Environment Conservation Journal 13(3) 95-103, 2012
ISSN 0972-3099 (Print) 2278-5124 (Online)
Abstracted and Indexed

A Review on isolation and molecular identification of *Aeromonas* Spp.

Shashi Yadav[1], Anoop K. Dobriyal ✉[2]

Received: 22.07.2012 **Accepted: 18.09.2012**

Abstract

This paper reviews the isolation and identification of *Aeromonas* spp. through biochemical tests and molecular typing with special reference to their infection in human beings and future prospective of research related to human health.

Keywords*: Aeromonas, **i**solation, biochemical properties, molecular typing*

Introduction

Genus *Aeromonas* are Gram-negative, non-spore forming, rod-shaped, facultative anaerobic bacilli. They are generally motile by polar flagella (Baron and Finegold, 1990; Villari *et al.*, 2003). They grow over a wide range of temperature 0-40^{o}C, with human (motile mesophilic) strains growing at between 10-40^{o}C, with 30^{o}C as the optimum temperature, while the non-motile psychrophilic species grow at between 22-28^{o}C in soil, food and animal body (Jatau and Yakubu, 2004; Cheesbough, 2005). Until recently, *Aeromonas* were classified in the family *Vibrionaceae* (Jawetz *et al.*, 2004). However, molecular genetic evidence (including 16s rRNA catalog, 5srRNA sequence, and rRNA-DNA hybridation) suggests they are not closely related to *Vibrio* species. Therefore in the latest edition of Bergey's Manual of Systematic Bacteriology, they are classified as a separate family the *Aeromonadaceae* (Sylvia *et al.*, 2004; Jawetz *et al.*, 2007). *Aeromonas* are ubiquitous in fresh and brackish waters (Jawetz *et al.*, 2004). These organisms have also been isolated from a wide variety of sources including soil, sea food and humans (Bishara, 1984; Michael *et al.*, 2000).

Author's Address

[1]National Bureau of Fish Genetic Resources, Canal Ring Road, Dilkusha, Lucknow-U.P.
[2]Department of Zoology and Biotechnology, HNB Garhwal University Campus, Pauri Garhwal, Uttarakhand
E-mail:anoopkdobriyal@rediffmail.com

The concentration of *Aeromonas* varies with environment in which they are found. In clean rivers, lakes, and storages reservoirs, their concentration is typically around 102 cfu/ml. The concentration in ground water is generally less than 1 cfu/ml. Drinking water immediately leaving the treatment plant may contain between 0-102 cfu/ml, with potentially higher concentration in drinking water distribution systems, attributed to growth in Biofilms (Payment *et al.*, 1988; United State Environmental Protection Agency, 2005). Higher density of 108 cfu/ml can be found in waste waters, treated sewage and crude sewage (Holmes *et al.*, 1996). They are also found in sinks, drain pipes and household effluent (Araujo *et al.*, 1991). *Aeromonas* species have been isolated from a variety of foods, including red meat (beef, pork and lambs) poultry produce, fish and shellfish (USEPA, 2005). *Aeromonas* species have been implicated in a variety of infections in humans such as gastroenteritis, wound infections (cellulitis), septicemia and occasionally others including urinary tract infection, meningitis, and peritonitis (Michael, 1991). *Aeromonas* infections are typically acquired through two routes, ingestion of contaminated water or food, or through contact of the organisms with a break in the skin (Jawetz *et al.*, 2004). Diseases associated with *Aeromonas* are intestinal and extra-intestinal. They are also implicated in colitis, meningitis, and are frequently isolated from wound infection sustained in aquatic

environments (Krovacek *et al.*, 1992). They are also being implicated in respiratory infection (Janda and Abbot, 1998). In recent years, *Aeromonas hydrophila* has gained public health recognition as an emerging pathogen (Bottarelli and Ossiprendi, 1999). Although food poisoning potential has not been reported, the association with human gastroenteritis strongly suggests that *A. hydrophila* plays a significant role in food borne diseases (Balaji *et al.*, 2004). The presence of these organisms in stools is significantly more often associated with diarrhea than with carrier state (Agger *et al.*, 1985; Aslani and Alikhani, 2004; Jawetz *et al.*, 2007; Kandakai-Olukemi *et al.*, 2007). *Aeromonas hydrophila* can be isolated with variable frequency from different foods (raw, refrigerated or frozen) of animal origin (Ventura *et al.*, 1998). Some preservative techniques seem ineffective in inhibiting the replication of *A. hydrophila*, which can multiply although at slow rate in products which are refrigerated and vacuum packed or packaged in modified atmosphere. The organism can also replicate at low pH (4.5) or at high sodium chloride (NaCl) concentration (up to 5%) in the environment (Bottarelli and Ossipnendi, 1999). The isolation of *A. hydrophila* from chlorinated water has been reported and it is less sensitive to chlorine compared to the coliforms (Chamorey *et al.*, 1999).

Medium for Isolation of *Aeromonas* Spp.

Shotts and Rimler (1973) proposed new differential medium, Rimler-Shotts and tested 109 isolates representing 13 genera of bacteria obtained from aquatic environments and animals. They found this medium to be effective in presumptive identification of the strains of *A. hydrophila* with 94% accuracy and this medium was designed to facilitate diagnosis of *A. hydrophila* infections in animals and humans. Mishra *et al.* (1987) compared five selective media for their effectiveness in primary isolation of *Aeromonas* spp. and found sheep blood agar with 30mg of ampicillin per litre (ASBA 30) in association with DNase-toluidine blue agar to be the most sensitive medium as it permitted more growth of *Aeromonas* colonies and effectively suppressed competiting microflora. Havelaar *et al.* (1987) reported satisfactory recoveries of *Aeromonas* spp. in a new medium, ampicillin dextrin agar at an ampicillin concentration of 10 mg/L and incubation for 24 hours at 30°C under aerobic conditions. They also observed that this medium had a greater confirmation rate along with its high specificity and no false negative colonies were encountered. Markwardt *et al.* (1989) assessed the applicability of Coomassie Brilliant Blue agar (CBB) as a differential medium for *A. salmonicida* and found this medium to be very valuable in diagnostic and epizootiological work and also in determining the presence of the pathogens in fish samples. Ribas *et al.* (1991) compared the properties of Starch Glutamate ampicillin penicillin-l0C agar with Ampicillin dextrin agar and m-Aeromonas medium for isolation of *Aeromonas* spp. in water samples. They found Starch Glutamate ampicillin penicillin-l0C agar to be the most adequate medium for *Aeromonas* spp. isolation due to its high specificity and selective composition. Holmes and Sartory (1993) considered Ampicilin Dextrin agar (ADA) to be highly satisfactory and selective, as this medium permitted good recovery of *Aeromonas* spp. in comparison to Ryan's medium, Bile-Salt-Irgasan-Brilliant Green agar (BIBA) and an agar medium containing xylose and ampicillin (XAA).

Von Graevenitz and Bucher (1993) reported that broth enrichment methods are frequently used to recover aeromonads from samples where they may be present in low numbers together with larger numbers of other bacteria. Also they found that use of Alkaline Peptone Water (APW) enrichment increased recovery of aeromonads from clinical specimens and APW with or without ampicillin (10 or 30 mg/L) may be used for qualitative detection of aeromonads when using the membrane filtration method for sample processing. Jenkins and Taylor (1995) compared the Rimler-Shotts (RS) medium and Starch-Glutamate-ampicillin-penicillin-based medium (SGAP-l0C) for the recovery of *Aeromonas* spp. Their studies indicated that, the recovery frequency of *Aeromonas* spp. was higher, efficient and specific on SGAP-l0C at 24°C for 48 hours, thus proving it to be a better choice of the laboratory for recovery of *Aeromonas* spp. from clinical fish samples. Gobat and Jemmi (1995) evaluated seven selected agar media and two enrichment broths for isolation of *Aeromonas* spp. from meat, fish and shellfish samples. Their findings revealed that Bile-salts-irgasan-brilliant green agar (BIBG) at 35^{0}□C was the most selective

medium and presumptive identification of *Aeromonas* on sheep blood agar supplemented with 30mg/L ampicillin (ASBA 30) was very easy. Singh (1997) compared two commercially available media, Ryan's *aeromonas* medium (RAM) and *pseudomonas aeromonas* selective agar base (GSP) and one laboratory prepared medium Starch ampicillin agar (SAA) for their ability to recover *Aeromonas* spp. from raw ground meats in Eastern Canada. He observed that in all instances, SAA was better than GSP and RAM with 100% of typical colonies confirming as *Aeromonas* spp. Sachan and Agarwal (2000) tested six selective agents (ampicillin, novobiocin, cephalothin, bile salts, brilliant green and ethanol) during the development of a selective enrichment broth for the isolation of *Aeromonas* spp. from chicken meat. They found that, of the six selected agents, cephalothin to be the best selective agent owing to its greater selectivity and efficiency in recovering stressed and lower cell concentrations of *Aeromonas* spp.

Biochemical Properties

Leblanc *et al.* (1981) isolated 195 strains of motile *Aeromonas* from fish which were characterized as *A. hydrophila* and *A. sobria.* They classified these organisms serologically and observed a relationship between heat-stable particulate antigens and virulence of *A. hydrophila*, also a cross-reaction between *A. hydrophila* and *A. sobria* was observed. Martinez-Murcia (1992) reported that *A. allosaccharophila* could not be identified in clinical laboratory since it did not possess unique biochemical characteristics which enable it to phenotypically separate this group from other mesophilic species. Janda *et al.* (1996) characterized 268 *Aeromonas* isolates upto genomospecies level by performing a series of biochemical tests. They biochemically separated the members of *A. hydrophila* complex *(A. hydrophila,* HG2 and *A. salmonicida)* and serogroups analysis of these 268 isolates indicated that, each genomospecies was serologically heterogenous and individual serogroups could be found in more than one species. Borrell *et al.* (1998) identified 983 isolates of *Aeromonas* upto the genomospecies level. The use of citrate and production of acid from sorbitol enabled them to separate the members of *A. hydrophila* complex and the most common genomospecies from intestinal sources encountered were *A. veronii* biotype *sobria* and *A. caviae.* On their result findings, they stated that prevalence of these pathogenic genomospecies should be regarded as an important threat to public health. Alavandi and Ananthan (2003) studied the differences between clinical and environmental *Aeromonas* spp. with respect to their biochemical properties, serogrouping and virulence factors. Their results did not reveal any significant differences between them, but differences were observed in respect to the ability of the *Aeromonas* isolates to produce the β-haemolytic where in higher percentage of environmental isolates were haemolytic. Awan *et al.* (2005) carried out biochemical characterization of *Aeromonas* spp. isolated from food and environment using seven types of API strips. They observed that these strips provided an extensive biochemical profile of the isolates and strip API 20E gave the most reliable results where as in all other strips some of the characteristics appeared as significant in differentiation of the various species.

Molecular Typing of *Aeromonas*

Although certain biochemical tests allowed for some improvements, phenotypic identification of the genospecies of *Aeromonas* was difficult. The molecular typing methods were used as taxonomic tools to discriminate among strains of *Aeromonas* for epidemiological purposes.

Phenotyping

Different phenotypic methods used to study *Aeromonas* strains are biotyping, phage typing, serotyping, chromatography of cell wall fatty acid methyl esters (FAME), multilocus enzyme electrophoresis (MEE), plasmid analysis and ribotyping. These phenotypic methods are based on phenotypical chracteristics of microorganisms.

1. Biotyping

Biotyping is based on activity patterns of metabolic enzymes of cells using enzymes with not more than 20 kinds and based on biochemical tests that differentiate *Aeromonas* to the species level. Different enzyme activity in the different microorganisms has the effect of gene expression in each strain for producing the various enzymes. The biotyping has low discriminatory power because it

is correct for 78% of all *Aeromonas* strains and is not sufficient to distinguish the different genospecies of *Aeromonas* and has little discrimination for epidemiological investigations (Havelaar *et al.*, 1992).

2. Phage typing

Phage typing is technically demanding and requires the maintenance of viable phages (which as lytic bacteriophages such as viruses are capable of infection and lysing bacterial cells) and control strains for propagating phage. A study was done using a total of 95 different phages to type clinical *Aeromonas* isolates from fecal specimens. These phages could type 81% of the *Aeromonas* strains (Altwegg *et al.*, 1988). A comparison between phage typing with three phenotypic *Aeromonas (A. hydrophila*, *A. sobria*, and *A. caviae)* and with DNA hybridization groups found that there was not strong association. These demonstrated that phage typing should be a conjunct study with other typing methods for typing and epidemiological study of *Aeromonas.*

3. Serotyping

Serotyping is based on the differences of antisera such as somatic O- and flagella H-antigens, somatic O- and K-antigens or lipopolysaccharide antigen. Serotyping was studied as the direct epidemiologic linkage between strains isolated from patients and strains isolated from the public water system, and it was found that serotyping could not demonstrate epidemiology with *Aeromonas* strains causing disease with patients and isolation from the environment (Guinee and Jansen,1987; Havelaar *et al.*, 1992; Moyer *et al.*, 1992).

4. Chromatography of cell wall fatty acid methyl esters (FAME)

FAME has low discrimination power to identify and type individual *Aeromonas* strains but is found useful to study the overall relationship between the *Aeromonas* groups which are isolated from different origins (Havelaar *et al.*, 1992).

5. Multilocus enzyme electrophoresis (MEE)

Multilocus enzyme electrophoresis is used to detect different metabolic enzymes, and the different protein profiles to identify the diversity of bacteria due to variations in genes encoding metabolic enzymes (Selander *et al.*, 1986). In addition, this method is highly reproducible and has discriminatory power (Picard and Goullet, 1985). Despite the genetic complexity of the genus *Aeromonas*, the use of MEE might be the sole method for species determination. For example, *A. hydrophila* complex (HG1, 2, and 3) was separated by using two enzymes: elastase and lysine decarboxylase, while *A. caviae* (HG 4, 5, and 6) was separated by using pyrazinamidase enzyme. This method might be suitable for typing each single *Aeromonas* strain (Abbott *et al.*, 1992). The diversity of enzymes produced by *Aeromonas* strains from the environment was more than *Aeromonas* strains from humans (Picard and Goullet, 1987). *Aeromonas* strains from humans have lower genetic distance than *Aeromonas* strains from the environment, demonstrating the variety of enzymatic systems produced by *Aeromonas* strains from the environment (Tonolla *et al.*, 1991).

Genotypic methods

These genotypic methods are based on genome analysis of microorganisms.

1. DNA-DNA hybridization

The deoxyribonucleic acid relationships among members of the genus *Aeromonas* found that variation of genome size and percentage of guanine and cytosine (G+C) ranged from 57.1 to 62.9%. The motile *Aeromonas* showed a wide variation in percentage homology, while in contrast the non-motile *Aeromonas* appeared to be a genetically homogenous group, with very high homology values (Maclnnes and Trust, 1979).

2. Plasmid analysis

Plasmid analysis is relatively simple and does not require very special equipment. Bacterial strains are lysed to prepare a plasmid, and tested with electrophoresis and ethidium bromide staining. Plasmid analysis is of little epidemiological value due to there being few plasmids in the genus *Aeromonas* and plasmids can be easily lost. In addition, plasmids might be conjugate between strains, and thus have low discrimination to identify *Aeromonas* strains (Chang and Bolten, 1987). The relationship between plasmid and capacity of

pathogenicity of *A. hydrophila* isolated from the environment indicated that the number of plasmids is different between *A. hydrophila* strains from the different environments and the number of *A. hydrophila* plasmids relates with the capacity for pathogenicity. *A. hydrophila* strains from the environment have more diversity of plasmids (Borrego *et al.*, 1991).

3. **Ribotyping**

Ribotyping is based on the hybridization of rRNA or of a DNA probe containing genes coding for rRNA to genomic DNA in the strains. The genomic DNA is digested with an appropriate restriction enzyme, and the digested fragments separated in an agarose gel are transferred onto a membrane by Southern blotting. This restriction pattern reflects the heterogeneity in the restriction sites. Reproducibility and stability of ribotyping patterns is excellent. Ribotyping is useful for epidemiological investigation of *Aeromonas* strains (Altwegg *et al.,* 1991).

4. Pulsed Field Gel Electrophoresis (PFGE)

PFGE is based on the different profiles generated from specific restriction endonuclease cutting to produce a large number of fragments. These fragments were separated in agarose gel by the influence of constant low electric field strength. Advantages of this method are that it is a rapid and discriminatory technique. Disadvantages are inconvenience or impossibility to compare a large number of fragments (Talon *et al.*, 1996).

5. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis uses separation of whole cell protein such as outer membrane protein (OMPs) according to size of protein by using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Protein profiles of the various organisms are investigated. However, the patterns produced are usually very complex, and thus it is difficult for interpretation (Hanninen, 1994).

6. Restriction endonuclease analysis

Restriction endonuclease analysis involves comparison of the number and the size of fragments produced by digestion of DNA with a restriction endonuclease (RE) as an enzyme that cuts DNA constantly within a specific recognition site. Usually, RE is composed of 4 to 6 bp fragment products ranging from 5-50 kb. The complete digestion of DNA with a specific RE gives a reproducible array of fragments. These fragments can be separated by agarose gel electrophoresis and visualized by staining with ethidium bromide. It is not easy to interpret restriction profiles because of the large number of bands. This problem can be improved by using a nucleic acid probe to reduce the number of bands after restriction endonuclease digestion (Kuijper *et al.*, 1989).

7. Restriction Fragment Length Polymorphism (RFLP-PCR)

RFLP-PCR is a technique to make restriction endonuclease profiles by using restriction endonuclease cutting PCR-products. The selection of a specific restriction endonuclease is important and is based on two criteria which are (1) the restriction fragment must be suitable for analysis in terms of size and frequency and (2) the fragments in this size range should not be too numerous, to avoid overlapping bands. Usually, 16S rRNA genes of all *Aeromonas* strains are highly similar and the difference of nucleotides has range of 1 to 32 bases (Matinez-Murcia *et al.*, 1992). The RFLP-PCR study of *Aeromonas* using l6S rRNA genes with endonuclease, *Alu*I and *Mbo*I, and using computer analysis provided the specific profiles in each species of clinical *Aeromonas* isolates (Borrel *et al.*, 1997), but *Nar*I and *Hae*III were used to differentiate *A. salmonicida* from *A. encheleia*. Figueras *et al.* (2000) added two additional endonucleases *Alw*NI and *Pst*I to this restriction fragment length polymorphism (RFLP) method to differentiate *A. salmonicida* and *A. bestiarum* and for recognition of *A. popoffii*.

8. Randomly Amplified Polymorphic DNA polymerase chain reaction (RAPD-PCR)

Williams and colleagues developed the RAPD-PCR technique in 1990. RAPD-PCR is a rapid and simple technique, which requires no previous knowledge of nucleotide sequences, and is not reliant on the actual transcription and translation. In addition, it is highly sensitive, requiring a minimum amount of template DNA and it potentially analyses

the whole genome, as well as being highly discriminative. RAPD-PCR was used to study the differentiation of seven *A. hydrophila* strains and thirteen *A. salmonicida* strains in genospecies and it was found that the scatter profiles of motile *A. hydrophila* isolates were different between isolates (Miyata *et al.*, 1995; Inglis *et al.*, 1996; Oakey *et al.*, 1996). These indicate the genomic diversity of *A. hydrophila* isolates, while the profiles of non-motile *A. salmonicida* isolates were homogeneous. RAPD-PCR may be useful for preliminary investigation of relatedness within *Aeromonas* groups because: (1) RAPD-PCR analysis has proved useful to demonstrate the similarity of isolates of *A. salmonicida* subspecies *salmonicida* from widely diverse geographical origins; (2) the technique allows discrimination of atypical strains and demonstration of like isolates within the heterogenous hydrophila-complex; (3) RAPD-PCR promises to be useful in epidemiological studies for rapid identification of bacteria for which a source of reference DNA is available and may be useful in preliminary investigations of relatedness within groups; but (4) the limitations of the method in comparative studies between systems must be borne in mind, at least within the current technical constraints (Inglis *et al.*, 1996).

9. Amplified Fragment Length Polymorphic-Polymerase Chain Reaction (AFLP-PCR)

For the AFLP-PCR analysis, the total genomic DNA of microorganism is digested with restriction endonucleases. Then restriction fragments are selectively amplified under high-stringency PCR conditions. The amplification products are separated by running polyacrylamide gel and visualized by autoradiography and the AFLP-profile or band patterns is useful to differentiate between strains of microorganisms. AFLP-PCR can separate the different 14 DNA hybridization groups (HGs) in the genus of *Aeromonas.* The digitized fingerprints of 13 AFLP corresponds with the DNA hybridization group and shows the significant genotypic heterogeneity of *A. eucrenophila* (HG6), but this method does not separate the difference between *A. veronii* (HG8/10) and *A. eucrenophila* (HG6) (Huys *et al.*, 1996). AFLP technique is a valuable high-resolution genotypic tool for classification of *Aeromonas* species.

Conclusion

Aeromonas causes traveller's diarrhoea affecting millions of people, particularly traveller's visiting less developed regions (Asia, Africa and Central and South America). *Aeromonas* spp. should be included in the list of possible enteric pathogens so that the organisms will not be overlooked. *A. hydrophila* is responsible for causing Motile Aeromonad Septicemia (MAS), Hemorrhagic Septicemia, Ulcer disease or Red-Sore disease in fresh water fishes. 'Stress' is the main underlying factor in addition to mishandling, overcrowding, transportation under poor conditions, poor level of nutrition and poor water quality. The presence of *Aeromonas* in fishes is the most common and troublesome cause of Motile Aeromonad Septicemia and treatment with terramycin and romet approved to be useful for control of Motile Aeromonad infections in fishes. The virulence factor of these isolates associated with EUS can be compared with of human diarrheal and environmental isolates. Modern methods like PCR, Plasmid profile are more affective to differentiate virulent and avirulent strains of *Aeromonas*.

Future Scope

Aeromonas hydrophila is a widespread representative of *Aeromonas* found in water, water habitants, domestic animals and foods (fish, shellfish, poultry, and raw meat). The microorganism has the potential to be a foodborne pathogen, especially strains from hybridization group (HGl), associated with clinical cases of illness. The pathogen produces different virulence factors including exotoxins, cytotoxins and others. As a psychrotroph, *A. hydrophila* grow in foods during refrigeration. The disease spectrum associated with this microorganism includes gastroenteritis, septicemia, traumatic and aquatic wound infections, and infections after medical leech therapy. Multiple resistance of the bacterium to many antimicrobials is a fact of high significance. The potential of *A. hydrophila* to become a food borne pathogen is a controversial issue. Many approaches are effective for control of the presence of *A. hydrophila* in food for human consumption. The serotypes of *Aermonas* should be studied thoroughly using the latest tools of molecular biology to get the detailed antigenic

profiles. This can be added to better understanding of the zoonotic nature and mutation patterns of the organisms. The epidemiological features of *Aeromonas* spp. infection should be vividly studied including the environmental factors, immunosuppressive factors and other adaptability factors of host and pathogen responsible for the establishment of pathogenic state. Public health and safety aspect of meat products sold in the market should always be the first priority and should taken into account strictly. Detailed characterization of various toxins of the organism can be further studied. Also, efforts should be directed to have better vaccines with specific portion of the immunogens to get better immunogenicity than the vaccines used now a days with variable efficacies. The multiple drug resistance phenomenon showed by these organisms should also be studied in details and the changes of transferable drug resistance and plasmid borne resistance phenomenon in order to invent newer antimicrobial substances which are cheap, safe and effective with newer mechanism of action. All slaughterhouse workers should be screened serologically. The pathogenicity can be studied in detail through histopathological examination to know the extent of pathogenesis of the disease.

References

Abbott, S. L., Cheung, W.K., Roske-Bystrom, S., Malekzadeh, T., and Janda, J.M., 1992. Identification of *Aeromonas* strains to the genospecies level in the clinical laboratory. ***Journal of Clinical Microbiology***. 30(5):1262-1266.

Agger, W.A., Mc Cormik, J.D. and Gurwith, M.J., 1985. Clinical and microbiological features of *Aeromonas hydrophila* associated diarrhoea. ***Journal of Clinical Microbiology.*** 21: 909-913.

Alavandi, S.V. and Ananthan, S., 2003. Biochemical characteristics, serogroups, and virulence. factors of *Aeromonas* species isolated from cases of diarrhoea and domestic water samples in Chennai. ***Indian. J. Med. Micrbiol***. 21(4): 233-238.

Altwegg, M., Altwegg-Bissig, R., Demarta, A., Peduzzi, R., Reeves, M.W. and Swaminathan, B., 1988. Comparison of four typing methods for *Aeromonas* species. ***J Diarrhoeal Dis Res***. 6(2):88–94.

Altwegg, M., Martinetti Lucchini, G., Luthy-Hottenstein, J., and Rohrbach, M., 1991a. *Aeromonas* associated gastroenteritis after consumption of contaminated shrimp. ***European Journal of Clinical Microbiology & Infectious Diseases***. 10(1):44-45.

Altwegg, M., Reeves, M.W., Altwegg-Bissig, R., and Brenner, D.J., 1991b. Multilocus Enzyme Analysis of the Genus *Aeromonas* and its use for Species Identification. ***Zentralblatt Fuer Bakteriologie***. 275(1):28-45.

Aslani, M.M., and Alikhani, M.Y., 2004.The role of *Aeromonas hydrophila* in diarrhea. ***Iranian Journal of Public Health.*** 33(3): 54-59.

Araujo, R.M., Arribas, R.M., Lucena, F. and Pare, R., 1991.Relation between *Aeromonas* and fecal coliforms in fresh waters. ***Journal of Applied Bacteriology***. 67: 213-217.

Awan, B.M., Ahmed, M.M., Bari, A. and Saad, M.A., 2005. Biochemical characterization of the *Aeromonas* species isolated from food and environment. ***J. physiol***. 1: 1-2.

Balaji, V., Mary, V.J. and Sridharan, G., 2004. Cytotoxin testing of environmental *Aeromonas* spp. In vero cell culture. ***Indian Journal of Medical Research***. 119:186-189.

Baron, J.O. and Finegold, M., 1990. *Diagnostic microbiology* 8th edition, Pp 435-433. The C.V Company, New York.

Bishara, J.F., 1984. *Aeromonas* biology of the organism and disease in children. ***Paediatric Infectious Disease Journal***. 3:164-175.

Borrego, J.J., Moringo, M.A., Martinez-Manzanares, E., Bosca, M., Castro, D., Barja, J.L. and Oranzo, A.E., 1991. Plasmid associated virulence properties of environmental isolates of *Aeromonas hydrophila*. ***J. Med. Microbiol***. 35(5):264-269.

Borrell, N., Acinas, S.G., Figueras, M.J. and Martinez-Murcia, A.J., 1997. Identification of *Aeromonas* clinical isolates by restriction fragment length polymorphism of PCR-amplified 16S rRNA genes. ***Journal of Clinical Microbiology***. 35(7):1671-1674.

Borrell, N., Figueras, J.M., and Guarro, J., 1998. Phenotypic identification of *Aeromonas* genomospecies from clinical and environmental sources. ***Can. J. Microbiol***. 44(2): 103-108.

Bottarelli, E. and Ossiprandi, M.C.,1999. *Aeromonas* infection: An update http:/www.unipr.it/apra/facvet/annali 1999/bottarilli 2 bottarelli:htm.

Chamory, E.J., Forem, M. and Drancourt, M., 1999. An invitro evaluation of chlorine against environmental and nosocomial isolates of *Aeromonas hydrophila*. ***Journal of Hospital Infection***. 41: 45.

Chang, B. and Bolton, SM., 1987. Plasmids and resistance to antimicrobial agents in *Aeromonas sobria* and *Aeromonas hydrophila* clinical isolates. Antimicrob. Agents. ***Chemother***. 31(8): 1281-1282.

Cheesbrough, M., 2005. District Laboratory Practice in Tropical Countries part 2, Pp 192-193 Cambridge University press low price edition.

Figueras, M. J., Guarro, J. and Martinez-Murcia, A., 2000. Use of restriction fragment length polymorphism of the PCR-amplified 16S rRNA gene for the identification of *Aeromonas* spp. ***Journal of Clinical Microbiology***. 38(5):2023-2024.

Gobat, P.F. and Jemmi, T., 1995. Comparison of seven selective media for the isolation of mesophilic *Aeromonas* species in fish and meat. ***Int. J. Food. Microbiol***. 24(3): 375-384.

Guinee, P.A., Jansen, W.H., Gielen, H., Rijpkema, S.G., Peters, P.W., 1987. Protective immunity against *Vibrio cholerae* infection in the rabbit. ***Zentralbl Bakteriol Mikrobiol Hyg*** [A]. 266 (3-4):552-562.

Hanninen, M.L.,1994. Phenotypic characteristics of the three hybridization groups of *Aeromonas hydrophila* complex isolated from different sources. ***Journal of Applied Bacteriology***. 76:455–462.

Havelaar, A H., During, M. and Versteegh, J.E. 1987. Ampicillin dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filteration. ***J. Appl. Bacteriol.*** 62(3): 279-287.

Havelaar, A. H., Schets, F.M., Van Silfhout, A., Jansen, W.H., Wieten, G. and Van Der Kooij, D., 1992. Typing of *Aeromonas* Strains from Patients with Diarrhoea and from Drinking Water. ***Journal of Applied Bacteriology***. 72(5):435-444.

Holmes, P. and Sartory, D.P., 1993. An evaluation of media for the membrane filteration enumeration of *Aeromonas* ,from drinking water. ***Lett. Appl. Microbiol***. 17(2): 58-60.

Huys, G., Coopman, R., Janssen, P. and Kersters, K., 1996. High-resolution genotypic analysis of genus *Aeromonas* by AFLP fingerprinting. ***Int. J. Syst. Bacteriol***. 46:572-580.

Inglis, V., Colquhoun, D., Pearson, M.D., Miyata, M. and Aoki, T., 1996 Analysis of DNA relationships among *Aeromonas* species by RAPD (randomly amplified polymorphic DNA) typing. ***Aquaculture International***. 4: 43-53.

Janda, J.M. and Abbott, S.L., 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. ***Clin. Infect. Dis***. 27:332–344.

Jatau, E.D. and Yakubu, S.E. , 2004. Incidence of *Aeromonas hydrophila* in tilapia obtained from Ahmadu Bello University Dam Zaria. ***Nigeria Journal of Scientific Research***. 4: 86- 91.

Jawetz, Melnick, and Adelberg's. ,2004. *Medical Microbiology*. In: Geo, F. B., Karen, C. C., Janet, S. B. and Stephen, A. M. 23rd Int. edition Pp. 267-272 McGraw Hill publisher.

Jawetz, Melnick, and Adelberg's., 2007. *Medical microbiology*. In: Geo, F.B., Karen, C.C., Janet, S.B. and Strephen, A.M. Pp 270-273, 24th ed. McGraw Hill International edition.

Jenkins, A.J. and Taylor, W.P., 1995. An alternative bacteriological medium for isolation of *Aeromonas* spp. ***J. Wildlife. Dis***. 31(2): 272-275.

Kandakai-Olukemi, Y.T., Mawala, J.D., Olukemi, M.A. and Ojumah, S.O., 2007. *Aeromonas* related diarrhoea in Nasarawa, Nigeria. ***Anal. of African Medicine***. 6(2): 76-79.

Kiujper, E.J., Steigerwalt, A.G., Schoenmakers. B.S.C.I., Peeters. M.F., Zanen, H.C. and Brenner, D.J., 1989. Phenotypic characterization and DNA relatedness in human fecal isolates of *Aeromonas* species. ***J. Clin. Microbiol.*** 27:132-138.

Krovocek, K., Dunontet, S., Ericksson, E. and Baladj, S.I., 1992. Isolation and virulence profile of *Aeromonas hydrophila* implicated in an outbreak of food poisoning in Sweden. ***Microbiology Immunology***. 39:5655-5661.

Leblanc, D., Mittal, R.K., Oliver, G. and Lallier, R., 1981. Serogrouping of motile *Aeromonas* species isolated from healthy and moribund fish. ***Appl. Environ. Microbiol***. 42(1): 56-60.

MacInnes, J. I., Trust, T. J. and Crosa, J. H., 1979. Deoxyribonucleic acid relationships among member of the genus *Aeromonas*. ***Can. J. Microbiol***. 25(5): 579-586.

Markwardt, M.N., Gocha, M.Y., and Klontz, W.G. 1989. A new application for Coomassie Brilliant Blue agar: Detection of *Aeromonas salmonicida* in clinical samples. ***Dis. Aquat. Org***. 6: 231-233.

Martinez-Murcia, A.J., Esteve, C., Garay, E. and Collins, D.M., 1992. *Aeromonas allosaccharophila* sp. *nov*., a new mesophilic member of the genus *Aeromonas.* FEMS. ***Microbiol. Lett***. 91: 199-206.

Micheal, J., 1991. Recent advances in the study of taxonomy pathogenicity and infections syndromes associated with the genus *Aeromonas*. ***Clinical Microbiology Review***. 4:397-409.

Michael, J.P.R., Chan, E. C. S., and Noel, R. K., 2000. *Microbiology*. 5th edition, Pp 274. Tata McGraw-Hill Publishing Company Limited. New Delhi.

Mishra, S., Balakrish, G., Bhadra, K.R., Sikdar, N.S., and Pal, C.S., 1987. Comparison of selective media for primary isolation of *Aeromonas* species from human and animal feaces. ***J. Clin. Microbiol***. 25(11): 2040-2043.

Miyata, M., Aoki, T., Inglis, V., Yoshida, T., and Endo, M. , 1995. RAPD analysis of *Aeromonas salmonicida* and *Aeromonas hydrophila****. J. Appl. Bacteriol***. 79(2): 181-185.

Moyer, N.P., Martinetti. G.J., Luthy-Hottenstein., and Altwegg, M., 1992a. Value of rRNA gene restriction patterns of *Aeromonas* spp. for epidemiological investigations. ***Current Microbiology***. 24(1):15-22.

Moyer, N.P., Luccini, G.M., Holcomb. L.A., Hall, N.H., and Altwegg, M., 1992b. Application of Ribotyping for Differentiating Aeromonads Isolated from Clinical and Environmental Sources. ***Applied and Environmental Microbiology***. 58(6):1940-1944.

Oakey, J.H., Ellis, J.T. and Gibson, L.F., 1996. Differentiation of *Aeromonas* genomospecies using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). ***J. Appl. Bacteriol***. 80(4): 402-410.

Picard, B. & Goullet, P.H. , 1985. Comparative electrophoretic profiles of esterases and of glutamate, lactate and malate dehydrogenases, from *Aeromonas hydrophila*, *A. caviae* and *A. sobria*. ***Journal of General Microbiology***. 131: 3385-3391.

Picard, B., Goullet, P.H., 1987. Epidemiological complexicity of hospital *Aeromonas* infections revealed by electrophoretic typing of esterases***. Epidemiol. Infect***. 98, 5–14.

Payment, P., Gamache, F. and Paquetta, G., 1988. Microbiology and virological analysis of water from two water filtration plants and their distribution system. ***Canadian Journal of Microbiology***. 34:1304-1309.

Ribas, F., Araujo, R., Frias, J., Huguet, M.J., Ribas, R.F., and Lucena, F. , 1991. *Comparison of different media for the identification and quantification of Aeromonas spp. in water*. L. Antonie van Leeuwenhoek. 59(4): 101-104.

Sachan, N. and Agarwal, K.R., 2000. Selective enrichment broth for the isolation of *Aeromonas* spp. from chicken meat. ***Int. J. Food. Microbiol***. 60(1): 65-74.

Selander, R.K., Caugant, D.A., Ochman, H., Musser, J.M., Gilmour, M.N., Whittam, T.S., 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. ***Appl. Environ. Microbiol***. 51:873-884

Singh, U. , 1997. Isolation and identification of *Aeromonas* spp. from ground meats in Eastern ***Canada. J. Food Proctec.*** 60(2): 125-130.

Shotts, B.E. and Rimler, R., 1973. Medium for isolation of *Aeromonas hydrophila.* Appl. Microbiol. 26(4): 550-553.

Sylvia, V., Cecillia, U., Susana, M., Matide, R.C., Laras, S., Graciela, C.E., Maria J.F. and Juan M.T., 2004. Complete type III secretion system of a mesospheric *Aeromonas hydrophila* strain. ***Applied Environmental Microbiology***. 70(11):6914-6919.

Talon, D., Dupont, M.J., Thouverez, M. and Michel-Briand, Y., 1996. Pulsed-field gel electrophoresis as an epidemiological tool for clonal identification of *Aeromonas hydrophila****. J. Appl. Bacteriol***. 80: 277-282.

Tonolla, M., Demarta, A., Peduzzi, R., 1991. Multilocus genetic relationships between clinical and environmental *Aeromonas* strains*. FEMS **Microbiol. Lett.*** 81:193–200.

USEPA, 2005. *Aeromonas* detection what does it means http/www. Epagor/safe water urenr/date Aeromonas. htmt.

Ventura, C., Civera, T. and Grassi, M.A., 1998. Aeromonas ailments; rishi sanitarie modalita di controllo. ***Indian Alim***. 37:982.

Villari, P., Crispino, M., Montuori, P. and Boccia, S. , 2003. Molecular typing of *Aeromonas* isolated in a natural mineral water. ***Applied Environmental Microbiology***. 69: 697-701.

Von Graevenitz, A. and Bucher, C., 1993. Evaluation of different selective media for isolation of *Aeromonas* and *Plesiomonas* spp. from human feces. ***J. Clin. Microbiol***. 17: 16-21.