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DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY PROFILES OF *PSEUDOMONAS AERUGINOSA* FROM VARIOUS TYPES OF DRINKING WATER SAMPLES IN AND AROUND OF THE HOLY CITY TIRUPATI.

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ABSTRACT

The present study was undertaken to investigate the spectrum of bacteria present in the River Gomati water before and after chlorination for drinking purposes. We observed that the strains of *Pseudomonas aeruginosa* that survived chlorination on three out of seven occasions were resistant to almost all the antibiotics tested. The chlorine-resistant bacteria had mucoid colonies and grew better at 24°C. All attempts to isolate the plasmid responsible for chlorine resistance were unsuccessful. Laboratory experiments using different strains of the *P. aeruginosa* in distilled water showed that only the resistant strain survived chlorine treatment at a dose of ⩽500 µg/L. Similar results were obtained when water collected from seven different sites on the River Gomati was treated with graded doses of chlorine. At the higher dose of chlorine, all the bacteria died in 30 min, whereas with lower doses all the bacteria survived. In this study, the water quality and antibiotic sensitivity profile of human enteric pathogens, a total of 24 drinking water samples were collected from Raw water, Alum mixing water, Filtered water, Bore water, Well water, Tap water, Hand pump, Tank water for the isolation and detection of thermo tolerant *Pseudomonas aeruginosa* from different localities of salinity affected villages of in and around Tirupati city and determined the identification and characterization of *Pseudomonas aeruginosa* from drinking water samples via. Gram staining, Biochemical analysis, Antibiotic susceptibility test profiles, and also the prevalence of *Pseudomonas aeruginosa* from drinking water samples.

The present study underscores the importance of measuring water chlorine concentrations to assure they are sufficiently high to remove pathogenic bacteria from drinking water. To our knowledge, this is the first report in the literature of the selection of multidrug-resistant bacteria by suboptimal chlorine treatment of water.

1. INTRODUCTION

The public health standards for safe drinking water for human consumption have the following guidelines. Drinking water should not contain any bacteria indicative of fecal pollution such as *Pseudomonas spp.* Microbial contamination is most common and widespread health risk associated with drinking water, either directly or indirectly, by animals or humans (Tambekar *et al.,* 2005). In developing nations, more than 250 million new cases of waterborne diseases are reported annually (Batt *et al.,* 2006). This has resulted in high morbidity and mortality rates, especially in young children and elders. In our laboratory extensive research work was carried on various health problems in relation to bacteria (Hari Prasad *et al.,* 2011; Pradeepkiran *et al.,* 2017), viral and microbial (Chiranjeevi *et al.,* 2014; Sudheer *et al.,* 2015; Bharathi *et al.,* 2019) oriented diseases. Oligotrophic bacteria such as forming biofilms under conditions that are usually considered nutrient restricted (Diab *et al.,* 2002). Such organisms are found in low nutrient environments such as drinking water, ground water, and surface water, attaining population densities of 106-107 cells per mL in drinking water.

Waterborne infections are most common causes of morbidity and mortality in the underdeveloped and developing countries and 80% of the infectious diseases are waterborne in India (Fewtrell *et al.,* 1997). Around 2.2 million people die due to basic hygiene related diseases like gastroenteritis, diarrhoea, typhoid, and dysentery (Kramer *et al.,* 1996). *Pseudomonas aeruginosa* are environmental bacteria frequently present in small number in normal intestinal flora of humans and animals and particularly good at forming biofilm and associated with deterioration of bacteriological quality of drinking water including test, odour and turbidity (Richard *et al.,* 1994; Lateef *et al.,* 2005).

A report by the United Nations says that more than three million people in the world die of water-related diseases due to contaminated water each year, including 1.2 million children (Servais *et al.,* 1992). In India, over one lakh people die of water-borne diseases annually. It is reported that groundwater in one-third of India's 600 districts is not fit for drinking as the concentration of fluoride, iron, salinity and arsenic exceeds the tolerance levels (Tamagnini *et al.,* 1997). About 65 million people have been suffering from fluorosis, a crippling disease due to a high amount of fluoride, and five million are suffering from arsenicosis in West Bengal due to high amount of arsenic. A World Resources Report says, about 70 per cent of India's water supply is seriously polluted with sewage effluents (WHO, 2003). The UN reported that India's water quality is poor - it ranks 120th among 122 nations in terms of quality of water available to its citizens (A.P.H.A, 1998).

Water-borne diseases like cholera, gastroenteritis and diarrhea erupt every year during summer and rainy seasons in India due to poor quality drinking water and sanitation. Hence, an attempt was made to determine the antibiotic susceptibility profiles of bacteria in various types of drinking water samples of Tirupati city.

2. MATERIALS AND METHODS

2.1 Chemicals and culture media:

Nutrient broth (Himedia) is composed by peptic digest of animal tissue, NaCl, Beef extract, yeast extract is used for bacterial enrichment. Cetrimide agar (Himedia), the specific medium used for isolation of *Pseudomonas aeruginosa*. Crystal violet, Gram's iodine, safranin, decolorizer (ethyl alcohol) are used for Gram Staining. In biochemical analysis, Tryptone broth (Himedia) and kovac's reagent are used for indole test. Triple sugar iron agar (Himedia) used to identify the lactose, sucrose, dextrose activity in bacterial cells. Luria agar (Himedia) and 11 different antibiotics are used to test antibiotic sensitivity profiles of the bacterium.

2.2 Site of selection:

The study area included a total of 6 site sampling locations, in these, 3 sites selected from R.Mallavaram village of Renigunta Mandal at Tirupati(rural) and 1 site selected from Mangalam village (from Telugu Ganga Tank), remaining 2 sites selected from Padmavathi Nagar and Lela mahal road of Tirupati city. Only Drinking water samples were collected from these sites.

2.3 Sample collection:

The total 24 samples were collected from (Public water supply areas in the month of April 2021) Raw water (1), Alum mixing water (1), Filtered water (1), Bore water (6), Tap water (8), Hand pump (4), Well water (1), Tank water (2) for the isolation and detection of thermo tolerant *Pseudomonas aeruginosa* bacteria from different localities of salinity affected villages in and around Tirupati city. The samples were collected by using sterilized borosil bottles (100 ml). The bacteriological examination was performed within the 24 hours of collection. Physicochemical and Microbiological analysis were done for the assessment of drinking water quality. Appearance, turbidity, pH, taste of water samples were recorded at the site during sampling period.

2.4 Enrichment of Bacteria:

For the enrichment of *Pseudomonas aeruginosa* bacteria, 1 ml of freshly collecting water sample was inoculated into 10 ml of Nutrient broth in a test tube. Nutrient broth (Himedia) is composed by peptic digest of animal tissue, NaCl, Beef extract, Yeast extract. The broth is used for 13 grams in 1000 ml. e.g., 1.3 grams Nutrient broth in 100 ml of distilled water. Each test tube is filled with 10 ml of Nutrient broth. After inoculation of water sample, it can be incubate overnight at 37°C for 24-48 hours in incubator for bacterial enrichment.

2.5 Measuring Activity Index:

Following formula was used to Measure Activity Index, Activity Index = (Zone of inhibition of antibiotic). Zone of inhibition of stocks against each bacterial species and similarly zone of inhibition of Antibiotics were measured.

3. RESULTS AND DISCUSSION:

3.1 Sample Collection:

A total of 24 Drinking water samples were collected from 8 different types of water resources. Various types of water resources and their physicochemical parameters of water samples are mentioned in (Table 1). Water collection proofs are seen in photographic picture.

Table-1: Physicochemical Parameters

S.NO	Sample Type	pH	Appearance	Turbidity	Taste
1	Raw water	7.0	Clear	5.9	Taste less
2	Alum mixed water	7.2	Clear	4.9	Taste less
3	Filtered water	7.1	Clear	3.9	Taste less
4	Bore water	7.2	Clear	0.9	Taste less
5	Tap water	7.1	Clear	0.6	Taste less
6	Well water	7.2	Clear	0.6	Taste less
7	Hand pump	7.2	Light brown	0.9	Sour taste
8	Tank water	7.1	Clear	0.6	Taste less

Table-2: Sampling Results

S.NO	Date of collection	Type of water sample	Positive bacteria	Negative bacteria
1	**27/03/2021**	Bore water	-	-ve
2	**27/03/2021**	Tap water	-	-ve
3	**28/03/2021**	Raw water	**+ve**	
4	**28/03/2021**	Aulum mixing water	**+ve**	
5	**28/03/2021**	Filtered water	**+ve**	
6	**02/o4/2021**	Well water	**+ve**	
7	**02/04/2021**	Tank water	**+ve**	
8	**02/04/2021**	Hand pump	**+ve**	
9	**02/04/2021**	Bore water	**+ve**	
10	**02/04/2021**	Hand pump	-	-ve
11	**02/04/2021**	Hand pump	-	-ve
12	**02/04/2021**	Tank water(LNK)	**+ve**	
13	**06/04/2021**	Tap water	**+ve**	
14	**06/04/2021**	Tap water	-	-ve
15	**06/04/2021**	Bore water	**+ve**	
16	**06/04/2021**	Bore water	**+ve**	
17	**06/04/2021**	Tap water	**+ve**	
18	**06/04/2021**	Bore water	**+ve**	
19	**06/04/2021**	Tap water	**+ve**	
20	**06/04/2021**	Bore water	**+ve**	
21	**06/04/2021**	Tap water	**+ve**	
22	**06/04/2021**	Tap water	**+ve**	
23	**06/04/2021**	Hand pump	**+ve**	
24	**06/04/2021**	Tap water	-	-ve

3.2 Enrichment of bacteria: For enrichment of bacterial growth, 1.3 grams of Nutrient broth is

taken into a conical flask and dissolved in 100 ml of distilled water. The flask is tightly packed to placed into autoclave for15 lbs pressure at 121°C. After autoclaving, test tubes are filled with Nutrient broth. Each test tube having 10 ml of NB. Then freshly collecting 1 ml of water samples are inoculate into a test tube and incubate for 24-48 hrs at 37°C temperature in an incubation. After incubation we observed bacterial cultures in test tubes as like shown in the picture.

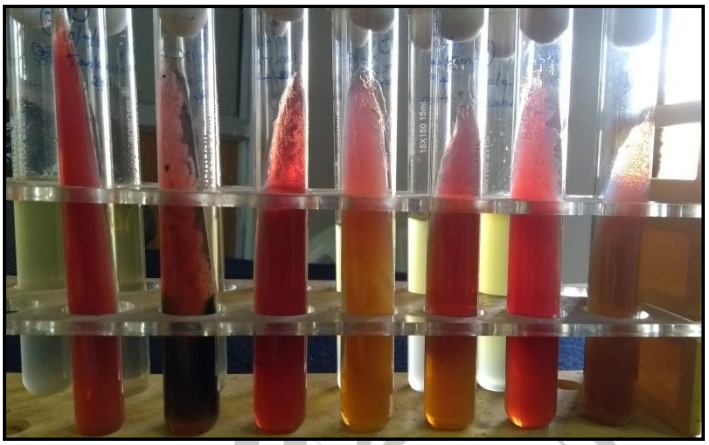
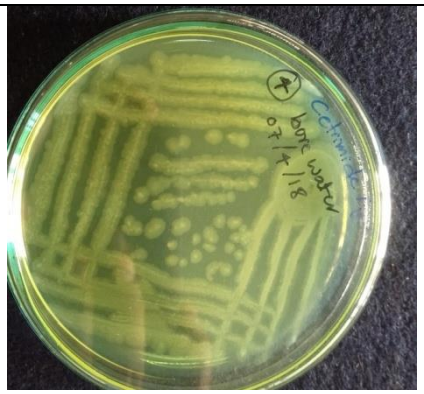


Fig-1: Enrichment Culture Tubes

3.3 Bacterial Isolation:

The autoclaved, 121°C for 15 lbs pressure of sterilized media is poured into petriplates and streaked with loop of bacterial culture from enrichment media on the surface of Cetrimide agar medium for the specific growth of *Pseudomonas aeruginosa* with the help of sterilized loop. Streaked petri plates are incubated for 24-48 hours at 37°C in an incubator. After that we are observed isolated group of bacterial colonies to individual colonies as like shown in the pictures. When Cetrimide agar media will be turned into light yellowish green colour medium. This colour indicates the positive results of *Pseudomonas aeruginosa* in drinking water sample. The colour pigment was secreted by bacterial colonies. The pigment is Pyoviredine (green), green coloured pigment. In this study 75% of *P. aeruginosa* bacteria were isolated.



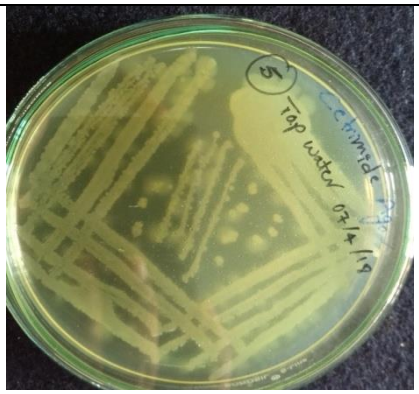


Fig-2.1: Bacterial colonies from bore water	Fig-2.2: Bacterial colonies from tap water

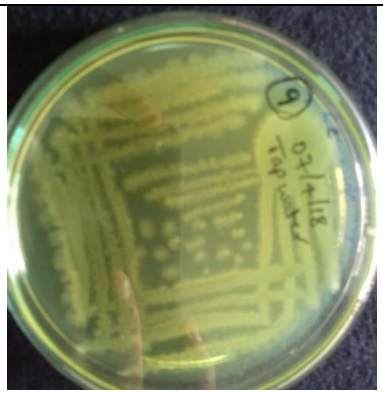
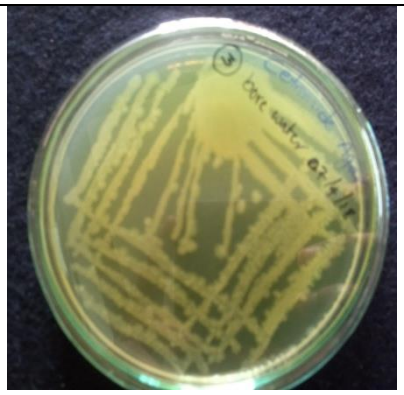

	

Fig-2.3: Bacterial colonies from tap water	**Fig-2.4: Bacterial colonies from bore water**
	
Fig-2.5: Bacterial colonies from bore water	

Fig-2: Bacterial Colonies of Different Samples

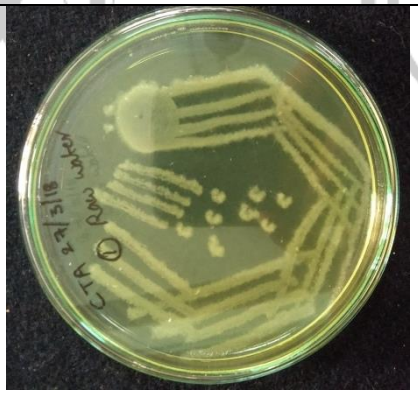
	
Fig-3.1: Bacterial colonies from filter water	Fig-3.2: Bacterial colonies from raw water

Fig-3.3: Bacterial colonies from Alum water

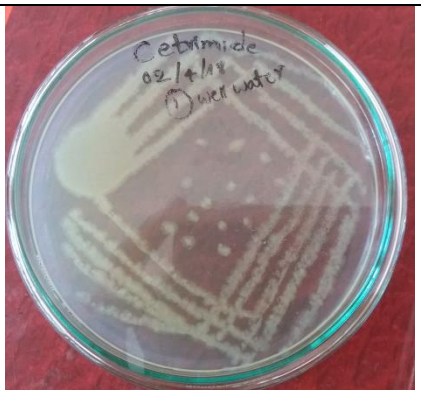


Fig-3.4: Bacterial colonies from well water

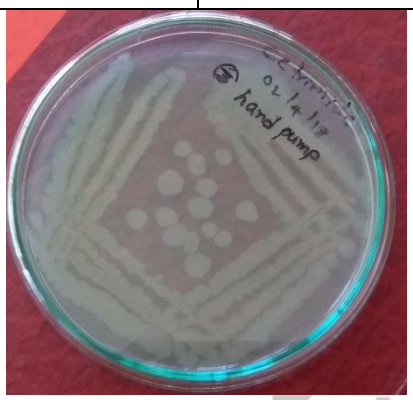


Fig-3.5: Bacterial colonies from Hand Pump

Fig-3: Bacterial Colonies of Different Samples

3.4 Gram staining:

Followed by the technique of Gram staining, after that we observed cleared Rod shaped bacterium under the fluorescent microscope by placing the immersion oil, the bacterial rods are pink coloured as like shown in the picture. The pink coloured rods are indicates Gram negative (-ve) *Pseudomonas* bacterial rods.

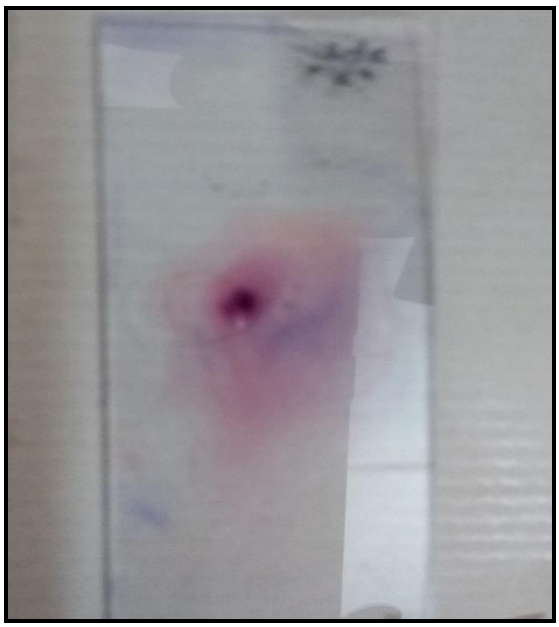


Fig-4.1: Smear Preparation

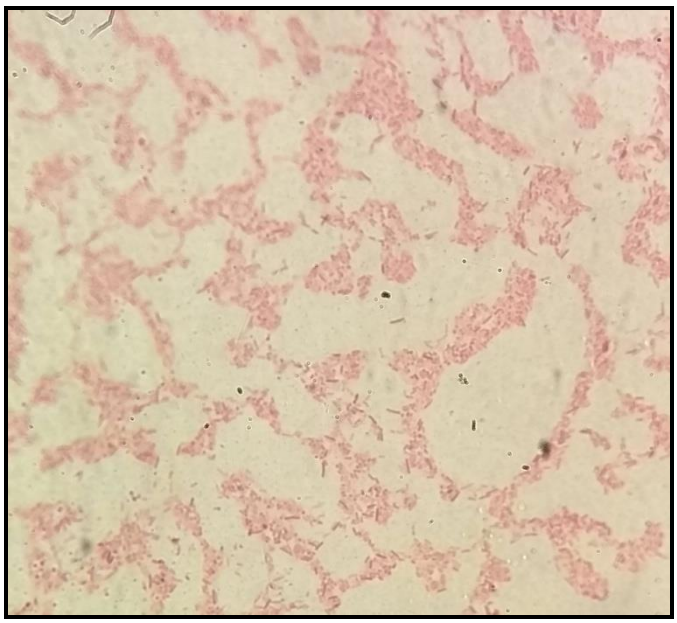


Fig-4.2: Microscopic Slide

3.5 Biochemical tests:

In this study biochemical tests are done by indole test and Triple sugar iron agar test. After adding Kovac's reagent of indole test, pink coloured ring does not formed. The absence of pink coloured ring indicates negative test of *Pseudomonas aeruginosa.*

The steaked and prepared Triple sugar iron agar slant was incubated in 24-48 hrs at 37°C in incubaror. After incubation we are observed colour difference of the slant. The slant differentiates the various biological parameters in various water samples. Triple sugar iron agar test is positive biochemical test for *P. aeruginosa*. The test is clearly shown in the (Table 2).

5. Sharing the colour variation in slant

Abbreviations: R- red, Y- yellow, glu ferm- glucose fermentation, lac- lactose, suc ferm- sucrose fermentation, gas- gas produced.

Table-3: TSI Agar Test Results

S.No.	Type of Sample	Observed characteristics
1	Tank water(2/4/18)	R/R: obligate aerobe
2	Well water(2/4/18)	R/Y: glu ferm, H_2s
3	Tank water, LNK(2/4/18)	R/R: obligate aerobe
4	Bore water(2/4/18)	R/Y: only glu ferm
5	Hand pump(2/4/18)	R/Y: only glu ferm
6	Bore water(6/4/18)	R/R: obligate aerobe
7	Tap water(6/4/18)	Y/Y: lac+/or suc ferm, gas

Abbreviations: R- red, Y- yellow, glu ferm- glucose fermentation, lac- lactose, suc ferm- sucrose fermentation, gas- gas produced.

3.6 Antibiotic Susceptibility Test:

After spreading and inoculating antibiotic discs of culture media plates were transferred into an incubator for 24 to 48 hrs at 37°C temperature. After incubation we are observed the 24 bacterial isolates were exposed to 10 antibiotic discs for susceptibility testing, and the zones of inhibition calculated by measuring a diameter in milli meter (mm). Table 3 shows the antibiotic susceptibility results. The studied antibiotics are commonly used as drugs for human beings. Depending on the Zone of inhibition formed, the isolates are categorized as resistant (R), sensitive (S) and intermediate (I).

Antibiotic Susceptibility Test Results:

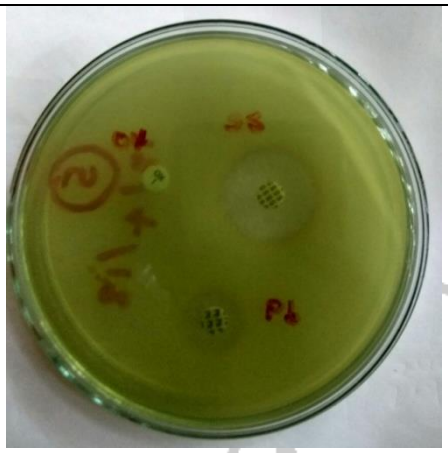


Fig-6.1: Sensitivity test for Tank water (LNK)

Fig-6.2: Sensitivity test for Bore water

Fig-6.3: Sensitivity test for Tap water

Fig-6.4: Sensitivity test for Tap water

Fig-6: Sensitivity Test for Different Samples

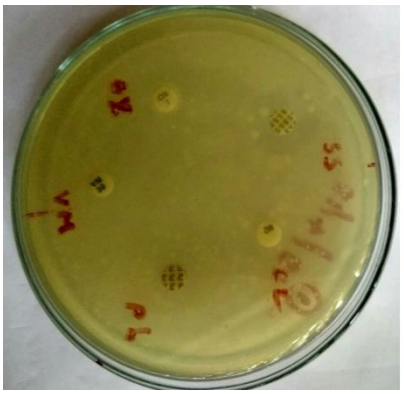


Fig-7.1: Sensitivity test for Bore Water

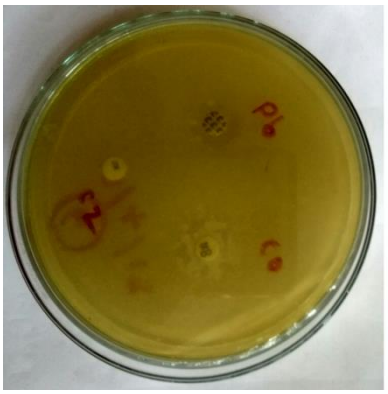


Fig-7.2: Sensitivity test for Tank Water (LNK)

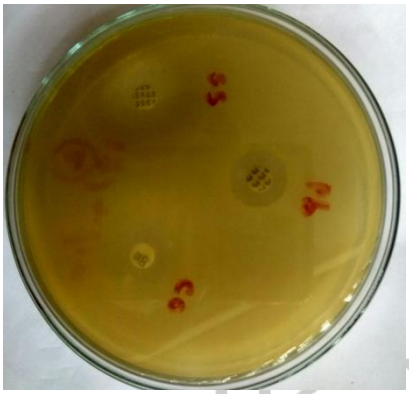


Fig-7.3: Sensitivity test for Tap Water

Fig-7.4: Sensitivity test for Tap Water

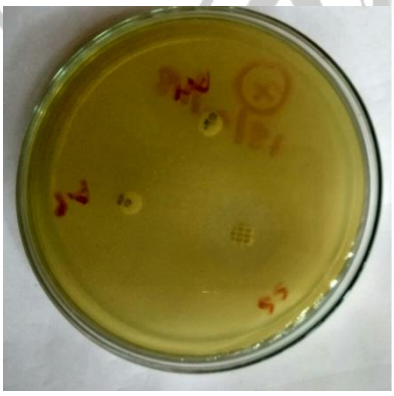


Fig-7.5: Sensitivity test for Bore Water

Fig-7: Sensitivity Test for Different Samples

Table- 3: Antibiotic test results

Antibiotics Used	Sensitive zone diameter measurement(mm)	Intermediate zone diameter measurement(mm)	Resistant in percentage (%)
1. Streptomycin(10µl)	100	40	-
2. Cefazolin(30µl)	-	-	100%
3. Polymixin-B(10µl)	60	40	-
4. Vancomycin(10µl)	-	-	100%
5. Oxacillin(10µl)	-	-	100%
6.CO-Trimoxazole (25µl)	50	40	-
7. Ampicillin(10µl)	40	-	80%
8. Amoxicillin(10µl)	-	-	100%
9. Kenamycin(10µl)	100	60	-
10.Rifampicin(10µl)	100	40	-

From this table, 50% of antibiotics are resistant and 50% of antibiotics are susceptible for *Pseudomonas aeruginosa* bacteria. In this Streptomycin, Kenamycin, Rifampicin are more susceptible antibiotics for *Pseudomonas aeruginosa*.

4. SUMMARY AND CONCLUSION:

A total of 24 samples were analysed from various sources such as Raw water(1), Alum mixing water(1), Filtered water(1), Bore water(6), Tap water(8), Hand pump(4), Well water(1), Tank water(2) for the presence of thermo tolerant *Pseudomonas aeruginosa* and recorded almost all (75%) samples contaminated with *P.aeruginosa bacteria*. It includes Raw water(4%), Alum mixing water(4%), Filtered water(4%), Bore water(20%), Tap water(20%), Well water(4%), Hand pump(8%), Tank water(8%). The study reported high incidence (75%) of *P.aeruginosa* in Drinking water showing health risks from microbial growth and biofilms in Drinking water distribution system.

In this study almost all samples of *Pseudomonas aeruginosa* were resistance to Cefazolin, Vancomycin, Oxacillin, Ampicillin, Amoxillin. These finding supported the observations of several previous studies, comparatively high antibacterial sensitivity observed due to rare or occasional use of the drug and could be attributed to the fact that these drugs were seldom used.

Study indicated high (75%) incidences of thetmo tolerant *P.aeruginosa* is common organism found in water and its growth can cause problems with colour, taste, odour, and turbidity to the Drinking water. It is a typical biofilm –producing organism that grows on tubing, fittings and showerheads as well as in sinks and sink drains. The study reported high incidences (75%) of *P. aeruginosa* in Drinking water indicating danger to human health due to its biofilm

producing ability. In present study *P. aeruginosa* showed 50% resistance and 50% sensitive to tested antibiotics.

All the isolated stains of *P. aeruginosa* were resistance to Cefazolin (10%), Vancomycin (10%), Oxacillin (10%), Ampicillin (10%), Amoxillin (10%). The higher –level resistance to this antibiotic might be attributed to antibiotic and antibiotics resistance bacterial emergence because of improper and extensive use of these antibiotics. *P. aeruginosa* were highly sensitive to Streptomycin (10%), Polymixin-B (10%), CO-Trimoxazole (10%), Kenamycin(10%), Rifampicin (10%). The finding recommended that Streptomycin, Polymixin-B, CO-Trimoxazole, Kenamycin, Rifampicin should be the drug of choice for diarrhoeal diseases caused by *Pseudomonas aeruginosa* bacteria. The study concluded that most of water is contaminated with *P. aeruginosa* bacterium and unsafe for Drinking purpose, hence properly treated water should be used for Drinking water.

5. REFERENCES:

A.P.H.A, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed, edited by Andrew D Eaton (American Public Health Association, Washington DC) Part 9000: 9- 140.

Batt, A. L., Bruce, I. B., and Aga, D. S. (2006). Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environmental Pollution, 142(2), 295–302.

Chiranjeevi T., Hari Prasad O., Venkateswara Prasad U., Kishor Kumar A., Praveen Chakravarthi V., Balaji Rao P., Venkata Gurunadha P., Krishna S., Nagi reddy Raveendra Reddy and Bhaskar M. (2014). Identification of Microbial Pathogens in Periodontal disease and Diabetic patients of South Indian Population. Bioinformation. 10(4); 241-244.

Diab, A.M., Abdel Aziz M.H., and Selim S.A. (2002). Plasmid encoded transferable antibiotic resistance in gram-negative bacteria isolated from drinking water in Ismailia city. Pak. J Biol. Sci., 5(7): 774-779

Fewtrell, L., Kay, D., Wyer, M., Godfree, A., and ONeill, G., (1997). Microbiological quality of bottled water. Water Sci. Tech. 35, 47–53.

Hari Prasad O., Sudheer A., Kishore Kumar A., Navya A., Hari Krishna O., Bhaskar M, Papa Rao A, and Ravindra Reddy N. (2011). Comparative evaluation of the antibacterial efficacy of P. juliflora and three commercially available mouthrinses: an in vitro study. J. Pharmacy Research. 4(7); 2149-2151.

Kramer, M.H., Herwaldth, B.L., Craun, G.F., Calderon, R.L., and Juraneck, D.D., (1996). Waterbone disease: 1993–94. J. Am. Water Works Assoc. 32, 66–80.

Lateef, A., Olake J.K., and Gueguimakan E.B. (2005). The prevalence of bacterial resistance in clinical, food, water and some environmental samples in southwest Hungeri. Environ Monit Assess, 100(1-3): 59-69.

P.Bharathi, Mamatha.C, Payani S., Chandraprakash C., Ramasamy P., and Bhaskar M. (2019). Bioreduction Potential of Ficus elastic rubra Aqueous extracts for synthesis of Silver Nanoparticles and their Antimicrobial Studies.Proc. Zool.Soc. India.18(2):1-7 IF:0.438.

Pradeepkiran J. A, Jagadish R., and Bhaskar M. (2017). Multiplex quantification of Escherichia coli, Salmonella typhi and

Vibrio cholera with three DNA targets in single reaction assay. Microbial Pathogenesis 110; 50-55.

Richard, P., Le Floch, R., Chamoux, C., Pannier, M., Espaze, E., Richet, H., (1994). Pseudomonas aeruginosa outbreak in a burn unit: role of antimicrobials in the emergence of multiply resistant strains. J. Infect. Dis. 170 (2), 377–383.

Servais, P., Billen, G., Laurent, P., Le´vi, Y., and Randon, G., (1992). Studies of BDOC and bacterial dynamics on the drinking water distribution system of the Northern Parisian suburbs. Revue des Sciences de l'Eau 5(spe´ cial), 69–89.

Sudheer A., Sagar A., Praveen V., Payani S., and Bhaskar M. (2015). Synthesis and Spectral characterization of Pyrimidine analogues: An antimicrobial activity against clinically isolated microbes. IJOMAS. 1(3); 5-11.

Tamagnini, L.M., and Gonzalez, R.D., (1997). Bacteriological stability and growth kinetics of Pseudomonas aeruginosa in bottled water. J. Appl. Microbiol. 83 (1), 91–94.

Tambekar, D.H. and Banginwar Y.S., (2005). Studies on potential Intervention for Control of water borne diseases: Promotion of storage, handling and serving practices of drinking water in hotels /restaurants. Poll. Res., 24 (2): 371-375.

World Health Organization (WHO)., 2003. Guidelines for Drinking-Water Quality. Third Ed., Geneva, /http:// www.who.int/water_sanitation_health/S.