Detection of coliforms from drinking water sources, their characterisation and checking their susceptibility against different common drinking water disinfectants

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ABSTRACT: Drinking water from different sources of Kolkata were tested and coliforms were detected from the samples. Two of the samples from the water supply contained way more numbers of organisms than permissible and were subjected to characterisation. In the characterisation they were found to be motile, very highly Catalase and Oxidase positive , Gram Negetive coliforms which were further tested on EMB agar plates and IMViC test and found out to be Escherichia coli and Enterobacter aerogenes which are both capable of causing enteric diseases. On checking antibiotic susceptibility against commonly prescribed drugs for different enteric diseases such as nalidixic acid, tetracycline, linezolid , clotrimoxazole and cefuroxime by Disc-diffusion method, tetracycline and linezolid had no effect to prevent the growth of the organisms , whereas, the others had very low effect characterised by very small zones of inhibition. Therefore the organisms were distinguished as Multidrug Resistant bacteria (MDR). Next the susceptibility of the organisms against commonly used disinfectants such as alum, chlorine , KMnO4 and camphor was tested by Cup Plate method, Optical density method and Colony Counting Method at different concentrations and it was found that only alum and chlorine had inhibitory effects on the growth of the organisms at quite a high concentration. On doing Fluorescent Microscopy by propidium iodide staining on the treated samples membrane damage was evidenced characterised by propidium iodide penetrated damaged cells at a very large number. This experiment successfully evidences that even routinely checked samples of water can be source of many pathogenic bacteria. The novel finding of this study is that there is no effect of alum camphor or extracts such as Neem or Tulsi on this kind of MDR bacteria. Only alum and chlorine has the capability of preventing their growth significantly and that also at a very high concentration.

Index Terms: drinking water, coliforms, disinfectants, MDR, inhibition

I.INTRODUCTION:

Water microbiology is the scientific discipline that is concerned with the study of all biological aspects of the microorganisms that exist in water. The type of microbial population in water depends on several physical and chemical conditions like temperature, hydrostatic pressure, light, salinity, turbidity and hydrogen ion concentration. It is also known that some microbes are specific to certain zones of lake water sources like littoral zones, limnetic zones, profundal zones and benthic zones. Apart from lake water sources, microbes are also associated with different sources like sea, streams, springs, etc.

Some water sources are also occupied by pathogens, which are definitely not good for our health. So, when the drinking water is supplied from such sources, obviously it is not safe .Water can act as a vector for the transmission of microbial agents which cause a variety of diseases (mainly intestinal).Water is responsible for approximately 80% of all infectious diseases not just water-borne but any disease where water plays a role. According to WHO reports, the water-related diseases like diarrhea occurs worldwide and causes 4% of all deaths and 5% of health loss to disability. The gastrointestinal infections kill around 2.2 million people globally each year, mostly children in developing countries. Cholera and dysentery cause severe, sometimes fatal forms of diarrhea. World-wide around 1.1 billion people lack access to better-quality water sources and 2.4 billion have no basic hygiene. In South-east Asia and Africa, diarrhea is responsible for as much as 8.5% and 7.7% of all deaths respectively. Hence, there is an urgent need to examine the drinking water before intake and to treat them if any undesired components or pathogens are detected.(6)

According to WHO Water safety and quality are fundamental rights to human development and well-being. Public water systems must provide with safe and reliable drinking water to their consumers. Presence of harmful organisms in the drinking water leads to serious illness in the community. An estimation of 842 000 deaths per year, is attributable to unsafe water supply(WHO). The presence of contaminants in drinking water can lead to adverse health effects, including gastrointestinal illness, reproductive problems as well as neurological ailments.(3)

Indicator organisms are normally used to determine the potential presence or absence of pathogens. According to the report by Water related diseases cause 3.4 million deaths each year.in order to check the presence of pathogens in

water sample presence of indicator organisms are checked. The use of indicators is due to the reason that it reduces the complexity and cost of analyzingsludges or water or soil etc for individual pathogens. Fecal coliform bacteria are indicators of fecal contamination and of the potential presence of pathogens associated with wastewater or sewage sludge, such as bacteria, viruses, and parasites. (2)

To proceed with the practical work, water samples from different drinking water sources of our college are collected and several experiments are done to check if it's safe or not for intake purposes. Detection of coliforms was done using Most Probable Number test (MPN).In case of those samples which were found to have presence of coliforms, confirmed and confirmatory tests were performed and they were distinguished as Escherichia coli and Enterobacteraerogenes .both of the organismas are capable of producing enteric diseases and certain strains of them can even result in epidemic situation. Further microscopic characterization was performed by Gram-staining method, IMViCtrest , catalase and oxidase test , Haemolytic test on blood agar plate etc. Besides this motility testing was also done and to our expectation was found to be motile.
Antibiotic assay was also performed by disk diffusion method with tetracycline, linezolid, nalidixic acid clotrimoxazole and cefuroxime. The result showed that these organisms were resistant to more than one antimicrobial drug. Hence, they were multi-drug resistant (MDR). Such pathogens are dangerous to our health as when we drink such un-treated water, coliforms often reach the gut microflora and by horizontal gene transfer they pass on the factors to other pathogens residing in our gut. The latter also becomes multiple-resistant in nature and as a consequence no medications would work on time. Thus, delay is there for the proper treatment of the patients leading to drastic health conditions.
To check the susceptibility of some commonly known disinfectants in water treatment plants (alum, chlorine, camphor, potassium permanganate) agar-cup method was applied. The test indicated that zone of inhibition was prominently observed in case of alum and chlorine. For camphor and potassium permanganate, no zone of inhibition was indicated. Thus, chlorine and alum are good disinfectants. For further testing Optical density, colony counting methods of susceptibility testing against these bacteria with the two disinfectants was followed at different concentrations . It was found that only at a very high concentration alum can coagulate and chlorine can prevent the growth of these MDR bacteria. This study was then supported with Fluorescence microscopy of the treated samples by means of propidiumiodode. Detection of numerous fluoresced bacteria was indicative of dead bacteria penetrated by propidium iodide compared to the control evidenced the effective killing of the bacteria.
As published in many experiments and publications it is suggested that tulsi and neem leaves are having antibacterial properties. So, slurry of tulsi and neem leaves are made and applied for agar-cup method to test their efficacy. Unfortunately, no zone of inhibition is observed indicating that they do not have the ability to prevent the growth of the particular bacterial load. (4)
Basically, the novel finding of this experiment is that only alum and chlorine has the capability of preventing the growth of these kind of MDR microorganisms significantly and that also at a very high concentration.

II.OBJECTIVES:

1. Detection of coliforms from drinking water sources
2. Characterisation of the bacteria
3. Checking their susceptibility against different common drinking water disinfectants

III.RESEARCH METHODOLOGIES:

EXPERIMENT NO. 1:

MOST PROBABLE NUMBER TEST:

This is a method used to estimate the probable number of viable microorganisms in a sample of soil, water etc. by means of replicate lactose broth growth in ten-fold dilutions. Here this technique is applied for quality testing of water to ensure if the water is safe or not in terms of presence of bacteria in it.(1)

MPN test is performed in the following 3 steps:

- Presumptive test
- Confirmatory test
- Completed test

PRESUMPTIVE TEST:

Test samples were diluted serially and inoculated in lactose broth; if coliforms are present in water they will utilize the lactose present in the medium to produce gas and acid. The presence of acid is indicated by change of color of the medium and the presence of gas is detected as gas bubbles accumulated in the inverted durham tubes inserted in the medium. The number of total coliforms in individual samples is determined by counting the number of tubes giving positive reaction and comparing the pattern of positive results with standard statistical tables, in this case a 3 tube set up.

CONFIRMED TEST:

Certain microorganisms other than coliforms may also produce acid and gas from lactose fermentation. Therefore in order to confirm the presence of coliform, confirmatory test is done. From each of the fermentation tubes with positive results one loopful of medium was transferred to lactose-broth. The tubes were then incubated at 37°C for 24hrs and 48 hrs.The gas formations in lactose broth indicates the presence of a member of the coliform group in the sample examined. Further microscopic characterisation was also done by gram staining of the samples. Presence of Gram negative, non-spore-forming bacteria is the positive result.

COMPLETED TEST:

In order to increase the efficiency it is desirable to do completed tests. To perform this, inoculum from each positive tubes of the confirmatory test is streaked on a plate of EMB agar. The streaked samples were then incubated at 37°C for 24 hrs .Presence of Coliforms produce colonies with greenish metallic sheen which differentiates it from non-coliform colonies with no metallic sheen.

EXPERIMENT NO. 2:

MICROSCOPIC CHARACTERISATION OF THE SAMPLES:

For microscopic characterisation, Gram staining was done, this technique was developed by Danish physician Hans Christian Gram in 1884. This staining procedure diffferentiates most bacteria into two groups depending upon cell wall composition:

1. Gram positive bacteria (thick layered peptidoglycan-90%)- stains purple
2. Gram negative bacteria (thin layeed peptidoglycan-10% of cell wall and high lipid content) –stains red/pink

When stained with a primary stain crystal violet and fixed by a mordant iodine, some bacteria are able to retain the primary stain by resisting decolorization by alcohol while others get decolorized. Those bacteria which retain the primary stain crystal violet are called Gram positive and those bacteria which get decolorized and then get counterstained by safranin are called Gram negative.

Crystal violet penetrates through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV+ ion interacts with negatively charged constituents of bacterial cells and stains the cells purple.

Iodine (I), used as mordant interacts with CV+ and forms into large complexes(CV–I) within the inner and outer layers of the cell.

When a decolorizer such as alcohol is added, it interacts with the lipopolysaccharides of the cell membrane. Since Gram negative organism posses thin peptidoglycan layer and have additional lipopolysaccharide layer which gets dissolved due to the addition of alcohol, so gram negative organism fails to retain the color and gets decolorized as the complex is washed away. In contrast, a Gram-positive cell becomes dehydrated and pores shrink due to an ethanol treatment. This prevents the stain from exiting the cell. The large CV–I complexes become entrapped within the Gram-positive and retaines the purplrcolor.

Following decolorization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Finally, a counterstain, which is usually positively-charged safranin, is applied to give decolorized Gram-negative bacteria a pink or red color.

EXPERIMENT NO. 3:

IMViC TEST:

E.coli and Enterobacteraerogenus are faecal and non faecal contamination of water respectively. They closely resemble in morphological and cultural characteristics. Biochemical tests are performed to differentiate them. Tests are collectively known as “IMViC” coined by Parr from first four letters of the four test:

I - Indole Test
M - Methyl Test
Vi - VogesProskauer Test
C - Citrate Test
Coliforms are grouped into faecal and non faecal types based on the following characteristics:

- Ability to produce Indole from tryptophan. E.coli can, E.aerogenus cannot.
- Amount of acidity produced in a special glucose broth medium and detection by the pH indication Methyl Red.
- Ability to produce the compound acetone in a glucose peptone medium.
- Utilisation of sodium citrate as carbon source.

INDOLE TEST:

Tryptophan is an essential amino acid that can undergo oxidation by way of enzymatic activities of some bacteria. Conversion of tryptophan into metabolic products is activated by the enzyme tryptophanase. In this experiment, tryptone broth is used. The process of indole is detectable by Kovac's reagent which produces a cherry red reagent layer. Indole is extracted from the medium into the reagent layer by acidifying butanol and form a complex with p-dimethyl amino benzaldehyde yielding a cherry red colour.

METHYL RED TEST:

The hexose monosaccharide glucose is the major substrate oxidised by all enteric organisms for energy production.In the test, pH indicators, methyl red detects the presence of large concentration of acid end products. Both E.coli&E.aerogenus produce organic acid end products during the early incubation. The low acidic pH 4 is stabilised and maintained by E.coli at the end of incubation. During the later incubation period E.aerogenus converts this acidic to non acidic end products such as 2,3-butanediol, thus resulting in elevated pH 6. Methyl red indicator in pH range of 4, will turn red which is indicator of positive test.

VOGES PROSKAUER TEST:

The VP test is used to determine the ability of some microorganisms to produce neutral end products such as acetyl methyl carbinol from the organic acids, those results from glucose metabolism.
The reagent used in the test is the Baritt reagent which consists of a mixture of alcoholic alpha-naphthol and 40% KOH solution of acetyl methyl carbinol requires that the end products is oxidised to a di-acetyl compound.
The reaction will occur in the presence of peptone of MRVP medium. As a result , a pink colour is formed imparting a rose colour to the medium.This represents a positive test and its absence a negative test.

CITRATE TEST:

In the absence of fermentable glucose or lactose , some organisms are capable of using citrate as a carbon source for their energy.This ability depends on the presence of citrate permease that facilitates the transport of citrate in the cell.
Citrate is the 1st major intermediate in the Kreb's cycle and is produced by the condensation of active acetyl coenzyme with oxalo acetic acid . Citrate is acted on by the enzyme, citrus which produces oxaloacetic acid and acetate. These products are the enzymatically transformed into pyretic acid and CO2 that is produced, combines with sodium and water to form sodium carbonate and alkaline product. The presence of Na,CO2 changes the bromothymol blue indicator is corporate onto the media from green to Prussian blue. Following incubation citrate positive cultures are identified by the presence of growth on the surface of start which is accompanied by the coloration . Citrate negative cultures will not show growth and the medium will remain green.

EXPERIMENT NO. 4:

MOTILITY TESTING:

Bacteria move by means of propeller like flagella or by special fibrils that produces gliding form of motility. The motility test can be easily carried out by using a cavity slide, cover slip and observation under microscope.

EXPERIMENT NO. 5:

BLOOD AGAR TEST:

Blood agar is an enriched, bacterial growth media and it is useful for determining the hemolytic capabilities of an organism. The bacteria having this hemolytic property can be easily identified by this method as they produces certain exo-enzymes that lyse red blood cells in the blood agar and degrade hemoglobin. Thus in order to check the pathogenicity of the organisms in our study, blood agar test was performed.

EXPERIMENT NO. 6:

CATALASE TEST:

Catalase is an enzyme that decomposes H2O2 into water and oxygen. Catalase is present in certain bacteria which helps in the breakdown of the toxic hydrogen peroxide into water, oxygen and produce effervescence.

EXPERIMENT NO. 7:

OXIDASE TEST:

The cytochromes are iron-comprising hemoproteins that act as the last link in the electro transport chain of aerobic respiration by transferring electrons to oxygen with the formation of water. Cytochrome C oxidase is an enzyme of the bacterial electron transport chain. The oxidase test is used to identify bacteria that produces this enzyme. The important reagent used for this test is di- and tetramethylparaphenylenediaminedihydrochloride. This reagent acts as an artificial substrate, donating electrons and becoming oxidized to a bluish- black compound in the presence of oxidase and free oxygen. Development of this blue-black coloration indicates cytochrome oxidase production and represents a positive test.

EXPERIMENT NO. 8:

INTERACTION BETWEEN ORAL MICROFLORA AND THE ISOLATED SAMPLES:

Microorganisms (including viruses, bacteria, archaea and protists) donot exist in isolation but form complex ecological interaction webs. Interactions within these ecological webs can have a positive impact, a negative impact or no impact on the species involved. Oral microbial habitat is composed of wide variety of organisms contributing significant role in retaining the health of the oral cavity by paying in various ways. The oral microbial community represents the best-characterized group associated with the human host. There are strong associations between the qualitative composition of the oral microbiota and clinically healthy or diseased states. Interactions between mucosa and normalmicrobiota are key to host defence, health, and disease. To check the interaction T streaking was done with the test samples and oral microorganism collected by swab.

EXPERIMENT NO. 9:

ANTIBIOTIC ASSAY BY DISK DIFFUSION :

In this method antibiotic discs are used to test the extent to which bacteria are affected by those antibiotics. In this test, paper disks containing antibiotics are placed on agar plate where bacteria have been spread, and the plate is left to incubate at 37°C for 24 hrs. If an antibiotic inhibits the bacteria from growing or kills the bacteria, there will be an area around the paper disk where the bacteria have not grown enough to be visible. This is called “Zone of Inhibition”. The diameter of zone of inhibition is proportional to the extent the antibiotic is susceptible against the bacteria.The antibiotic chosen were nalidixic acid, tetracycline, linezolid, cotrimoxazole, cefuma, cetil and cefakine

EXPERIMENT NO. 10:

CHECKING SUSCEPTIBILITY OF DIFFERENT COMMON DISINFECTANTS FOR TREATMENT OF DRINKING WATER.

AGAR–CUP METHOD:

In this method cups or holes are dug in the agar plates using cork borer after spreading bacteria on the agar surface. Solutions of the disinfectants are prepared in optimum concentration permissible to treat drinking water and in high concentration .the solutions were then poured into the cups and incubated . then the zone of inhibition is measured to check the efficacy of the disinfectants.as in case of disk diffusion method thee diameter of zone of inhibition is proportional to the extent the disinfectants are susceptible to the bacteria.

Table1.dosage of disinfectant:

	Optimum	High	Very high
Alum	5mg/L	100mg/L	200mg/L
Chlorine (5%)	50µL	100µL	200µL
Camphor	10mg/mL	100mg/mL	200mg/mL
KMnO4	1.0 mg/L	20mg/L	40mg/L

EXPERIMENT NO. 11:

CHECKING SUSCEPTIBILITY OF DIFFERENT COMMON DISINFECTANTS FOR TREATMENT OF DRINKING WATER.

COLONY COUNTING METHOD:

However the treatment was actually being suggested to treat drinking water therefore the efficacy of the disinfectants were also checked in broth cultures and then plated onto agar plated to count the number of colonies. Lessening of the number of colonies on the agar plates would suggest the effectivity of the disinfectants towards the organisms. Optimum, high as well as 5times higher concentration of the disinfectants were added in each test samples and incubated at 37°C for 24 hrs.

The next day 50µl of the samples were then spread on the surface of agar plates and again incubated for 24hrs at 37°C and the following day the number of colonies were then counted.

EXPERIMENT NO. 12:

CHECKING SUSCEPTIBILITY OF DIFFERENT COMMON DISINFECTANTS FOR TREATMENT OF DRINKING WATER

OPTICAL DENSITY METHOD:

In this method sample organisms were incubated for 24 hrs at 37°C in LB broth along with increasing concentration of disinfectants the same as used in colony count.then the OD was measured in spectrophotometer at 600nm and then plotted in graph.

EXPERIMENT NO. 13:

PRODIUM IODIDE STAINING:

PI or propidium iodide is an intercalating and fluorescent agent. Staining with propidium iodide is a very well-known method to check the viability of cells under fluorescent microscope and flow cytometer. PI stains only dead cells as a result of porous membrane, since PI cannot diffuse into intact viable cells or dead cells still having an intact membrane.

EXPERIMENT NO. 14:

TREATMENT OF WATER USING ANTIBACTERIAL PLANT EXTRACTS:

To check the effectivity of medicinal plant extracts against the bacteria saturated solutions of 1gm/ml and high concentration of 5gm/ml of neem and tulsi were prepared and poured into holes dug in the surface of agar plates. Then the plates were incubated at 37°C for 24 hrs. the next day the zone of inhibition was measured.

EXPERIMENT NO. 15:

CONFIRMATION OF WATER QUALITY AFTER TREATMENT FROM THE SAME SOURCES:

According to the findings of the experiment the most potent mean of disinfection of treatment was chosen and the reservoirs of the contaminated sample of water were treated. Following the treatment the water was again tested by means of "Most Probable Number "tests.

IV. RESULTS:

MOST PROBABLE NUMBER (MPN):
PRESUMPTIVE TEST:

Table 2.the formation of gas bubbles in the test tubes were as follows,

Sample name	No. of tubes showing gas formation (.1ml) 1X lactose broth	No. of tubes showing gas formation (1ml) 1X lactose broth	No of tubes showing gas formation (10ml) 2X lactose broth	MPN index (per ml)
SW1	0	0	0	-
SW2	0	1	3	0.43
SW3	0	0	0	<0.03
SW4	0	0	2	0.092
SW5	1	0	0	0.030
SW6	0	0	0	<0.030

However, the maximum permissible limit of coliforms in per100ml of water is 0.
Presence of coliforms in the test samples were detected in SW2,SW4 and SW7.

CONFIRMATORY TEST:

Table 3.formation of gas bubbles in lactose broth and microscopic characteristics,

Sample name	Formation of gas bubbles in 5ml lactose broth	Microscopic characteristics
SW2	+	Gram –ve, non sporeforming , short rods
SW4	+	Gram –ve, non sporeforming, bacilli
SW7	+	Gram +ve

According to the results obtained in confirmatory test the SW2 and SW4 were identified as true coliforms and tests with them were carried out further , however for being gram positive the SW7 was discarded.

COMPLETED TEST:

Table 4.the streaked pattern of the bacteria were as follows;

Sample number	EMB agar plates	Prediction
SW2	Colonies along the streaking line with Metallic sheen	Escherichia coli
SW4	Colonies along the streaking line with Pink colonies	Enterobacteraerogenes

On EMB plates Escherichia coli appears as large, blue-black colonies, often with a green metallic sheen. Therefore the SW2 contained E. coli.

Again in SW4 presence of brown to blue-black, mucoid colonies with no sheen is indicativeof presence of Enterobacteraerogenes. The acid production from lactose fermentation results in the eosin-methylene blue dye complex to be taken up by bacterial cells and appear as a brown to blue-black colony .

MICOSCOPIC CHARACTERISATION:

Table 5.gram character and the morphology of the bacteria were as follows

Sample name	Microscopic characteristics
SW2	Gram –ve, non sporeforming , short rods
SW4	Gram –ve, non sporeforming, bacilli
SW7	Gram +ve

SW2 and SW4 were identified as true coliforms and tests with them were carried out further , however for being gram positive the SW7 was discarded. And it also confirmed that the sources of contaminants were different.

IMViC TEST:

Table 6.results of IMViC test,

Sample number	Indole Test	Methyl Red Test	VogesProskauer	Citrate	Inferred Organism
SW2	+	+	-	-	Escherichia coli
SW4	-	-	+	+	Enterobacteraerogenes

The characteristic results again confirmed the presence of coliforms of fecal origin. And the bacterial isolate in SW2 and SW4 were confirmed as E.coli and Enterobacteraerogenes.

MOTILITY TEST:

Table 7.motility of test samples were as follows,

Sample number	motility
SW2	+
SW4	+

The bacteria were motile. Both of the bacteria Escherichia coli and Enterobacteraerogenes were motile by means of their flagella.

CATALASE OXIDASE TEST:

Table8: results of catalase test,

Sample number	Formation of gas bubbles	Blue coloration
SW2	+	+
SW4	+	+

Both of the samples were catalase as well as oxidase positive , therefore aerobic in nature.

HAEMOLYSIS TEST ON BLOOD AGAR PLATE:

Table 9.haemolytic nature of the bacteria

Sample number	Presence of hallow zone	
SW2	-	Non haemolytic
SW4	-	Non haemolytic

Therefore the bacteria do not produce any kind of haemolytic by product. Thus incapable of causing Haemolytic Uremic Syndrome (HUS).

INTERACTION BETWEEN ORAL MICROFLORA AND THE MICRO ORGANISMS ISOLATED FROM THE WATER SAMPLE :

Table 10.results of interaction study

Sample number	Interaction
SW2	No interaction
SW4	No interaction

We can say that the organisms were not being inhibited by the oral microbes.

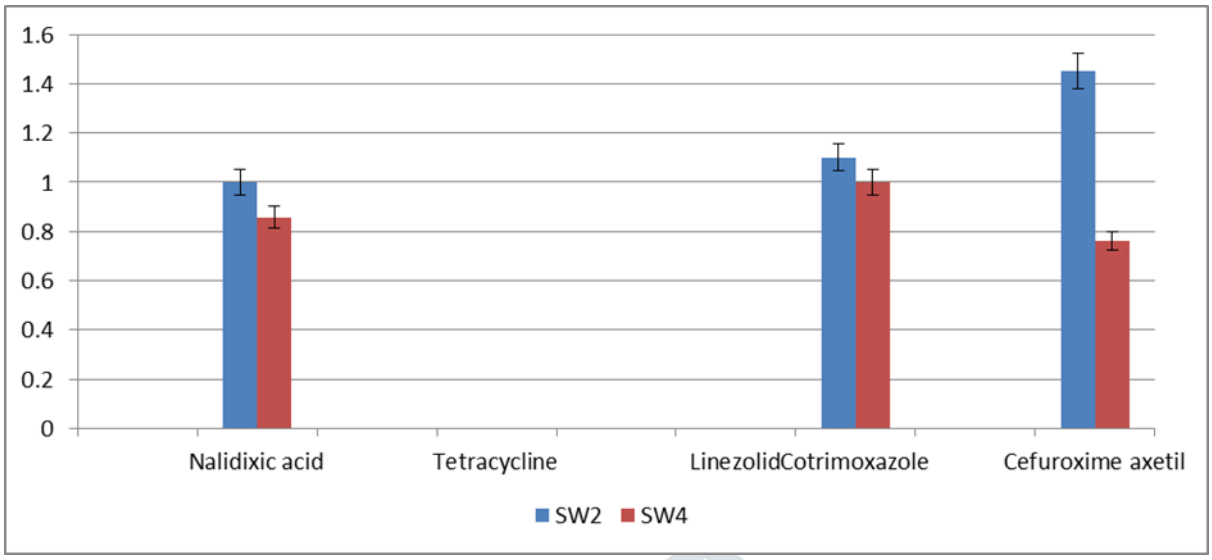
ANTIBIOTIC ASSAY BY DISK DIFFUSION METHOD:

Table 11.diameters of zone of inhibition formed by different antibiotics

Sample name	SW2				SW4				Inference
Nalidixic acid	1	1	.9	1	0.8	0.9	0.9	0.858	Very low
Tetracycline	-	-	-	-	-	-	-	-	resistant
Linezolid	-	-	-	-	-	-	-	-	resistant

Sample name	SW2				SW4				Inference
Nalidixic acid	1	1	.9	1	0.8	0.9	0.9	0.858	Very low
Tetracycline	-	-	-	-	-	-	-	-	resistant
Linezolid	-	-	-	-	-	-	-	-	resistant

figure 1. graph representing the diameter of zone of inhibition formed by the drugs against sw2 and sw4 in antibiotic assay by disk diffusion method.



Therefore, we can conclude that the bacteria were resistant to tetracycline and linezolid. Nalidixic acid and Cotrimoxazole have very low and cefuroxime has negligible effect against the bacteria.

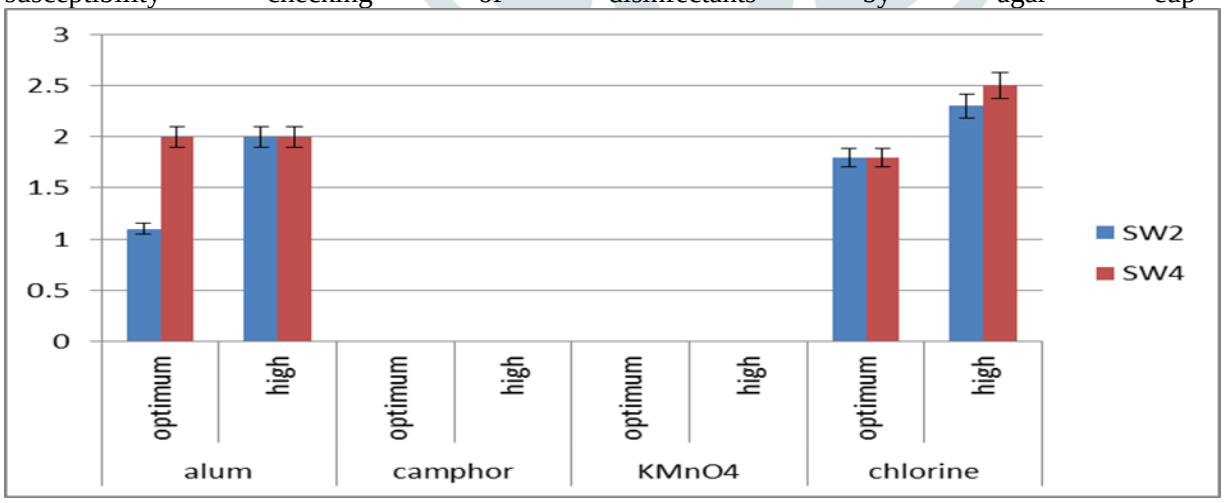
CHECKING SUSCEPTIBILITY OF DIFFERENT COMMON DISINFECTANTS FOR TREATMENT OF DRINKING WATER:

AGAR–CUP METHOD:

Table 12.diameters of zone of inhibition formed by different disinfectants

Sample name	alum		camphor		KMnO4		chlorine	
	optimum	high	optimum	high	optimum	high	optimum	high
SW2	1.1	2	-	-	-	-	1.8	2.3
	1	1.9	-	-	-	-	1.9	2.3
SW4	1	1	-	-	-	-	1.8	2.4
	2	2	-	-	-	-	1.8	2.5

figure2. graph representing the diameter of zone of inhibition formed by the disinfectants against sw2 and sw4 in susceptibility checking of disinfectants by agar cup method.



Therefore, we see that in broth culture camphor and KMnO4 remains uneffective as they do not inhibit the growth of the bacteria evidenced by presence of no zone of inhibition around the corresponding holes. However only alum and chlorine can prevent the growth as they show zone of inhibition around the holes.

COLONY COUNTING METHOD:

Table 13.numbers of colonies formed by different disinfectants

Sample name	Alum			Camphor			KMnO4		
	optimum	high	Very high	optimum	high	Very high	optimum	high	Very high
SW2	TNTC	TNTC	10	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	TNTC	TNTC	8	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
SW4	TNTC	TNTC	17	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	TNTC	TNTC	12	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC

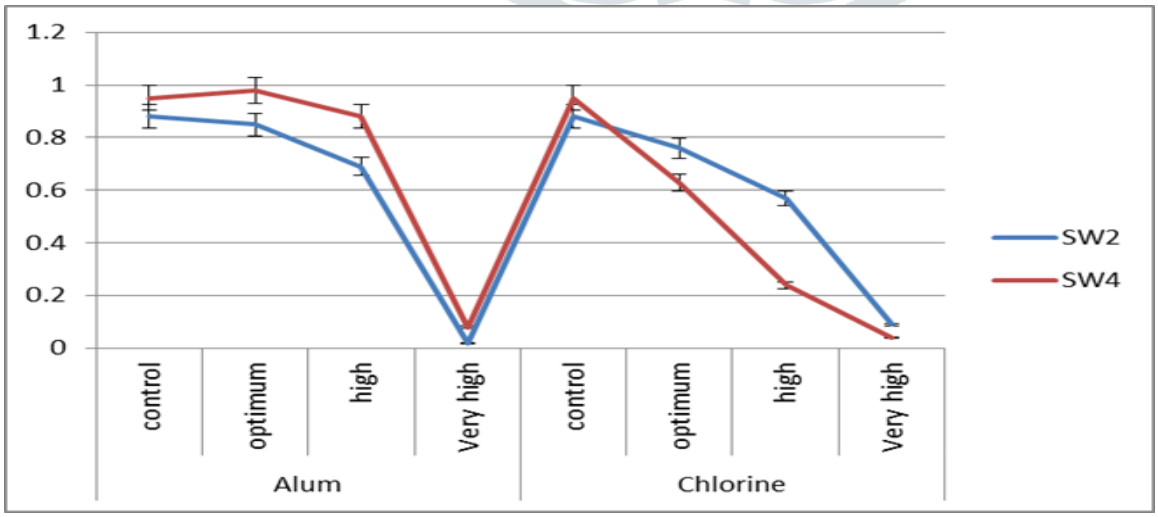
According to the results in colony counting method no significant decrease in the number of bacteria was observerd for alum camphor or KMnO4. Only chlorine at a very high concentration was able to kill the bacteria significantly.

OPTICAL DENSITY METHOD:

Table 14.optical density in broths after being treated by different disinfectants

Sample name	Alum				Chlorine			
	control	optimum	high	Very high	control	optimum	high	Very high
SW2	0.88	0.85	0.69	0.02	0.88	0.76	0.57	0.09
SW4	0.95	0.98	0.88	0.08	0.95	0.63	0.24	0.04

figure3. graph representing the optical density of samples when incubated with optimum, high and very high concentration of the most effect tive disinfectants.



Therefore we can see that with the increasing concentration of the disinfectants the rate of growth of the bactereia was decreasing in case4 of alum as well as chlorine. However the effect of both chlorine and alum was considerably satisfactory at a very high concentration.

TREATMENT OF WATER USING ANTIBACTERIAL PLANT EXTRACTS:

Table 15.diameters of zone of inhibition formed by neem and tulsi extracts

Sample name	Neem		Tulsi	
	1mg/ml	10mg/ml	1mg/ml	10mg/ml
SW2	-	-	-	-
SW4	-	-	-	-

No zone of inhibition was obtained for either Neem or Tulsi . Therefore Neem and Tulsi had no antibacterial effect against the bacteria.

PRODIUM IODIDE STAINING:

Table 16.number of dead cells counted per field

Sample name	No of dead cells in control (per field)	No of dead cells in alum treated samples (per field)	No of dead cells in chlorine treated samples (per field)
SW2	20	44	>300 (TNTC)
SW4	16	53	>300 (TNTC)

The increased number of dead cells indicates the effective killing of the bacteria by the treatments compared to control.

CONFIRMATION OF WATER QUALITY AFTER TREATMENT FROM THE SAME SOURCES:
MOST PROBABLE NUMBER (MPN):
PRESUMPTIVE TEST:

Table 17.The formation of gas bubbles in the test tubes were as follows,

Sample name	No. of tubes showing gas formation (.1ml) 1X lactose broth	No. of tubes showing gas formation (1ml) 1X lactose broth	No of tubes showing gas formation (10ml) 2X lactose broth	MPN index (per ml)
SW2	0	0	0	-
SW4	0	0	0	-

The increased number of dead cells indicates the effective killing of the bacteria by the treatments compared to control.

V.DISCUSSIONS:

Our study includes the testing of water samples from different places in Kolkata. Surprisingly we found that most of the sources were detected to have the presence of coliforms as contaminants. However 2 of them contained the number much higher. Further study revealed the coliforms to be Escherichiacoli and Enterobacteraerogenes. According to The Canadian Drinking Water Quality Guideline the number of total coliforms is none detectable per 100 ml. Whereas in our samples it was detected to be 43 coliforms in 100 ml (SW2) and 9 coliforms (SW4) in 100 ml that was very high than considerable range in the presumptive test.The confirmed test and the IMViC tests were performed in order to identify the bacteria as Escherichiacoli and Enterobacteraerogenes. Therefore the drinking water sources were contaminated with fecal contaminants and most probably contained pathogens in them.

Certain strains of these organisms show virulence due to their capability to invade mucus membrane and the epithelial membrane of the gut and other luminal cavities. Such as enteroinvasive E. coli. Recent extensive studies of the colonization factors of enteropathogenic and uropathogenic bacteria have shown that fimbriae, flagella and motility of the bacteria are important factors for colonization Therefore the motility of the organisms were teasted and very obviously they were found to be motile.(7)

Again both of the organisms are evidenced of being causative agents of haemolytic uremic syndrome. To check the ability of the bacteria to produce haemolysin the bacteria were streaked on sheep blood agar plates. However none of the bact3eria were found to be producing any kind of hallow zone around their growth colonies, and therefo4re concluded to be non-haemolytic.

Reactive oxygen species (ROS) are by-products of normal cellular metabolism. Low and moderate amounts of ROS have advantageous effects including killing of invading pathogens, wound healing, and tissue repair processes. Thus, these organisms were identified as catalase and oxidase positive at a very fast rate. Due to this property they are resistant to the ROS generated in the gut and can efficiently survive there.(8)

One very interesting observation in our study was that, the organisms we obtained from the sources were very much resistant to many commonly used antibiotics. Susceptibility of both of the bacteria was tested against the drugs Nalidixic acid, Tetracycline, linezolid, clotrimoxazole and also Cefuroxime Axetil.

Nalidixic acid is a quinolone used for the treatment of urinary tract infections caused by gram-negative microorganisms, including the majority of Escherichia coli and Enterobacter species. (Drug Bank)

Tetracycline is a broad spectrum polyketide antibiotic active against all kinds of Enteric bacteria including E. coli, V. cholera as well as Shigella. (Drug Bank)

Linezolid is a synthetic antibiotic, the first of the oxazolidinone class, used for the treatment of infections caused by multi-resistant bacteria including streptococcus and methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin resistant Enterococcus. (Drug Bank)

Co-trimoxazole is used for treating bacterial infections, such as pneumonia , bronchitis , UTI, and infections of intestines. It also is used to treat 'travelers' diarrhea. Co-trimoxazole is a mixture of trimethoprim and sulfamethoxazole and is in a class of medications, sulfonamides. (Drug Bank)

Cefuroxime is a broad-spectrum antibiotic cephalosporin resistant to beta-lactamase, suggested for infections caused by gram-negative and gram-positive organisms, gonorrhea, and haemophilus. Mainly they are used for the treatment of many different types of bacterial infections such as bronchitis, sinusitis, tonsillitis, ear infections, skin infections, gonorrhea, and urinary tract infections.(Drug Bank)

According to the data obtained in antibiotic susceptibility test by disk diffusion method a very small zone of inhibition was observer to occur in case of nalidixic acid , cotrimoxazole and cefuroxime, and completely resistant against linezolid and tetracycline. Escherichiacoli and Enterobacteraerogenes are pathogens capable of showing horizontal gene transfer to other pathogens.therefore on consumption these bacterias could efficiently transfer these properties to other organisms in the system. These bacteria would then become untreatable by the antibiotics.

Finally the susceptibility of the organisms against certain disinfectants were checked .the disinfectants were the ones which are commonly used in wastewater treatment plants. Camphor is being used as disinfectants form very primitive times and also mentioned in Ayurveda. KMnO4 is used as a disinfectant of water in many waste water treatment plants as well as water supplies. The findings in the experiment suggest that these disinfectants had almost no effect against these multidrug resistant kind of bacteria. Next the trial was to check the susceptibility of the bacteria against alum and chlorine. This study revealed that at a very high concentration alum and chlorine has satisfactory results, and thus concluded to be used as a disinfectant.

To check the extent of damage the membrane damage of the the organism due to the disinfectants was studied under fluorescence microscope.

Escherichiacoli and Enterobacteraerogenescan efficiently do horizontal gene transfer and can acquire new genes as well as transfer the genes to other organisms . Considering the multidrug resistant property of the bacterias this is a question of major concern to the microbioloogists.

Therefore we can come to the conclusion after the whole study that although claimed as safe source of drinking water major water sources are contaminated with many harmful organisms. Theoretically they themselves may not be considered as pathogen but they may be the carrier of certain genes that when transmitted to pathogens due to several gene transfer mechanisms can render the pathogenic organisms even more dangerous.

VI.SUMMARY:

Presence of coliforms in the test samples were detected in SW2,SW4 and SW7. According to The Canadian Drinking Water Quality Guideline the number of total coliforms is none detectable per 100 ml. Whereas in our samples it was detected to be 43 coliforms in 100 ml (SW2) and 9 coliforms (SW4) in 100 ml that was very high than considerable range in the presumptive test.

According to the results obtained in confirmatory test the SW2 and SW4 were identified as true coliforms and tests with them were carried out further.
On EMB plates Escherichia coli appears as large, blue-black colonies, often with a green metallic sheen. Therefore the SW2 contained E. coli.
Again in SW4 presence of brown to blue-black, mucoid colonies with no sheen is indicativeof presence of Enterobacteraerogenes.
It also confirmed that the sources of contaminants were different.
The characteristic results obtained I IMViC again confirmed the presence of coliforms of fecal origin. And the bacterial isolate in SW2 and SW4 were confirmed as E.coli and Enterobacteraerogenes
The bacteria were motile. Both of the bacteria Escherichia coli and Enterobacteraerogenes were motile by means of their flagella.
Both of the samples were catalase as well as oxidase positive, therefore aerobic in nature
The bacteria do not produce any kind of haemolytic by product. Thus, incapable of causing Haemolytic Uremic Syndrome (HUS).
The bacteria were resistant to tetracycline and linezolid. Nalidixic acid and Cotrimoxazole have very low and cefuroxime has negligible effect against the bacteria.
In cup plate method camphor and KMnO4 remains ineffective as they do not inhibit the growth of the bacteria evidenced by presence of no zone of inhibition around the corresponding holes. However, only alum and chlorine can prevent the growth as they show zone of inhibition around the holes.
The results in colony counting method also shows no significant decrease in the number of bacteria for alum camphor or KMnO4. Only chlorine at a very high concentration was able to kill the bacteria significantly.
According to Optical Density method we can see that with the increasing concentration of the disinfectants the rate of growth of the bactereia was decreasing in case4 of alum as well as chlorine as evidenced by reducing optical density at 600nm.
Therefore, the effect of both chlorine and alum was considerably satisfactory at a very high concentration.
Neem and Tulsi had no antibacterial effect against the bacteria.
The effective killing of bacteria was evidenced by propidium iodide stained bacteria in large number in the treated sample when observed under fluorescence microscopy.
Therefore we come to the conclusion as a novel finding of this study is that there is no effect of alum camphor or extracts such as Neem or Tulsi on this kind of MDR bacteria. Only alum and chlorine has the capability of preventing their growth significantly and that also at a very high concentration.

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