Journal of Advanced Zoology
ISSN: 0253-7214
*Volume **43** Issue **- 1 Year 2022** Page 971**-978***

Quality And Potability Assessment Of Drinking Water In Midnapore Town And Siromoni, Paschim Medinipur

Dr. Subhankari Prasad Chakraborty*

**Department of Physiology, Ramananda College, Bishnupur, Bankura-722 122, West Bengal, India.*

****Corresponding Author:** Dr. Subhankari Prasad Chakraborty*
**Assistant Professor, Department of Physiology, Ramananda College, Bishnupur, Bankura- 722 122, West Bengal, India. Tele-fax: (91) 3244 – 254427, E-mail: subhankariprasad@gmail.com*

ABSTRACT

The microorganisms in tested water sample mainly include bacteria and viruses. The disease, which are caused by those bacteria, are Typhoid, Dysentery (bacillary) and Cholera, Paratyphoid and Diarrhea are also water borne diseases. About 80% people suffered mainly by stomach troubles and most of the people from skin diseases, Kidney troubles. Jaundice due to composition of contaminated drinking water. The present study was attempted to record the distribution pattern of the "fecal coliform" of water samples collected from tap water from Khaprailbazar, Midnapore town and shallow water from Siromoni, Paschim Medinipur. The isolated bacteria from drinking water of two different sources were characterized by different biochemical and physiological tests. Screening of isolated bacteria with blood agar plate, it has been concluded that all the isolated bacteria were non-pathogenic. By the antibiogram, it has been concluded that some of these isolated bacteria were found to be resistant towards Chloramphenicol antibiotic but other were mild sensitive towards this antibiotics. All the isolated bacteria were sensitive towards Streptomycin and Tetracycline. Most of the isolates were sensitive towards Ampicillin and Norfloxacin.

Key-words: Assessment of water quality, pathogenicity, antibiogram, norfloxacin

1. Introduction

Water is one of the most important elements for all forms of the life and is indispensablein the maintainance of life on earth and essential for the composition and renewal of cells. A regular plentiful supply of water is essential for the existence of living organisms. Water constitutes about 90% of the total weight of living cell and all the metabolic processes are carried out in presence of water. Normally water contains many types of bacteria. Drinking water has to be visually acceptable being clear and colorless without disagreeable taste or odor. The presence of bacteria and pathogenic organisms consider when considering the safety of drinking water (Dott et al., 1982). As a potential carrier of pathogenic microrganisms, water performs as a vector of diseases like Typhoid, Paratyphiod, Dysentry, Cholera and many others. The causative organisms of these diseases are present in faeces and urine of infected human being as well as in animal faeces and are released in water bodies either directly or after being washed through the main water (Rivat and Quinton, 1997).

The term "water quality" expresses the suitability and potability of water for various purposes. Good quality of water can insure sustainable socio-economic development and good human health. Monitoring on the other hand is meant for programmed process or sampling measurement and recording of various water parameters in accordance with definite objections. Water quality monitoring is very much necessary for the sake of water quality improvement and human heath consciousness.

The bacteriological tests of drinking water simply establish the fact and the degree of fecal contamination of the water sample by demonstrating the presence of so-called indicator organisms. The most frequently used indicator organisms for fecal contamination is the normally nonpathogenic. The coliform bacteria are being principally recognized as a vital indicator of microbial contamination of water. This group includes a huge number of species belonging to various genera like Escherichia, Enterobacter, Citrobactor, Streptococcus, Salnovella, Clostedium, Sigella, Klebsilla etc. Only pathogens introduce into water by excremented or sewage pollution poses a risk to human health (Dott et al., 1982). The coliform group includes all the facultative anaerobic gram negative non spore forming rod shaped bacteria that ferments lactose with gas formation within 24-48 hr at 37^0C (Nataro and Kaper, 1998). There probably is no universal indicator organism for determining water quality. Under different conditions, different population may be batter indicators than others (Atlas and Bartha, 1998). The present study was attempted to record the distribution pattern of the "fecal coliform" of water samples collected from tap water from Khaprailbazar, Midnapore town and shallow water from Siromoni, Paschim Medinipur.

2. Materials and Methods

2.1 Culture media and Chemicals

Luria broth, nutrient agar, agar powder, beef extract, pancreatic digest of casein, blood agar, crystal violet, lugol's iodine, safranine were purchased from Himedia, India. Sodium chloride (Nacl), potassium dihydrogen phosphate (KH_2PO_4), di potassium hydrogen phosphate (K_2HPO_4), sodium hydroxide (NaOH) were procured from Merck Ltd., SRL Pvt. Ltd., Mumbai, India. All other chemicals were from Merck Ltd., SRL Pvt., Ltd., Mumbai and were of the highest grade available.

2.2 Collection and transport of sample

Samples were collected from the specific source during a three month period from January 7, 2015 to March 6, 2015. Samples were obtained using sterile glass tubes and were transported to the laboratory within 1 hr of collection.

2.3 Culture of microorganisms

Samples were kept in LB and were shaken in a shaking incubator at 37°C for overnight. Bacterial cultures were grown on Nutrient agar (NA) media and purified by a single colony isolation technique on NA containing 10% sodium chloride (Saha et al. 2008). Isolates were sub-cultured from primary media to NA plates and incubated at 35°C in 5 to 7% CO_2 for 16 to 24 hr.

2.4 Gram staining

Gram staining of isolates was performed according to Duguid 1996. Briefly, on a glass slide bacterial smear was prepared from broth culture and heated gently to fix. The slide was flooded with 0.5% crystal violet and left for 30 sec. The slide was tilted and poured sufficient 1% Lugol's iodine to wash away the excess stain. The slide was covered with fresh iodine and allowed to act for 30 sec. The slide was tilted and washed off the iodine with 95 - 100% ethanol until colour ceases to run out of the smear. The slide was rinsed with water and 0.1% safranin was poured on it and left to act for 2 min. The slide was washed with water and blotted to dry and observed under microscope.

2.5 Lactose fermenting test

Mac Conkey media was prepared by suspending 5.15 gm% Mac Conkey media in sterile distilled water, followed by autoclaving at 15lb (121ºC) pressure for 15 minutes, cooled to 45-50ºC and poured into sterile petridish and checked overnight at 37ºC. On the next day, overnight growing isolates in MHB were streaked on overnight checked Mac Conkey agar plates and plates were kept at 37ºC for overnight. A positive result indicates the appearance of pinkish or whitish or raddish colony (Hass and Defago, 2005).

2.6 Sucrose fermenting test

Tris Citrate Bile Salts Sucrose media was prepared by suspending 8.9 gm% TCBS media in sterile distilled water, followed by heating to boil to dissolve the medium completely, cooled to 50ºC and poured into sterile petridishes and check overnight at 37ºC. On the next day, overnight growing isolates in MHB were streaked on overnight checked TCBS plates and plates were kept at 37ºC for overnight. A positive result indicates the appearance of yellow colony (Hass and Defago, 2005).

2.7 Peptone water media for peptone hydrolysis test

Peptone water media was prepared by suspending 10.0 gm% peptone and 5.0 gm% Nacl in sterile distilled water, followed by autoclaving at 15lb (121ºC) pressure for 15 minutes, cooled to 45-50ºC and poured into sterile petridish and checked overnight at 37ºC. On the next day, overnight growing isolates in LB were inoculated on overnight checked peptone water media and were kept at 37ºC for overnight. This media is selective media for Vibrio (Hass and Defago, 2005).

2.8 Peptone hydrolysis test by peptone agar media

Peptone agar media was prepared by suspending 10.0 gm% peptone, 5.0 gm% Nacl and 20 gm% agar in sterile distilled water, followed by autoclaving at 15lb (121ºC) pressure for 15 minutes, cooled to 45-50ºC and poured into sterile petridish and checked overnight at 37ºC. On the next day, overnight growing isolates in LB were inoculated on overnight checked peptone agar media and were kept at 37ºC for overnight. This media is specific for Vibrio (Hass and Defago, 2005).

2.9 Protease hydrolysis test

2.0 gm% Luria broth and 1.5 gm% nutrient agar were dissolved in a same conical flask but 1 gm% casein was dissolved in another conical flask, sterilized by autoclaving at 15lb (121ºC) pressure for 15 minutes, cooled both autoclave mixtures to 45-50ºC, mixed in a same conical flask properly, poured in sterile petridishes and checked overnight at 37ºC. On the next day, overnight growing isolates in MHB were streaked on overnight checked caesine plates and plates were kept at 37ºC for overnight. A positive result indicates the appearance of clear zone around the bacterial growth (Hass and Defago, 2005).

2.10 Haemolysis test

All isolates were inoculated onto blood agar and plates were incubated at 37°C for 24 hr. Appearance of a clearing zone surrounding the bacterial colony indicates the positivity of this test (Bannerman, 2003).

2.11 Antibiogram assay

Antibiogram is screening of bacterial isolate by exposing the bacterial cells to antibiotic environment. This experiments shows the bacterial sensitivity and resistance toward different antibiotics. Antibiogram of bacterial isolates from water samples were done with seven antibiotics e.g. Amphicillin, Tetracyclin, Chloramphenical, Streptomyocin and Norfloxacin. The working concentrations of the antibiotics used in the experiments were as follows: Amphicillin-50µg/ml, Tetracyclin-40µg/ml, Streptomycin-100 µg/ml, Chloramphenical-20 µg/ml, Norfloxacin-50 µg/ml. 100 µl of bacterial solution was spreaded over two sterile Nutrient Agar plates for overnight of a bacterial isolate. In one Nutrient Agar plate with bacterial lawn five small holes were inserted at equal distance and another plate with bacterial lawn two small holes were inserted at equal distance. 100 µl of each of the above mentioned antibiotic in their respective working concentration was added in each of the holes. The plate was incubated at 37^0C for 16 to 18 hours. Positivity of this test indicated by the clear zone production with no bacterial growth around the whole cointaining the antibiotic. This is known as inhibition zone. It shows the sensitivity of the bacteria toward the antibiotic (Hass and Defago, 2005).

3. Results and Discussion

1ml of each water sample has collected from and mixed with 5ml of sterilized distilled water mixed well 100µl of the water sample is sterilized distilled water was taken directly and also a 1:1, 1:10, 1:100, 1:1000, 1:10000 dilution was prepared 100µl from each of the sample and each of the dilutions were spread on Nutrient Agar plate and incubated at 37^0C for 16-18 hrs and more different types of colonies obtained on the plate were counted and recorded. The results are represented in Table 1.

Water and water bodies are one of the vital components for the survival of life. Now-a-days, pollution of water is severe and fatal problem of all over the world, which is a matter of great concern. Bacteria cause disease in humans and animals; infact relatively few of the thousands of bacterial species that occur in nature are pathogenic. Drinking water is one of the way by which bacteria can enter into humans and animals. The drinking water of most communities and also municipalities is obtained from surface sources, river, streams and lakes. Such natural water supplies are likely to be polluted with domestic and industrial wastes (Pelczer et al., 1993a). In this study, drinking water was collected from two sources; tap water from Midnapore town (Khaprailbazar) and shallow water from Siromoni, Pashim Medinipore. From this drinking water bacteria were isolated and there characters were determined.

In the present study it has been shown that isolated bacterial colonies from drinking water are maximum whitish and opaque. Out of five isolated bacterial colonies; four isolated colonies (1a, 1b, 1c, and 2a) were whitish colony and one (2b) yellowish colony and three (1b, 1c and 2b) opaque colony and two (1a and 2a) were transparent colony (Table-1).

The cell wall of Gram negative bacteria is generally thinner than Gram positive bacteria. Gram negative bacteria contain a higher percentage of lipids than Gram positive bacteria. Experimental suggests that during staining of Gram negative bacteria the alcohol treatment extract the lipid, which results in increased porosity of permeability of the cell wall. Thus crystal violet iodine (CV-I) complex can be extracted and the Gram negative organisms is decolorized. These cells subsequently take on the colour of the saffranine counter stain. The cell wall of Gram-positive bacteria because of their different composition (lower lipid content) becomes dehydrated during treatment with alcohol. The pore size decreases permeability is reduced and the CV-I complex cannot be remaining purple violet (Pelczer et al., 1993a).

Out of five isolated bacterial colonies (1a, 1b, 1c, 2a and 2b) all were appeared in red color. So, all bacteria were Gram-negative bacteria (Table 2). MacConkey Agar test was done to identify lactose-fermenting bacteria present in collected water sample. The experiment shows that lactose fermenting bacteria can produce reddish or pinkish colony, but non-lactose fermenting bacteria express colorless or transparent colony (Pelczer et al., 1993a). In this study, out of five isolated bacterial colonies three colonies(1a, 1b and 1c) grew on MacConkey medium as reddish or pinkish colony but two colonies (2a and 2b) grew on these media as colorless, transparent colony. So, these three bacteria were lactose-fermenting bacteria and other two bacteria were non-lactose fermenting bacteria (Table 3). TCBS (Tris citrate Bile salts sucrose Agar) test was done to identify the sucrose fermenting bacteria presence in collected sample. Sucrose fermenting bacteria will grow in this medium producing either yellow or blue green colonies (Pelczer et al., 1993a). Out of five isolated bacterial colonies two colonies (2a and 2b) bacteria can react in TCBS medium. The colour of these media becomes changed to yellow but other three bacteria cannot react in this media. So, these two bacteria were sucrose fermenting bacteria and others were not sucrose fermenting bacteria (Table 4). As, we know, TCBS media is selective media for Vibrio genus, so, these two isolated bacterial colonies may be in vibrio. In the present study, the two bacterial colonies give the positive results in TCBS media. So, peptone water media and peptone agar test was done to further confirm of the presence of vibrio genus because peptone water and peptone agar media is one of the selective media for vibro. Out of five bacterial colonies two (2a and 2b) bacteria can grow in peptone water and peptone agar but other three bacteria cannot grow in these media. So, among such isolated bacterial colonies only these two bacteria may be in vibrio genus (Table 5 and 6).

Protien hydrolysis test (casein plate) was done to identify protease activity of the bacteria present in collected water sample. Protease enzyme causes hydrolysis of casein (Peppler and Perlman, 2004). The experiment showed that positive control produced a clear zone around the bacterial growth. Out of five isolated bacterial colony only one bacterium (1b) produced a clear zone in the casein plate but other four bacterial colonies (1a, 1c, 2a and 2b) cannot produced any clear zone. So, only one (1b) bacteria have protease enzymes and it can express protease activity but other four cannot express any protease activity (Table 7).

Experiment for Blood agar test to identify whether any pathogenic bacteria present in the collected water sample or not. Pathogenic bacteria can grow on the blood agar media and by their hemolysin activity they can destroy or hemolyze the red blood cells produce a clear zone around bacterial colony. In this study; out of five isolated bacterial colony (1a, 1b, 1c, 2a and 2b) all the bacteria were not grew in this blood agar media

and connot hemolysis the red blood cells. So, all the bacteria were non pathogenic and they cannot express hemolysin activity (Table 8).

Antibiogram of the isolated bacterial colony was performed to identify the bacterial sensitivity and resistance towards different antibiotics. A clear zone is produced with isolated bacterial growth around the hole containing the antibiotic. This is known as inhibition zone, it shows the sensitivity of the bacteria towards the antibiotic. No such inhibition zone is produced around the antibiotic containing hole shows the resistance of the towards antibiotic. Out of five isolated bacterial colonies three bacteria were found to be resistant towards Chloramphenicol (1a, 1b and 1c) antibiotic but rest two bacteria (2a and 2b) shows mild sensitivity towards these antibiotics. Three isolated bacterial colonies (1a, 1b and 1c) were sensitive towards Ampicillin except two bacteria (2a and 2b) these bacteria were resistant towards Ampicillin. Most of the isolated bacterial colonies were sensitive towards Norfloxacin except only one (1c). All the isolated bacteria (1a, 1b, 1c, 2a and 2b) were sensitive towards Streptomycin and Tetracycline antibiotics (Table 9).

4. Conclusion

Drinking water samples were collected from two sources and the bacteria were isolated from this collected water sample. By this experimental study it is concluded that that the Midnapore town drinking water (Khaprailbazar area) contain very few bacteria than the drinking water collected from Siromoni, Paschim Medinipore. Among five isolated bacterial colonies, four colonies formed whitish and only one was formed as yellowish colonies and three colonies were formed opaque and two colonies were formed transparent colony. The isolated bacteria from drinking water of two different sources were characterized by different biochemical and physiological tests. By the Gram staining, it has been concluded that all the isolated bacteria from drinking water were Gram-negative bacteria. Screening of isolated bacteria with MacConkey agar plate, it has been concluded that some of these bacteria are lactose-fermenting bacteria and some of these bacteria are non-lactose fermenting bacteria. Screening of isolated bacteria with TCBS agar plate, it has been concluded that some of these isolated bacteria are sucrose fermenting bacteria but others are not. By the protein hydrolysis test, it has been concluded that some of these isolated bacteria have protease enzyme and exhibit protease activity. Screening of isolated bacteria with blood agar plate, it has been concluded that all the isolated bacteria were non-pathogenic. By the antibiogram, it has been concluded that some of these isolated bacteria were found to be resistant towards Chloramphenicol antibiotic but other were mild sensitive towards this antibiotics. All the isolated bacteria were sensitive towards Streptomycin and Tetracycline. Most of the isolates were sensitive towards Ampicillin and Norfloxacin.

Acknowledgement

Author express gratefulness to Vidyasagar Institute of Health, Midnapore for providing the facilities to execute this work. Author is highly obliged to the Principal, Ramananda College, Bishnupur, Bankura for constant help and encouragement for article writing.

Declaration of Interest

The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

References

1. Atlas MD and Bartha R, Quantative ecology: Numbers,biomass and activities . In: Microbial Ecology: Fundamental and Application, 4th Eds. Pearson Education, India. 218-263, (1998)
2. Bannerman TL. Staphylococcus, Micrococcus, and other catalasepositive cocci that grow aerobically, In Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (ed.), Manual of clinical microbiology, 8th ed. ASM Press, Washington DC, pp 384-404, (2003).
3. Dott W, Tuschewitzki G J and Thoferm E (1982), "Methylotropic bacteria in the sphere of drinking water. 2. communication: biochemical/physiological and morphological characterization of the isolated bacteria", Zentralbl Bakteriol Mikrob ol Hyg[B], vol. 176, pp. 189-201.

4. Duguid JP. Staining methods. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone, pp 793-812, (1996).
5. Hass D and Defago G (2005), Nature review microbial, vol 3: 369-372.
6. Nataro JP and Kaper JB (1998), "Diarrhoeagenic *Escherichia coli*", Clin Microbiol Rev. vol, 11, pp. 142-201.
7. Pelczer Jr, Chan ECS and Krieg NR. Microbiology of domestic water and wastewater. In: Microbiology, 5th Eds. Tata McGrow-Hill, 7west Patel Nagar, and New Delhi,India.P.P 593-617, (1993a).
8. Pelczar JR, Chan ECS and Krieg NR. The Microscopic Ezamination of Micoorganisms . In: Microbiology, 5th Eds. Tata Mcgraw. Hp11, 7west Patel Nagar, and New Delhi, India. P.P. 50-69, (1993b).
9. Peppler HJ and Perlman D. In: Microbial technology. 2nd edition. Elcvier India, New Delhi. (2004).
10. Rivat MS and Quinton DN. (1997), "Tap water as a wound cleansing agent in accident and emergency", J acid Emerg Med, vol. 14(3), pp. 165-166.
11. Saha B, Singh AK, Ghosh A and Bal M. 2008. "Identification and characterization of a vancomycinresistant *Staphylococcus aureus* isolated from Kolkata (South Asia)". Journal of Medical Microbiology. Vol. 57, 72-79.

Tables:

Table 1: Bacterial isolate obtained from drinking water sample.

Sample No	Dilution	Water spread	No. of colony in 100 µl sample	Types of colony	No of isolates outer look and colour colony
1.	1:1 1:10 1:100 1:1000 1:10000	100 µl 100 µl 100 µl 100 µl 100 µl	8 2 No colony No colony No colony	3	**1a.** Medium size, rough surface, white,nucleated transparent. **1b.** Medium siae, opaque, white,nucleated, smooth surface **1c.** Small, round, smooth surface, white
2.	1:1 1:10 1:100 1:1000 1:10000	100 µl 100 µl 100 µl 100 µl 100 µl	Lawn culture 124 30 12 No colony	2	**2a.** Large, rough surface, nucleated, oval shaped, transparent **2b.** Small, nonnucleated, opaque, smooth surface. Yellowish.

Table 2: Gram staining of bacterial isolates obtained from drinking water sample.

No: of isolates	Gram positive(+)stain (crystal violet)	Gram negative(-)stain (red)
1a		(-)
1b		(-)
1c		(-)
2a		(-)
2c		(-)

(+)=Gram positive bacteria, (-)=Gram negative bacteria.

Table 3: Screening of bacterial isolate with MacConkey agar to identify lactose fermenting bacteria.

No: of isolates	Growing in MacConkey Agar	Colour of colony
1a	(+)	Redish
1b	(+)	Redish
1c	(+)	Redish
2a	(+)	Transperent
2b	(+)	Transperent

(+)= Bacteria grow on MacConkey Agar.

Table 4: Screening of bacterial isolates with TCBS Agar to identify Sucrose fermenting bacteria.

No: of isolates	Grown in TCBS	Colour of colony (yellow colonies)
1a	(-)	(-)
1b	(-)	(-)
1c	(-)	(-)
2a	(+)	(+)
2b	(+)	(+)

(-)= Negative, (+)= Positive

Table 5: Screening of bacterial isolates with Peptone Water Media to identify Vibrio genus.

No; of isolates	Grown in Peptone water media
1a	-
1b	-
1c	-
2a	+
2b	+

(-)= Negative, (+)= Positive

Table 6: Screening of bacterial isolates with Peptone Agar Media to identify Vibrio genus.

No: of isolates	Grown on Peptone Agar
1a	-
1b	-
1c	-
2a	+
2b	+

(-)= Negative, (+)= Positive

Table 7: Protien Hydrolysis test identify the bacteria which produced protease enzyme.

No: of isolates	Protease activity
1a	(-)
1b	(+)
1c	(-)
2a	(-)
2b	(-)

(+)=Presence of protease activity, (-)=Absence of protease activity.

Table 8: Screening of bacterial isolates with Blood Agar Media to identify Pathogenic bacteria.

No: of isolates	Grown in Blood Agar	β - hemolysis	α - hemolysis
1a	-	-	-
1b	-	-	-
1c	-	-	-
2a	-	-	-
2b	-	-	-

(-) =Negative

Table 9: Antibiogram of bacterial isolates obtained from drinking water sample.

No. of isolates	Amphicilin 50 µg/ml	Streptomycin 100 µg/ml	Chloramphenicol 20 µg/ml	Norfloxacin 50 µg/ml	Tetracycline 40 µg/ml
1a	S (10.2mm)	S (14.0mm)	R	S (9.5mm)	S (7.4mm)
1b	S (15.2mm)	S (17.0mm)	R	S (10.5mm)	S (8.5mm)
1c	S (12.0mm)	S (18.0mm)	R	R	S (16.0mm)
2a	R	S (18.0mm)	S (4.0mm)	S (10.0mm)	S (26.0mm)
2b	R	S (16.0mm)	S (2.0mm)	S (10.5mm)	S (25.0mm)

S= Sensitive, R= Resistant

Abbreviations:

ATP	: Adenosine triphosphate
BHI	: Brain heart infusion
CFU	: Colony formation unit
DAD	: Disc agar diffusion
DNA	: Deoxyribonucleic acid
E. coli	: *Eschrichia coli*
LB	: Luria broth
MHB	: Mueller-Hinton broth
NA	: Nutrient agar
NaOH	: Sodium hydroxide
SDS	: Sodium dodecyl sulfate
TSB	: Tryptic soy broth