesophilic aeromonads in human disease: Current taxonomy, laboratory identification, and infectious disease spectrum. *Review of Infectious Diseases* **10**: 980–96.

Jay J M, Loessner M J and Golden D A (Eds.) 2005. *Modern Food Microbiology*. Pp. 241–84. Springer.

Kaznowski A and Konecka E. 2005. Identification of *Aeromonas* culicicola by 16S rDNA RFLP. *Polish Journal of Microbiology* **54**: 335–38.

Khan A A and Cerniglia. 1997. Rapid and sensitive method for the detection of *Aeromonas caviae* and *Aeromonas trota* by polymerase chain reaction. *Letters in Applied Microbiology* **24**: 233–39.

Khan I U, Loughborough A and Edge T A. 2009. DNA-based real-time detection and quantification of aeromonads from fresh water beaches on Lake Ontario. *Journal of Water and Health* **7**: 312–23.

Khurana R and Kumar A. 1997. Prevalence of motile aeromonads in foods of animal origin. *Journal of Food Science and Technology India* **34**: 228–229.

Khushiramani R, Girisha S K and Karunasagar I. 2009. Evaluation of a digoxigenin-labelled probe for detection of *Aeromonas* spp. *Letters in Applied Microbiology* **48**: 383–85.

Kingombe C I B, Huys G, Howald D, Luthi E, Swings J and Jemmi T. 2004. The usefulness of molecular techniques to asses the presence of *Aeromonas* spp. harboring virulence markers in foods. *International Journal of Food Microbiology* **94**: 113–21.

Kirov S M, Anderson M J and McMeekin T A. 1990. A note on *Aeromonas* spp. from chickens as possible food-borne pathogens. *Journal of Applied Bacteriology* **68**: 327–34.

Kirov S M. 1993. The public health significance of *Aeromonas* spp. in foods. *International Journal of Food Microbiology* **20**: 179–98.

Korbsrisate S, Trakulsomboon S, Damnin S, Gatedee J, Ratchtrachenchai O A, Leelaporn A, Eampokalap B, Gongviseisoog C and Suparak S. 2007. Genetic variations in *Aeromonas hydrophila* isolates from clinical and environmental sources in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **38**: 721–27.

Kostiæ T, Weilharter A, Rubino S, Delogu G, Uzzau S, Rudi K, Sessitsch A and Bodrossy L. 2007. A microbial diagnostic microarray technique for the sensitive detection and identification of pathogenic bacteria in a background of nonpathogens. *Annals of Biochemistry* **360**: 244–54.

Kostrzynska M and Bachand A. 2006. Application of DNA microarray technology for detection identification, and characterization of food-borne pathogens. *Canadian Journal of Microbiology* **52**: 1–8.

Kühn I, Huys G, Coopman R, Kersters K and Janssen P. 1997. A 4–year study of the diversity and persistence of coliforms and *Aeromonas* in the water of a Swedish drinking water well. *Canadian Journal of Microbiology* **43**: 9–16.

Kumar A. 1998. 'Studies on aerolysin of motile aeromonads' Ph.D. thesis, Deemed University, IVRI, Izatnagar, India.

Lee C, Cho J C, Lee S H, Lee D G and Kim S J. 2002. Distribution of *Aeromonas* spp. as identified by 16S rDNA restriction fragment length polymorphism analysis in a trout farm. *Journal of Applied Microbiology* **93**: 976–85.

Lee D Y, Lauder H, Cruwys H, Falletta P and Beaudette L A. 2008. Development and application of an oligonucleotide microarray and real-time quantitative PCR for detection of wastewater bacterial pathogens. *Science of Total Environment* **398**: 203–11.

Ljungh A and Wadstrom T. 1985. *Aeromonas hydrophila* – an accepted enterotoxigenic "neoenteropathogen"? *Journal of Infectious Diseases* **151**: 972–73.

Lund V, Jenssen L M and Wesmajervi M S. 2002. Assessment of genetic variability and relatedness among atypical *Aeromonas salmonicida* from marine fishes, using AFLP-fingerprinting. *Diseases of Aquatic Organisms* **50**: 119–26.

Martínez-Murcia A J, Soler L, Saavedra M J, Chacón M R, Guarro J, Stackebrandt E and Figueras M J. 2005. Phenotypic, genotypic, and phylogenetic discrepancies to differentiate *Aeromonas salmonicida* from *Aeromonas bestiarum*. *International Microbiology* **8**: 259–69.

Merino S, Camprubi S and Tomas J M. 1993. Detection of *Aeromonas hydrophila* in food with an enzyme-linked immunosorbent assay. *Journal of Applied Bacteriology* **74**: 149–54.

Merino S. Rubiers X. Knochel S. and Tomas J.M. 1995. Emerging pathogens: *Aeromonas* spp. *International Journal of Food Microbiology* **28**: 157–68.

Nash J H, Findlay W A, Luebbert C C, Mykytczuk O L, Foote S J, Taboada E N, Carrillo C D, Boyd J M, Colquhoun D J, Reith M E and Brown L L. 2006. Comparative genomics profiling of clinical isolates of *Aeromonas salmonicida* using DNA microarrays. *BMC Genomics.* **7**: 43.

Nayduch D, Honko A, Noblet G P and Stutzenburger F. 2001. Detection of *Aeromonas caviae* in the common housefly *Musca domestica* by culture and polymerase chain reaction. *Epidemiology and Infection* **127**: 561–66.

Nayduch D, Noblet G P and Stutzenberger F J. 2002. Vector potential of houseflies for the bacterium *Aeromonas caviae*. *Medical and Veterinary Entomology* **16**: 193–98.

Oakey H J, Ellis J T and Gibson L F. 1996. Differentiation of *Aeromonas* genomospecies using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). *Journal of Applied Bacteriology* **80**: 402–10.

Okrend A J G, Rose B E and Bennett B. 1987. Incidence and toxigenicity of *Aeromonas* species in retail poultry, beef and pork. *Journal of Food Protection* **50**: 509–13.

Ormen O, Granum P E, Lassen J and Figueras M J. 2005. Lack of agreement between biochemical and genetic identification of *Aeromonas* spp. *Acta Pathologica Microbiologica Immunologica Scandinavia* **113**: 203–07.

Palumbo S A, Beneicengo M M, Del Corral F, Williams A C and Buchanan R L. 1989. Characterization of the *Aeromonas hydrophila* group isolated from retail foods of animal origin. *Journal of Clinical Microbiology* **27**: 854–59.

Palumbo S A, Maxino F, Williams A C, Buchanan R L and Thayer D W. 1985. Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophila. Applied Environmental Microbiology* **50**: 1027–30.

Pollard D R, Johnson W M, Lior H, Tyler S D and Rozee K R. 1990. Detection of aerolysin gene in *Aeromonas hydrophila* by the polymerase chain reaction. *Journal of Clinical Microbiology* **28**: 2477–81.

Porteen K, Agarwal R K and Bhilegaonkar K N. 2006. PCR-Based detection of *Aeromonas* from milk samples. *Journal of Food Technology* **4**: 111-15.

Porteen K, Agarwal R K and Bhilegaonkar K N. 2007. Detection of *Aeromonas* sp. from chicken and fish samples by polymerase chain reaction. *American Journal of Food Technology* **2**: 30–37.

Pund R P and Theegarten D. 2008. The importance of aeromonads as a human pathogen. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* **51**: 569–76.

Rahman A F M S and Willoughby J M T. 1980. Dysentery-like syndrome associated with *Aeromonas hydrophila*. *British Medical Journal* **281**: 976.

Rahman M, Huys G, Rahman M, Albert M J, Kühn I and Möllby R. 2007. Persistence, transmission, and virulence characteristics of *Aeromonas* strains in a duckweed aquaculture-based hospital sewage water recycling plant in Bangladesh. *Applied Environmental Microbiology* **73**: 1444–51.

Rasooly A and Herold K E. 2008. Food microbial pathogen detection and analysis using DNA microarray technologies. *Food-borne Pathogens and Diseases* **5**: 531–50.

Sachan N and Agarwal R K. 2000. Selective broth for the isolation of *Aeromonas* sp. from chicken meat. *International Journal of Food Microbiology* **60**: 65–74.

Sachan N and Agarwal R K. 2002. A simple enzyme linked immunosorbent assay for the detection of *Aeromonas* spp. *Veterinarski Archiv* **72**: 327–34.

Saiki R K, Gelfand D H, Stoffel S, Scharf S J, Higuchi R, Horn G T, Mullis K B and Erlich H A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**: 487–91.

Santos J A, González C J, Otero A and García-López M L. 1999. Hemolytic activity and siderophore production in different *Aeromonas* species isolated from fish. *Applied Environmental Microbiology* **65**: 5612–14.

Schubert R H W. 1991. Aeromonads and their significance as potential pathogens in water. *Journal of Applied Bacteriology* **70**: 131S–5S.

Sechi L A, Deriu A, Falchi M P, Fadda G and Zanetti S. 2002. Distribution of virulence genes in *Aeromonas* spp. isolated from Sardinian waters and from patients with diarrhoea. *Journal of Applied Microbiology* **92**: 221–27.

Seethalakshmi I, Subashkumar R and Saminathan P. 2008. Distribution of putative virulence genes in *Aeromonas hydrophila* and *Aeromonas* salmonicida isolated from marketed Fish Samples. *Journal of Fishery and Aquatic Science* **3**: 145–51.

Sendra R M, Esteve C, Alcaide E. 1997. Enzyme-linked immunosorbent assay for detection of *Aeromonas hydrophila* serogroup O:19. *FEMS Microbiology Letters* **157**: 123–29.

Shankar K, Narasimhan R and Habibullah-Khan M M. 1994. Incidence, isolation and characterisation of psychrotrophs in ice cream. *Indian Veterianry Journal* **71**: 1202–06.

Singh B R, Kapoor K N, Yadav A S, Tiwari A K, Singh Y, Kumar A and Bhilegaonkar K N. 1997. Characterization of virulence factors among enterotoxigenic strains of *Aeromonas hydrophila* isolated from fish. *Journal of Food Science and Technology India* **34** : 443–45.

Soejima T, Iida K, Qin T, Taniai H, Seki M, Takade A and Yoshida S. 2007. Photoactivated ethidium monoazide directly cleaves bacterial DNA and is applied to PCR for discrimination of live and dead bacteria. *Microbiology and Immunology* **51**: 763–75.

Soler L, Figueras M J, Chacón M R, Guarro J and Martinez-Murcia A J. 2003. Comparison of three molecular methods for typing *Aeromonas* popoffii isolates. *Antonie Van Leeuwenhoek* **83**: 341–49.

Soler L, Marco F, Vila J, Chacón M R, Guarro J and Figueras M J. 2003. Evaluation of two miniaturized systems MicroScan W/A and BBL Crystal E/NF, for identification of clinical isolates of *Aeromonas* spp. *Journal of Clinical Microbiology* **41**: 5732–34.

Swale G V, Das B C, Acland P R and Heath D A. 1986. Fatal infection with *Aeromonas sobria* and *Plesiomonas shigelloides*. *British Medical Journal* **292**: 525–26.

Tacão M, Moura A, Alves A, Henriques I, Saavedra M J and Correia A. 2005b. Evaluation of 16S rDNA- and gyrB-DGGE for typing members of the genus *Aeromonas*. *FEMS Microbiology Letters* **246**: 11-18.

Talon D, Mulin B and Thouverez M. 1998. Clonal identification of *Aeromonas hydrophila* strains using randomly amplified polymorphic DNA analysis. *European Journal of Epidemiology* **14**: 305–10.

Trakhna F, Harf-Monteil C, Abdelnour A, Maaroufi A and Gadonna-Widehem P. 2009. Rapid *Aeromonas hydrophila* identification by TaqMan PCR assay: comparison with a phenotypic method. *Letters in Applied Microbiology* **49**: 186–90.

Tsai G J and Chen T H. 1996. Incidence and toxigenicity of *Aeromonas hydrophila* in seafood. *International Journal of Food Microbiology* **31**: 121–31.

Vila J, Ruiz J, Gallardo F, Vargas M, Soler L, Figueras M J and Cascon J. 2003. *Aeromonas* spp. and traveler's diarrhoea: clinical features and antimicrobial resistance. *Emerging Infectious Diseases* **9**: 552–55.

Villari P, Crispino M, Montuori P and Stanzione S. 2000. Prevalence and molecular characterization of *Aeromonas* spp. in ready-to-eat foods in Italy. *Journal of Food Protection* **63**: 1754–57.

Wang G, Tyler K D, Munro C K and Johnson W M. 1996. Characterization of cytotoxic, hemolytic *Aeromonas caviae* clinical isolates and their identification by determining presence of a unique hemolysin gene. *Journal of Clinical Microbiology* **34**: 3203–05.

Wang X W, Zhang L, Jin L Q, Jin M, Shen Z Q, An S, Chao F H and Li J W. 2007. Development and application of an oligonucleotide microarray for the detection of food-borne bacterial pathogens. *Applied Microbiology and Biotechnology* **76**: 225–33.

Yanez M A, Catalan V, Apraiz D, Figueras M J and Martinez-Murcia A J. 2003. Phylogenetic analysis of members of the genus *Aeromonas* based on *gyrB* gene sequences. *International Journal of Systematic and Evolutionary Microbiology* **53**: 875–83.

Yu C P, Farrell S K, Robinson B and Chu K H. 2008. Development and application of real-time PCR assays for quantifying total and aerolysin gene-containing *Aeromonas* in source, intermediate, and finished drinking water. *Environmental Science and Technology* **42**: 1191-1200.