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Occurrence, Survival and Regrowth of Potentially Pathogenic Bacteria in a Potable Public Water Supply

*Sonu Chouhan
**Ph.D. Scholar, Biotech dept., PAHER University, Udaipur, Rajasthan*

Abstract: *For the control of hygienic quality of the water supply, it is of utmost importance that the bacteriological examination of both the water entering the distribution water and the water in the distribution system itself be carried out frequently and regularly. Present study was an effort to study the survival and after-growth of the source water pathogens, in alum treated-chlorinated finished water at the plant and community tap water at the point of consumption, respectively. Towards this, samples from the Dam (N=24), Finished water (N=24) and Community tap (N=24) were comparatively tested for the presence and concentration of pathogens, fortnightly for one year. Pathogens isolated from source water included commonly found potential enteric pathogens; E.coli, Salmonella, Shigella and V.cholerae and P.aeruginosa and S.aureus. Complete elimination of all the detected pathogens was not observed in any sample of finished water analyzed throughout the study period; 66.6% samples were contaminated with all the six pathogens, 29.1% samples showed presence of five pathogens and 4.16% samples harboured four pathogens, however, the counts reduced after treatment, with a significant difference than those obtained in source water. The microbiological quality at the point of use, was worse than at the outlet of plant; 95.8% community water samples was having presence of all the six pathogens and the rest 4.16% samples were contaminated with five pathogens while free of P.aeruginosa. The concentration of pathogens in community water samples was surprisingly far greater than in the finished water samples (P<0.05). Furthermore, the concentration of pathogens in all the tested finished and community water samples was significantly higher than the prescribed WHO and BIS standards (P<0.05).*

Keywords: *Concentration, Counts, Pathogen, Regrowth, Water*

I. Introduction

Drinking water can be life threatening if it carries water-borne pathogens. Water safety is a concern for countries all over the world. Interest in the quality of drinking water supplies has been stimulated by the enactment of the Safe Drinking Water Act of 1974 and the dramatization of recent waterborne epidemics, which occurred in recent years. The goal of this act is to improve the quality of drinking water supplies throughout the nation. To date, most of the research on this topic has been devoted to municipal supplies since the population at risk from any treatment failure is much greater for public water supply systems than for smaller, private water supplies.

People living in our country have always been facing problem for safe drinking water. In many places, this problem is made harder by the fact that many of the available water sources have been adulterated with agricultural as well as animal and human waste. Such adulterated waters contain vast amounts of organic matter that serve as an excellent nutritional source for growth and multiplication of the contaminating microorganisms. Secondly, even the most common treatments are not available to most of the local population, if available and raw water is processed at the plant to produce potable water, bacterial recontamination of treated water is another huge problem. The growth of bacteria in water distribution systems and water treatment devices has been recognized for many years. Such growth is affected by many different factors, including the types of bacteria present in water released from a water treatment plant, the temperature, disinfectant concentration, the presence of sediment in the pipe work, the types and amount of nutrients present and the rate of flow of the water. Many of these factors cannot be controlled, and thus microbial regrowth will continue to be investigated. The organisms involved in microbial regrowth are those that have been released from the water treatment plant or that have been introduced into the distribution system at some point downstream of the water treatment plant. If it is assumed that the water treatment plant is performing adequately, then the numbers of bacterial pathogens released into the water distribution system will be low, and those that are present are likely to be killed during transport in systems where residual disinfectant is present. However, a break in the integrity of the distribution system (e.g., burst water main) can lead to the ingress of contaminated water. Such water may contain organisms that are potentially pathogenic for humans. Consequently, numbers of people suffering with common diseases like diarrhea etc. are major causes of mortality. It is therefore of great importance to study the occurrence of pathogens in a raw water source, their complete removal at treatment plant and at last, to ensure the delivery of safe water at the point of use. The present research focused on these objectives and reported the comparative presence and densities of pathogens in source, finished and community water.

II. Material & Methods

Samples from the Jaju sagar dam (Source water), Hingoria plant outlet (Finished water) and point of use (Community water) were collected twice a month using standard methods of sampling for a period of 1 year (Jan 2013 to Dec 2013). The samples were subjected to **Direct Plating Method** using various selective and differential media to study presence and density of potential waterborne pathogens; for *V.cholerae* TCBS agar (selective) was used, likewise, *S.aureus* was enumerated on Mannitol salt agar, *Salmonella* and *Shigella* on Salmonella and Shigella agar, *P.aeruginosa* on Cetrimide agar and *E.coli* on EMB agar. The isolated pathogens were further subjected to some biochemical tests to confirm their identity.

III. Results

Two third of the total drinking water consumed worldwide is derived from various surface water sources that may easily be contaminated microbiologically by sewage discharges or fecal loading by domestic or wild animals defecation, malfunctioning of septic trenches, storm water drainage, municipal wastes and industrial effluents. Thus, occurrence of potential waterborne pathogens in these is very common. The results from the present study also reflect this; *P.aeruginosa, S.aureus, E.coli, V.cholerae, Salmonella* and *Shigella* constantly and abundantly shown their occurrence in each and every sample (100%) from Jaju Sagar Dam, throughout the whole study period (Fig.1). Non-significant differences were found in the total viable counts of two samples of a month ($P>0.05$), hence, average log counts of pathogens, obtained in source water samples are depicted monthwise in Table:1 and their range, frequency and density are mentioned in Table:2. The raw water from the dam is treated at Hingoria treatment plant through traditional methods of alum flocculation, rapid sand filtration and final disinfection by chorination; the final finished water which is ready to be distributed must be free of any pathogen (WHO [1] and BIS [2]), however, the results of this study are in contrast to this, no sample of finished water showed complete absence of all the six tested pathogens- 66.6% samples were positive for all the six pathogens, 29.1% samples showed presence of five pathogens and 4.16% samples were contaminated with four pathogens. However, the concentration of pathogens reduced significantly after the treatment ($P<0.05$) (Table:1) but their presence itself, present potential health hazard. The microbiological quality of finished water further deteriorated within the distribution system up to the point of use and was not according to WHO and BIS standards; 95.8% community water samples was having presence of all the six pathogens and the rest 4.16% samples were free of *P.aeruginosa* only but contained rest of the pathogens. It was noticeable that the concentration of pathogens at the point of use exceeded to that, observed in finished water at the plant (Table:1).

IV. Discussion

Meeting the goal of clean, safe drinking water requires a multi-barrier approach that includes: protecting source water from contamination, appropriately treating raw water, and ensuring safe distribution of treated water to consumer's taps. Therefore, for complete microbiological monitoring of drinking water, testing of water must be done at the source, treatment plant and municipal tap. With this in mind, the study compared the presence and concentration of six selected pathogens in source water, finished water and community water supplies to evaluate their removal after treatment, and survival and after-growth within the distribution system.

In Jaju Sagar Dam, the possible source of *P.aeruginosa* and *S.aureus*, might be environmental because they are widely distributed in nature (water, soil, plants) and not fecally transmitted as the other intestinal pathogens do. The extremely hazardous sources of contamination may be excessive monsoon rains, floods, herbicides, fungicides, untreated municipal waste, medical waste and coastal water pollution due to waste discharges and oil spills. The origin of *S.aureus* may also be due to the animals and human activities in the Jaju Sagar Dam, because *S.aureus* is the normal flora of skin. Presence of *E.coli* in the dam indicates continuous fecal contamination. As it is improbable that *E.coli* can be recovered from an environmental source, unless there is a continuous source of fecal contamination. Being an intestinal normal flora, it always enters a water body through fecal-oral route. The presence of *Shigella, Salmonella* and *V.cholerae* may be due to the fecal contamination by the infected humans and animals or their origin may be environmental. This indicated that poor sanitation and hygiene conditions and lack of, or little environmental awareness among the nearby residents could be considered as the major causes of dam water contamination. There are many factors that can influence the growth of bacteria in aquatic environments; suitable temperature and pH, and nutrients availability are some of important factors. Each bacterial species has its own set of environmental conditions, some are normal flora found persistently, and some are human pathogens which find their way to water body through carriers and are transient, shortly leave when find another host. Contamination of raw water sources with potential pathogens has been reported worldwide by many researchers. Occurrence of *S.aureus, P.aeruginosa* and *E.coli* in source water was also observed by Manji et al., [3] in untreated well and stream water sources of Calabar South Local Government Area. Lechevallier et al., [4] isolated *S.aureus, Pseudomonas* spp. and *E.coli* from raw water. Rahim et al., [5] found *S.aureus* in all the tested raw waters of Atbara River, of Al Gedarif city, Sudan. Clark et al., [6] detected *E.coli* (11.6–39.7%) in all the water samples from municipal raw water.

Kolawole et al., [7] isolated *E.coli, P.aeruginosa, Salmonella* sp., *Shigella* sp. and *S.aureus* from raw water of Oyun river. Edberg et al., [8] also isolated *E.coli* from source water. Hatha et al., [9] observed increased prevalence of *V.cholerae, E.coli* and *Salmonella* in Vembanadu Lake, along west coast of India. Ahmed et al., [10] recovered *S.aureus, E.coli, Salmonella* spp. and *Shigella* spp. from water samples of different dams of Rawalpindi, Islamabad region in Pakistan. According to Mthembu et al., [11] *E.coli* and *Pseudomonas* spp. were constantly detected at all five sites of Mhlathuze River in KwaZulu-Natal. Ekhaise et al., [12] obtained *Escherichia, Pseudomonas, Staphylococcus, Salmonella* and *Shigella* in Ebutte River, Uhunmwonde Local Government Area, Edo State, Nigeria, above the WHO standards. Sati and Faidah [13] detected *P.aeruginosa* and *E.coli* in different drinking water resources of Makkah City, Saudi Arabia. Chandra et al., [14] obtained *E.coli, P.aeruginosa, S.aureus* and *V.cholerae* in Gola river water, Uttaranchal, India. Yongsi et al., [15] identified 1,242 isolates of enteric bacteria from a variety of drinking water sources of Yaounde, which included *Shigella* (0.24%), *Salmonella* (1.30%), *E.coli* (5.15%), and of the 461 aerobic bacteria, he recovered 71.80% *Psuedomonas*. Their 95% tested samples crossed WHO limits. Ihejirika et al., [16] recovered *Escherichia coli* (100%), *Shigella* spp. (71.0%), *Salmonella* spp. (71.0%), *Vibrio* spp. (42.9%), *Pseudomonas* spp. (42.9%) and *Staphylococcus* spp. (85.7%) from Imo River, Nigeria. Obasi et al., [17] detected *E.coli* sp. and *Pseudomonas* sp. from Ero and Ureje Dams, the municipal water supply of Ekiti State, Southwest, Nigeria. He found *E.coli* counts above the recommended standards of WHO 2002. Mwajuma et al., [18] recovered *Shigella* spp., *Salmonella* spp. (non-typhi) and *Pseudomonas* spp. from selected drinking water sources in Samburu South. Smruti and Sanjeeda [19] identified *E.coli, Salmonella* and *Shigella* from surface waters in Indore. Obi et al., [20] obtained *E.coli, Vibrio, Shigella* and *Salmonella* in water sources of the Venda Region of the Limpopo Province, with a higher tendency in the months of summer in comparison to other seasons ($P<0.05$). Different kinds of enteric bacteria were isolated by Jayana et al., [21] from different drinking water sources of Madhyapur Thimi which included *E.coli* (24.6%), *P.aeruginosa* (2.1%), *S.typhi* (2.1%), *S.paratyphi* (1.4%), *Shigella dysentery* (2.8%) and *V.cholerae* (0.7%). Antony et al., [22] observed that drinking water of Ananthanar channel water of Kanyakumari district, Tamil Nadu, was contaminated with *P.aeruginosa.*

In the present study, the failure in complete removal of pathogens at the plant may be due the inefficiency of the filtration and chlorination steps at the Hingoria Treatment Plant to produce the water of an acceptable quality. The drinking water supplier has to provide the consumer potable water of a quality identical to that leaving the treatment plant. However, it has been well documented that water that reaches the consumer's tap is often of worse microbiological quality than that which left the plant. The case with the present study is implausible. As the finished water at the outlet of plant itself, does not comply with the prescribed standards of zero pathogen count (WHO 2011 & BIS 2012) then the microbiological status of water at the point of use is obviously expected to be of worst quality. The decline in microbial water quality within the distribution system may be attributed to the recovery and subsequent growth of sub-lethally damaged bacteria or due to the depletion of the residual chlorine or due to the infiltration of contaminated water through cross-connection, leakage points and back siphonage. In piped supplies, discontinuity increases the likelihood of contamination as the risk of back siphonage into the distribution network is increased when pipes are at lower pressure than the surrounding soil, which often contains leaked out effluent from leaking sewers. The problem is worsened by the old distribution networks having corroded and aged pipelines and the existing biofilms on pipelines, which can act as the reservoir of the coliforms and pathogenic bacteria, and protect them from the attack of residual chlorine. Hence, occurrence of pathogens and other opportunistic flora within the distribution system and at the point of use is usually reported worldwide. September et al., [23] isolated *E.coli, Salmonella, Shigella, Pseudomonas* and *Vibrio* spp. from biofilms of drinking water distribution systems, South Africa. Shakya et al., [24] also recovered *E.coli* (24.6%), *P.aeruginosa* (2.1%), *S.typhi* (2.1%), *S.paratyphi* (1.4%), *Shigella dysentery* (2.8%) and *V.cholerae* (0.7%) from distributions systems of Kathmandu Municipality. According to Abed et al., [25] tap water obtained from different areas of Riyadh Saudi Arabia, was contaminated with *Staphylococcus*. Felfoldi et al., [26] found the presence of several bacteria in drinking water distribution system of a hospital in Hungary, which included *Legionella* and *Pseudomonas aeruginosa, Escherichia albertii, Acinetobacter lwoffi* and *Corynebacterium tuberculostrearicum.* Pindi et al., [27] also reported *P.aeruginosa* in drinking water samples of different rural Health Centers, Mahabubnagar District. Shittu et al., [28] isolated E.coli, *Pseudomonas* spp., *S.aureus, Salmonella typhosa, Shigella* spp. and *V.cholerae*, from drinking water of Abeokuta, Nigeria. Kazmi et al., [29] isolated *E.coli, Salmonella, Shigella, Stapylococcus* and *Pseudomonas* from local drinking water of Islamabaad, Pakistan. Omezuruike et al., [30] identified *S.aureus, Salmonella* sp., *E.coli* and *P.aerugionosa*, from different drinking water samples of Abeokuta and Ojota, Lagos state Nigeria. Kurup et al., [31] found Coagulase Negative *Staphylococcus* sp., *Salmonella* sp. and *Pseudomonas* sp. from all the tested drinking water samples of Georgetown, Guyana. According to Vagarali et al., [32] *Pseudomonas* (20%) was the common cause of contamination in the drinking water samples tested by him, while *E.coli* was present in 10% samples. Suthar et al., [33] also found *E.coli, P.aeruginosa* and *S.aureus*, in his study on the detection of potentially pathogenic bacteria in the drinking water in Northern Rajasthan. Ojo et al., [34] studied

occurrence of *E.coli, P.aeruginosa* and *S.aureus* in potable public water supply within Lagos University, Ojo. Manji et al., [3] also reported incidence of *S.aureus, P.aeruginosa* and *E.coli* in treated tap water from Calabar South Local Government Area. Lechevallier et al., [4] isolated nearly 700 SPC bacteria from distribution water, raw water, drinking water and distribution water during a chlorine failure including *Staphylococcus aureus, Pseudomonas* spp. and *E.coli.* Rahim et al., [5] found *S.aureus* in all the tested treated waters of Atbara River, of Al Gedarif city, Sudan. Kolawole et al., [7] isolated *E.coli, P.aeruginosa, Salmonella* sp., *Shigella* sp. and *S.aureus* from chlorinated tank, storage tank, male hostel tap and female hostel tap. Ahmed et al., [10] reported occurrence of *S.aureus, E.coli, Salmonella* spp. and *Shigella* spp. from water samples of different filtration plants of Rawalpindi, Islamabad region in Pakistan. Sila et al., [35] recovered *E.coli* (61%), *S.aureus* (48%) and *Shigella* sp. (4%) from the Lamingo Dam Jos Nigeria, its water filter tanks and water taps, but in contrast to our findings (Source>Community>Finished), he concluded that tap water samples showed the highest frequency of occurrence of bacterial isolates followed by the dam water samples, while the treated water samples showed the least. Lamka et al., [36] identified several bacteria from standard plate count agar in rural drinking water supplies, which included *Pseudomonas acidovorans, P.alcaligenes, P.mallei, S.aureus, P.maltophilia* and total coliforms- *Citrobacter freundii, Klebsiella pneumoniae* and *E.coli.* Clark et al., [6] obtained *E.coli* (11.6–39.7%) in all the potable drinking water samples analyzed. Based on major findings, it is concluded that if the raw water is not effectively or adequately treated at the treatment plant, it serves as a source of infections to the end-point users through distribution systems. The proportion of waterborne disease outbreaks associated with the distribution system failures has been increasing over the years. Water authorities throughout the world are thus dedicated to ensure that the water that reaches the consumer is safe for consumption and free from any substances that may be harmful to health. At treatment plants the raw water must be effectively treated and adequately disinfected and the finished water must comply the drinking water standards (**HPCs - <100 CFU/ml, TC - 0 CFU/100ml, any pathogen- 0 CFU/ml**). In addition, the treatment plant must ensure no further deterioration of finished water quality within the distribution system, so that the quality of final water in the tap of consumers must be identical to that leaving the treatment plant. Drinking water distribution systems provide an oligotrophic environment, and post-treatment recovery and growth of bacteria is therefore a concern because of the effect the environment can have on public health.

Table 1: Viable counts (Log CFU/ml) of the pathogens

Months	Sample	*E.coli*	*S.aureus*	*P.aeruginosa*	*Salmonella*	*Shigella*	*V.cholerae*	Highest Colony counts
January	SW	1.87	2.04	1.95	1.82	1.93	2.27	*V.cholerae*
	FW	1.61	1.50	0.90	1.21	1.68	1.38	*Shigella*
	CW	1.70	1.81	1.74	1.82	1.97	1.95	*Shigella*
February	SW	1.98	2.25	2.02	1.95	1.85	2.05	*S.aureus*
	FW	0.65	0.99	1.58	0.80	0.65	1.11	*P.aeruginosa*
	CW	1.53	1.76	1.87	1.90	1.60	1.90	*Salmonella,V.cholerae*
March	SW	2.10	2.41	2.38	2.04	2..02	2.33	*S.aureus*
	FW	1.70	1.83	1.85	1.26	1.65	1.63	*P.aeruginosa*
	CW	1.94	1.84	2.05	1.90	2.05	2.10	*V.cholerae*
April	SW	2.23	2.43	2.43	1.85	2.13	2.18	*S.aureus,P.aeruginosa*
	FW	1.04	1.42	1.93	0.75	1.15	0.97	*P.aeruginosa*
	CW	1.61	1.97	2.04	1.88	1.95	2.09	*V.cholerae*
May	SW	2.22	2.36	2.51	2.19	2.07	2.44	*P.aeruginosa*
	FW	1.56	0.71	1.83	1.34	1.65	1.72	*P.aeruginosa*
	CW	1.82	1.68	1.65	1.93	2.07	2.22	*V.cholerae*
June	SW	2.25	2.43	2.15	2.05	2.16	2.34	*S.aureus*
	FW	1.70	1.30	1.83	1.58	1.48	1.65	*P.aeruginosa*
	CW	1.85	1.77	1.92	1.88	1.99	2.11	*V.cholerae*
July	SW	2.36	2.53	2.25	2.16	2.25	2.63	*V.cholerae*
	FW	0.94	1.93	0.50	1.46	1.76	1.78	*S.aureus*
	CW	1.94	1.97	1.50	2.07	2.07	2.29	*V.cholerae*
August	SW	2.28	2.55	2.28	2.10	2.21	2.57	*V.cholerae*
	FW	1.79	1.92	1.70	0.80	1.30	1.58	*S.aureus*
	CW	1.94	1.98	1.84	2.01	1.99	2.23	*V.cholerae*
September	SW	2.32	2.26	2.21	2.05	2.20	2.54	*V.cholerae*
	FW	1.78	1.69	0.68	1.15	1.58	1.28	*E.coli*
	CW	2.03	1.77	1.73	1.96	1.94	1.97	*E.coli*
October	SW	2.35	2.37	2.28	1.97	2.12	2.36	*S.aureus*
	FW	1.95	1.59	1.34	0.81	1.10	1.61	*E.coli*
	CW	1.89	1.73	2.01	1.67	1.92	2.02	*V.cholerae*
November	SW	2.01	2.12	1.97	2.03	2.08	2.29	*V.cholerae*
	FW	1.75	1.81	0.86	0.97	1.43	1.50	*S.aureus*
	CW	1.99	2.06	1.96	1.86	1.90	2.19	*V.cholerae*
	SW	2.00	2.37	2.13	2.08	2.02	2.29	*S.aureus*

December	FW	1.68	1.76	1.84	1.57	0.76	1.55	*P.aeruginosa*
	CW	1.96	2.01	0.68	1.82	1.61	2.08	*V.cholerae*

SW- Source water, FW- Finished water, CW- Community water. Each value is an average of two readings (S1 & S2)

Table 2: Comparative percentage occurrence and densities of pathogens

Parameter	Sample	*E.coli*	*S.aureus*	*P.aeruginosa*	*Salmonella*	*Shigella*	*V.cholerae*
Range	SW	1.82-2.38	1.98-2.59	1.88-2.53	1.75-2.23	1.84-2.27	2.02-2.65
	FW	0.51-1.98	0.51-2.01	0.51-1.96	0.51-1.69	0.51-1.82	0.51-1.82
	CW	1.36-2.11	1.52-2.16	1.30-2.17	1.60-2.14	1.30-2.19	1.86-2.31
Percentage Occurrence (N=24)	SW	100%	100%	100%	100%	100%	100%
	FW	95.8%	95.8%	87.5%	87.5%	91.6%	100%
	CW	100%	100%	95.8%	100%	100%	100%
Density	SW	*V.cholerae > S.aureus > P.aeruginosa > E.coli > Shigella > Salmonella*					
	FW	*S.aureus > E.coli >P.aeruginosa >V.cholerae >Shigella >Salmonella*					
	CW	*V.cholerae >Shigella >Salmonella >S.aureus >E.coli >P.aeruginosa*					

Table 3: Biochemical results of isolated pathogens

Biochemical Characteristics	*E.coli*	*S.aureus*	*P.aeruginosa*	*Salmonella*	*Shigella*	*V.cholerae*
Indole	+	-	-	-	+	V
Methyl Red	+	+	-	+	+	V
Voges- proskauer	-	V	-	-	-	V
Simmon Citrate	-	-	+	+	-	+
Oxidase	-	-	+	-	-	+
Catalase	+	+	+	+	+	+
Coagulase	NT	+	NT	NT	NT	NT
O/F test	F	F	O	F	F	F
Nitrate Reduction	+	+	+	+	+	+
Mannitol Fermentation	+	+	-	+	+	+
Amylase	-	-	-	-	-	+
Gelatinase	-	+	+	-	-	+
Urease	-	-	-	-	-	-
Arginine	NT	NT	NT	NT	NT	-
Lysine	NT	NT	NT	NT	NT	+
Ornithine	NT	NT	NT	NT	NT	+
Growth at 42^0C	NT	NT	+	NT	NT	NT
TSIA- 1. Glucose	+	+	-	+	+	+
2. Gas from Glucose	+	-	-	+	-	-
3. Sucrose	+	+	-	-	-	+
4. Lactose	+	+	-	-	-	V
5. H_2S	-	-	-	+	-	-

+ = 90 to 100% of the isolates were positive;
- = 0 to 10% of the isolates were positive; V = variable reaction
NT = Not tested, O = Oxidative, F = Fermentative

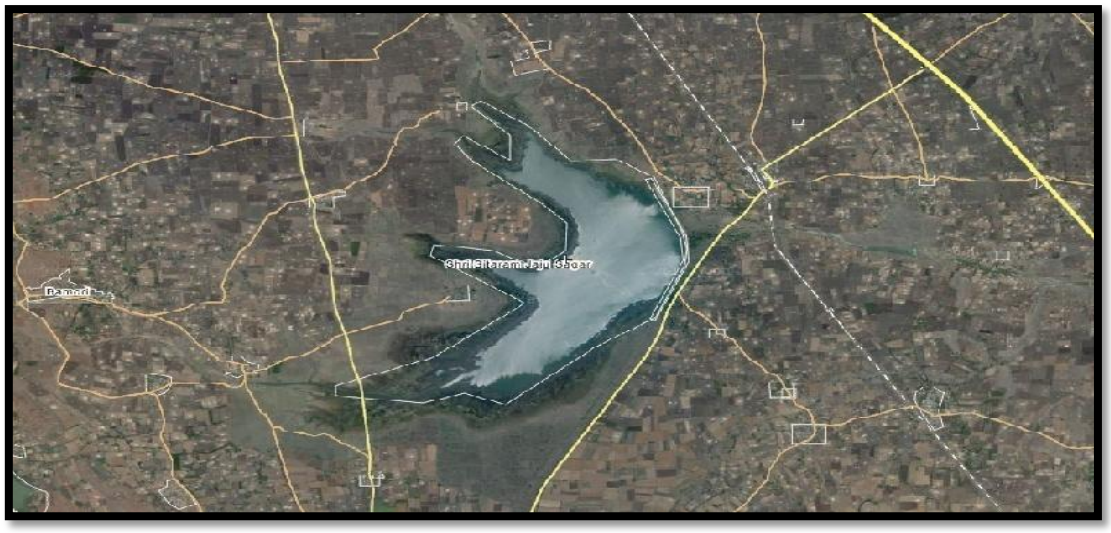

Fig 1: Map showing Jaju Sagar Dam, Neemuch

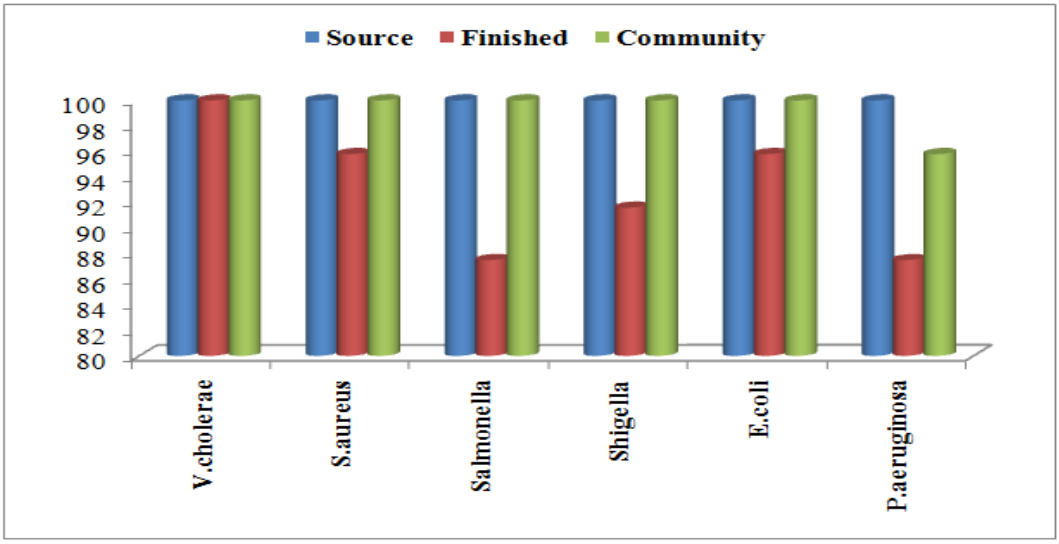

Fig 2: Percentage Occurrence of pathogens

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