Biophysico-Chemical Examination of Potable Water and Their Antibiotics Resistance Profile at Rural Area of Meerut Region

[1]Ayush Tomar, [2]Nisha Dhillon, [3]Shalu Chaudhary

[1]Research Assistant, [2,3]Assistant Professor
[1]Chaudhary Charan Singh - National Institute of Animal Health (CCS-NIAH), Baghpat
[2,3]College of Applied education and Health Sciences, Meerut

Abstract: The biophysico-chemical parameters of potable water depend on its sources such as fresh water rivers, ponds, tube wells and lakes etc.Now days, these water bodies are under stressed due to anthropogenic activities, that's why the quality of potable water might be polluted with the use of pesticides, pathogens, heavy metals, and fertilizers etc.These all degrade the water quality and affect human health.The present study was aimed to analyse the Physico-chemical and bacteriological parameters of potable water and determine their antibiotics resistance profiles. Water sample were analysed for the presence of faecal indicator bacteria as well as aeromonas, Coliform, staphylococcus, pseudomonas, and bacillus species. Most of the bacteria were resistant towards bacterial antibiotics such as ampicillin, amoxicillin, doxycycline, penicillin and caftazidime but ciprofloxacin were against *e.coli, pseudomonas* and *bacillus*.
this paper, focus is t source of water for human being comes Most of the water bodies disappeared due to depends on the nature and extent of the industrial, agricultural, and other anthropogenic activities in the encroachment and pollution as mentioned by Yogendra and Puttaiah (2008). The surface water quality in a region largely.

Keywords: Antimicrobial resistance, Drinking water, biophysico chemical parameters, bacteria, antibiotics.

Introduction
Water is an essential requirement for life on earth because it functions as a universal solvent thus helps to regulateour body fluid. Water is the second vital resource for survivalof living beings after air. Pure drinking water is the basic need for good health, and also a basic right of humans.If any potable water source is contaminated with pathogenic microbes, and toxic substances, it might have serious health hazards on human health. All living organisms need water for their survival. As of now only earth which contain 70% of water. Drinking water is an important source for trace microbes' intake into human body. The presence of water has always been of great importance for life &for living microorganisms. Thus, the drinking water quality has a great impact on human health.Water has not any nutritional value such as calories, fat, carbohydrates, gives, sugar, and protein etc. Drinking water also contains various types of pathogenic and non pathogenic microbes which cause infectious diseases in human body. From drinking water, gram positive and gram negative bacteriawere isolatedwhich are harmful for human health. Isolated gram positive bacteria were Bacillus spp., Staphylococcusspecies and gram negative bacteria such as pseudomonas sp, coliform, aeromonas bacteria and some unidentified fungi also present in water. Ground water is used primarily as drinking water by vast majority (85%) of rural areas. Ground water playsan important role in various motives such as domestic use, commercial use & flooding in all over the world. In developing countries, due to increased community, mechanization, and self made activity,it has become contaminated. with different harmful contaminants. The objective of this study was to analyze the microbiological flora of potable water source and checks the antibacterial resistance activity against antibiotics .And also analyzing some important physico-chemical characteristics of water such as pH, temperature, TS,TDS ,TSS, chloride content, Hardness, alkalinity, DO, BOD5, COD, SO4, PO4 etc.

Materials and Methods
2.1 Study Area
In this study, Drinking water sample were collected from rural area. These sampling area were chosen for the study because water from well is used as drinking water as well as agricultural purposes. This is major source of pathogens, it's important to analysis the bacterial quality of water at this point.

Figure 1. collection of different water samples in sterilized bottles.

2.2 Collection of Sample

The drinking water used for this work, were collected from different sources of Meerut regions. Drinking water taken in sterilized bottles. The sample were labelled according to location & sample transferred into microbiology laboratory.

2.3 Determination of water pH

Medium pH play a major role in the growth of micro-organisms. Determination of medium pH is essential for examining the growth of microbes. The pH of medium is between the range of 7.2 to 7.5.

2.4 Preparation of Media

Composition of nutrient agar media

- Peptone   - 5.0 gm
- Nacl   – 5.0 gm
- Beef extract   - 3.0 gm
- Yeast extract   - 3.0 gm
- Agar   - 15.0 gm
- Distilled water   - 1000 **ml**

Prepared the media and autoclaved at 121℃ for 15PSI. After autoclave the media put in laminar air flow and poured the media in each petri plates and left for solidification.

2.5 Serial dilution of water sample and Culturing

The water sample was collected in sterile bottles from different sources (town). The water sample was serially diluted by taking 10 sterilized test tubes labelled from $10^1 – 10^9$ containing 9 ml of autoclaved distilled water. 1ml of water sample was dissolved in tube no.1 and this process repeat till last test tube. 0.1ml sample from dilution factor 10^1, 10^3, 10^5, and 10^7were spreaded on NAM (nutrient agar media) plates for the growth of bacteria and incubated at 37℃ for 24 hours.

2.6 Sub-Culturing of Isolated colonies

After 24 hours of incubation for bacteria sub-culturing of obtained colonies was accomplished by picking up the colonies with the help of sterile loop and streaking on nutrient agar media respectively and incubated in the same manner.

And last after 24 hours isolated colonies transfer on NAM Slant and incubated at 37℃ for 24 hours.

3. Colonial Morphology of Bacteria

Microorganisms produce definite patterns on culture media plates as they grow and divide. Colonial morphology is size, shape, colour, texture and the general structure of an individual colony of a particular microorganism on a culture media plate that support it's growth. Colonial morphology which can also be referred to as cultural characteristics pertains to the microscopic appearance of a bacterium on different kinds of growth culture media. In this study, the morphology of colonies were isolated such as irregular, spindle , round, and entire in shape and these colonies were appeared brown, white, milky, and orange in colour.

4. Morphological Characteristics

For morphological characterization one staining was performed for identification of bacteria.

4.1 Gram's staining for bacteria

Dr. Hans Christian gram's developed gram's staining in 1884 on the basis of colour. In this staining, the bacteria are classified into two groups on the basis of colour that bacteria are gram positive (purple colour) and gram negative (pink colour). We used four reagent in this staining such as crystal violet (primary stain), gram's iodine (mordent dye), decolorized, and safranin (counter stain). A smear was made on a clean slide and heat fixed, 1-2 drop of crystal violet for 60seconds were added and washed by distilled water drop wise. Then 1-2 drop of gram's iodine for 60seconds were added and washed by distilled water. Decolonizer or ethanol for 20-30 seconds were added and washed by distilled water. Then Safranin was added fir 60seconds and washed. Extra water was removed with the help of tissue paper and left the slide to dry. Examine the slide under microscope at 100X.

8. Biochemical Characterization for bacteria

For biochemical characterization some test were performed for identification of bacteria.

8.1 Catalase test

Pour 1-2 ml of hydrogen peroxide solution on slide. Using a sterile inoculating loop, take single colony of the 18 to 24 hours test organism and immerse in the hydrogen peroxide solution. Observe for immediate bubbling. The catalase test performs for identification of bacteria which found in drinking water. Some bacterial test are positive and some bacteria negative after performed catalase test.

8.2 Coagulase Test

 Coagulase test also an biochemical test for confirmation of bacteria. In coagulase test, 1-2 drop plasma was placed on clean grease free slide. About one single colony of the bacteria picked up with the help of sterilized inoculated loop and properly mixed in plasma. Presence of bubbles indicated positive result.

9. Antibacterial Susceptibility Test

Antibiotics are medicines used to fight bacterial infections. There are different types of antibiotics. Each type is only effective against certain bacteria. An antibiotic sensitivity test can help find out which antibiotic will be most effective in treating your infection. Antibiotics play a major role for the prevention of bacterial infections. it inhibit or kill the growth of microbes. Different types of antibiotics were used against different micro-organisms.

In antimicrobial susceptibility test, the fresh culture broth of different cultures spread on agar plates. After that antibiotics disc was aseptically placed over the agar plates by the help of sterilized forceps. The plates were incubated at 37℃ for 24 hours.

Biophysico-chemical parameters

It is very important and key point to test the water before it used for any purpose like drinking and agriculture etc. Water must be tested with different biophysico-chemical parameters. Selection of parameters for testing of water is depends on that what is the

purpose going to use. Some biophysico-chemical tests should be perform for its such as temperature, colour, pH, TDS, DO, alkalinity and other characters. Following different biophysico-chemical parameters are tested explain below –

Estimation of pH

pH is the most important in determining the corrosive nature of water. Lower the pH value hiher is the corrosive nature of water. pH was positively correlated with electrical conductance and total alkalinity (Gupta 2009). The higher values observed suggests that carbon dioxide, carbonate, bicarbonate equilibrium is affected more due to change in physic-chemical condition (Karanth 1987).

Estimation of temperature

In an established system the water temperature controls the rate of all chemical reactions, also it affects growth, reproduction and other characteristics of aquatic life. Drastic temperature changes can be harmful and sometimes lead to fatal of aquatic life.

Estimation of Total Dissolved Solid

The total dissolved of water sample were determined by evaporating method.

The evaporating porcelain basin was cleaned thoroughly with concentrated nitric acid and washed with distilled water. The basin was dried in an oven at 200ºC for 1 hour. The basin was cooled, desiccated, weighed and stored in desiccators. 25ml of water sample was transferred to the reweighed basin and evaporated to dryness steam bath. When the sample in the basin was dried then basin left for cooled in desiccators and weighed.

Estimation of Colour

The colour of water sample were determined by platinum cobalt standard method (spectrophotometer).

Coloured is determined by comparison of sample with known concentration of coloured solution. It is the standard method, until of colour being that produced by 1mg platinum/L in the form of the chloroplatinateion. The colour of water is extremely pH dependent and invariable increases as the pH of water is raised.

25 ml of water sample was placed in the sample cell and the colour was determined at 455 nm of dematerialized water as blank.

Estimation of Alkalinity

Alkalinity acts as stabilizer of water pH. It affect the toxicity of many substances in the water. It composed from carbonate and bicarbonate.

Estimation of Dissolved Oxygen

Dissolved oxygen is one of the most important parameter. Its correlation with water body gives direct and indirect information i.e. bacterial activity and photosynthesis etc.

Results &Discussion

In this study, drinking water of tube well collected in sterile container. The sample was taken from rural area in Meerut region. Different bacteria were isolated from tube well water on nutrient agar media. These bacteria were identified by gram's staining & on the basis of biochemical analysis.

Among the isolated bacteria *the* Bacillus was present in high amount as compared to other bacteria, such as *Pseudomonas* , *streptococcus*, *shigella,* and *e.coli.* these bacteria were characterized on the basis of physical properties analysis as per table 1. On the basis of gram's staining bacteria were differentiated as gram positive and gram negative bacteria show in table 2. The bacteria were characterized on the basis of morphological and biochemical analysis as per table 3. & Table 4.

In this study, antibiotics also play a major role for the Identification that bacteria a sensitive or resistance against antibiotics. In present investigation show that only one antibiotics which were ciprofloxacin against *bacillus* , *pseudomonas* and *e.coli* as per table 5.

Table 2. The Result of Growth of bacteria of Drinking water sample (August 2021).

S.No.	Shape	Gram's staining	Bacteria
1.	Rod shape	Pink colour	Pseudomonas
2.	Coccus & rod shape	Purple colour	Streptococcus & Bacillus
3.	Rod shape	Purple colour	Bacillus
4.	Rod shape	Pink colour	Escheria coli
5.	Rod shape	Pink colour	Shigella

In this present investigation gram positive and gram negative bacteria were found in water. Which are some of pathogenic & non-pathogenic bacteria such as *Shigella*, *pseudomonas*, *E.coli*, *staphylococcus* & *bacillus*.

figure 2. The result of isolated Pure Culture Plates of same pH by Streak Plate Method.
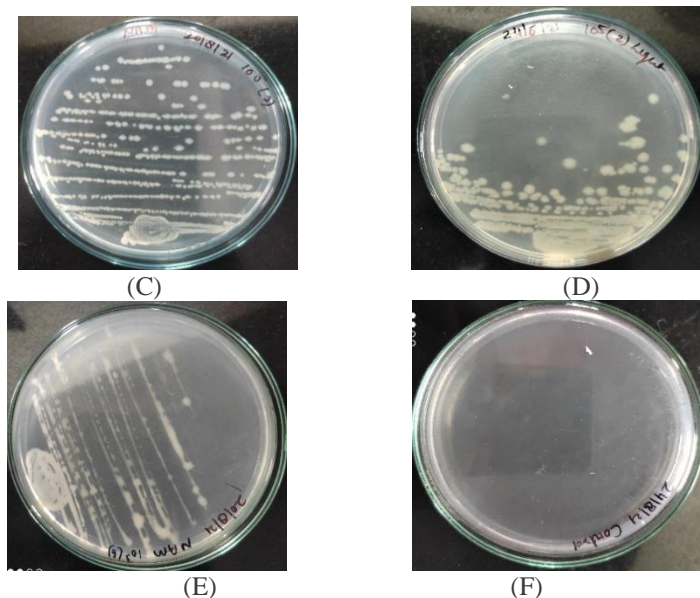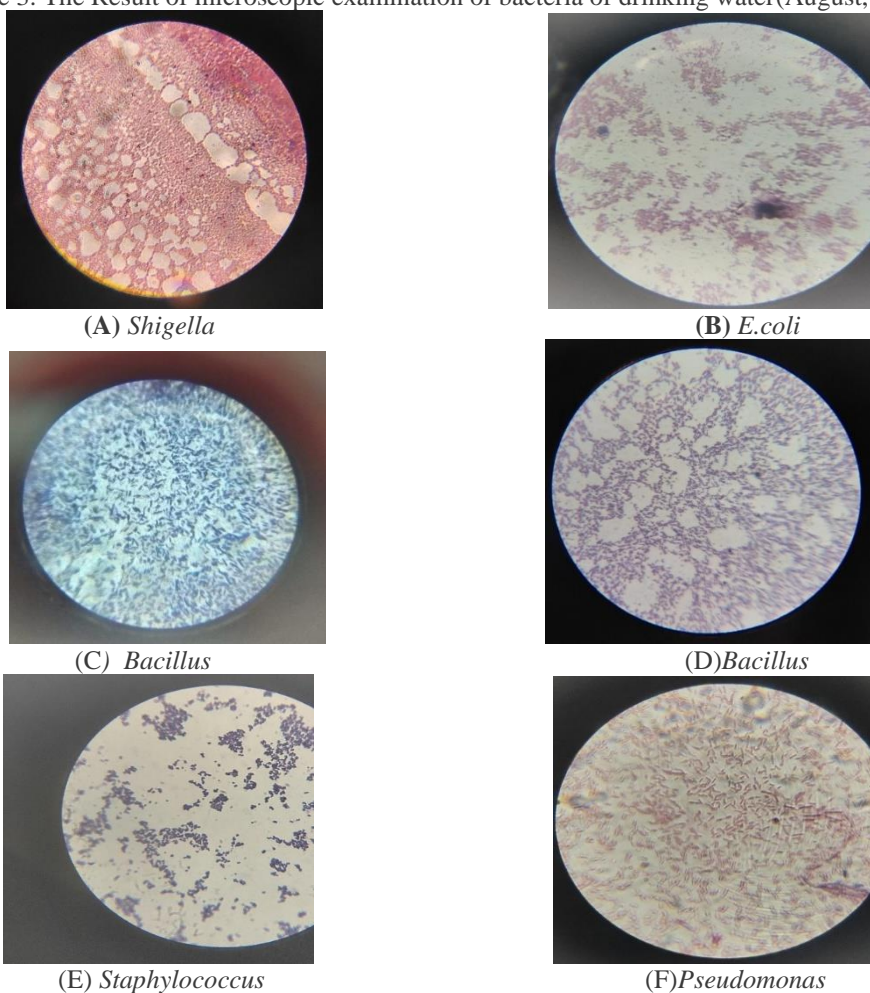

(A)　　　　　　　　　　　　　　　　　(B)

(C)   (D)

(E)   (F)

Figure 3. The Result of microscopic examination of bacteria of drinking water(August, 2021).

(A) *Shigella*   **(B)** *E.coli*

(C) *Bacillus*   (D)*Bacillus*

(E) *Staphylococcus*   (F)*Pseudomonas*

Different types of bacteria were identified such as A: *Shigella*; B: *E.coli*; C: *Bacillus*; D: Bacillus; E: *Staphylococcus*; F: *Pseudomonas.*

Table 3. The Result of Morphological Characteristics of bacteria of Drinking water sample (August, 2021).

S. No.	Morphological Shape	Colony Colour	Margin	Elevation
1.	Irregular	Opaque or White	Lobate	Pulvinate
2.	Spindle	Milky	Erose	Some growth in culture media

	Filamentous	Brown	Undulate	Convex
3.	Round	Brown	Entire	Punctiform
	Irregular	Opaque or white	Labot	Pulvinate (Cushion shaped)
4.	Entire (smooth)	White or pink or red	Punctiform	Umbonate
	Round	Orange	Curled	Raised

Table 4.The Result of Biochemical Analysis of Bacteria of Drinking water (August, 2021).

S. No.	Bacteria	Catalase test	Coagulase test
1.	*Pseudomonas*	Positive	Negative
2.	*Staphylococcus*	Positive	Positive
3.	*Bacillus*	Negative	Positive
4.	*Escherichia Coli.*	Positive	Negative
5.	*Shigella*	Negative	Negative

Figure 4A. The result of Coagulase Test of Potable water.

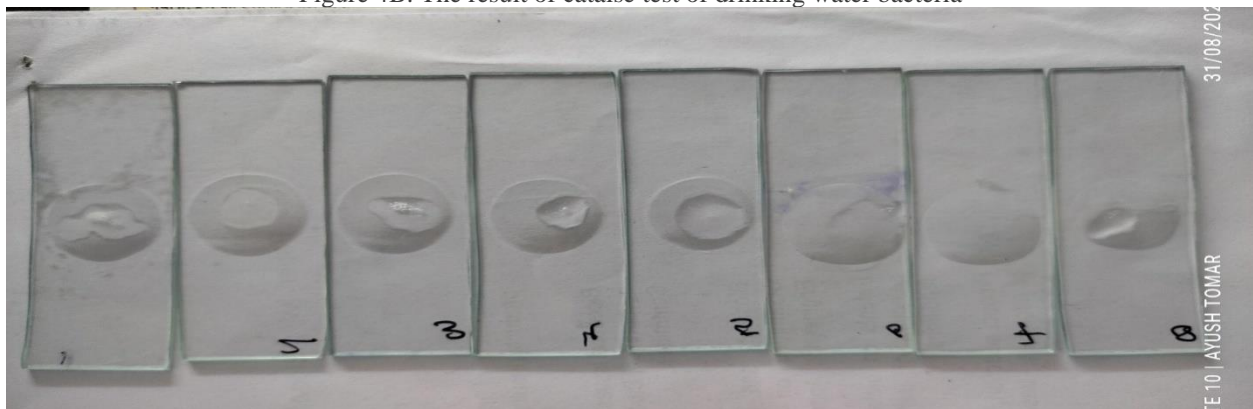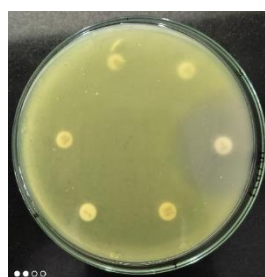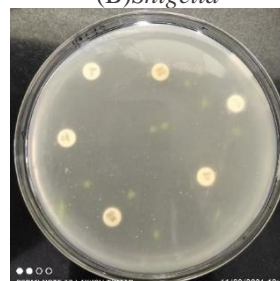Figure 4B. The result of catalse test of drinking water bacteria

Table 5. The result of Antibiotics Susceptibility test of drinking water bacteria.

S. No.	Bacterial Antibiotics	*Shigella*	*E.coli*	*Staphylococcus*	*Pseudomomas*	*Bacillus*
1.	Ampicillin	-	-	-	-	-
2.	Amoxicillin	-	-	-	-	-
3.	Doxycycline	-	-	-	-	-
4.	Ciprofloxacin	-	+	-	+	+
5.	Penicillin	-	-	-	-	-
6.	Ceftazidime	-	-	-	-	-

(A)*E.coli*

(B)*Shigella*

(C) *Pseudomonas*

(D) *Bacillus*

(E) *Staphylococcus*
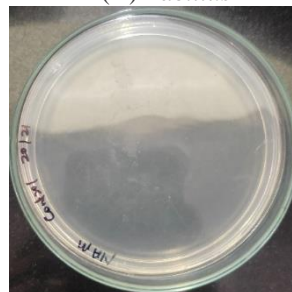
(F) Control

Biophysico-chemical parameter

According to biophysico-chemical parameters, potable water samples which collected from different sources are not more polluted. This drinking water is also unable to spread various ypes of diseases.

Table 6. Final concentration and observed value given below table.

S. No.	Physico-chemical parameters	Tab water	RO water	Tube well water	Hand pump water	Boring water
1.	pH	7.3	7.4	7.6	7.6	7.5
2.	Colour	0.001	0.019	0.027	0.0238	0.017
3.	Temperature	30°C	24°C	21°C	23°C	21°C
4.	Total Dissolved solids	328	97	234	228	246
5.	Dissolved oxygen	6.8	6.5	6.4	6.6	6.4
6.	Alkalinity	6.9	6.5	7.3	6.8	7.1

Conclusion

In conclusion, it was found that various species of gram positive & gram negative bacteria were present in drinking water such as *Bacillus, E.coli, Shigella, Pseudomonas*, & *streptococcus*. In present investigation pH, temperature, colour and total dissolve solid etc, were beyond the permissible limit. So, the potable water considered as not more polluted water. The antibiotics resistance profile of bacteria isolates from drinking water of tube well and resistance pattern of organisms in drinking water in ruler area that there has been an indiscriminate use of the antibiotics tested. Ciprofloxacin were against *e.coli, bacillus, shigella* and *pseudomonas* but other antibiotics don't work against bacteria of drinking water. Therefore, this proves that all the bacteria found in the water do not cause infectious diseases.

Acknowledgment

References

1. Aneja, K.R. (2003). Experiments on microbiology, Plant pathology and Biotechnology. New Age International (P) Limited Publishers, New Delhi: 4th edition.
2. S.P. Gautam (2005). Guide Manual: water and waste water Analysis. Standard Methods for the Examination of water and Wastewater, APHA, AWWA and WEF, 21st Edition.

3. J Arun Raj,(2018). A study on physico-chemical parameters of Urinjikulam Pond, Thiruthangal(Virudhunagar District, Tamil Nadu). International Journal of Agriculture and Fisher Sciences, Vol:4(1), pp. 010-012.
4. Suma George Mulamattathi., Carols Bezuidenhout., Moses Mbewe., and Collins NjieAteba(2014)., Isolation of Environmental Bacteria from Surface and Drinking Water in Mafikeng, South Africa, And Characterization Using Their Antibiotics Resistance Profiles. Journal of Pathogens. Vol:2014, pp 11pages.
5. Tassadaq Hussain., AneelaRoohi., ShehzadMunir.,Iftkhar Ahmed.,JafarKhan., Veronique Edel-Hermann., Kil Yong Kim., Muhammad Anees. Biochemical Characterization and Identification of bacterial strains isolated from drinking water sources of kohat, Pakistan. African Journal of Microbiology Research, Academic Journal 2013, Vol:7(16), pp. 1579-1590.
6. Yinn Kay Khaing., KhinHtay Win., ThidarKhaing (2019). Water Quality Assessment and Treatment of Tube Well Water from Selected Areas in Mandalay Region, Myanmar. International Journal of Scientific and Research Publications, Vol:9(7). pp. 744-749.
7. Dr. Shailpreet., Dr. Sita Malhotra., Dr. Pushpa Devi., Dr. Anuj Gupta (2016). Bacteriological Analysis of the drinking water from different schools in Northern India: A concern in developing countries. Int J Med Res 2016; Vol:4(4). pp. 630-634.
8. M. S. Islam., A. Siddika., M. N. H. Khan.,M. M. Goldsr., M. A. Sadique.,A. N. M. H. Kabir., Anwar Huq., and R. R. Colwell. Journal of Applied and Environmental Microbiology(2001). Vol:67(7). pp. 3328-3330.
9. Devangee P. Shukla., Krishna kumar B., Vaghela & Nayan K. Jain. Assesment of physic-chemical and bacteriological water quality parameters: A review. International journal of pharmacy and integrated life Sciences (2016). Vol;5(2). Pp- 1-17.