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Detection of *Escherichia coli* and total microbial population in River Siran water of Pakistan using Emb and Tpc agar

Sahibzada Waheed Abdullah[1*], Sahibzadi Aalia Gul[2], Sana Farheen[2], Tayba Kibrea[2], Adila Saleem[2], Sahibzadi Sana Gul[3] and Umair Ahmad[2]

[1]State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (LVRI, CAAS), Xujiaping I, Lanzhou, Gansu, 730046, The People's Republic of China.
[2]Department of Microbiology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan.
[3]Department of Zoology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan.

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There is an increased ratio of gastrointestinal diseases in the area of Mansehra, Pakistan, which depends on the Siran River for drinking. These diseases drew our attention to analyze the microbial population. For detection of *Escherichia coli* and other microbial population, samples were collected from different regions of Mansehra, Pakistan. Two samples were collected from each site for proper detection of microorganisms and repeated the process 5 times. The microbial counts were performed using total plate count agar (TPC) and eosin methylene blue (EMB). The samples were cultured on EMB and TPC agar at 37^{0}C for 24 h. The findings revealed that Baffa and Bajna had high microbial count confirmed by EMB and TPC. To this end, we confirmed that Siran River has high microbial count. The *E. coli* detection in water indicates the fecal contamination and the presence of *E. coli* in water makes the water unfit for drinking.

Key words: *Escherichia coli*, eosin methylene blue, total plate count, drinking water.

INTRODUCTION

Every year around the globe millions of people die due to gastrointestinal diseases; the leading contributor to this death toll is drinking contaminated water (Hrudey et al., 2006). Contaminated water quality, hygiene and sanitation are responsible for approximately 1.7 million deaths each year around the world. Water-borne diseases can be defined as, those diseases which spread and can be transmitted through water; few of them are cholera, typhoid, bacillary dysentery, infectious hepatitis, leptospirosis, giardiasis, gastroenteriris etc. (Ashbolt, 2004). The quality of recreational and drinking water was analysed through indicator bacteria belonging to certain genera. However, the culture tests used to determine these bacteria require a long time to complete and do not differentiate between human and animal fecal material sources (Glassmeyer et al., 2005). *Escherichia coli*

*Corresponding author. E-mail: drwaheed149@gmail.com. Tel: +86-13141113229.

phylogenetic group determination and its application in the identification of the major animal source of fecal contamination have been used as an indicator of water fecal contamination (Carlos et al., 2010). In early 1890s, the total coliform and fecal coliform test were used as assessing the quality of water for drinking purpose. After 1980s with the advancement of technology the new easy cheap and affordable methods were discovered for analysing the drinking water for *E. coli* (Edberg et al*.,* 2000). Total direct counts of aquatic bacteria from natural samples were estimated by epifluorescence microscopy after acridine orange staining (Francisco et al., 1973). Fecal pollution in water can indicate the presence of waterborne pathogens, such as *Salmonella* and *Giardia* (Carlos et al., 2010). The monitoring of microbiological water quality is usually based on the enumeration of indicator bacteria as total coliforms (TC), faecal coliforms (FC), *E. coli* and intestinal enterococci (Howard et al., 2003). The rise in microbiological flora of the rivers is more intense during sewer overflow and heavy rainfall or accidental pollution. It is of outmost importance to assess the microbiological quality of river water, especially if the water is used for drinking and recreational purposes. Therefore, it is very necessary to either treat the water or to restrict the people from bathing in such a contaminated water (Garcia-Armisen et al., 2005). Just like *E. coli* there are other many pathogens that are present in drinking water for example *Legionella*, *Shigella*, Salmonella and Pseudomonas (Hardalo and Edberg, 1997). Frequency of dividing cells is suggested to be an indirect measure of the mean growth rate of an aquatic bacterial community (Larsson et al., 1979). In view of the increasing interest in the possible role played by hospital and municipal waste-water systems in the selection of antibiotic resistant bacteria, biofilms were investigated using *Enterococci*, *Staphylococci*, *Enterobacteriaceae*, and heterotrophic bacteria as indicator organisms (Schwartz et al., 2003). At the point of consumption drinking water quality is routinely monitored in distribution network but not inside household. Fluctuating temperatures, residence times (stagnation), pipe materials and decreasing pipe diameters can promote bacterial growth in buildings (Lautenschlager et al., 2010). This project was designed to find out the microbial quality of the Siran River water. This study will help us to formulate the control strategies for water borne diseases in the area.

MATERIALS AND METHODS

This study was carried out at Department of Microbiology, Hazara University during the period of February 2017 to August 2017 on Siran, a river in Mansehra District, Khyber Pukhtunkhwa, Pakistan. Samples were taken from different sites of the river. Study organism was basically *E. coli* as well as determination of total microbial population. Two water samples were collected from each site. Total sites were 7 named Sum, Bajna, Shinkiari, Ichrian, Dadar, Baffa and Kolegah. The process was repeated seven times. Total samples from seven sites were 70 till the end. The study included collection of surface water samples over time at various locations within the watershed. The primary sampling point was in the surface water layer (0 to 5 cm from the layer) at the centre of the main flow. However, the top 1 to 2 cm of this surface layer was avoided so as not to collect floating dust, oil, etc. The water samples were collected in small- leak proof glass bottles with a highly sealed lid and all the bottles were autoclaved previously. Each water sample was cooled immediately after collection to approximately 1.5°C by storing in crushed ice; they were transported as soon as possible and hand delivered. Two media were used; Eosin Methylene Blue Agar and Total Plate Count Agar. EMB is the best medium for determination of *E. coli,* while total plate count media was used to observe the growth of whole microbial population. In general, cultivation based heterotrophic plate count (HPCs) is also used as a common microbial quality parameter in drinking water treatment and distribution (Hammes et al., 2008).

Media preparation

Thirty five grams of powdered EMB medium was suspended in one litre of distilled water then mixed well and dissolved by heating, while the protocol for TPC media is to suspend 23 g of medium into distilled water. The solutions were boiled for 1 min and then sterilized in autoclave at 121°C for 15 min (Omezuruike et al., 2008). The materials were packaged in individual water-tight bags in order to avoid cross contamination (Pernier et al*.*, 2005).

Pouring into plates

We kept the medium to cool to 50°C; it was mixed well and poured into the sterilized petri plates in hood to prevent any kind of contamination. As the medium solidified, we inverted the plates to evade additional moisture. The prepared medium was stored in refrigerator.

Making dilutions of samples

The samples taken were diluted before the process of culturing to minimize the strength of microbial population. 1:10, 1:100 and 1:1000 dilutions were made.

Culturing

After making dilutions of 1:10, 1:100, and 1: 1000, respectively, 200 µL of water sample was taken and inoculated through an incinerated spreader on both media e.g. EMB and TPC. The inoculation was performed through a spreader inside flow hood in a restricted condition to avoid any casual contamination present in the air.

Incubation

The EMB plates are placed in an incubator with a set temperature of 37°C for 24 h. Plate count agar is presently the suggested medium for the standard bacterial plate count (35°C, 48 h incubation) of water and wastewater so each type of bacteria grow on TPC and the bacteria can easily be enumerated using microscope or total plate counter (Reasoner and Geldreich, 1985).

Observations

During the period of incubation different bacterial strains have grown in sufficient colonies that can easily be counted on bacterial

Table 1. Growth on EMB.

Sample sites	Original	1/10	1/100	1/1000
Dadar	No growth	Blue 20 Pink 138	Blue 9 Pink 0	No growth
	Less but not countable	Blue 76 Pink 84	Blue 0 Pink 54	No growth
Sum	Heavy growth (Uncountable)	Blue 65 Pink Numerous	Blue 28 Pink 72	Blue 8 Pink 5
	Heavy growth (Uncountable)	Blue 23 Pink Numerous	Blue 0 Pink 56	Blue 2 Pink 0
Ichrian	No growth	Blue 14 Pink 36	Blue 2 Pink 14	Blue 0 Pink 33
	Less but not countable	Blue 2 Pink 18	Blue 0 Pink 6	Blue 0 Pink 24
Kulegah	Less but not countable	Blue 13 Pink Numerous	Blue2 Pink 28	Blue 11 Pink 14
	No growth	Blue 92 Pink 227	Blue 44 Pink35	Blue 12 Pink 8
Bajna	Heavy growth	Blue 06 Pink 70	Blue 0 Pink 02	Blue 08 Pink 16
	Heavy growth	Blue 0 Pink Numerous	Blue 18 Pink 118	Blue 17 Pink 77
Buffa	No growth	Blue 41 Pink 122	Blue 09 Pink 45	Blue 19 Pink 94
	Less but not countable	Blue 25 Pink 176	Blue 0 Pink 82	Blue 0 Pink 133
Shinkiari	No growth	Blue 84 Pink 43	Blue 18 Pink 111	Blue 08 Pink 122
	No growth	Blue 09 Pink 154	Blue 0 Pink 65	Blue 0 Pink 0

colony counter. On TPC, lot of bacterial strain can be seen with different morphology while on EMB being a selective medium for Gram negative rods, grow only gram negative bacteria. A comparison of counts obtained on EMB and TPC agar slide surfaces is useful as a control on the extent of contamination in the water sample other than *E. coli* (Cohen and Kass, 1967). The contaminants growing on TPC include some pathogenic microorganism.

RESULTS

Suitable incubation period for samples was 37°C for 24 h. After the incubation period, it was observed each plate showed different microbial count from different sites. *E. coli* detected on EMB showed water faecal contamination. The rate of growth of microbial population on EMB and TPC is shown in Tables 1 and 2, respectively.

DISCUSSION

From practical work it was concluded that microbial population was maximum in Baffa and Bajna as shown in Table 2 (the bacterial contamination increases as we move from upper stretch to lower stretch) during summer. This may be because large number of tourist visiting the area in summers or may be due to temperature; as temperature is directly co-related with the faecal coliform

Table 2. Growth on TPC.

Sample sites	Original	1/10	1/100	1/1000
Dadar	32	Numerous	15	10
	Numerous	Numerous	16	06
Sum	Numerous	Numerous	14	10
	Numerous	Numerous	19	11
Ichrian	19	Numerous	20	22
	46	Numerous	36	11
Kulegah	08	Numerous	17	31
	40	Numerous	10	26
Bajna	Numerous	Numerous	Numerous	55
	Numerous	Numerous	Numerous	44
Baffa	57	Numerous	Numerous	60
	116	Numerous	Numerous	55
Shinkiari	Numerous	Numerous	90	41
	Numerous	Numerous	96	20

count, which is in accordance with prior studies (Vinay et al., 2005; Khan et al., 2014).

The study conducted by LeChevallier et al. (1980) indicated that there were approximately 700 bacteria isolated from the surface, and untreated drinking water using SPC (standard plate count) and further performed the taxonomic classification of the identified bacteria. While we compared the microbial population of the original sample to the microbial population followed by dilutions has different microbial counts. The difference between the present study and previous is we used EMB plate for the detection of Pink colonies and confirmed the presence of *E. coli* in the river water as elaborated in Table 1.

According to the study performed in Lahore for water contamination the results revealed that samples collected from Bagarian, Multan Road, Burdwood Road, Green town and Band Road were contaminated with faecal coliforms that showed, respectively, 1600, 920, 540, 240 and 240 MNP per 100 ml. All positive tubes from presumptive test were streaked on Eosin methylene blue (EMB) agar for the detection of coliform colonies especially *E. coli*. Positive confirmed samples that showed typical coliform colonies, that is metallic green sheen colonies of *E. coli* on EMB agar were then tested individually. A total of 42 *E. coli* strains were isolated in 2014 (Zareen et al., 2014) .In our study, it is shown that the microbial population was numerous and *E. coli* counts were more than 42.

It is also a fact that microbial population is more and many micro-organisms maybe present in water other than *E. coli;* but our concern is the total count of microbes on culture plate. The presence of fecal indicator organisms provides warning of waterborne problem, which is a direct threat to human and animal health.

Conclusion

The current study concluded that water of Siran River is highly contaminated. A number of microbial population were detected including *E. coli* which indicates faecal contamination in water. The ability to drink water that is delivered into households without fear of becoming ill may be one of the key defining characteristic of nations that are in developing state in relation to the majority of the world. *E. coli* causes highly lethal effects on human health. So the water of Siran is not appropriate for drinking purpose. For safe drinking water, the public health must follow these parameters: Firstly, a maximum of 500 colony forming unit (CFU) per ml of heterotrophic plate count (HPC) free from coliforms; secondly, drinking water should not contain any bacteria that present any indication of faecal pollution such as pseudomonas spp; thirdly, more than 250 million new cases waterborne diseases in developing nations are reported per year which increases morbidity and mortality rates especially in children.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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