



# The Open Microbiology Journal

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## RESEARCH ARTICLE

### Virological Quality of Urban Rivers and Hospitals Wastewaters in Addis Ababa, Ethiopia

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#### Abstract:

##### Background:

Polluted rivers and hospital wastewater become a greater concern because of their public health and environmental hazards with high tendency to result in epidemics.

##### Methods and Materials:

The current study investigated 84 samples of Urban rivers and 30 samples of hospitals wastewaters in Addis Ababa, Ethiopia between February and April, 2017. The simultaneous detection of male-specific and somatic Coliphages from the samples was carried out using *Escherichia coli* CB390 as the host according to the single agar layer plaque assay at public health microbiology laboratory of Ethiopian Public Health Institute.

##### Results:

Of the total 114 samples tested, coliphages were detected in 44 (52.4%) and 3 (10%) samples of urban rivers and hospital waste waters, respectively. Total coliphages enumerations ranged from <1pfu/100ml to 5.2×10<sup>3</sup>pfu/100ml for urban rivers and <1pfu/100ml to 4.92×10<sup>3</sup>pfu/100ml for hospitals wastewaters.

##### Conclusion:

The detection of total coliphages in our study settings warrants the possibility that the pollution of urban rivers and hospital wastewaters may be a source for pathogenic viral infections. Unless coliphages, viral and fecal indicators are also examined in the waters by public health agencies, waterborne infections cause a major risk to public health.

**Keywords:** Coliphages, Virological, Hospital wastewater, Indicators, Fecal pollution, Agar.

#### Article History

Received: February 23, 2019

Revised: May 15, 2019

Accepted: May 22, 2019

## 1. INTRODUCTION

Quality of river water is essential to humans, animals and for a good environment [1]. However, river waters are getting polluted by indiscriminate disposal of sewerage, industrial waste and excess of human activities, which affect water quality globally [2]. The degradation of river water depends mainly on wastewater quality. The harmful effects of these effluents on the quality of river waters are diverse and rely on the volume of the discharge and microbiological load of the effluents [3].

In under developed countries, insufficient wastewater management systems can lead to increase waterborne diseases [4]. The sanitary related risk for humans linked to the presence of pathogens is associated with the use of the water such as for drinking, recreational activities, bathing, irrigation and shellfish harvesting [5].

Wastewater generated mainly from hospital may also pose serious health risks to human beings and animals when contaminated with these wastes [6]. Hospital wastewater where individuals with enteric illnesses are hospitalized is a principal problem during diarrheal illnesses outbreaks [7]. The problems faced related to the management of wastewater are due to high population growth, urbanized societies and industrialization [8].

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In Addis Ababa, Ethiopia due to insufficient and inefficient solid and liquid waste management services, pollutants including some of the clinical wastes are discharged directly or indirectly to the nearby rivers [9]. These polluted waters are used by downstream residents to grow vegetables, which are sold and consumed in the city [10]. Such polluted waters can also contaminate water supplies [11].

Fecal pollution in water sources is a public health risk and surrogates of fecal contamination are utilized widely to regulate water quality [12]. The monitoring of river water and wastewater for fecal pollution is becoming increasingly significant as the world's population has become more urbanized. To reduce the risk of diseases in public, good fecal indicators are required. Conventionally, *bacterial indicators* have been used as microbial indicators to monitor fecal pollution in waters. Nevertheless, it has been recommended that these bacterial indicators are not good for predicting enteric viruses [13]. The detection of all pathogenic microbes potentially present in water bodies is very difficult due to the large diversity of pathogens, low abundance of each species and the absence of standardized for their detection [5].

No information concerning the occurrence of phages in urban rivers and hospitals' wastewaters is available for most countries including Ethiopia in general and in Addis Ababa in particular. The objective of this study was to assess the contamination level of urban rivers and hospitals wastewaters in Addis Ababa, Ethiopia using coliphages.

## 2. MATERIALS AND METHODS

A cross sectional study was carried out on a total of 114 urban rivers and hospital wastewaters samples at Public Health Microbiology Research Team Laboratory in Ethiopian Public Health Institute between February and April, 2017.

Thirty selected urban rivers and streams (R1-R30) that flow through nine sub cities (SC 1-SC 9) of Addis Ababa and four hospitals wastewaters in the city were used for collecting samples for the current study. Eighty-four samples were collected using a grab sampling technique from the rivers that have large water flow. Three discrete samples from the first sampling point, 100 meter downstream and 200 meter downstream, were collected from 11 rivers (R1, R3, R8, R9, R11, R12, R16, R17, R28, R29 and R30) in the first round and processed independently. Two discrete samples from the first sampling point and 100 meter downstream were collected in the second round except for three rivers. The samples were processed independently. A single discrete sample was collected from 15 rivers and streams (R2, R4, R5, R6, R10, R13, R14, R15, R18 - R21, R24, R25 and R27) in the first and second round and for the remaining four rivers (R7, R22, R23 and R26) only in the first round (Table 1). The second round samples were collected at 15 day intervals. For hospital waste waters, a total of 30 untreated samples were collected from various cafeterias, laundries, Wards and medical laboratories units of four government hospitals. For all the samples, about 150 ml samples were collected using sterilized glass bottles and transported on ice to the laboratory. The samples were maintained at 4 °C. Microbiological screenings were performed within 24 hours after collection. All the samples were tested for

total coliphages using standard EPA method [14, 15].

**Table 1. Number of samples collected from rivers in nine sub-cities of Addis Ababa between February and April 2017.**

Rivers and streams in nine sub-cities of Addis Ababa	No. of Samples
R7, R22, R23 and R26	1
R2, R4, R5, R6, R10, R13, R14, R15, R18, R19, R20, R21, R24, R25 and R27	2
R8 and R30	3
R28	4
R1, R3, R9, R11, R12, R16, R17 and R29	5
Total	84

### 2.1. Detection of Coliphages

Simultaneously, both types of male-specific and somatic Coliphages from polluted rivers, streams and hospitals wastewaters samples were detected using *Escherichia coli CB390* [obtained from University of North Carolina, Chapel Hill] as the host bacterium according to the single agar layer plaque assay. The Host log phase containing 0.15% ampicillin was applied with magnesium chloride in double strength tryptic soy agar [Difco]. All the plaques, 1 to 10 mm diameter lysis zone formation in the lawn of host bacteria, were counted after 16 to 24 hours of incubation at 37 °C per plates from a single sample for total coliphages positive; no circular zone of clearing or an intact lawn of bacteria identical to the background lawn of bacteria was found for coliphage negative. The coliphage enumerated was computed per 100 mL of the sample [14, 15].

### 2.2. Data Analysis Procedures

The data was analyzed using SPSS version 20 for Windows [SPSS Inc. Version 20, Chicago, Illinois. The Kruskal-Wallis test was used to observe the differences in total coliphages values by sub-cities and water sources. The significance level was set at  $p$  value  $\leq 0.05$ .

## 3. RESULTS

Of 84 river and streams water samples tested between February and April 2017 in Addis Ababa, Ethiopia, total coliphages were observed in 44 (52.4%) samples ranging from  $<1$ pfu/100ml to  $5.2 \times 10^3$ pfu/100ml. All the nine sub-cities included in the study contained coliphages in one or more of their river water samples. Out of 30 rivers and streams in the sub-cities, 24 (80%) rivers had coliphages. The distributions of total coliphages detected in the river water samples in the sub-cities SC-1 to SC- 9 were 50% (4), 25% (2), 83.3% (10) 10% (1), 37.5% (3), 54.5% (6), 62.5% (5), 85.7% (6) and 87.5% (7), respectively (Table 2).

The maximum recovery of the phages in the urban river water samples was 5200 pfu/100ml (Table 3). Of 30 waste water samples tested from four hospitals in Addis Ababa, Ethiopia, total coliphages were detected in 3 (10%) samples ranging from  $<1$ pfu/100ml to  $4.92 \times 10^3$  pfu/100ml. In the hospital wastewaters, total coliphages were detected in wastewater from the ward, medical laboratory and laundry

facilities units with the concentrations of  $4.92 \times 10^3$ ,  $4.2 \times 10^2$  and  $2.7 \times 10^2$ , respectively. P- value for coliphages using the

nonparametric, Kruskal-Wallis test for the samples by the sample type (urban rivers and hospitals wastewaters) was 0.003.

**Table 2. Enumeration of total coliphages in river water samples using single agar layer in nine sub-cities with their maximum enumeration of Addis Ababa between February and April 2017.**

Coliphages [pfu/100ml]	Addis Ababa Sub-cities' Rivers and Streams Water Samples									
	SC- 1	SC- 2	SC-3	SC-4	SC-5	SC-6	SC-7	SC-8	SC-9	Total
<1	8	6	2	9	5	5	3	1	1	40
11-100	1	0	5	0	1	4	1	1	0	13
100-500	2	2	5	1	2	2	2	0	1	17
501-1000	1	0	0	0	0	0	0	2	3	6
>1000	0	0	0	0	0	0	2	3	3	8
<b>Total</b>	12	8	12	10	8	11	8	7	8	84

SC-Sub-City

**Table 3. The number of river water samples positive and negative for coliphages, minimum and maximum for total coliphages in nine sub-cities of Addis Ababa using single agar layer between February and April 2017.**

Rivers	Sub-Cities	Phage +ve Samples	Phage -ve Samples	Min. [pfu/100ml]	Max. [pfu/100ml]
R1	SC-1	2	3	0	600
R 2	SC-1	0	2	0	0
R 3	SC-1	2	3	0	400
R 4	SC-2	1	1	0	300
R 5	SC-2	0	2	0	0
R 6	SC-2	1	1	0	200
R 7	SC-3	1	0	150	150
R 8	SC-3	3	0	20	70
R 9	SC-3	4	1	0	200
R 10	SC-3	1	1	0	80
R 11	SC-4	1	4	0	360
R 12	SC-4	0	5	0	0
R13	SC-5	0	2	0	0
R14	SC-5	1	1	0	400
R15	SC-5	0	2	0	0
R16	SC-6	1	4	0	50
R17	SC-6	4	1	0	290
R18	SC-7	1	1	0	60
R19	SC-7	1	1	0	290
R20	SC-7	1	1	0	5200
R21	SC-7	2	0	250	3100
R22	SC-3	1	0	80	80
R23	SC-6	1	0	70	70
R24	SC-2	0	2	0	0
R25	SC-5	2	0	100	120
R26	SC-9	1	0	1960	1960
R27	SC-9	2	0	870	2200
R28	SC-9	3	1	0	1500
R29	SC-8	4	1	0	1600
R30	SC-8	3	0	60	1700

R-river, SC-Sub-city, min.-minimum, max. -maximum

**Table 4. Detection of total coliphages using single agar layer in various units of four hospitals' wastewaters in Addis Ababa between February and April 2017.**

Hospital	Hospital's Units	No. of Negative Samples	No. of Positive Samples	Total Number of Samples
H1	Caffe	1	0	2
	Laundry	1	0	
H2	Laboratories	6	1	12
	wards	3	0	
	OPD	1	0	
	laundry	0	1	
H3	Hostel	2	0	9
	Wards	4	1	
	Laundry	2	0	
H4	Mixed	7	0	7
<b>Total</b>				30

OPD– Out-Patient Department

#### 4. DISCUSSION

This study was intended to investigate the incidence of total coliphages in polluted urban rivers and hospitals waste water. The coliphages were monitored using suitable *E. coli* strain CB 390 by the single agar layer plaque assay. The detection of coliphages in environmental water samples can be carried out by plaque assay using single-layer agar methods [16]. Even if the two main types of coliphage are examined separately on different *E. coli* host bacteria; with the right choice of *E. coli* host, it can be possible to measure both somatic and male-specific coliphages together on a single *E. coli* host. This can reduce the cost and work load in detecting and quantifying total coliphages in waste water. *E. coli* CB390 is the only *E. coli* host for simultaneous detection of total coliphages that give similar concentrations as the sum of coliphages detected by the individual somatic (CN13) and male-specific [Famp] *E. coli* hosts [17].

The occurrence of total coliphages in 52.4% of the urban rivers and 10% hospitals' wastewater sources could indicate fecal pollution and hence the presence of enteric viruses and possibly also other pathogens [18, 19]. The excellent indicators of pathogenic viruses in wastewater and fecally contaminated water are because of compositional similarity, structural similarity, site of replication, size similarity, morphological similarity, their resistance to environmental changes [20] and different water treatments similarities [21].

Contamination by pathogenic viruses can be predicted by specifically detecting the viruses or by assessing the level of fecal contamination using some indicators [22]. However, specific detection of enteric viruses is not adapted to routine analysis. Culturing, which is the reference method for the detection of environmental viruses, is time-consuming and does not allow the detection of all viral serotypes and no information is achieved regarding viral infections using molecular techniques [23]. Seventy percent of sequences obtained from the environment had no match with any database [24]; therefore a suitable and cheap technique is needed [25].

As indicated by different researchers, total coliphages concentrations are correlated with pathogens [26]. In somatic and male-specific coliphages in polluted rivers, viruses and

protozoan parasites were detected in one study [27]. In another study, the presence of coliphages and pathogenic viruses at different locations was observed [28]. Quality of river water samples positive for enteric viruses enhances with increasing concentration of coliphages [22]. The review of the EPA report shows that coliphages exist when fecal pathogens are present, but are likely to be absent in non-fecally contaminated water. They are a better surrogate for viruses than enterococci or *E. coli* in the effluent. In most cases, these organisms are present in greater numbers than the pathogen in this case, human viruses [29].

Nowadays, viral infections being the communicable diseases caused by bacteria and parasites are at the forefront [30]. The extremely small size of the enteric viruses allows them to infiltrate soils and reaching aquifers to contaminate groundwater after being shed in large quantities in feces of infected individuals [31]. They are commonly more resistant to treatment, more infectious; and do not need to be large in number to cause a disease than most of the other pathogens [32]. These pathogenic viruses and also protozoa are major threats to human health in all freshwater supplies [33] and wastewaters [34].

Fecal pollution is highly problematic to both developing and developed countries, though the levels of pollution and contamination type vary among nations. Micro-organisms of fecal origins largely transfer to the water bodies through industrial and domestic wastewater discharges [35]. This fecal pollutant contamination of water resources may pose significant health risks to human beings and animals since many pathogens are frequently associated with feces [36].

The recovery of total coliphages in 52.4% of river water samples collected from Addis Ababa, Ethiopia was nearly in agreement with the study conducted in Malaysia by Foong who analyzed some coliphages in various rivers heavily polluted with sewage and animal fecal matter from residential areas [37]. The detection rate of total coliphages in more than half of urban rivers water samples was lower than the study conducted in South Africa found at least one coliphage in 97.2% of the rivers samples in all rivers with maximum recovery of  $2 \times 10^4$  pfu/100ml [38].

The contamination of urban rivers waters in Addis Ababa, Ethiopia with coliphages (52.4%) in all of the nine sub-cities was higher as compared to hospitals wastewater (10%) with higher maximum recovery. This may indicate the degree of pollution of urban river waters. The pollution of the rivers can be due to damaged septic tanks, runoff from agricultural lands [39] and wastes discharged directly or indirectly to the nearby rivers because of insufficient and inefficient solid and liquid waste management services [9].

The detection of total coliphages in the hospitals especially in the medical laboratories showed pathogenic microbes that can pose a serious health risk for patients in the healthcare units, health professionals, community, and the environment [29]. Hospital wastewater pollutants including viruses can simply reach the water resources in the environment causing aquatic pollution and human health crisis [40].

The detection of coliphages in the laundry facilities may indicate the impurity of healthcare textiles with enteric viruses or other microbes. Healthcare textiles such as gowns, bed sheets, towels, blankets, personal clothing, patient apparel and uniforms can be contaminated with large numbers of microbes from stool [41].

All the wastewater samples collected in the study were discharged to the environments without any pretreatment. Discharge of infectious agents in hospital wastewater to the groundwater and other environments might pose a high risk for hospital personnel and the nearby communities [42]. Therefore, medical waste management is of greater significance because of its potential public health risks and environmental hazards with high tendency to result in epidemics [43].

The wastewater effluents are responsible for the degradation of the water bodies, rivers or streams [3]. Wastewater is used as a source of irrigation for farming in many urban areas in the world [44]. As a result of easy access or no alternative source, this water is causing severe negative impacts on health [45, 46]. The water pollution is leading to cause large scale deaths of humans across the world [47]. Furthermore, consumption of crops irrigated with wastewater causes 99.7% of deaths as the the consumption of such contaminated crops leads to diarrhea in developing countries and 90% of the deaths occur in children [48]. These wastewaters contain numerous pathogenic microbes that may get eroded into drinking water supplies or receiving water bodies [49]. Majorly, pathogenic viruses are extensively dispersed in waters and the environment and are consumed by humans and animals *via* drinking water, water used in food irrigation, and shellfish production [50 - 52].

The nonparametric Kruskal-Wallis test revealed that total quantity of coliphages differed statistically in different the sample types i.e urban rivers and hospitals wastewaters, (p values 0.003). The maximum recoveries of total coliphages in the three sub-cities were higher than other sub-cities.

## CONCLUSION

The detection of coliphages in all the sub-cities and one-half of the hospitals' samples might be a source for pathogenic viral infections. Unless the level of contamination of the rivers

and hospital wastewaters is not properly monitored for coliphages by the scientific community, poor quality of the water will continue to cause a major health risk and will result in more number of deaths and also will affect the aquatic life, production of different crops, other sources, and drinking water.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

## FUNDING

None.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

We thank Ethiopian Public Health Institute for all the support and Lydia S Abebe from the University of North Carolina, Chapel Hill for providing coliphage and bacterial strains.

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