WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 2116-2121. Case Study ISSN 2277– 7105

ISOLATION AND CHARACTERIZATION OF FAECAL COLIFORMS IN DIFFERENT WATER SAMPLES: A CASE STUDY

Pranjali Yadav and Anjana Pandey*

Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Allahabad-India 211004.

Article Received on
10 May 2015,

Revised on 05 June 2015,
Accepted on 28 June 2015

***Correspondence for Author**
Anjana Pandey
Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Allahabad-India 211004.

AIM

The current study focuses on isolation of different coliforms from different water samples from different site locations in Allahabad (U.P.), India.

INTRODUCTION

Water is a vital part of mostly all organisms' sustenance. Water acts as a carrier for distributing essential nutrients to all parts of the body, helps in chemical and metabolic reactions, transport of nutrients, regulation of body temperature, etc. Biological parameters are perhaps of greatest importance from human point of view. All natural waters contain a variety of organisms, both plants and animals as the natural flora and fauna. Almost all the world's water (97%) is located in the oceans, but as might be expected, the high concentration of salts renders the oceans virtually unusable. 1.74% is present as glaciers and permanent snow and 0.3% is present as groundwater, stored the aquifer both as confined and unconfined and as perched aquifers the available freshwater content in terms of direct use is only thus limited to 1%. India is a developing nation facing a serious problem of natural resource scarcity, especially that of water in view of population growth and economic development.

In India, water bodies are contaminated with sewage, domestic waste, industrial wastes, etc. The vehicles of water born diseases include virus, bacteria, protozoa and helminthes. The bacteria causing cholera, typhoid fever and bacillary dysentery may be present in sewage water or polluted water. Polluted water also contains several pathogenic viruses, out of which the prominent one are the one causing infections such as hepatitis (jaundice) and poliomyelitis, etc. One should always keep a check on the presence of biological as well as

chemical pollutants present in water. Municipal water and food products are routinely monitored for the presence of pathogens, but there are many challenges in doing so. Even non pathogenic bacteria, if present in large enough numbers, can cause deterioration of food products and water.

The alternative for the testing of many different types of pathogens is to test for those bacteria that indicate the presence of pathogens. The most common source of pathogens in food and water is faecal contamination, of either animal or human origin. Faeces also contain enumerable non-pathogenic bacteria, and the presence of these other bacteria in food or water indicates that faecal contamination has occurred (undesirable enough) and the potential presence of pathogens. The most commonly used "indicator bacteria are faecal coliform and faecal *Enterococci*.

COLIFORMS

Coliforms reside among the intestinal flora of humans and other warm-blooded animals, and are thus found in fecal wastes. Coliforms, detected in higher concentrations are used as an index of the potential presence of enteropathogens in water environments. Some of them are pathogenic and cause diseases like typhoid, dysentery and enteric fever etc. Thus, the presence of these organisms in water and fruit juices is dangerous for human consumption (Salle, 2000). *E. coli*, acts as an indicator of microbiological water quality. They themselves are non pathogenic but are citizens of the digestive systems of animals, and thus abundant in faeces. Coliform are defined as "Gram-negative aerobic or facultative anaerobes, nonspore-forming, rod-shaped bacteria that ferment lactose with acid and gas production." *E. coli*, the most abundant bacterium of the human colon, is the most important indicator of human faecal contamination.

However, some coliform bacteria, such as Enterobacteraerogenes, are of non-faecal origin and may be present in uncontaminated samples. Thus testing for coliform bacteria involves several tests to minimize the possibility of false positive results. Bear in mind that the presence of indicator bacteria does not mean that human pathogens are definitely present, but their presence suggests that the faecal contamination has occurred, and that pathogens may be present.

This group comprises the entire facultative and aerobic gram negative; non-spore forming rod shaped bacteria that ferment lactose with gas formation within 48 hrs at 37 ^{o}C. The coliform

bacteria include the genera *Escherichia coli (E.coli), Citrobacter, Enterobacter* and *Klebsiella* (Ashbolt, 2004). It includes lactose fermenting bacteria, such as *Enterobacter cloacae* and *Citrobacter freundii*, which can be found in both faeces and the environment (nutrient-rich waters, soil, decaying plant material) as well as in drinking-water containing relatively high concentrations of nutrients, as well as species that are rarely, if ever, found in faeces and may multiply in relatively good-quality drinking-water, e.g. *Serratia fonticola, Rabnella aquatilis,* and *Buttiauxella agrestis. Escherichia coli* are a member of the family Enterobacteriaceae, and characterized by possession of the enzymes β-galactosidase and β-glucuronidase.

The MPN technique is a statistical method of estimating the concentration of bacteria. Imagine a hypothetical situation where a sample of water contains 10 bacteria evenly dispersed in 100 ml of water.

MATERIALS & METHODOLOGY

COLLECTION OF SAMPLES

The different samples collected for analysis were Sewage Water, Drinking water (MNNIT Allahabad), Tap Water (MNNIT Allahabad), The Yamuna river water (Allahabad, U.P., India) The Ganges river water (Allahabad, U.P., India). All the samples were stored at 4 $^{\circ}$C in a sterile container.

MPN

In this test 1X (single strength) and 2X (double strength) Bromocresol Purple Broth (containing lactose) was prepared for the presumptive test and Lactose Bile Broth was prepared for the confirmatory test. The water sample was used undiluted i.e. 10mL, 1mL and 0.1mL of sample was added to the tubes (Aneja 2002).

ISOLATION and CHARACTERIZATION

All the tubes that showed positive results were transferred on selective media such as Eosin Methylene Blue Agar (EMB) and Mac Conkey Agar (MCA). The plates were incubated at 37 °C for 18-24 hrs. Prominent colonies were picked and studied further morphologically and microscopically. Gram staining and motility test were also performed.

BIOCHEMICAL TEST KIT

RAPID Biochemical test kits were purchased from Himedia laboratories. The different kits purchased were HiSalmonella Biochemical test kit, HiVibrio Biochemical test kit, HiListeria Biochemical test kit, Hi*E.coli* and Coli-nella Water testing kit. The instructions were followed as mentioned in the product information and results were compared with the result interpretation chart given in the same.

RESULTS

Table 1: MPN result sheet.

SAMPLES	DILUTION	INCUBATION PERIOD (hrs.)	TUBES				
			1st	2nd	3rd	4th	5th
Sewage water	Presumptive	24	+	+	+	+	+
	Confirmatory		+	+	+	+	+
	Complete		+	+	+	-	+
Ground Water	Presumptive	24	+	+	+	+	+
	Confirmatory		+	+	-	+	+
	Complete		-	+	+	-	+
Tap Water	Presumptive	24	+	+	-	+	+
	Confirmatory		+	+	-	+	-
	Complete		-	+	-	-	-
Distilled Water	Presumptive	24	-	-	-	+	-
	Confirmatory		-	-	-	-	-
	Complete		-	-	-	-	-
Drinking Water	Presumptive	24	-	-	-	+	-
	Confirmatory		-	-	-	-	-
	Complete		-	-	-	-	-
Yamuna Water	Presumptive	24	+	+	+	+	+
	Confirmatory		+	+	+	-	+
	Complete		+	+	+	-	+
Ganges Water	Presumptive	24	+	+	+	+	+
	Confirmatory		+	+	+	+	+
	Complete		+	+	+	+	+

Table 2: Colony characteristics of coliforms of water sample grown on different selective medium.

Selective media	EMB Agar	MCA
Size of colony	Small	Small
Shape	Round	Round with raised margins
Surface	Smooth	Smooth
Elevation	Raised/ flat	Raised/ flat
Color	Opaque green metallic sheen/ dark centered metallic sheen	Pink colonies/ transparent colonies
Interpretation	Lactose fermenting	Lactose fermenting

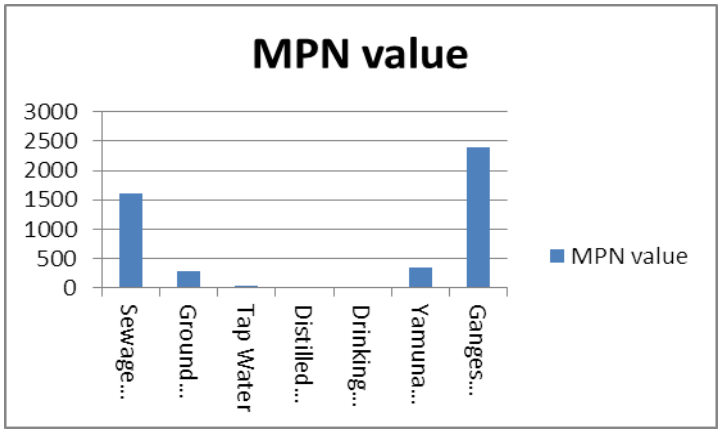

Figure 1: MPN results for water samples.

Table 3: Results obtained from HiMedia RAPID Biochemical test kits.

S. No.	Sample Name/ Kit used	HiSalmonella Biochemical test kit	HiVibrio Biochemical test kit	HiListeria Biochemical test kit	Hi*E.coli* Biochemical test kit	Coli-nella Water testing kit
1	Sewage Water	*S. choleraesius*	*V. aestuarians*	*Listeria spp.*	*E. coli*	+
2	Ground Water	_	_	_	Variable	+
3	Tap Water	_	_	_	_	+
4	Distilled Water	_	_	_	_	_
5	Drinking Water	_	_	_	_	_
6	Yamuna Water	*Paratyphi subspp. indica*	Variable	*Listeria spp.*	*E. coli* inactive	_
7	Ganges Water	*S. choleraesius*	*V. cholerae / V. parahaemolyticus*	*Listeria spp.*	*E. coli*	_

DISCUSSION

MPN results showed that the Ganges water is most contaminated followed by Yamuna water and then Sewage water. These three water samples were also found to be totally contaminated with a count of 1,100+ microorganisms per 100 ml. Based on the above studies, it has been confirmed that the water samples were highly contaminated with faecal coliforms. The results of the present studies also indicated the presence of four different types of fecal coliforms namely, *Klebsiella pneumonia, Citrobacter freundii, Enterobacter aerogens* and *Escherichia coli.* These organisms are highly pathogenic and may cause serious diseases in human beings. *Klebsiella pneumonia* species cause urinary tract infections, chronic broncho-pulmonary diseases, pneumonia, septicemia, meningitis etc. *Citrobacter freundii* causes urinary tract infections, infections in gall bladder, middle ear etc. *Enterobacter aerogens* are responsible for urinary tract infections and hospital sepsis etc. *Escherichia coli* causes diarroea, urinary infections, pyogenic infections and septicemia etc. (Ananthanarayan and Jayaram Paniker, 1996; Matthew *et al.*, 2007; Samonis *et al.*, 2009).

CONCLUSION

The MPN analysis showed high levels of contamination in river water. The study indicated the Ganga water, Yamuna water and Sewage water contains a wide variety of enteropathogenic bacteria namely, *Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii* and *Enterobacter aerogens.* The members that were identified were *S. choleraesius, V. aestuarians, Listeria spp.* and *E. coli* in sewage water sample, *Paratyphi subspp. indica* in Yamuna water sample and *S. choleraesius, V. cholerae* / *V. parahaemolyticus, Listeria spp.* and *E. coli* in the Ganges water.

The above results are really shocking as the river water is found to be a lot contaminated. The Government should take appropriate measures to decontaminate the river as it is affecting the aquatic life as well as the Siberian migratory birds that visit here each and every year.

ACKNOWLEDGEMENT

The authors are thankful to Dr. P. Chakraborty, Director, MNNIT Allahabad for allowing us to utilize the resources of the institute and TEQIP grant of the MNNIT Allahabad.

REFERNCES

1. Ananthanarayan R., Jayaram Paniker CK. 1996. Text Book of Microbiology, Orient Longman Limited, 160- Anna Salai, Chennai. 40-43: 250-261.
2. Aneja KR. 2004. Experiments in microbiology, plant pathology and biotechnology (4th ed.). New Delhi: New AGE International (P) Ltd. Pub.
3. Ashbolt NJ. 2004. Microbial contamination of drinking water and disease outcome in developing regions. *Toxiclology,* 198: 229-238.
4. Matthew E. Falagas, Petros I. Rafailidis, Diamantis Kofteridis, Simona Virtzili, Fotini C. Chelvatzoglou, Vassiliki Papaioannou, Sofia Maraki, George Samonis, Argyris Michalopoulos. 2007. Risk factors of carbapenem-resistant *Klebsiella pneumoniae* infections: a matched case-control study. *Journal of Antimicrobial Chemotherapy.* 60: 1124-1130.
5. Salle AJ. 2000. Fundamental principles of Bacteriology. TMH edition,Tata Mc Graw Hill Publishing Co Ltd., New Delhi. 691-699.
6. Samonis G, Karageorgopoulos DE, Kofteridis DP, Mattaiou DK, Sidiropoulou V, Maraki S, Falagas ME. 2009. Citrobacter infections in a general hospital: characteristics and outcomes. *Eur J Clin Microbiol Infect Dis.* 28(1): 61-68.