Year: 2014; Volume: 1; Issue: 1
Article ID: MI14 11; Pages: 1-8

BioMed Research

The Open Access Publisher

www.bmrjournals.com

# BMR Microbiology

*Research Article*

## Effect of water purification methods on waterborne pathogen Aeromonas spp. from Surat water distribution system

**Desai B.A.* and Desai, P.B.**

Department of Microbiology, Shree Ramkrishna Institute of computer education and Applied Sciences, Athwalines, Surat-395 001

Correspondence should be addressed to Desai B.A.

Received 9 July 2014; Accepted 23 July 2014; Published 1 August 2014



### Abstract

The occurrence of Aeromonas spp. in water supply of Surat city was monitored from several samples like four main water works, nine different pumping station and different customer usage water samples for a period of 1 year. The selective isolation was carried out by using membrane filter technique using M-Aeromonas selective media and selective isolation was done on Rippey Cabelli agar plate. Total 199 isolates were obtained. There are 22 different species of Aeromonas known till date, out of which 11 species are commonly found in aquatic environment. According to the research work, 9 different isolates of Aeromonas spp. were isolated from the water samples under study they are Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae Aeromonas Salmonicida subsp. salmonicida, Aeromonas schubertii, Aeromonas veronii, Aeromonas euccrenophila, Aeromonas trota and Aeromonas popoffii. The growth range of Aeromonas shows seasonal variation more often during the warmer months.

**Key words:** Membrane Filter Technique, *Aeromonas spp.* Seasonal variation.

### Introduction

Species of *Aeromonas* are Gram negative, non-spore-forming, rod-shaped, facultative anaerobic bacteria which are widely spread in the aquatic ecosystem like seawater, irrigation water, river water, brackish water, fresh water, ground water, spring water, sewage water etc [9,14,19]. *Aeromonas spp.* have frequently been found in water including chlorinated and unchlorinated drinking water and from bottled mineral water, which shows that they are able to withstand long periods of nutrient limitation [6,19,21]. Although historically the genus *Aeromonas* has undergone a number of taxonomic and nomenclature revisions over the past 15 years. Originally it was placed in the family *Vibrionaceae*, due to similarities in there characteristics. But later *Aeromonas* from removed from the family *Vibrionaceae* and transfer to a new family *Aeromonadaceae* [1,13]. The survival of

ISSN 2349-4603

Aeromonads in these ecosystems can be correlated with their capacity to produce and live within Biofilm on the surface of pipes and bottles [17]. *Aeromonas spp.* have been associated with diarrheal illness and can cause infection and septicemia .the infections can occur in healthy people of all ages and are often acquired through foreign travel. Wound infections are relatively uncommon but can progress rapidly if not treated [8]. The main species causing human infection are *Aeromonas hydrophila, Aeromonas caviae, Aeromonas veronii subsp. sorbia and Aeromonas media*. Some motile species associated with hemorrhagic septicemia in freshwater fish and amphibians [7]. Exposure to water contaminated with *Aeromonas spp.* has been reported to precede some human *Aeromonas* infections which are particularly hazardous in patients with impaired immunity. The purpose of present study was to isolate *Aeromonas* spp. from aquatic ecosystem (chlorinated and unchlorinated drinking water).This serves to check the quality of water and for the purpose of public health [5].

## Material and methods

**Sampling:**

Every month samples were collected from four main water works, nine different pumping station and different domestic water samples from June 2013 to May 2014. Here three seasons were considered viz. June 2013 to September 2013-monsoon season, October 2013 to January 2014-winter and February 2014 to May 2014-summer season. As *Aeromonas spp.* are found in drinking water; the samples need to be collected from the similar sites every month to check seasonal variations. (Table 1)

The water samples are collected from different sites as mention in the table(**Table 1: Sampling sites for water samples)**

| **Water works:** | |
|---|---|
| **WW1** | NANAWARACHHA |
| **WW2** | SARTHANA |
| **WW3** | KATARGAM |
| **WW4** | RANDER |
| **PUMPING STATION:** | |
| **WPS1** | KATARGAM |
| **WPS2** | UMARWADA |
| **WPS3** | KHATODARA |
| **WPS4** | UDHNA |
| **WPS5** | ATHWA |
| **WPS6** | JOGANINAGAR |
| **WPS7** | PANDESARA |
| **WPS8** | DUMBHAL |
| **WPS9** | ALTHAN |
| **T1** | SMC tap water |
| **DOMESTIC WATER SAMPLES:** | |
| **PB1** | AQUA GUARD CRYSTAL WATER PURIFIER (power boiling + ) |
| **R.O** | AQUA GUARD REVIVA WATER PURIFIER |
| **U.V** | AQUA GUARD CLASSIC WATER PURIFIER |
| **R.O+U.V** | AQUA GUARD DUO WATER PURIFIER |

**Isolation**

The selective isolation was carried out by using Membrane Filter Technique (0.45 µm) to get enumerate *Aeromonas* in drinking water by using M-*Aeromonas* selective media, incubated at $25^0$ C [16]. 100 ml of water sample was filtered for enumeration. Isolated colony is selected for further screening. For selective isolation of *Aeromonas*

ISSN 2349-4603

*hydrophila*, Rippey Cabelli agar plate (Hi media, Mumbai) was used at 25$^0$ C [15].

**Phenotypic Identification**

The morphological and colony characteristics were studied using Nutrient agar plate and Mac conkey's agar plate along with highly selective medias M-*Aeromonas* selective media and Rippey Cabelli agar plate. The physiological characteristics of all the obtained isolates were studied. The biochemical characteristics (Oxidase, Catalase, Indole, Methyl Red, V-P Test, Citrate (Simmons), H2S Prodn , Brown Soluble Pigment, Gelatin, Urea, Lipase, Bile esculin, Lysine Decarboxylase, Nitrate, Phenylalaine, and various Sugars) were also carried out from them, using standard references. [W.H.O. manual-2007, Brenner 2005 and Macfadin 2000].

## Results

The selective isolation was carried out by using membrane Filter Technique using M-*Aeromonas* selective media.

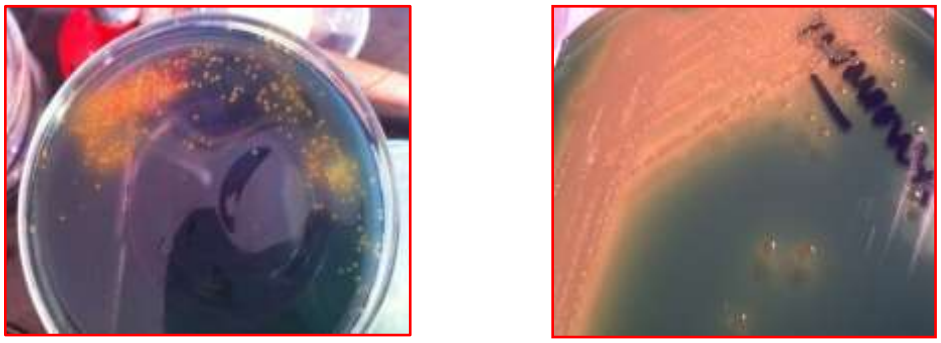

**Fig-1: M-Aeromonas selective medium**

The microbiological analysis of the water samples were done by membrane filter technique using M-Aeromonas selective media, which gives bright yellow color colonies but older colonies showed greenish hue. (Fig. 1) Another highly selective media is Rippey Cabelli which is used for identification of Aeromonas *hydrophila,* as it is the most pathogenic bacteria from all *Aeromonas spp.* which gives blue color colony of *Aeromonas hydrophila.* (Fig. 2)

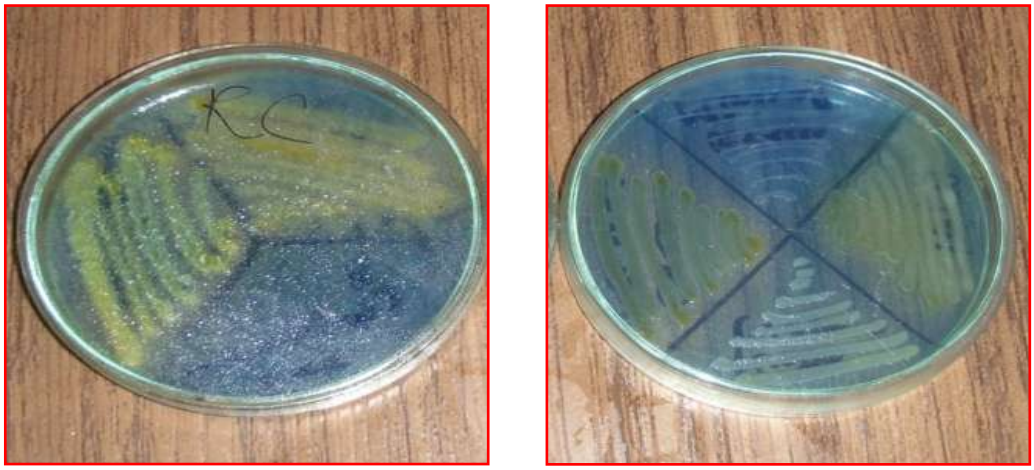

**Fig-2: Rippey Cabelli Agar Plate**

The total number of isolates was counted and further proceeded for their morphological and motility test. The colony characteristics of the obtained isolates were studied using Nutrient agar plate and Mac Conkey's agar plates which indicated small pin point colonies, mostly lactose fermented after 24 h of incubation. They were Gram negative, short rods and appeared singly. All isolates grow well at $25^0$C . Following table 2 shows results of various biochemical characteristics of the isolates.

| Test | Isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 96 | 26 | 25 | 11 | 12 | 12 | 08 | 05 | 04 |
| **Oxidase** | + | + | + | + | + | + | + | + | + |
| **Catalase** | + | + | + | + | + | + | + | + | + |
| **Indole** | + | + | + | - | - | + | + | + | + |
| **Methyl Red** | + | - | + | + | + | + | + | + | + |
| **V-P Test** | + | + | - | + | - | + | - | - | - |
| **Citrate (Simmon)** | - | - | - | - | - | + | - | - | - |
| **$H_2S$ Prod$^n$** | + | - | - | + | - | - | - | - | - |
| **Brown Soluble Pigment** | - | - | - | + | - | - | - | - | - |
| **Gelatin** | + | + | + | + | + | + | + | + | + |
| **Urease** | - | - | - | - | - | - | - | - | - |
| **Lipase** | + | - | + | + | + | + | - | + | + |
| **Bile esculin** | + | - | + | + | - | + | + | + | + |
| **Lysine Decarboxylase** | - | + | - | - | + | + | - | - | - |
| **Nitrate** | + | + | + | + | + | + | + | + | + |
| **Phenylalanine deaminase** | - | + | - | - | - | + | - | - | - |
| Carbohydrates | | | | | | | | | |
| **Glucose** | A | - | A | A | - | A | A | + | + |
| **Lactose** | V | - | V | - | - | - | - | - | - |
| **Adonitol** | - | - | - | - | - | - | - | - | - |
| **Arabinose** | A | - | A | A | - | - | A | - | + |
| **Cellobiose** | - | V | V | - | - | V | A | + | - |
| **Dulcitol** | - | - | - | - | - | - | - | - | - |
| **Erythritol** | - | - | - | - | - | - | - | - | - |
| **Galactose** | A | A | A | A | A | A | A | + | + |
| **Glycerol** | A | V | V | V | V | A | A | + | + |
| **Inositol** | - | - | - | - | - | - | - | - | - |
| **Maltose** | A | A | A | A | A | A | A | + | + |
| **Mannitol** | A | V | A | A | - | A | A | + | + |
| **Mannose** | V | A | V | A | A | A | A | + | + |
| **Melibiose** | - | - | - | - | - | - | - | - | - |
| **Raffinose** | - | - | - | - | - | - | - | - | - |
| **Rhamnose** | - | - | - | - | - | - | - | - | - |
| **Salicin** | A | - | A | V | - | A | A | - | - |
| **Sorbitol** | - | V | - | - | - | - | - | - | - |
| **Sucrose** | A | V | A | - | - | A | V | + | - |
| **Trehalose** | A | V | A | A | A | A | A | + | + |
| **Xylose** | - | - | - | - | - | - | - | - | - |

**Table 2 : Biochemical characteristics of the isolates.**

Along with morphology and motility characteristics isolates bacterial species were identified on the basis of their biochemical characteristics. We found various spieces of Aeromonas viz. *Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae Aeromonas Salmonicida subsp. salmonicida, Aeromonas schubertii, Aeromonas veronii, Aeromonas euccrenophila, Aeromonas trota and Aeromonas popoffii* respectively. The total number of isolates and its types are shown in fig 3.

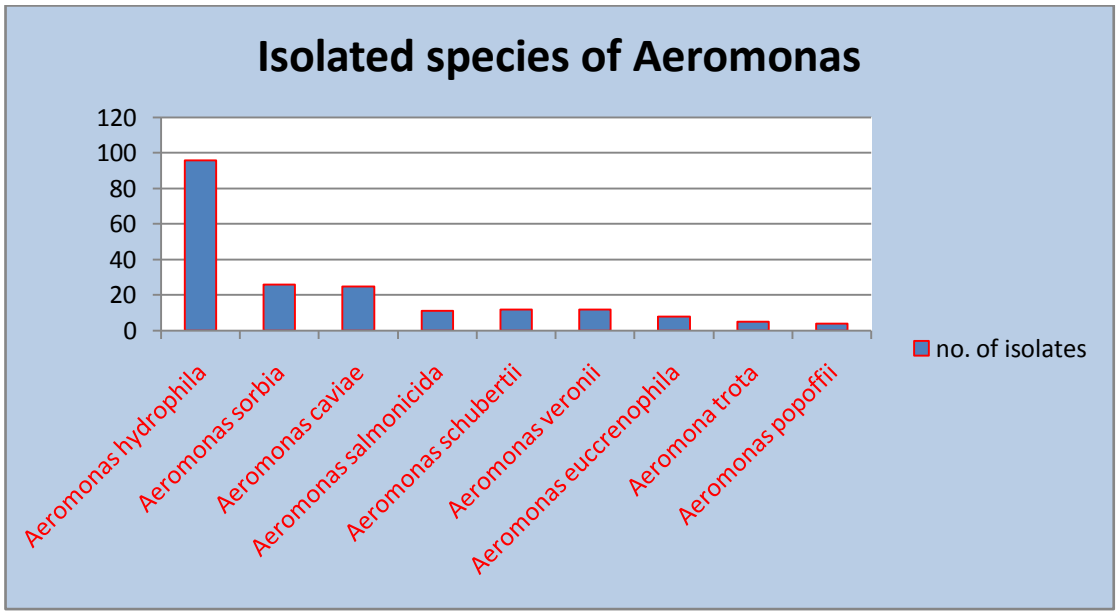


**Fig: 3 : Isolated species of Aeromonas.**

The monthly count of Aeromonas is considered as three seasons i.e. Monsoon (June 2013 to September 2013), winter (October 2013 to January 2014) and summer (February 2014 to may 2014). Our analysis as per seasons of variations bacteria under study is shown in fig 4.

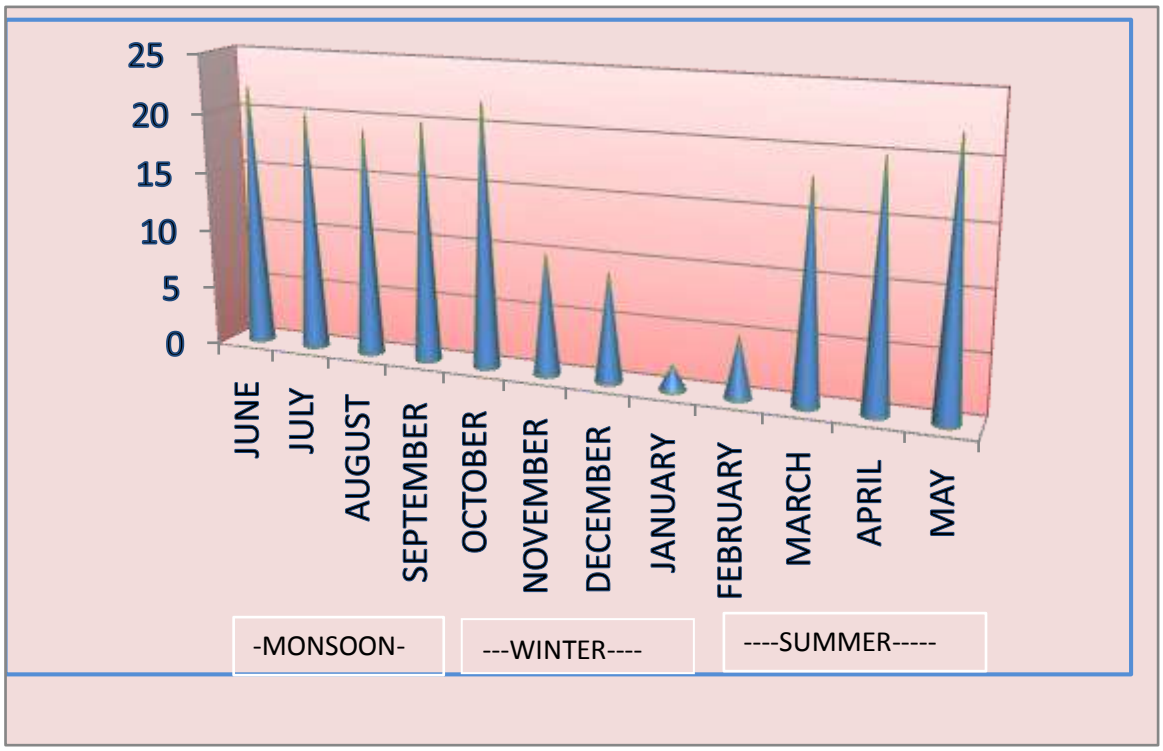


**Fig 4: Seasonal variation in number of bacteria.**

## Discussion

From the above study nine different species of *Aeromonas* from 199 isolates. This suggests the presence of *Aeromonas spp.* in drinking water. It is of concern with regard to human health. There are 22 different species of *Aeromonas* known till date, out of which 11 species are commonly found in aquatic environment [1]. According to the research work, 9 different isolates of *Aeromonas spp.* were isolated from the water samples under study. We found different species of *Aeromonas* viz. *Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae Aeromonas Salmonicida subsp. salmonicida, Aeromonas schubertii, Aeromonas veronii, Aeromonas euccrenophila, Aeromonas trota and Aeromonas popoffii.* from the different water samples. The highest number of isolates obtained was of *Aeromonas hydrophila* (96), *Aeromonas sobria* (26) and *Aeromonas caviae* (25) during June 2013 to May 2014. Burke (1984) studied seasonal variation and he reported that the count of *Aeromonas* spp. were more in warmer months; Same findings were achieved with our study, as the number of isolates are more in warmer months than in winter, and thus the seasonal variation was observed. *Aeromonas hydrophila, Aeromonas sobria and Aeromonas caviae* are the three species of *Aeromonas* which were found to have maximum number during summer seasons whereas *Aeromonas euccrenophila, Aeromonas trota and Aeromonas popoffii* were the least during the seasons. Cavari (1981), Chauret (2001), and Clark (1982) reported in their study that, *Aeromonas hydrophila Aeromonas trota and Aeromonas popoffii* are present in drinking-water distribution system. Same results were found with our research work. Le Chevallier reported the presence of *Aeromonas sobria* in chlorinated water. In our study it also showed prences of same spp.

## Conclusion

Different *Aeromonas spp.* were isolated from different water samples within the distribution systems. *Aeromonas hydrophila* which is the main common spieces which can cause diarrheal illness and can cause infection and septicemia in frog, amphibians and most comely in fish. Indirectly fish is a diet food of human, so infection can spread through fish to human's .Thus it serves to check the quality of water and for the purpose of public health. They are able to withstand long periods of nutrient limitation and have capacity to produce and live within Biofilm on the surface of pipes and bottles. Thus a mear suggestion is that the presence of Aeromonas spp. in drinking water.

## References


1.Brenner J. D, Krieg R. N. Staley T. J. 2005. Bergey's manual of Systematic Bacteriology, second edition,volume 2 The Proteobacteria, Part B The Gammaproteobacteria.

2. Cavari, B. Z., D. A. Allen, and R. R. Colwell. 1981. Effect of temperature on growth and activity of Aeromonas spp. and mixed bacterial populations in the Anacostia River. Appl. Environ. Microbiol. 41:1052-1054.

3. Chauret C., Volk C., Creason R., Jarosh J., Robinson J. and Warnes C. (2001). Detection of *Aeromonas hydrophila* in a drinking-water distribution system: a field and pilot study. 47: 782-786(5).

4. Clark, J. A., G. A. Burger, and L. E. Sabatinos. 1982. Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. Can. J. Microbiol. 28:10021013.

5. Denis de Oliveria Scoaris, Jean Colacite, Celso V. Nakamura, Tania Ueda-Nakamura, Benicio A. de Abreu Filho and Benedito P. Dias Filtho. (2007). Virulence and antibiotic susceptibility of *Aeromonas Spp.* isolated from drinking water. 93: 111-122.

6. Desai B. A. and Desai P.B., 2011, Isolation and seasonal correlation with isolates of Bacterial water borne pathogens *Aeromonas* spp. in water distribution system- current and emerging organisms of concern. Proceeding of International Conference on Innovative Science and Engineering Technology.pg no. 226-230.

7. Desai B. A. and Desai P.B., 2012, Study of pathogenic microorganisms from infected fish. Proceeding of National Conference on Advanced Trends in Applied Sciences and Technology.pg no.579-583.

ISSN 2349-4603

8. Hanson, P. G., J. Standridge, F. Jarrett, and D. G. Maki. 1977. Freshwater wound infection due to Aeromonas hydrophila. J. Am. Med. Assoc. 238:1053-1054.

9. Khalifa Sifaw Ghenghesh, Khalifa Belhaj, Amna Algaui, Enas Alturki, Amal Rahouma and Salaheddin Abeid. (2007). Bacteriological quality of drinking water obtained from Mosques in Tripoli, Libya.

10. Le Chevallier, M. W., T. M. Evans, R. J. Seidler, 0. P. Daily, B. R. Merrell, D. M. Rollins, and S. W. Joseph. 1982. *Aeromonas sobria* in chlorinated drinking water supplies. Microb. Ecol. 8:325-333.

11. M.W.Lechevallier, T.M. Evans, R.J. Seidler, O. P. Daily, B. R. Merrell, D. M. Rollins, and S. W. Joseph. (2005). *Aeromonas Sobria* in chlorinated drinking water supplies. 8: 325-333.

12. Macfadin F. Jean. 2000. Biochemical tests for identification of medical bacteria-3rd edition.

13. Marisa Di Bari, Hachich Elayse M., Melo Adalgsia M. J., Sato and Maria I. Z. (2007). *Aeromonas Spp.* and Microbial indicators in raw drinking water sources. 38: 516-521.

14. Massa, S.; Altieri, C.; D'Angela, A.A.A. (2001). The occurrence of Aeromonas spp in natural mineral water and well water. Intern. J. Food Microbiol., 63, 169-173.

15. Rippey, S. R., and V. J. Cabelli. 1980. Occurrence of *Aeromonas hydrophila* in limnetic environments: relationship of the organism to trophic state. Microb. Ecol. 6:45-54.

16. Sharon L. Abbott, Wendy K. W. Cheung and J. Michael Janda. (2003). The genus *Aeromonas:* Biochemical characteristics, Atypical reactions and Phenotypic identification schemes. 41: 2348-2357.

17. Sylvia M. Kirov, Marika Castrisios and Jonathan G. Shaw. (2004). *Aeromonas* flagella (Polar and Lateral) are enterocyte adhesions that contribute to Biofilm formation on surfaces. 72: 1939-1945.

18. V Burke, J Robinson, M Gracey, D Peterson and K Partridge. (1984). Isolation of *Aeromonas hydrophila* from a metropolitan water supply: seasonal correlation with clinical isolates. 48: 361-366.

19. V Burke, J Robinson, M Gracey, D Peterson, N meyer and V. haley. (1984). Isolation of *Aeromonas Spp.* from an unchlorinated domestic water supply. 48: 367-370.

20. World Health Organization. 2007. International standards for drinking water, 3rd ed. World Health Organization. Geneva.

21. Warburton, D.W.; Dodds, K.L.; Burke, R.; Johnston, M.A.; Laffey, P.J. (1992). A review of the microbiological quality of bottled water sold in Canada between 1981 and 1989. Can. J. Microbiol., 38, 12-19.

ISSN 2349-4603