African Journal of Biotechnology Vol. 11(9), pp. 2287-2295, 31 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2056
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Bacterial community patterns of municipal water of Sukkur city in different seasons

Abdul Hussain Shar*, Yasmeen Faiz Kazi, Nisar Ahmed Kanhar and Irshad Hussain Soomro

Department of Microbiology Shah Abdul Latif University Khairpur, Sindh, Pakistan.

Accepted 2 December, 2011

The objective of this study was to determine whether there was an association of seasonal variation and bacterial communities of municipal water. The sampling was carried out fortnightly after a flow time of 5 min to eliminate any contaminant present in the mouth of tap in sterilized screw caped 500 ml white glass flasks (Pyrex), containing 0.1 ml of a 1.8% solution of sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) per 100 ml of sample. Samples were placed in ice boxes and brought to laboratory within 1 h of collection. Samples were analyzed for bacterial communities using standard microbiological method (membrane filtration technique). The suspected colonies were then further purified and identified using API 20E (BioMerieux) commercial identification kit. Twelve pathogenic bacterial species were isolated and identified from municipal water on conventional and selective media. Their prevalence was higher in summer season. The average isolation rate was as follows: *Escherichia coli* 69.4%, *Proteus mirabilis* 65.2%, *Providencia rettgeri* 65.2%, *Providencia stuarti* 61%, *Klebsiella oxytoca* 54.1%, *Citrobacter youngae* 60%, Non fermenter species 57%, *Chryseobacterium meningosepticum* 51.3%, *Vibro mimicus* 39%, *V. cholerae* 38%, *Aeromons hydrphilia* 65.2% and *Pseudomonas aeruginosa* 78%. It is important to mention that water samples were positive for the above pathogens throughout the study period (2005 to 2007). The temperature of water samples was reported highest in July to September and the pH of water samples ranged 7 and 7.8. The bacteriological quality of drinking water under study was very poor. In summer, the isolation rate of bacterial communities was higher than in winter

Key words: Waterborne pathogens, drinking water, contamination.

INTRODUCTION

Waterborne diarrheal diseases are the primary causes of water related health problems. Studies have linked yearly out breaks of gastroenteritis among children to seasonal freshwater algal blooms with cyanobacteria (blue green algae) in the reservoir that supplies water to the people (Chorus and Bartram, 1999). In Brazil in 1988, an out break of gastroenteritis that killed 88 people living near the Itaparcia dam was linked to a large bloom of cyanobacteria in dammed lake (Chorus and Bartram, 1999). In developing countries, the quality of drinking water is unsatisfactory. In Pakistan, the unchecked disposal of sewage and industrial wastewater in water sources and excessive use of fertilizers and insecticides are the major sources of contamination of drinking water reservoirs. Bacteriological contamination of drinking water has been reported to be one of the most serious problems throughout the country (Kahlown et al., 2004). Such contamination is attributed to leakage of pipes, pollution from sewerage pipes and faulty water distribution systems. Pakistan's drinking water quality ranks as 80th of 122 Nations (UNESCO, 2002). It is calculated that in 2004 to 2005 approximately 38.5 million people lacked access to safe drinking water source and approximately 50.7 million lacked accesses to improved sanitation in Pakistan (Khan and Jawed, 2007). The government of Pakistan estimated with regard to diarrhea that this mainly water related disease accounts for 14% of illnesses for children under five and 7% of all disease

*Corresponding author. E-mail: abdulhussainshar@yahoo.com.

in people age of five and older and 40% of all reported illnesses are also water related (Pakistan Council of Research and Water Resources (PCRWR), 2005). It has also been estimated that 200,000 children in Pakistan die every year due to diarrheal diseases alone. Unsafe water affects mainly rural and urban poor, who suffer above the average from water related diseases and 60% of infants deaths are caused by waterborne diarrhea in Pakistan (Pakistan National Human Development Report, 2003). Keeping in view the reported cases of diarrhea and gastroenteritis in different seasons in local hospitals of Sukkur, this study was undertaken to investigate the presence of waterborne and water-related bacterial pathogens in municipal water reservoirs in different seasons from 2005 to 2007.

MATERIALS AND METHODS

Water sampling sites

The study sites comprised main storage reservoir of municipal water of Sukkur city. The source of this water was surface water from River Indus. The sampling was carried out fortnightly after a flow time of 5 min to eliminate any contaminant present in the mouth of tap in sterilized screw caped 500 ml white glass flasks (Pyrex), containing 0.1 ml of a 1.8% solution of sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) per 100 ml of sample, in order to neutralize the effect of residual free chlorine which could inhibit the growth of target bacteria. Collected samples were placed in ice boxes and brought to laboratory within 1 h of collection. Samples were processed within 2 h of sampling.

Microbiological analysis

Samples were analyzed for members of bacterial communities using standard microbiological method (membrane filtration technique). The suspected colonies were then further purified and identified by using API 20E (BioMerieux) commercial identification kit. The following culture media were used: Xylose lysine deoxycholate (XLD) agar, MacKonkey's agar, nutrient (NA) agar, Eosin-methylene blue (EMB) deoxycholate citrate (DCA) agar, thoisulphate citrate bile salt sucrose TCBS agar, pseudomonas F agar and ampicillin dextrin agar.

Sample processing (method for the examination of waters and associated materials, 2002)

The sterile filtration assembly (Barant, USA) was placed in a position and connected with source of vacuum. The funnel was removed and holding the edge of the membrane filter of pore size 0.45 µm and the diameter 47 mm (Micropore). With the help of sterile smooth tipped forceps, the sterile membrane filter was placed onto the porous disc of the grid base. The sterile funnel was replaced securely on the filter base. A 100 ml water sample in duplicate was poured into the funnel by applying the vacuum of 65 kpa (500 mm of mercury) sample was filtered slowly through membrane filter. The stopcock was closed as sample was filter to prevent the air to be drawn through the membrane filter. The funnel was removed and the filter was carefully transferred to 47 mm diameter, Petri plates containing appropriate agar media which were left at 37°C temperature for 30 min prior to use. To remove the air bubbles trapped between the membrane filter and the medium, the membrane filter was rolled onto the medium. The Petri plates were incubated at 44.5°C for 24 h for thermo-tolerant fecal colifrom and at 37°C for other bacterial species. The process was repeated to other replicates of water samples. After incubation, the membrane filters were examined for typical colonies. Further, the isolates were sub-cultured on nutrient agar for final confirmation by API 20E biochemical kit (BioMerieux), according to manufacturer's instructions. Typical growth of each bacterial species was recorded and confirmed by gram staining, motility and oxidase test .

Statistical analysis

A Pearson correlation between water temperature and bacterial isolates was analyzed by using SPSS version 10. Software.

RESULTS

Keeping in view the public health impact of drinking water quality in Sukkur, the present study was carried out to isolate bacterial pathogens together with indicator bacteria of fecal contamination especially, *Escherichia coli* present in the municipal water which is used for drinking, cooking, bathing and other domestic uses by the population. The comparison of bacteriological quality of drinking water of Sukkur city with the WHO guideline values for drinking water was also intended in present study.

Escherichia coli

In drinking water (Municipal water) samples collected from Sukkur, *E. coli* was isolated in the present study during 2005 and 2007. The isolation rate of *E. coli* was recorded quarterly throughout the study period. The mean quarterly isolation rate in January to March was 44.4%, in April to June it was 89%, in July to September it was 100% and in October to December it was 44% as shown in Table 1. The pattern of quarterly isolation rate of *E. coli* in this study was: July to September>April to June>January-March>October to December. In 2005, the mean yearly isolation rate of *E. coli* was 75%, in 2006 the mean yearly isolation rate was 71% and in 2007 the mean yearly isolation rate was 62.5%. The mean isolation rate of three years was 69.4% as given in Table 2. The mean yearly isolation pattern of *E. coli* remained thought study was 2005>2006>2007, indicating decrease in isolation rate with passage of time.

Proteus mirabilis

In drinking water of Sukkur city, *P. mirabilis* was isolated during 2005 to 2007. The quarterly isolation rate in different quarters was different. The mean quarterly isolation rate in January to March was 55.5%, in April to June it

Table 1. Mean Quarterly Isolation Rate of Bacterial Pathogens from Drinking Water of Sukkur City during (2005-2007).

Bacteria	January-March	April-June	July-September	October-December
E. coli	44.4%	89%	100%	44.4%
P. mirabilis	55.5%	83.3%	83.3%	39%
P. rettgeri	44.4%	94.4%	78%	44.4%
P. stuarti	50%	61%	94.4%	39%
K. oxytoca	33.3%	72.2%	83.3%	28%
C. youngae	39%	72.2%	78%	61.1%
Non-fermenter Species	28%	50%	94.4%	55.5%
C. meningosepticum	39%	78%	55.5%	33.3%
V. mimicus	38.6%	61.3%	61%	38.6%
V. cholerae	0%	50%	55.6%	44.5%
A. hydrophilia	17%	67%	89%	28%
p. aeruginosa	78%	83.3%	89%	61.1%

was 83.3%, in July to September it was 83.3% and in October to December isolation was 39% as shown in Table 1. The isolation rate was found increased in 2nd and 3rd quarters when compared with 1st and 4th quarter giving a prevalence pattern as April to June = July September>January to March>October to December. In 2005, the mean yearly isolation rate was 71%, in 2006 the mean yearly isolation rate was 75% and in 2007 the mean yearly isolation rate was 50%. The mean isolation rate of three years was 65.2% as shown in Table 2. The year wise isolation pattern was 2006>2005>2007.

Providencia rettgeri

In drinking water of Sukkur city, *P. rettgeri* was isolated during 2005 to 2007. The quarterly isolation rate in different quarters throughout the study period was different. In January to March, the mean quarterly isolation rate was 44.4%, in April to June it was 94.4%, in July to September it was 78% and in October to December, the isolation rate was 44.4% as shown in Table 1. *P. rettgeri* has shown very high isolation rate in 2nd and 3rd quarter where as 1st and 4th quarter showed equal isolation rate. As shown in the results that this organism exhibited the quarterly pattern of isolation rate as April to June>July to September>October to December=January to March. In 2005, the mean yearly isolation rate was 62.5%, in 2006 the mean yearly isolation rate was 71% and in 2007 the mean yearly isolation rate was 67%. The mean isolation rate of three years was 65.2% as is given in Table 2. The mean yearly isolation rate pattern shown by this bacterial species was 2006>2007>2005.

Providencia stuarti

P. stuarti was isolated during 2005 to 2007 from drinking water (Municipal water) of Sukkur city. The isolation rate of these bacteria in drinking water was also varying in different quarters. The mean quarterly isolation rate in different quarters of three years that is, in January to March was 50%, in April to June it was 61%, in July to September it was 94.4% and in October to December the isolation rate was 39% as shown in Table 1. As shown in the results, this organism exhibiting the isolation rate pattern as July to September>April to June>January to March>October to December. The mean yearly isolation rate in 2005 was 71%. In 2006, the mean yearly isolation rate was 62.49% and in 2007 the mean yearly isolation rate was 62.49%. The mean isolation rate of three years was 61% as shown in Table 2. The yearly isolation rate pattern was 2005>2006=2007

Klebsiella oxytoca

The *K. oxytoca* was isolated from drinking water (Municipal water) of Sukkur city during 2005 and 2007. The isolation rate was different in different quarters. The mean quarterly isolation rate in different quarters that is, in January to March was 33.33%, in April to June it was 72.2%, in July to September it was 83.3%, in October to December the mean quarterly isolation rate was 28% as shown in Table 1. The *K. oxytoca* was isolated at high isolation rate in summer months and the pattern of prevalence showed by these bacteria in different quarter was July to September>April to June>January to March>October to December. In 2005, mean yearly isolation rate was 62.4%, in 2006 isolation rate was 54.1% and in 2007 the mean yearly isolation rate was 46%. The mean isolation rate of three years was 54.1% as shown in Table 2. The yearly prevalence pattern of this organism was 2005>2006>2007.

Table 2. Mean Yearly Percentage of Isolation Rate of Bacterial Pathogens from Drinking Water of Sukkur City during (2005-2007).

BACTERIA	2005 (%)	2006 (%)	2007 (%)	Total average (%)
E. coli	75	71	62.5	69.4
P. mirabilis	71	75	50	65.2
P. rettgeri	62.5	71	67	65.2
P. stuarti	71	62.4	62.4	61
K. oxytoca	62.4	54.1	46	54.1
C. youngae	58.3%	67	62.4	60
Non-fermenter Species	58.3	62.4	50	57
C. meningosepticum	46	50	58.3	51.3
V. mimicus	58.4	50	42	50.1
V. cholerae	33.3	46	33.4	38
A. hydrophilia	50	58.3	42	50
p. aeruginosa	83.3	79.1	71	78

Citrobacter youngae

The isolation rate of *C. youngae* was different in different quarters. The mean quarterly isolation rate in different quarters of three years that is in January-March was 39%, in April to June it was 72.2%, in July to September it was 78% and in October to December the mean quarterly isolation rate was 61.1% as shown in Table 1. *C. youngae* showed the same prevalence pattern of other members of *Enterobacteriaceae* isolated in this study. This organism was isolated from the water samples in the different quarters as July to September>April to June>October to December>January to March. The mean yearly isolation rate in 2005 was (58.3%), in 2006, it was 67% and in 2007 it was 62.49%. The mean isolation rate of three years was 60% as shown in Table 2. In the three years of study, these organisms were isolated and the pattern they exhibited was 2006>2007> 2005.

Non-fermenter species

The drinking water of Sukkur city was also investigated for waterborne bacterial pathogens. The non-fermenter species was isolated during the study period. The mean quarterly isolation rate in different quarters throughout study period was observed as: in January-March the mean isolation rate was 28%, in April- June it was 50%, in July-September it was 94.4% and in October-December the mean quarterly isolation rate was 55.5% as shown in Table 1. The non fermenter species showed the pattern isolation rate that is July-September> October-December> April- June> January-March. The mean yearly isolation rate was also recorded. In 2005, the mean yearly isolation rate was 58.33%, in 2006 the mean yearly isolation rate was 62.4% and in 2007 the mean yearly isolation rate was 50%. The mean isolation rate in three years was 57% as shown in Table 2. The pattern of prevalence in three years of this organism was 2006>2005>2007.

Chryseobacterium meningosepticum

The isolation rate of *C. meningosepticum* was different in different quarters in drinking water of Sukkur city. The mean quarterly isolation rate in different quarters throughout the study period was observed as: in January-March, April-June, July-September, October- December, the mean isolation rate was 39, 78, 55.5, 33.3% respectively as shown in Table 1, April-June> July-September>January-March>October-December. The mean yearly isolation rate was also recorded throughout study period. In 2005, in 2006 and in 2007 the mean yearly isolation rate was 46, 50, 58.3% respectively. The mean isolation rate of three years was observed according to these results the mean isolation rate was 51.38% as shown in Table 2. The yearly pattern of isolation rate of this organism was that is 2007>2006>2005.

Vibrio mimicus

The isolation rate of *V. mimicus* was different in different quarters in drinking water samples. The mean quarterly isolation rate in different quarters throughout the study period was observed as: in January-March, April-June, July-September, October- December, the mean isolation rate was 39, 61, 78 and 33.4% respectively. The mean yearly isolation rate was also recorded throughout the

study period as shown in Table 1. *V. mimicus* showed the quarterly pattern of isolation rate that is July-September> April-June> January-March >October-December. In 2005, in 2006 and in 2007 the mean yearly isolation rate was 58.3, 62.5 and 39%, respectively. The mean isolation rate of three years was observed according to these results. The mean isolation rate was 53% as shown in Table 2. The yearly pattern of isolation rate showed by this organism was 2006>2005>2007.

Vibrio cholerae

The isolation rate of *V. cholerae* was different in different quarters in drinking water samples. The mean quarterly isolation rate in different quarters throughout the study period was observed as: in January-March, April-June, July-September, October- December, the mean isolation rate was 0, 50, 56 and 44.4% respectively as shown in Table1. Quarterly pattern of isolation rate showed by this organism in drinking water samples was that is July-September>April-June>October- December>January-March. The mean yearly isolation rate was also recorded throughout study period. In 2005, in 2006 and in 2007 the mean yearly isolation rate was 33.3, 46 and 33.3% respectively. The mean isolation rate of three years was observed according to these results the mean isolation rate was 39% as shown in Table 2. The year wise pattern of isolation rate showed of this organism was 2006>2005=2007.

Aeromons hydrophila

In drinking water samples from Sukkur city, *A. hydrophila* was isolated. The mean quarterly isolation rate in different quarters throughout the study period was observed as: in January-March, April-June, July-September, October- December, the mean isolation rate was 17, 67, 89 and 28% respectively as shown in Table 1. *A. hydrophila* was isolated at high isolation rate in warmer months than summer month and showed a prevalence pattern that is July-September>April-June>January-March>October-December. The mean yearly isolation rate was also recorded throughout the study period. In 2005, in 2006 and in 2007, the mean yearly isolation rate was 50, 58.3 and 42% respectively. The mean isolation rate of three years was 50% as shown in Table 2. This organism showed the yearly prevalence pattern as 2006>2005>2007.

Pseudomonas aeruginosa

The isolation rate of *P. aeruginosa* was different in different seasons in drinking water samples from Sukkur city. The mean quarterly isolation rate in different quarters throughout the study period was observed as: in January-March, April-June, July-September, October-December, the mean isolation rate was 78, 83.3, 89 and 61.1% respectively as shown in Table 1. *P. aeruginosa* was isolated in all seasons at high isolation rate; the pattern it showed by these bacterium was July-September>April-June>January-March>October-December. The mean yearly isolation rate was also recorded throughout the study period. In 2005, in 2006 and in 2007, the mean yearly isolation rate was 83.3, 79.1 and 71% respectively. The mean total isolation rate of three years was observed; according to these results, the mean total isolation rate was 78% as shown in Table 2. In the three years of study, *P. aeruginosa* was isolated from drinking water samples, it showed little bit different prevalence pattern that is 2005>2006>2007 (Tables 1 and 2).

Temperature measurement

Temperature of 72 drinking water samples (municipal water; n =72) that is 18 samples in each quarter was measured during 2005-2007. January-March minimum temperature was 16.0°C, the maximum temperature was 23.3°C, and the mean temperature was 20.6°C; April-June minimum temperature was 26.0°C, maximum temperature was 30.2°C and the mean temperature was 28.1°C; July-September, the minimum temperature was 30.0°C, the maximum temperature was 31.0°C and the mean temperature was 30.7°C and in October-December the minimum temperature was 20.0°C, maximum temperature was 29.5°C and mean temperature was 25.70°C as shown in Table 3.

Correlation between isolated waterborne bacterial pathogens and water temperature

The correlation matrices among isolated waterborne bacterial pathogens and their correlation with water temperature from study area were analyzed. The isolated waterborne bacterial pathogens showed moderate to strong correlation with water temperature throughout the study period (r ranging from 0.60-0.98). *E. coli* (r = 0.81), *P. mirabilis* (r = 0.85), *P. rettgeri* (r = 0.66), *P. stuarti* (r = 0.70), *K. oxytoca* (r = 0.75), *C. younga* (r = 0.90), *Nonfermeter spp.*(r = 0.65), *V. mimcus* (0.91), *V. cholera* (0.73), *A. hydrophilia* (r = 0.98). However, *C. meningosepticum* showed weak (r = 0.35) *and P. aeruginosa* showed moderate (r = 0.60) correlation with water temperature.

pH analysis of drinking water of Sukkur city

In the present study, the pH of water was analyzed in order to observe the quality of drinking water of Sukkur

Table 3. Temperature and pH of water sample in different quarters of study years (2005-2007).

Parameter	Time period	Minimum	Maximum	Mean	Standard deviation
Temperature (°C)	Jan-Mar	16.0	23.3	20.6	1.9
	Apr-Jun	26.0	30.2	28.1	1.3
	Jul-Sep.	30.0	31.6	30.7	0.5
	Oct-Dec.	20.0	29.5	25.2	2.9
pH	Jan-Mar.	7.2	8.3	7.8	0.3
	Apr-Jun.	7.0	8.2	7.81	0.2
	July-Sep.	7.1	8.5	7.7	0.3
	Oct-Dec.	7.0	8.5	7.8	0.4

city. It was observed that the pH of drinking water was in rage (6.5-8.5) fixed by World Health Organization guidelines for drinking water.

The pH of drinking water (municipal water) of Sukkur city during 2005-2007 in January-March, the minimum pH was 7.2, maximum pH was 8.3, and the mean pH was 7.8. In April-June, the minimum pH was 7.0, maximum pH was 8.2 and mean pH was 7.81. In July- September, the minimum pH was 7.1, the maximum pH was 8.5, and the mean pH 7.7. In October- December minimum pH was 7.0, maximum pH was 8.5 and the mean pH was 7.8 as given in Table 3. The pH value in different quarters did not show any significant variation; therefore, the bacterial species were isolated in all quarters with some fluctuations throughout the study period (Table 3).

DISCUSSION

This study was aimed to analyze the bacterial communities in municipal water of Sukkur city Sindh Pakistan. Further it determined the prevalence of different bacterial species in different months at different temperatures. This study shows the presences of 12 bacterial species in municipal water of Sukkur city during three years of study period (2005-2007). From the order of prevalence, *E. coli* was the most abundant after *P. aeruginosa* and followed by *P. mirabilis* and *P. rettgeri* through out the study period. Among these bacterial species, most are known human pathogens. *E. coli* causes diarrhea and enteritis (Zamxaka et al., 2004). *P. mirabilis* has been reported as an opportunistic pathogenic bacterium causing neonatal meningoencephalitis (Grahnquist et al., 1992). Presence of *P. mirabilis* during three consecutive years with different isolation rates (Table 2) indicates the unhygienic drinking water quality in the area. The source of contamination by this organisms may be anthropogenic and from animals. Isolation of *P. rettgeri* from municipal water is alarming because *P. rettgeri* can cause diarrhea (Yoh et al., 2005). *P. stuarti* has long been recognized as a pathogen for nursing home patients with chronic in-dwelling urinary catheters. The organism may have the etiologic role in diarrheal diseases; Yoh et al. (2005) isolated the *P. stuarti* from patients with traveler's diarrhea. In the present study, this species have also been isolated from drinking water of Sukkur city. It is important to mention that the source of water reservoirs via River Indus where clinical waste is also dumped which could enhance the number of bacterial communities in the water investigated. *Klebsiella* sp. *a*re opportunistic human pathogens that can be isolated from various animal and human clinical specimens (Podschun et al., 1998). The infection in neonatal and pediatric-Intensive care units have been reported and are frequently associated with serious systemic infection or death (Kayyali et al., 1972). In the present study, the *K. oxytoca* has been isolated from municipal water of Sukkur city. This bacterium is usually present in the fecal material of human and warm blooded animals. The presence of this organism in municipal water may be indicating fecal contamination of municipal water. *Nonfermenter* species though primarily regarded as contaminants or incidental organisms, they are becoming increasingly important as opportunistic pathogens in immunocompromised patients. They can also cause infection by gaining access to normally sterile body sites through trauma (Baron and Finegold, 1990). Presence of non-fermenter species may cause major health problems in people who come in contact with such contaminated water and may suffer from water related infections.

Citrobacter species causes gastroenteritis and opportunistic infection. These bacteria can also cause urinary and respiratory tract infection, especially in immune-

compromised individuals which may be associated with meningitis, brain abscess and neonatal abscess (Badger *et al* 1999). In the present study, *C. youngae* has been isolated from drinking water samples. This organism may be present in fecal material of human and animals and may enter in the sources of water of the Sukkur city through activities of human and animals.

C. meningosepticum is a ubiquitous, waterborne saprophytic bacterium. It is an opportunistic pathogen; the intermittent epidemics in Neonatal Intensive Care Units (NICUs) have been reported. Environmental studies show that this organism can survive in chlorine treated water often colonizing sink basins and taps and creating potential reservoirs for infections inside hospital environments. (Hoque et al., 2001). *Vibrio mimicus* was isolated from drinking water of Sukkur city, Sindh, Pakistan with different levels of isolation rate. The importance of this bacterium as a pathogen was elaborated when in 1991 a large cholera outbreak started in Latin America and etiologic agent was *V. cholerae* O1 but during this epidemic cases of severe diarrhea associated with the *V. mimicus* were reported in Costa Rica. Campos et al. (1996) and Fanning et al. (1981) suggested that both *V. cholera*-O1 and non-O1 as well as *V. mimicus* are potentially important in terms of public health in areas where sanitation and personal hygiene are very poor. Association between *V. mimicus* and fresh water and aquatic plants has been described. The factors responsible for adhesion of *Vibrio* are lectin fibronectin, collagen binding and the presence of bacterial fimbriae or microbial surface antigens which bind to a specific receptor of the host cells (Matthysse, 1992). Several bio-physico-chemical parameters are also involved in growth of *V. cholerae* in water. Along with bacterial adhesion factors *V. cholerae* secrets the degrading enzyme that degrades mucin and mucin like substances in plant cells and contributes to the association between *Vibrios* and plant surfaces especially in aquatic plant root. The plant surface may act as a habitat or a reservoir for *V. cholerae* through a non-specification association or by commercial interaction (Islam et al., 1990). It was observed that reservoirs under study were showing plant growth. *Aeromonas* species are widely distributed in the aquatic environment including raw and processed drinking water and have been isolated from various food products such as fish, shellfish, raw meat, vegetables and raw milk (Holmes et al*.,* 1996). In present study, *Aeromonas hydrophila* was isolated from drinking water of Sukkur city as it is mentioned that this organism can be pathogen, the presences of small fishes in storage pond of municipal water of Sukkur city were observed during this study which may be the reason of source of this organism in these waters.

Pseudomonas aeruginosa is mostly a nosocomial pathogen. According to the National Nosocomial Surveillance System of the CDC, the overall incidence of *P. aeruginosa* infection in US hospital between 1985-and 1991was 4.0 per 100 discharges, being the fourth most frequently isolated nosocomial pathogen. The presence of pathogenic bacterial species in municipal water of Sukkur city was an indication of elevated bacterial contamination of drinking water of Sukkur city. Results on the distribution of bacterial communities in drinking water during different quarters showed that Enterobacteriaceae was dominating with high isolation rate in hot months. In winter months, the isolation rate of these species was low. The *A. hydrophila* was very low in winter months and high in summer months. The *P. aeruginosa* was isolated in all seasons at a moderate isolation rate. The high prevalence of bacterial species in summer months in municipal water may be due to contamination of water of (River Indus) which is the source of drinking water of Sukkur city. The seasonal distribution of bacterial communities may be influenced by the human recreational activities in the region. In summer, the recreational activities get increased as compared to the cold season. The hot season may also influence the animal to come in close contact with river water which is the sources of drinking water in urban Pakistan including Sukkur. Huge efforts to explore the diversity of the bacterial kingdom have been made very little understanding of the factors that drive the actual composition of bacterial communities in water. In the studies of lakes, multivariate analysis showed that factors such as the biomass of plankton groups may play part in diversities of bacterial communities (Jardillier et al., 2004). The fresh water algal blooms and increased concentrations of agricultural chemicals and heavy metals in drinking water sources have all been linked to increased temperatures, greater evaporation and heavy rain events (Chorus and Bartram, 1999), which can increase microbial nutrients (Yannarell and Triplett, 2005). To investigate the impact of temperature on bacterial communities, the temperature of water sample was analyzed. The temperature range was 17-23°C, during January-March, 26-29°C during April-June, 29-31°C during July-September and 21-29°C during October-December reported in this study as shown in Table 3. The increased temperature of the environment may influence the temperature of river water. Environmental factors and particularly the incubation temperature has an influence on the survival ability of the bacteria in nutrient poor-water (Sautour et al., 2003; Gavriel et al., 1998) reported that Aermonads were found in high numbers in summer when the temperature was around 20-25°C and were rarely detected during the cold months (Gavriel et al., 1998). The results of our study showed the consistency with the reports of Kertsters et al*.* (1995) and Gavriel et al*.* (1998) in the prevalence timing of *A. hydrophila*. Temperature is widely recognized as an important controlling factor in influencing bacterial growth. In climate where water temperatures are warm,

bacterial growth may be very rapid, however, the minimum temperature at which microbial activity has been observed varies from system to system. The bacteria respond more quickly and at greater extent to dissolved organic matter (DOM) additions at higher temperatures (Kirchman and Rich, 1997). In the present study, high frequency of waterborne bacterial pathogens was isolated in summer months than in winter which indicates the possible effect of temperature on prevalence of waterborne bacteria inside biofilm environment. Furthermore, high temperatures facilitate the growth of bacteria by increasing solvent quality of water thereby increasing the organic matter and other element that results in enhancing the growth and multiplication of bacteria in water under study. It was observed during this study that the pH of water samples investigated ranged 6.5-8.5. The results show that there was no significant change observed in drinking water samples. The pH may also co-vary with bacterioplakton in aquatic environment. Temperature will also affect the equilibrium and the pH. In pure water, a decrease in pH of about 0.45 occurs as the temperature is raised by 25°C (APHA, 1989). The water flow may influence the bacterial communities in the fresh water environment. Such association between environmental factors and bacterial communities suggests that these environmental factors are important in determining the distribution of the microorganisms in freshwater environment. It was observed that the pH of drinking water was in the range recommended by World Health Organization. It was shown however that the pH of water did not fluctuated significantly during different seasons which may be also a reason of high rate of bacterial population in the water of area under study. Above mentioned factors, Sewage, agricultural and industrial wastes are the primary sources of contamination of drinking water sources of area under study. The microbes may be transported to a treatment plant and pass through the treatment process or enter the water supply system through cross contamination with sewage water and or combined sewer systems overflow (Rose et al., 2001).

REFERENCES

APHA. (1989). *Standard methods for the examination of water and wastewater,* 17th ed. Washington, DC, American Public Health Association.

Badger JL, Stins M F, Kim K S (1999). *Citrobacter freundii* invades and replicates in human brain microvascular endothelial cells. Infect. Immun. 67: 4208-4215.

Baron EJ , Finegold SM (1990) Bailey & Scott's diagnostic microbiology. St. Louis: Mosby Company 9. Forbes, B.A., et al., Bailey and Scott's *http://www.pdfqueen.com/bailey-&-scott's-diagnostic-microbiology.*

Campos E, Bolanos H, Acuna MT, Díaz G, Matamoros MC, Raventós H, Sánchez LM (1996). *Vibrio mimicus* Diarrhea following Ingestion of Raw Turtle Eggs. Appl. Environ.l Microbiol. 62: 1141-1144.

Chorus I, Bartram J. (1999). Toxic cyanobacteria in water. A Guide to their Public Health Consequences, Monitoring and Management. E & FN Spon *(*on behalf of World Health Organisation), London.

Fanning GR, Davis BR, Madden JM, Bradford Jr HB, Steigerwalt AG, Brenner DJ (1981). *Vibrio mimicus*: a newly recognized cholera like organism. Abstr.Am. Sci. Microbiol. p.50.

Gavriel AA, Lander JP, Lamb JP. (1998). Incidence of mesophilic *Aeromonas* within public water supply in north-east Scotland J.Appl.Microbiol. 84: 383-392.

Grahnquist L, Lundberg B, Tullus K. (1992). Neonatal *Proteus* meningoencephalitis Case report. Acta Pathol. Microbiol. Immunol. Scand. 100: 734-736.

Holmes P, Niccolls LM, Sartory DP (1996). The Ecology of Mesophilic *Aeromonas* in the Aquatic Environment. In: The *Aeromonas*. Austin B.M.Altwegg,P.J. Gosling and S. Joseph(Eds) Chrichester, UK: Willy and Sons. pp. 127-150.

Hoque SN, Graham J, Kaufmann ME, Tabaqchali S (2001). *Chrysobacterium (Flavobacterium) meningosepticum* outbreak associated with colonization of water taps in neonatal intensive care unit. J. Hosp. Infection. 47: 188-92.

Islam MS, Drasar BS, Bradley DJ. (1990). Survival of toxigenic *Vibrio cholera*-O1 with common duckweed *Lemna minor* in artificial aquatic exosystems. Trans. R. Soc. Trop. Med. Hyg. 93: 133-139.

Jardillier I, Basset M, Domaizon I, Belan A, Amblard_C, Richardot_M, Debroas D (2004). Bottom up and top down control of bacterial community composition in euphotic zone of a reservoir. Aquat. Microb. Ecol. 35: 259-273.

Kahlown MA, Tahir MA, Sheikh AA (2004). Water Quality Status in Pakistan: Second Report 2002 – 2003, Pakistan Council of Research in Water Resources, Islamabad. 2004 ISBN: 969-8469-13-3146.

Kayyali MZ, Nicolson DP, Smith IM (1972). A Klebsiella outbreak in a pediatric nursery: emergency action and preventive surveillance, *Clin Pediatr.* 11: 422-426.

Khan FJ, Javed Y (2007). Delivering Access to Safe Drinking Water and Adequate Sanitation in Pakistan. Pakistan Institute of Develpoement Economics (PIED) Working Papers. p. 30.

Kirchman DL, Rich JH (1997). Regulation of Bacterial Growth Rates by Dissolved Organic Carbon and Temperature in the Equatorial Pacific Ocean. Microbial Ecology. 33: 11-20.

Kertsters I, Van Vooren, L, Huys, GP, Kristers K , Verstraete W (1995). Influence of temperature and process technology on the occurrence of *Aeromonas* species and hygienic indicator organisms in drinking water production plants. Microbiol Ecology, 30: 203-218.

Matthysse AG (1992). Bacterial Adhesions. In: Laderberg J. (ed.). "Encyclopedia of Microbiology". Academic press, Inc, New York. 1: 29-33.

Methods for the Examination of Waters and Associated Materials, Environment Agency. (2002) : dwi.enquires@defra.gsi.gov.uk.

Pakistan Council of Research and Water Resources (PCRWR). (2005). National Monitoring Program, Report 2004. Islamabad.

Pakistan National Human Development Report. (2003). (Poverty Growth and Governance Karachi. p.23.

Podschun R, Ullmann U (1998). *Klebsiella species* as nosocomial pathogens: epidemiology, taxonomy, typing methods and pathogenicity factors Clin.Microbiol.Rev. 11: 589-603.

Rose JB, Epstein PR, Lipp EK**,** Sherman BH, Bernard SM, Patz JA (2001). Climate Variability and Change in the United States: Potential Impacts on Water- and Foodborne Diseases Caused by Microbiologic Agents. Environ. Health Perspect, 109: 211-221.

Sautour M, Mary P, Chihib NE, Hornez JP (2003). The effects of temperature, water activity and pH on the growth of Aeromonas hydrophila and subsequent survival in microcosm water. J. Appl. Microbiol. 95: 807-813.

UNESCO. (2002). Water quality indicator values in selected countries.. http://www.unesco.org/bpi/wwdr/WWDR_chart2_eng.pdf .

Yannarell AC, Triplett EW (2005). Geographical and environmental sources of variation in lake bacterial community composition. Appl. Environ. Microbiol. 71: 227-239.

Yoh M, Matsuyama J, Ohnishi M, Takagi K, Miyagi H, Mori K, Park KS, Ono T , Honda T (2005).Importance of *Providencia* species as a major cause of travelers' diarrhea J. Med. Microbiol. 54: 1077-1082.

Zamxaka M, Pironcheva G, Muyima NYO (2004). Bacterial community patterns of domestic water sources in the Gogogo and Nkonkobe areas of Eastern Cape, Province, South Afferica. Water SA. 30: 341-346.