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RESEARCH ARTICLE

COLONIZATION OF OPPORTUNISTIC BACTERIA WITHIN DISTRIBUTION SYSTEM

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ABSTRACT

The opportunistic bacteria including *P.aeruginosa* are ubiquitous to a wide variety of aquatic environments including drinking water distribution systems. Concern over the survival and spread of opportunistic pathogens in public drinking water supplies has been expressed in recent years because of the public health risk associated with their presence in properly treated water. A municipal drinking water distribution system in Neemuch was monitored over a 12 month period to investigate population shifts of opportunistic bacteria at various sampling sites. Data generated during this study indicated that samples collected from storage reservoir, DS1, DS2 and end points contained *P.aeruginosa* in substantial amounts significantly higher than the health standards ($P<0.05$). However, *Acinetobacter, Aeromonas, Moraxella* and *Flavobacterium* were detected in negligible amounts with lower isolation rate. Differences in the concentration of opportunistic bacteria among the four sampling sites; Dam, Storage Reservoir, Distribution System and Point of use were significant throughout the year ($P<0.05$), except between the two sampling sites; DS1 and DS2 within the distribution system. Reductions in cell counts and isolation rate of opportunistic bacteria during the treatment method from surface raw water to finished water reflected an improvement in its trophic status, indicating that the treatment practices principally fulfilled their function. However, their presence in samples after chlorine disinfection during distribution presents potential threat to Neemuch community. Collectively, our results show the need to develop best management practices for municipal water to control bacterial regrowth and deterioration of water before it is utilized at the point of consumption.

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INTRODUCTION

Water utilities in India most often add free chlorine or monochloramine at the end of the drinking water treatment process to maintain a disinfectant residual in the Water Distribution Systems. However, an increase of HPC values in treated water during distribution is usually reported even after chlorination, which is described as regrowth or aftergrowth. Despite of several efforts by water treatment authorities to improve drinking water quality, sporadic cases and point of use outbreaks of waterborne diseases continue to occur. Over 80 genera of bacteria that are nonpathogenic to humans have their natural habitat in water. In addition, some opportunistically pathogenic bacteria (*Pseudomonas, Serratia, Acinetobacter, Chromobacterium, Achromobacter, Aeromonas, Moraxella, Flavobacterium* etc.) occur naturally in water.

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Unlike the classic waterborne pathogens, such as *Salmonella*, *Shigella*, and *E.coli*, these opportunistic premise plumbing pathogens are indigenous to the premise plumbing environment and ideally adapted to survival, growth, and persistence in drinking water distribution systems. Furthermore, the presence of these potential opportunistic pathogens shows no correlation with conventional indicator bacterial counts. In recent years, opportunistic pathogens, including *Legionella pneumophila*, Nontuberculous Mycobacteria (NTM), *Pseudomonas aeruginosa*, and *Acanthamoeba* spp. have become a leading source of waterborne disease in developed countries. To address concerns about the quality of final water, regrowth of these pathogens in such water, in addition to regrowth of the indicator bacteria, has to be examined. This study was such an attempt to assess the growth potential of treated water within the distribution system. This was accomplished by specifically determining abundance of opportunistic pathogens with special reference to *P.aeruginosa* in the samples taken just after treatment (storage reservoir), during the distribution (DS1 & DS2) and after the distribution (end points).

MATERIALS AND METHODS

The town of Neemuch enjoys a sub tropical climate, and the weather is dry almost throughout the year. Summers and winters in Neemuch are dry, and the monsoon season brings sparse rainfall. The highest maximum temperature of 46°C reaches in May and June and remains up to last week of June. In winters, the minimum temperature reaches 2°C in month of December & January. The average rainfall of Neemuch City is 812 mm and maximum rainfall occurs in month of July & August.

Sampling sites

The water distribution system investigated is served by Hingoria treatment plant which receives raw water from the dam Jaju Sagar situated at 17 kms from Neemuch and is sole source of municipal water for the city. The raw water pumping station, 700 meters away from the dam, pumps the dam water to the Hingoria treatment plant through different C.I. pipelines where water is given conventional treatment involving coagulation settlement by aluminium sulphate, sedimentation, and rapid gravity sand filtration to improve color and turbidity and then is chlorinated at the service reservoir using dry calcium hypochlorite. Finished water from the treatment plant is pumped to various storage reservoirs situated in the city by two Centrifugal pump sets. There are 8 storage reservoirs in the Neemuch city to distribute treated water to different areas. In the present study, to examine the biostability of disinfected water from the outlet up to the point where the distribution network terminates, chlorinated samples were obtained every fortnight from storage reservoirs, DS1 and DS2 (two middle points in the distribution system) and the end points for one complete year. DS1 was nearer to the reservoir, and DS2 was farther from the reservoir but nearer to the end points.

Sample Collection

Raw surface water samples were collected from the four corners of the dam and composite samples were prepared to analyze numbers of opportunistic bacteria before treatment. Samples from the disinfected supplies were collected in sterile 250-ml glass bottle fitted with a glass stopper containing 0.1 ml of 2% sodium thiosulfate to quench the disinfectant (APHA 2005). The samples were shipped on ice to the laboratory.

Sample Analysis

Isolation & Identification of Opportunistic Bacteria

Serial dilution agar plating method was adopted for the enumeration of viable bacterial populations on Plate Count Agar (Himedia, Mumbai, Maharashtra, India). Identification of opportunistic bacteria was done by applying the scheme developed for the rapid and easy identification of standard plate count bacteria from drinking water (Lechevallier *et al.* 1980). The use of these protocols enabled bacterial isolates to be rapidly identified as representing 1 of 17 genera or species. Once the organism was identified as a genera or species, further confirmation could be carried out by routine identification procedures presented in Bergey's Manual.

Characterization of *P.aeruginosa* Isolates

Morphological and Cultural Characterization

Morphological characteristics were studied by using conventional microbiological techniques i.e., light microscopic observations of gram-stained smears. Motility was tested by stabbing the culture into deep tubes of mannitol motility test medium (Himedia, Mumbai, Maharashtra, India), appearance of cloudiness was evident for motility. To study cultural characteristics, colony morphology was observed on cetrimide agar as well as on nutrient agar (Himedia, Mumbai, Maharashtra, India). Type of growth on nutrient agar slant and in nutrient broth was also studied. Oxygen requirement was determined by inoculation in fluid thioglycollate medium (Himedia, Mumbai, Maharashtra, India) deep tubes.

Physiological Characterization

Presumptive *P.aeruginosa* colonies were purified on cetrimide agar and a variety of biochemical assays were carried out to have a comprehensive view of the phenotypic characteristics of the isolates and to confirm their identity.

Statistical Analysis

The statistical significance of differences between *P.aeruginosa* concentrations at various sampling locations; before treatment, just after treatment, during distribution and after distribution was determined using analysis of variance (ANOVA) with Excel using log 10 transformed data.

RESULTS AND DISCUSSION

The spread of opportunistic pathogens via municipal water systems is of growing concern. Present study evaluated the potential of municipal drinking water distribution system in Neemuch, to be a reservoir of opportunistic bacteria, which has demonstrated that *P.aeruginosa* in considerable numbers and other pathogens (*Aeromonas, Acinetobacter, Flavobacterium* and *Moraxella*) in insignificant amounts were recovered after the treatment during the supply of water. The comparative log densities of *P.aeruginosa* obtained in present study in the water supply before and after treatment, during and after distribution are presented in Table 1.

Prevalence of opportunistic pathogens

Before treatment

Surface freshwater is widely used as a source for drinking water production; the majority of the world's human population uses surface water as drinking water. In Neemuch, all of the drinking water consumed is originated from *Jaju Sagar Dam* after being treated through conventional methods at Hingoria Treatment Plant and the inhabitants of Neemuch totally rely on *Jaju Sagar* water for drinking and other domestic activities. Surface water is always contaminated and subject to frequent, dramatic changes in microbial quality as a result of a variety of activities, because discharges of municipal raw water, treated effluents from processing

Table 1. Concentration of *P.aeruginosa* in public water supplies before & after treatment and regrowth during distribution

Sampling Months	Dam		Storage Reservoir		Distribution System			Point of Use	
	Mean* CFU/ml	Log CFU/ml	Mean* CFU/ml	Log CFU/ml	DS1 Mean* CFU/ml	DS2 Mean* CFU/ml	Mean Log FU/ml	Mean* CFU/ml	Log CFU/ml
January	10.6	2.02	0.33	0.51	2.00	4.00	1.47	7.66	1.88
February	13.3	2.12	3.66	1.56	4.00	7.00	1.74	8.00	1.90
March	23.0	2.36	6.33	1.80	8.00	10.0	1.95	13.0	2.11
April	29.0	2.46	7.33	1.86	10.0	8.33	1.96	15.0	2.17
May	31.6	2.50	5.00	1.69	9.33	3.33	1.80	12.3	2.08
June	17.0	2.23	6.00	1.77	7.66	8.00	1.89	9.00	1.95
July	21.0	2.32	0.00	0.00	1.00	5.00	1.47	8.66	1.93
August	20.3	2.30	4.33	1.63	6.00	4.66	1.72	10.6	2.02
September	19.0	2.27	0.00	0.00	2.33	5.00	1.56	6.00	1.77
October	22.0	2.34	1.66	1.22	3.00	9.66	1.80	11.0	2.04
November	15.0	2.17	0.00	0.00	5.33	7.00	1.78	12.0	2.07
December	12.3	2.09	2.33	1.36	8.00	0.00	1.60	14.3	2.15
Frequency of Isolation (n=24)		100%		75.0%	DS1=100%		DS2=91.6%		100%
Range (Log CFU)		2.02-2.50		0.51-1.86			1.00-2.00		1.77-2.17
% of samples crossed detection limit (0 CFU/ml)		100%		75.0%	DS1=100%		DS2=91.6%		100%

*Mean= Average of two fortnight readings (viable count/ml)

facilities, storm water runoff, or other non-point source runoff all affect surface waters. These contaminants are reflected in the highest bacterial load obtained in this study from the dam water samples; which contained opportunistic bacteria in significant amounts with *P.aeruginosa* being the densest of all. The results revealed that the rate of isolation of *Acinetobacter* was 91.6%, *Flavobacterium* 75%, *Aeromonas* 83.3%, *Moraxella* 58.3% and *P.aeruginosa* was present in 100% samples (Fig 2), ranging from 2.02 (Jan) to 2.50 (May) (Table 1).

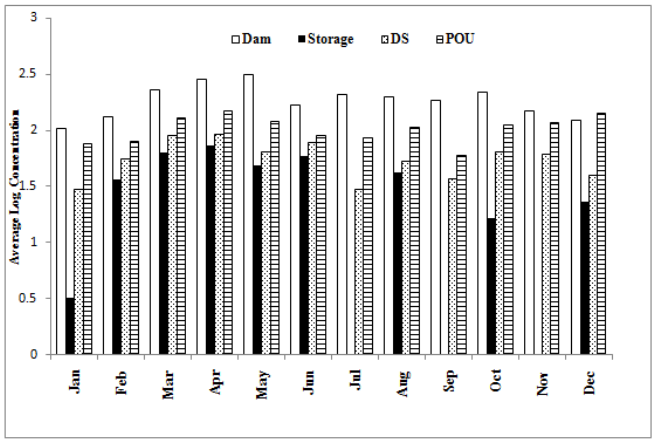


Fig. 1. Fluctuations in densities of *P.aeruginosa*

Some early data from similar studies, support results of this study. *Pseudomonas* sp. was the most abundant bacteria isolated by Hussain *et al.* (2013) who identified and characterized a total of 79 bacterial strains isolated from various drinking water sources including tap water, tube well water, home-well, bore-well and springs. *Aeromonas hydrophila* and *Acinetobacter* sp. was also isolated by him. Similarly, occurrence of *P.aeruginosa* in raw water samples was also observed by Osman *et al.* (2011) in Nile water Egypt and Bifulco *et al.* (1989) detected *Acinetobacter* spp. in 38% of the groundwater supplies investigated by him. Lye *et al.* (1997) detected significant amounts of opportunistic bacteria in groundwater and potable water. According to him, *Legionella* spp. and especially *Legionella pneumophila* are adapted to warm water systems, where they grow most effectively. In a study on bacteriological analysis of Ebutte River in Ebutte Community, Nigeria Ekhaise *et al.* (2011) isolated eleven bacterial genera from Ebutte river water, including *Pseudomonas* opportunistic pathogen (11%) and other pathogens, predominantly members of Enterobacteriaceae; *Escherichia, Salmonella, Shigella, Klebsiella, Proteus, Enterobacter, Bacillus, Staphylococcus, Streptococcus,* and *Clostridium.* Kolawale *et al.* (2013) found fifteen types of bacterial flora including *P.aeruginosa* from different treated and untreated water samples. The primary sources of these bacteria in natural water bodies may be animal and human wastes including surface runoff, pasture, and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil/plant bacteria.

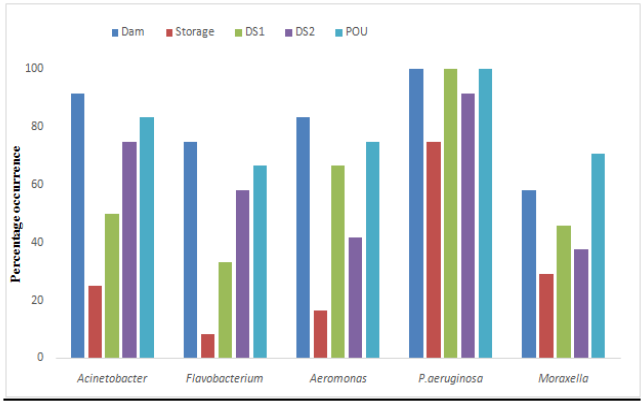
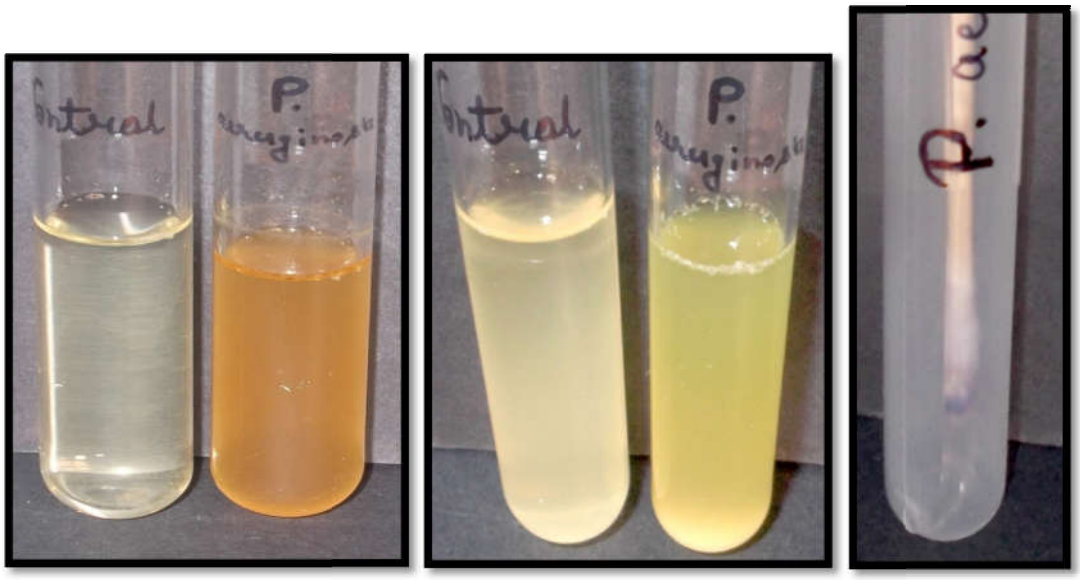
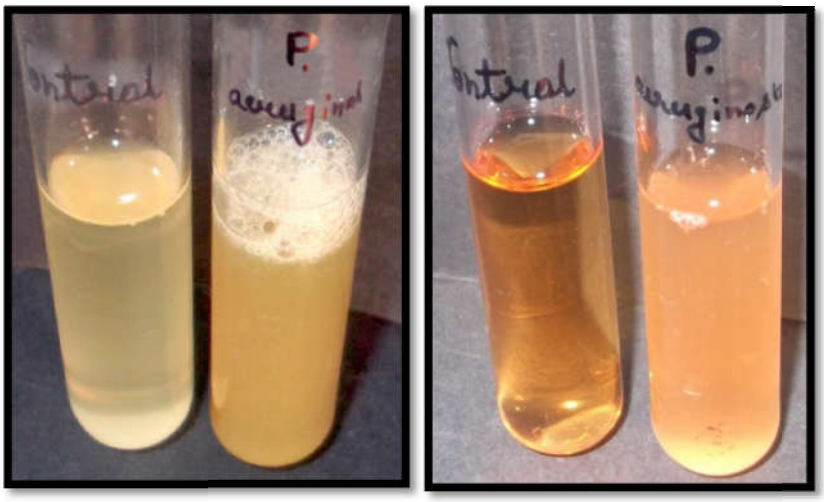


Fig. 2. Prevalence of Opportunistic Pathogens

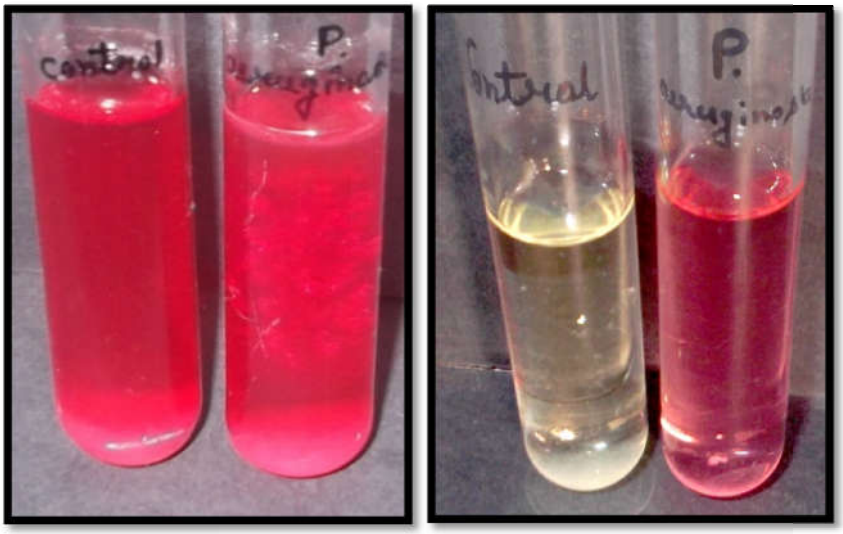
A seasonal pattern in the incidence of opportunistic bacteria emerged with infrequent isolation during the winter period increasing to a peak during the summer, with most isolates recovered during months of rainy season. Summers in the Neemuch are during the months of March, April, May and June. These months experience a maximum temperature of around 45°C and a minimum temperature of around 35°C.



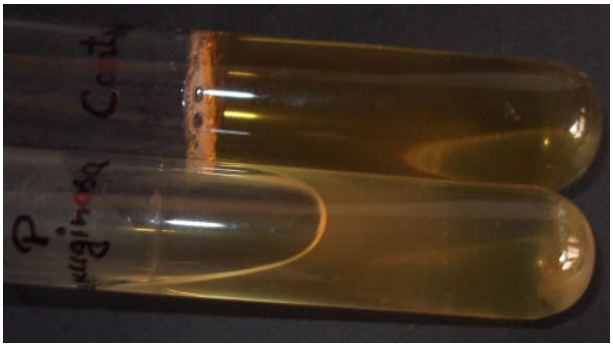
Negative Indole Test **Negative MR Test** **Positive Oxidase**



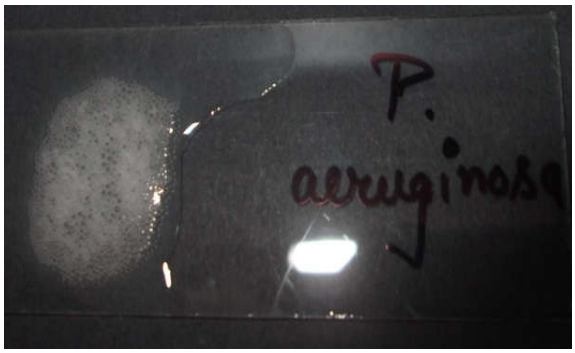
Negatve VP Test **Negative Urease Test**



Negative Mannitol Test **Positive NR Test**



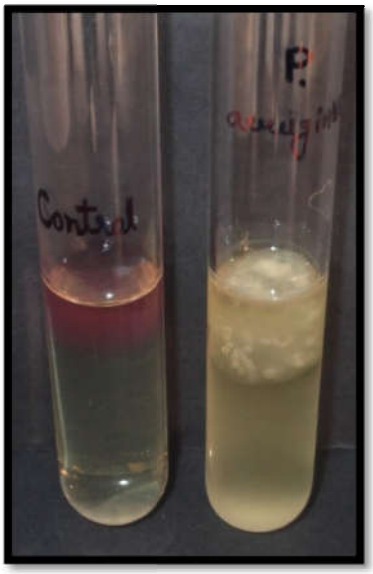
Positive Gelatinase Test



Positive Catalase Test

Positive SC Test

Negative TSIA Test



FTG Test- Aerobic

Negative Starch Test

OF Test - Oxidative Test

Fig. 3. Biochemical results of *P.aeruginosa*

In contrast to other opportunistic bacteria, concentration of *P.aeruginosa* was significantly higher in summer months (Mar-June) followed by rainy (July-Oct) and winter months (Nov-Feb). During the hottest month of the year i.e., May, *P.aeruginosa* was obtained in its highest concentration of the year and the average log count in summer season was 2.38, this clearly supports the fact that *P.aeruginosa* grows well at and around the temperature of 42^0C. Shar *et al.* (2010) also observed the isolation rate of *P.aeruginosa* to be significantly greater in summer months than in winter months in drinking water of Rohri city. The monsoon season in Neemuch is during the months of July, August, September and October. These months experience very sparse rainfall. The overall rainfall in the region range around 1500 mm. The average seasonal log count during rainy season was 2.30. Winters in Neemuch are during the months of November, December and January. The month of February experience pleasant weather. Temperature during winter is around a maximum of approximately 25°C and a minimum of approximately 2°C. The average log count during winter was recorded to be 2.10.

After treatment: Chlorination has been the most widely practiced method of disinfection for potable waters since the turn of the century and the principal means by which the microbial quality of water is maintained in developing countries. Despite such preventive measures, regrowth of HPC and opportunistic bacteria in bulk water and biofilms has yet to be controlled completely. No approach has shown complete success in eliminating biofilms or HPC bacteria from bulk water and pipe surfaces. Moreover, current public health standards for drinking water based on the coliform index fail to accurately predict large numbers of secondary opportunistic pathogens which these systems can sometimes harbor. In Neemuch, the water from the dam is disinfected by chlorination before being released into the drinking water supply. It appears that the purification and disinfection practices were not efficient in entire elimination of the opportunistic pathogens, though reduced their concentrations to some extent as evidenced by their lower appearances in samples just after the treatment; throughout the sampling months, disinfected water samples had a much lower incidence of opportunistic pathogens than untreated samples and their densities differed significantly between the two locations ($P<0.05$). The frequency of occurrence was reduced to 25% in case of *Acinetobacter*, 75% for *P.aeruginosa*, 8.33% for *Flavobacterium*, 16.6% for *Aeromonas*, and 29.1% for *Moraxella* (Fig 2). The total viable counts of *P.aeruginosa* in those samples ranged from 0.51 (Jan) to 1.86 (Apr) (Table 1), *P.aeruginosa* concentration was highest of all.

In the present study, isolation of *Aeromonas*, *Flavobacterium*, *Acinetobacter* and *P.aeruginosa* from treated water effluent is supported by different studies. *Aeromonas* spp. are ubiquitous bacteria found in diverse aquatic environments worldwide such as bottled water, chlorinated water, well water, and heavily polluted waters. Gavriel *et al.* (1998) also isolated *Aeromonas* from 21 of the 31 reservoirs investigated by him in a public drinking water supply in north-east Scotland. Aeromonads cause serious diseases of aquatic animals and represent an economic threat to the aquaculture industry. The motile aeromonads have emerged as a serious microbial threat to human populations, especially the immunocompromised (Janda *et al.,* 1996). Numerous cases and outbreak investigations of water and food-transmitted illnesses caused by aeromonads have been reported (Joseph *et al.* 1996). *Aeromonas* contamination of drinking water has been documented as a cause of traveler's diarrhea (Hanninen *et al.* 1995). *P.aeruginosa, Flavobacterium* and *Acinetobacter* are natural inhabitants of aquatic environments worldwide, which constitute a significant proportion of the HPC values in treated water and have been isolated from groundwater, treated drinking water, surface waters, wastewater, sludge, and sediment. A total of 55 bacterial strains were isolated by Pindi *et al.* (2013) who identified opportunistic pathogenic bacteria in drinking water samples of different rural health centers. The isolated strains belonged to *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Aeromonas*, *Methylobacterium, Pantoea, Cupriavidus*, *Delftia*, *Exiguobacterium*, *Kocuria,* and *Lysinibacillus.* The presence of pathogenic microorganisms in treated water samples usually is because they are able to survive the treatment process. Additionally, in most developing countries, water-treatment plants are usually faced with maintenance problems and a lack of qualified personnel.

During distribution: Drinking water distribution systems are a well-recognized reservoir for *P.aeruginosa* and other opportunistic microorganisms, which provide an oligotrophic environment by availing organic carbon to the organisms. Assimilable organic carbon is readily available for consumption by microorganisms, which in turn can enhance the regrowth of bacteria in the treated water. Their persistence is also due to favorable nutrient conditions provided by sediments and protection from sunlight inactivation and protozoan grazing. The fact is reflected from present study also; the rate of isolation and regrowth of opportunistic bacteria were even greater during distribution. At DS1, frequency of occurrence of *Acinetobacter* increased to 50%, *Flavobacterium* 33.3%, *Aeromonas* 66.6% and *Moraxella* was having 45.8%. In the same way, higher rate of isolation was also reported at DS2 as compared to that of Storage Reservoir; *Acinetobacter* was isolated from 75% samples, *Flavobacterium* 58.3%, *Aeromonas* 41.6% and *Moraxella* from 37.5% (Fig 2). *P.aeruginosa* was having 100% isolation rate at DS1 site with log concentration ranging between 1.00 (July) to 2.00 (Apr) and at DS2 it was isolated from 91.6% samples in which the log counts fluctuated between 1.52 (May) to 2.00 (Mar) (Table 1). The most frequent opportunistic bacteria detected in densest amounts was *P.aeruginosa* followed by *Acinetobacter, Aeromonas, Flavobacterium* and *Moraxella.* These bacteria are generally the predominant bacterial genera in drinking water system (Block *et al.,* 1997; Berry *et al.,* 2006).

Although treatment at Hingoria treatment plant effectively reduced the population of opportunistic bacteria in the finished water, bacteria might have regrown after the treatment during storage and distribution. Work of Jjemba *et al.* (2010) also met the same incident while investigating the quality of reclaimed water in treated effluent, after storage and in distribution system of four plants in California, Florida, Massachusetts, and New York. He reported elevated counts of *Aeromonas* spp., enteropathogenic *E.coli* O157:H7, *Legionella* spp.,

Mycobacterium spp. and *Pseudomonas* spp. in the reservoir and distribution systems because of the loss of residual disinfectant and high assimilable organic carbon levels and found that opportunistic pathogens, particularly *Aeromonas*, *Legionella*, *Mycobacterium*, and *Pseudomonas*, occurred more frequently than indicator bacteria (*Enterococci*, Coliforms and *E.coli*). Several of the reasons seem to be responsible for this after-growth of opportunistic bacteria, in addition to the possible loss of residual chlorine and high assimilable organic carbon levels as observed by Jjemba *et al.* (2010) in his study. Rise in the counts may also be due to the fact that bacteria which survived the treatment might have started proliferation during the distribution of water or sometimes some opportunistic bacteria (*Bacillus*, *Enterobacter, Klebsiella, Actinomyces, Streptomyces,* etc.) are washed into distribution system from their natural habitat in soil or vegetative matter, as a result of a large ingress of contaminated water.

The survival of opportunistic premise plumbing pathogens within distribution systems is based upon interactions of many variables, including temperature, pipe surface, nutrient levels and type and concentration of disinfectants. They are not merely transported through pipes, but are actually adapted to growth and persistence in drinking water, especially in building plumbing systems. Their emergence is due to the fact that conditions resulting from drinking water treatment select for them. The common features of this group of waterborne pathogens include: disinfectant-resistance, pipe surface adherence and biofilm formation, growth in amoebae, growth on low organic concentrations, and growth at low oxygen levels. Disinfectant-resistance property of these bacteria is also supported by the work of Ridgway *et al.* (1982), who studied chlorine resistance patterns of bacteria from two drinking water distribution systems and found relatively higher numbers of microorganisms from the chlorinated Irvine system water suggesting that certain bacteria may possess mechanisms enabling them to survive in highly chlorinated environments. Hence, it is concluded that disinfection provides selection pressures on chlorine-tolerant microorganisms in chlorinated water distribution systems that promote a wide range of survival strategies.

The data from the present study highly corroborates the earlier views. Felfoldi *et al.* (2008) investigated drinking water distribution system of a hospital and reported several opportunistic pathogenic bacteria, such as *Escherichia albertii*, *Acinetobacter lwoffi*, *Corynebacterium tuberculostrearicum*, *Legionella* and *P.aeruginosa*. He also emphasized that drinking water systems, especially those with stagnant water sections, could be the source of nosocomial infections. Bahry *et al.* (2013) detected *Aeromonas, Legionella, Pasteurella, Pseudomonads*, *Salmonella* and *Yersinia* when investigated municipal water quality in the distribution system and household water tanks relative to the presence and regrowth of opportunistic and potential pathogens. Wielen *et al.* (2012) found *Legionella pneumophila*, *P.aeruginosa*, *Stenotrophomonas maltophilia*, and *Aspergillus fumigates* from distributed unchlorinated drinking water from different treatment plants in the Netherlands, with the lowest numbers reported in the finished water. Thus, he also came to same conclusion that some of the opportunistic pathogens can multiply in the distribution and premise plumbing systems, as the case with present study.

Bacteria in drinking water systems either grow in bulk water or as biofilms attached to the walls of pipes. The purified water system that produces, stores, and circulates water under background conditions is susceptible to the establishment of adhesive biofilms, which can be the source of undesirable levels of viable microorganisms or endotoxins in the effluent water. Bacterial regrowth and formation of biofilms in drinking water distribution pipes has been detected even in countries with advanced water-treatment and health-care facilities. Recent studies have shown that nearly all large water purification systems can cause the formation of biofilm in the piping. This biofilm can spread microorganisms within the system and contribute to an increase in particles, bacteria, and the level of total organic carbon. The growth potential of the water, pipe material, temperature, and hydraulics influence biofilm growth on each location in the drinking water distribution system and premise plumbing system and might influence the growth of some opportunistic pathogens in drinking water. Different materials such as cast iron galvanised steel, stainless steel, copper and polyethylene have been used to manufacture water distribution pipes and these materials favour biofilm formation in the water distribution systems. Differences in the pipe materials greatly favour the survival of different bacterial species. Biofilms consisting of *P.aeruginosa* and different fecal bacterial species have been detected in water distribution systems, even in countries that have more advanced water-treatment facilities (Kilb *et al*. 2003, Werner *et al*. 2004). Previous studies of other distribution systems have also shown that distribution system communities differ according to the water conditions and pipe material in each system, Williams *et al*. (2004) identified different bacterial groups including *Legionella pnuemophila* when studied phylogenetic diversity of drinking water bacteria in a drinking water simulator using 16S rDNA analysis. He reported a predominance of alpha-proteobacteria in distribution system under two different disinfectant residuals; chlorine and monochloramine.

Bacterial colonization of the surfaces of drinking water distribution pipes has been recently documented by the use of scanning electron microscopy (Allen *et al*. 1980, Ridgway *et al*. 1982, Seyfried *et al*. 1980). Studies done by Ridgway *et al*. (1982) have demonstrated that such microbial colonization can occur even in highly chlorinated water systems (>1.0 mg of applied chlorine per liter). Microbial adhesion to solid surfaces is frequently mediated by extracellular mucopolysaccharides or glycoprotein polymers (Marshall *et al*. 1976), the manufacture and secretion of which by certain strains of *P.aeruginosa* has been shown to dramatically enhance resistance to disinfection by combined chlorine in swimming pool waters (Seyfried *et al*. 1980). The extracellular material observed on the surfaces of the particle-associated cells in the study of Ridgway *et al*. (1982) may also perform a similar protective function.

Drinking-water distribution pipe biofilms contain numerous species resistant to the applied disinfectant residual, including opportunistic pathogens. *Legionellae* and *Mycobacteria* are prominent among those that flourish in the niches provided in

biofilm growth and, perhaps, benefit from a reduced competition for nutrients when a disinfectant residual is present. A flow chamber study verified that the presence of high concentrations of disinfectants was not sufficient to eliminate the survival of pathogens, including *Legionella pneumophila* and *E.coli* (Williams *et al.* 2003). Therefore, a more comprehensive study on these local factors in the distribution system can identify mechanisms that are responsible for the growth of multiple opportunistic pathogens in drinking water distribution systems. In spite of preceding filtration and UV disinfection, *P.aeruginosa*, atypical *Mycobacteria* and *Legionella* spp. were found by Emtiazi *et al.* (2004) in biofilms of drinking water, during the production of drinking water from surface water embankment filtration. Through his results he concluded that saprophytic as well as facultatively pathogenic atypical *Mycobacteria*, *Legionella* spp., and *P.aeruginosa* may have been transferred from their natural habitat of surface water to the drinking water distribution system. Hence, surface water appeared to have a direct influence on the composition of biofilms in the drinking water distribution system.

Opportunistic premise plumbing pathogens are responsible for a significant number of infections and represent an emerging water borne disease problem with a major economic cost of more than 1 billion annually. As such, there is a need for novel approaches to reduce exposure to these pathogens. Pathogenic strains of certain opportunistic bacterial species can present special risks for the immunocompromised when biofilm fragments are shed into drinking water. *P.aeruginosa* is an important pathogen in nosocomial infections and its frequent presence in recreational and drinking water is a significant threat to public health (Trautamann *et al.* 2001). *Pseudomonas* spp. and *Aeromonas* were detected in biofilms from the POU devices by Mulamattathil *et al.* (2014) who investigated biofilm growth in the drinking water distribution systems in Mafikeng, South Africa. His results also indicated that bacteria present in the water have the ability to colonize as biofilms and drinking water biofilms may be a reservoir for opportunistic bacteria including *Pseudomonas* and *Aeromonas* species. *Aeromonas* is a common component of the bacterial population of drinking water in distribution systems but comprises only a small fraction of the heterotrophic population. *Aeromonas hydrophila* survives easily in waters polluted by feces and seems resistant to various disinfectants, insecticides, and chemicals. Burke *et al.* (1984) isolated *Aeromonas hydrophila* from a metropolitan water supply. According to him, water within the distribution system conformed to international standards for drinking water but contained *Aeromonas* spp. in numbers comparable to those in raw surface water.

Despite the recent advances in knowledge concerning the physiological mechanisms of chlorine disinfection, all of the physicochemical and biological parameters which influence the bactericidal properties of chlorine in the environment are not yet fully understood. Large numbers of human secondary opportunistic pathogens, can often be recovered from potable water distribution systems maintaining free chlorine residuals (i.e., HOCl+OCI-) of 0.5 to 1.0 mg liter (LeChevallier *et al.,* 1980, Means *et al.* 1981). Thus, specific mechanisms may exist for the survival of certain bacteria and viruses in waters containing relatively high concentrations of chlorine (Haas *et al.* 1979, Hoadley *et al.* 1977). Some proposed mechanisms by which bacteria and viruses may develop resistance to chlorine include: (i) modification of cell surface structures which may lead to increased aggregation or clumping of cells in situ (ii) microbial adhesion to pipe surfaces or to suspended particulate matter such as detritus or clay particles (iii) extrusion of protective extracellular capsular or slime layers and (iv) formation of resistant spores. Conceivably, some of these mechanisms may favorably influence the survival of various opportunistic pathogenic microorganisms which persist in drinking water distribution systems.

After distribution: Many studies have been conducted on the growth of bacteria on POU devices; in general, all studies have shown that the number of bacteria in tap water after distribution increases over that found in the treated water before distribution. This condition typically results from poor water quality at source, lapses in disinfection and filtration processes, or compromised distribution systems. Findings from present study also revealed a significant decline in the quality of water after distribution; opportunistic flora was found most frequently and in greatest numbers at the point of consumption, as compared to that of storage reservoir, DS1 and DS2. *Acinetobacter* was isolated with 83.3% rate, *Flavobacterium* with 66.6%, *Aeromonas* with 75% and *Moraxella* with 70.8% (Fig 2). *P.aeruginosa* is frequently found in drinking-water, where it is considered to be a nuisance organism rather than a pathogen. The presence of this organism in potable water indicates a serious deterioration in bacteriological quality and is often associated with complaints about taste, colour, odor, and turbidity linked to low rates of flow in the distribution system and a rise in water temperature (WHO, 1997). In present study also, presence of *P.aeruginosa* was reported from all the drinking water samples collected from the point of consumption (100%), in which the monthly counts ranged from 1.77 (Sep) to 2.17 (Apr) (Table 1). These results are not surprising considering that this bacterial group has been isolated in many oligotrophic aquatic environments including mineral drinking water, due to its ability to grow in water containing only traces of nutrient. These results give support to findings of similar investigations, which strengthen the fact that *P.aeruginosa* grows well in waters enriched with organic material; prevalence of *P.aeruginosa* in tap water was also observed by Behrends *et al.* (2003), who tested the piping of a new hospital in German and showed that the drinking water was contaminated with *P.aeruginosa.* Likewise, Manji *et al.* (2012) also reported incidence of *P.aeruginosa* in treated tap water from Calabar South Local Government Area.

Because of the ability of *P.aeruginosa* to live in both inanimate and human environments it has been characterized as a ubiquitous microorganism. In inanimate environments *P.aeruginosa* is frequently detected in water reservoirs polluted by animal and human waste such as sewage and sinks inside the hospital. It is also found in faeces, soil, water (swimming pools and whirlpools), sewage, plants and animals. Colonization in human occurs mostly at moist places such as perineum, axilla and the ear. The moisture loving characters contributes to the presence of *Pseudomonas aeruginosa* in respiratory equipments, antiseptic solutions, soaps, sinks,

mops, vegetables, flowers, hydrotherapy equipments, tap fittings, drains and shower heads, especially in health care settings and swimming pools. *P.aeruginosa* does not harm a healthy individual, being an opportunistic pathogen; it may cause problems in individuals with weak immune systems. However, it is more reliable and safe if the drinking water does not show the presence of *P.aeruginosa.*

Bacterial regrowth, whether in a municipal distribution system, POU device or bottle of water, reflects the initial flora, the temperature, available nutrients and water characteristics. However, the organisms that predominate are those opportunistic organisms that are adapted to growing in an aquatic environment with low temperatures and low nutrient concentrations. Regrowth can be managed under some circumstances and can be controlled to some extent through providing a disinfectant residual and by reducing the biodegradable organic matter, but it is almost impossible to prevent the growth of these opportunistic organisms that are adapted to the aquatic environment.

Characteristics of *P.aeruginosa* Isolates

Verified isolates were gram-negative, non-spore former, motile and aerobic rods. Colonies on nutrient agar were smooth, large, translucent, and 2-4mm in diameter. Most of the colonies were round while irregular forms were also observed. The colour of most of the colonies was grayish and cream, while isolates forming pigment were fluorescent green. The surface characteristics of bacterial isolates were found to be smooth, rough, dry, glistening and shiny. Margin of colonies were found to be entire, undulated, irregular and lobate. Most of the colonies had convex elevations while flat and raised elevations were also observed. Opacity of the colonies was observed mostly to be opaque or translucent. Filiform type, opaque, fluorescent green growth was seen in abundance on nutrient agar slants. Pellicle type of surface growth, greenish turbid type of sub-surface growth and flocculent type of sediment growth were seen in abundance in nutrient broth tubes. Biochemical analysis of isolates (Fig.3) gave negative results for Indole, Methyl red, Voges-proskaeur, Mannitol, Amylase, Urease and Triple sugar iron agar test and positive results for Simmon citrate, Oxidase, Catalase, Gelatinase and Nitrate reduction. All the isolates were found to have oxidative type of metabolism as confirmed by Oxidative-Fermentative test.

Conclusion

Historically, the evaluation of the sanitary quality of drinking water has dealt primarily with enteric bacteria associated with fecal pollution. More recently, studies have been extended to include isolation and identification of nonenteric bacteria. The results of this study provided some insight into the presence of opportunistic pathogens, particularly *P.aeruginosa*, in untreated dam water and distribution of conventionally treated municipal water supplies. This finding is a cause for concern, particularly for infants, the elderly and immunocompromised individuals in Neemuch community. Earlier investigations on bacterial contaminations of drinking water in municipal water distribution systems also suggested risk of opportunistic pathogens to the consumers. Survival of opportunist organisms in distribution systems is based upon complex interactions between physical, chemical and operational factors, and microbial ecology. Standard chlorination strategies are sometimes inadequate for controlling regrowth in the distribution system, and can be improved upon with a better understanding of microbial ecology. Our understanding of the mechanisms of microbial growth in the presence of disinfectants is superficial, and the elucidation of resistance mechanisms will allow the distribution system to be modeled accurately and will provide insights into novel control strategies.

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