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Contamination Status of *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. in Surface and Groundwater of the Kelani River Basin, Sri Lanka

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Abstract: Waterborne diseases are a global problem that causes more than 2.2 million deaths annually. Therefore, the present study was focused on microbiological contamination of both ground and surface water by means of total coliform, *Escherichia coli* (*E. coli*), *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. Seventy two groundwater and 45 surface water sampling locations were selected to collect water from the head, transitional and meandering regions of the Kelani River Basin for a period of one year (both dry and wet seasons). The results of the study revealed that the entire Kelani River basin was contaminated with total coliform and *E. coli* bacteria and almost all the sampling locations exceed Sri Lanka Standards Institute (SLSI) guideline value given for drinking water (0 CFU/100 mL). Further, in groundwater, 17 locations were positive for *Salmonella* spp., whereas only 2 locations were positive for *Campylobacter* spp. In surface water, 26 and three sampling locations were positive for *Salmonella* spp. and *Campylobacter* spp., respectively. In this study, 23 different human pathogenic serovars were isolated and the *Salmonella enterica serovar* Kentucky was identified as the commonest type. Thus, the result of the study revealed that the consumption of raw water from the Kelani River Basin is unsafe and possible to cause gastrointestinal diseases.

Keywords: Kelani River Basin; ground and surface water; *Salmonella* spp.; *Shigella* spp.; *Campylobacter* spp.

1. Introduction

Globally, since 1990, 2.3 billion people have gained access to improved sources of drinking water [1]. Recent World Health Organization (WHO) statistics have shown that 87% of the developing countries have access to safe drinking water, leaving 748 million people unable to access the luxury and the amount is largely regional specific [1]. A recent study discovered that nearly one billion people in the world still practice open defecation and this situation is severe in many developing countries with poor sanitation practices following discharge of approximately 95% of their untreated sewage directly into surface waters [2,3]. In Sri Lanka, total annual water resources (AWR) are 50 km^3 and only 20% is used in agriculture, industry and domestic purposes [4]. Sri Lanka comprises of 103 major river basins and the calculated total drain area is around 59,245 km^2 [5]. Among the river basins in Sri Lanka, Kelani River Basin plays a major role providing water for irrigation, recreation, agriculture, industry and drinking [6] flowing through the capital cities of the country and it is providing around 80% of

drinking water for a greater part of Colombo. However, the river is immensely exposed to pollutants via land use and anthropological activities. Thus, the Kelani River ranks as the most polluted river in Sri Lanka [7,8]. The Kelani River Basin (KRB) receives an average annual precipitation of 3718 mm and producing a surface runoff volume of about 8600 million m^3 in which around 65% discharges into the Indian Ocean [7]. The river flows through seven districts and caters to 25% of the population of the country and provides water for major industrial zones [9]. Thus, the Kelani River could be the largest recipient of the industrial effluents among all the other rivers in Sri Lanka [10,11].

WHO reports [12] revealed that, two and a half billion people in the world have no access to improved sanitation and that more than 1.4 million children die each year from diarrheal diseases. In 2012, it was estimated that 842,000 deaths happen in middle and low-income countries due to contaminated drinking water. Further, more than 2.2 million annual deaths were attributed due to waterborne diseases, thus it has become a major global health problem in the world [12]. According to the WHO report in 2003, recreational water in most part of the world is contaminated with pathogenic and non-pathogenic microorganisms and major causes of contamination were discharges of sewage, industrial effluent, agricultural and livestock wastes to surface runoff. A recent study has revealed that those water-borne and enteric diseases including acute gastrointestinal disease, cholera, dysentery, hepatitis-A and typhoid to be about 66% during a year [13]. Salmonellosis is one of the most common and widely distributed foodborne diseases, characterized by acute onset of fever, diarrhea, abdominal pain, nausea and vomiting that leads to tens of millions of human cases annually worldwide and more than hundred thousand deaths in the world [14,15]. *Salmonella* is transmitted by many ways on its resistance to environmental factors, which controls its survival and its capacity to be carried by water as it moves through the environment. Crump et al. [16] recorded that specifically the Indian subcontinent is vulnerable for typhoidal isolates and cause gastrointestinal diseases with an estimated 21.6 million annual positive cases and two hundred thousand deaths. Shigellosis is a worldwide endemic disease with millions of infections reported every year [17] and Bardhan et al. [18] calculated that nearly 125 million cases occur annually in Asia and around 14,000 cases result in death. Children <5 years of age are at highest risk for *Shigella* spp. related illness and death with symptoms of fever, anorexia, fatigue and malaise [19]. Further, *Campylobacter* is a major cause of foodborne diarrheal illness in humans and are the most common bacteria which cause gastroenteritis worldwide [12]. In both developing and developed countries, they contribute to more cases of diarrhea than foodborne *Salmonella* [12]. In 2008, Campylobacteriosis was the major cause of zoonotic disease in humans and around two hundred thousand confirmed cases were recorded [20]. Total coliforms (TC) and *E. coli* were accepted microorganisms are the standard microbial indicator to show faecal or pathogenic contamination in water. Therefore, identification and quantification of TC and *E. coli* in water is important to accept the water source for drinking purpose.

Thus, the present study was aimed to identify pathogenic microbial contamination in ground and surface water of the Kelani River Basin with special emphasis of *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. This is the first report on pathogenic *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. bacteria contamination status in the Kelani River Basin along with *Salmonella* serovars specificity with potential human pathogenicity.

2. Materials and Methods

2.1. Study Area

The Kelani River Basin drains an area of 2230 km^2 initiating at levels above 1500 m on the steep slopes of the western border of the central highlands. It is the fourth longest river in Sri Lanka (144 m) and its number of tributaries travel through deep and structurally controlled valleys in the basin [7]. River basin covers nearly seven districts (Colombo, Gampaha, Kaluthara, Kegalle, Rathnapura, Nuwara Eliya and Kandy) starting from the central highlands (Nallathanniya) and ends in the western part of the country (Mattakkuliya). Sampling locations to collect surface and groundwater were set up along the

head, transition and meandering zones in the Kelani River Basin (6.746°–7.234° N, 79.851°–80.780° E) (Figures 1 and 2).

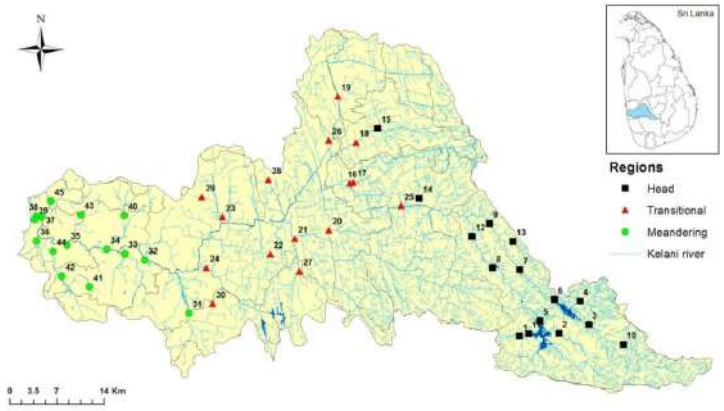

Figure 1. Surface water sampling locations in the head, transitional and meandering regions of the Kelani River Basin.

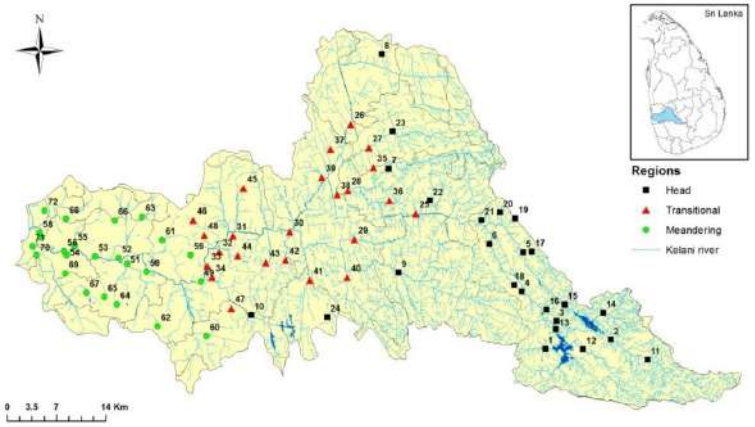

Figure 2. Groundwater sampling locations in the head, transitional and meandering regions of the Kelani River Basin.

2.2. *Water Sampling*

Sampling was conducted in both dry (February–March 2015) and wet (May–June 2015) seasons for 45 surface and 72 groundwater sampling locations (one sample from each location) in the Kelani River Basin (Figures 1 and 2; Tables 1 and 2). River water samples were collected from the river banks and middle of the river where reachable to collect. In total, 2.5 liters of water sample was collected to pre-cleaned sterilized amber color glass bottles with the help of sampling iron and transported to the laboratory in an ice box and kept in a cold room (4–8 °C). Microbiological analysis was performed within 24 hours of sample collection. Media preparation and sterilization were done according to the manufacture's instructions and prepared media was stored in the cold room at 4–8 °C until analysis [21]. Sampling controls were used to identify, measure and control sources of contamination or error that may be introduced from the time of sample collection through sample analysis. Therefore, field and media blanks were subjected for data evaluation and authentication.

Table 1. Surface water sampling locations of the Kelani River Basin.

No	Head Region	No	Transitional Region	No	Meandering Region
1	Kelani river (Nallathanniya)	16	Kelani river (Yatiyanthota)	31	Pusseli Oya
2	Sami male canal	17	We oya (Amanawala)	32	Kelani river (Ranala)
3	Kehelgamu oya (Norwood)	18	Ritigaha oya (Warawala)	33	Pahala bomariya ela
4	Dic oya	19	Gurugoda oya	34	Raggahawaththa oya
5	Maskeliya tank	20	Kahanawita canal (Dehiovita)	35	Kelani river (Kohila waththa)
6	Castlereigh tank	21	Seethawaka oya (Thalduwa)	36	Sebastian canal (Kelanithissa)
7	Norton tank	22	Eswaththa oya	37	Dutch canal
8	Kandura (Koththellena)	23	Pugoda oya (Pugoda)	38	Hamilton canal
9	Kelani river (Nagampitiya)	24	Wak oya (Kaluaggala)	39	Kelani river (Mattakkuliya)
10	Keselgamu oya (Tientsin)	25	Kelani river (Thaligama)	40	Raggahawaththa oya (Meegawaththa)
11	Mohini ella	26	Athalawa ela	41	Thalangama lake
12	Bokarabevila tributary	27	Getaheththa tributary	42	Diyawanna oya
13	Vidulipura tributary	28	Amthirigala tributary	43	Mahara
14	Alagal Oya (Gonagamuwa)	29	Pugada river (Mandawala)	44	Kittampahuwa ela (Wellampitiya)
15	Ritigaha oya (Bulathkohupitiya)	30	Pusseli Oya (Wewelpanawa)	45	Kalu oya

Table 2. Groundwater sampling locations of the Kelani River Basin.

No	Head Region	No	Transitional Region	No	Meandering Region
1	Wana male	25	Thaligama	49	Pollaththawela
2	Norwood	26	Kotiyakumbura	50	Ranala
3	Lakham	27	Warawala	51	Pahalabomariya
4	Koththellena	28	Kabulumulla	52	Biyagama
5	Kalaweldeniya	29	Kahanavita	53	Bollagala
6	Bokarabevila	30	Kudagama	54	Kohilawaththa
7	Malalpola	31	Kananpella	55	Kelaniya
8	Pitagaldeniya	32	Akarawita	56	Pilapitiya
9	Deraniyagala	33	Kahatapitiya	57	Paliyagoda
10	Waga	34	Kaluaggala	58	Aliwaththa
11	Tientsin	35	Kelaniwaththa	59	Palugama
12	Samimale	36	Levent	60	Moragahahena
13	Maskeliya	37	Siyabalawa	61	Dekatana
14	Dicoya	38	Karawanaella	62	Godagama
15	Castlereigh	39	Nawagammane	63	Udupila
16	Norton junction	40	Batangala	64	Hokandara
17	Athis	41	Getaheththa	65	Arangala
18	Koththellena 2	42	Puwakpitiya	66	Mawaramandiya
19	Ginigathhena	43	Hingurala	67	Thalangama
20	Badupola	44	Kosgama	68	Ederamulla
21	Kalugala	45	Viharakumbura	69	IDH
22	Gonagamuwa	46	Delgoda	70	Nawagampura
23	Bulathkohupitiya	47	Pinnawala	71	Mahawaththa
24	Thoranakada	48	Lunugama	72	Bangalawaththa

2.3. Isolation and Identification of Pathogenic Bacteria

To enumerate of *E. coli* and coliform bacteria (Membrane Filtration method) 100 mL of the sample was filtered through 0.45 µm cellulose acetate membrane filter (Whatman Cat No: 7001 0004, D-47mm)

using a manifold system (Elements, Australia, Reg DES 85628). The filter membrane was kept on the Membrane Lactose Glucuronide Agar (MLGA) plate and incubated at 37 ± 1 °C for 22 ± 2 h (Sri Lanka Standards Institution, 2013). For *Salmonella* and *Shigella* identification, *Salmonella typhi* and *Shigella sonnei* were used as positive controls obtained from the Medical Research Institute (MRI) Sri Lanka. The strains were maintained on nutrient agar (Oxoid CM003) slants under cold room condition at 4–8 °C. Buffered peptone water (BPW) (Oxoid CM0509) was used as primary enrichment for both *Salmonella* spp. and *Shigella* spp. One liter of water samples was filtered through 0.45 µm cellulose acetate membrane filter (Whatman Cat No: 7001 0004, D-47 mm) and the filter was dipped in sterile 90 mL buffered peptone water and incubated at 37 °C for 18 ± 2 hours. After incubation enriched broth (BPW) was inoculated to selective enrichment media. In total, 0.1 mL of enrichment broth into 10 mL of Rappaport Vassiliadis soya peptone broth (RVS) (Oxoid CM0866) and incubated at 41.5 °C for 24 ± 3 h and then 1 mL of enrichment broth was inoculated into 10 mL of Selenite cystine broth, (SCB) (Oxoid CM0395 and LP0121) and incubated at 37 °C for 24 ± 3 h. After the selective enrichment, colonies appeared on the disk was isolated onto Salmonella-Shigella agar (SSA) (Oxoid CM0533) and Xylose lysine deoxycholate agar (XLD) (Oxoid CM0469) following incubation at 37 °C for 24 ± 3 h. Suspected colonies of *Salmonella* spp. and *Shigella* spp. were identified based on colony appearances and the suspected colonies of *Salmonella* spp. and *Shigella* spp. were further subjected to the biochemical tests (Kligler iron agar, indole, urease, lysine decarboxylase, motility test) for confirmation [22–24].

Campylobacter jejuni sub sp. Jejuni (ATCC 33560) obtained from the Medical Research Institute (MRI) Sri Lanka and used as a positive control to evaluate presence *Campylobacter* spp. The strains were maintained on tryptose blood agar (Oxoid CM233) containing 10% (*v*/*v*) fresh sheep blood at 37 °C in the anaerobic jar with micro aerobic conditions created using Oxoid Gas Pack (CAMPYGEN CN0035). Bolton and Preston broths were used for the enrichment of the bacteria. Bolton broth (Oxoid SR183E and CM0983) and Preston (LAB 014, LAB M X114 and LAB M X115) enrichment broths were supplemented with 5% (*v*/*v*) fresh sheep blood (Medical Research Institute). One liter of water sample was filtered through 0.22 µm filter paper (Whatman Ltd, Japan, Cat No: 70010004, D-47 mm) and the filter disk was dipped in the sterile Preston/Bolton broth which contained 90 mL of broth in 100 mL glass bottles. Bolton broth contained sample was incubated at 37 °C for 44 ± 4 h while Preston broth contained sample was kept in the Incubator (EYELA, Japan, SLI-1000D) at 37 ± 1 °C for 22 ± 2 h and then kept at 41.5 ± 1 °C for further 22 ± 2 h. After 44 ± 4 h of incubation, a loop (10 µL) of bacterial was streaked on modified blood-free charcoal cefoperazone deoxycholate agar (mCCDA) (LAB 112) with selective supplement (LAB M X112) and then kept in the anaerobic jar (TOMY, SEIKO. Co. Ltd., Tokyo, Japan) with campy pack, (CAMPYGEN, Oxoid, CN0035) incubated at 41.5 °C for 44 ± 4 h under micro-aerobic condition [25]. Suspected colonies of *Campylobacter* spp. were identified following colony appearances. Further identification of suspected colonies of *Campylobacter* spp. was performed by gram staining and oxidase test.

2.4. Serotype Identification-Serological Identification Method

For serotype identification, isolated *Salmonella* spp. samples were sent to WHO National *Salmonella* and *Shigella* Center, Thailand.

2.5. Statistical Analysis

Data set was processed using Minitab version 15 statistical software. Two-way ANOVA and Pearson correlation tests were carried out for microbiological parameters for both ground and surface water sampling locations.

2.6. GIS Thematic Mapping

Collected data on microbiological parameters in the entire Kelani river basin were stored in a geographic information system (GIS) database. These data were digitized with a Sri Lanka survey

department digital map created in 1999 at 1:50,000 scale. The inverse distance weighted interpolation (IDW) under spatial analyst tool in the ArcGIS 10.0 software was employed for the interpretation of data.

3. Results and Discussion

Water borne diseases due to various types of pathogenic bacteria, viruses and protozoa were recorded in many parts of the world and the majority of cases have been recorded from developing countries [26]. WHO and UNICEF (2014) reported that 4000 annual child deaths in some developing countries in the world due to consumption of contaminated surface and groundwater without proper treatment. Mahagamage and Manage [27] documented that the majority of respondents in head and transitional regions in the Kelani River Basin consumed spring water for their day to day activities such as drinking, bathing, cooking and washing. Specially, central highlands are rich in spring waters and most of the people who live in hilly regions depend on their own spring near homeland or public water schemes which supply water from spring sources. However, people live in the meandering region mainly depend on well water and treated-water, which is provided by National Water Supply and Drainage Board (NWSDB) where several intakes in the Kelani River Basin including Ambathale, Biyagama, Labugama tank and Kalatuwawa tank.

The results of the study revealed that almost all surface and groundwater samples collected were contaminated with total coliform and *E. coli* bacteria. In total, 83% of drinking groundwater sources recorded greater than 200 CFU (100 mL) of total coliform (TC) where 28% contaminated with *E. coli*, during the dry season (Figure 3) following 83% and 25% TC and *E. coli* contamination during wet season (Figure 4). It was detected that *E. coli* contamination in surface water was high during the wet season compare to dry season (Figure 4). Improper sanitary facilities, inadequate draining of wastewater and sewage, inefficient sewage and waste management, land use application of the fertilizer mix with sewage and contamination of groundwater due to toilet pits are major pathways of contamination of ground and surface water with faecal microorganisms in the Kelani River Basin.

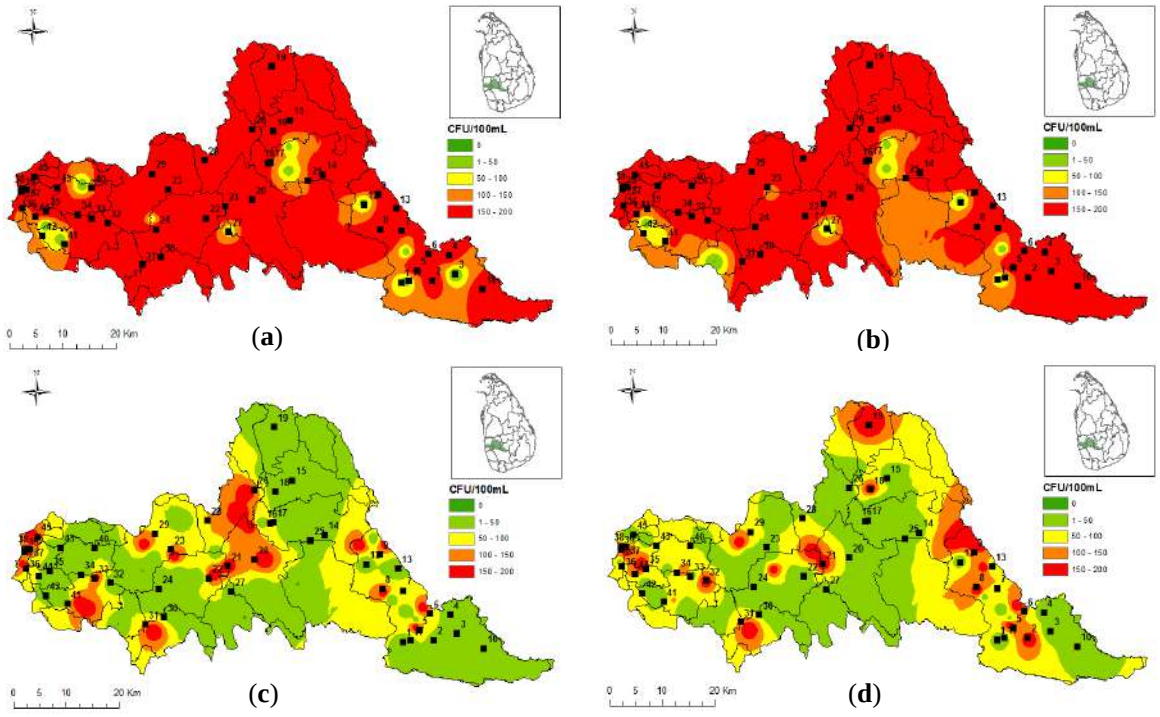

Figure 3. Spatial distribution pattern of total coliform and *E. coli* in groundwater of the Kelani River Basin (**a**) total coliform in dry period, (**b**) total coliform in wet period, (**c**) *E. coli* in dry period, (**d**) *E. coli* in wet period.

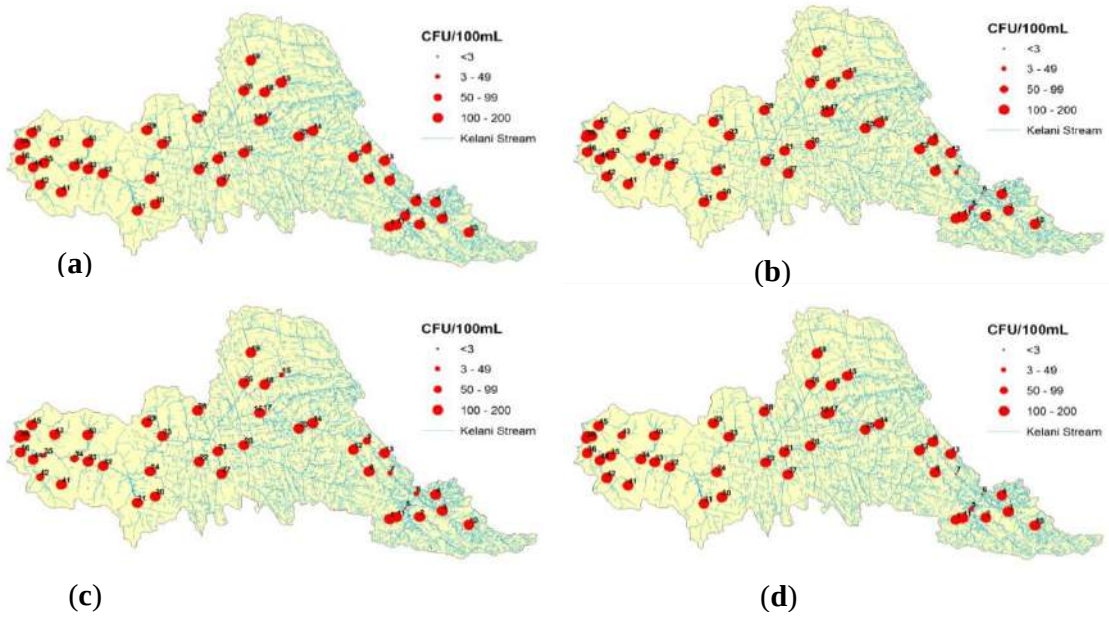

Figure 4. Spatial distribution pattern of total coliform and *E. coli* in surface water of the Kelani River Basin (**a**) total coliform in dry period, (**b**) total coliform in wet period, (**c**) *E. coli* in dry period, (**d**) *E. coli* in wet period.

Salmonella, *Campylobacter* and *Shigella* are common pathogenic bacteria find in the environment where sanitation is poor, since they are excreted by faeces of humans, farm animals and wild animals [26]. *Salmonella* cause salmonellosis, gastroenteritis, typhoid fever while *Campylobacter* cause Guillain–Barré syndrome and *Shigella* spp. causes shigellosis due to consumption of pathogenic contaminated water [19]. Numerous studies have shown that microbial pathogens, such as *Salmonella* and *E. coli* are relatively stable in groundwater [28–30] and surface water [31–34] and the contamination prevalence depends upon the vulnerability of water sources to anthropological activities such as bathing, washing and defecating [33,34]. The results of the study showed the occurrence of *Salmonella* and *Campylobacter* in some ground and surface water sources which may alarm possible root causes of susceptibility of gastrointestinal disease of people who consume contaminated water.

Among the other river basins in the Sri Lanka, the Kelani River Basin is the most urbanized and industrialized river basin and it has polluted by various point and non-point sources of pathogenic contaminates via municipal sewage and agricultural pollutants. Improper and poorly managed draining systems of wastewater and sewage into the environment and accidental sewage contamination of groundwater are the major pathways of contamination of aquifers by pathogenic or facultative pathogenic microorganisms [35]. In the present study, it was discovered that seventeen groundwater sampling locations were positive for *Salmonella* spp. where, two locations were positive for *Campylobacter* spp. (Table 3) and none of the locations were positive for *Shigella* contamination. Curiously, out of forty-five surface water sampling locations, twenty six sampling locations were contaminated with *Salmonella* spp. where only three locations were positive for *Campylobacter* spp. (Table 4). Further, it should be noted that 41% of the *Salmonella* spp. and *Campylobacter* spp. contaminated water sources are being directly used for drinking and other human consumption. Moreover, 23 types of different human pathogenic *Salmonella* serovars were isolated during the study and the *Salmonella enterica* serovar Kentucky was identified as the commonest. All the recorded serovars are human pathogens responsible to cause salmonellosis, gastroenteritis, typhoid fever and many more health impacts. Thus, awareness and provide safe drinking water is a must to safeguard people who consume such water sources in the river basin to prevent gastrointestinal diseases.

Table 3. Pathogenic bacteria in groundwater sampling locations in the Kelani River Basin.

Location	Dry Season	Wet Season
Maskeliya Ground		*Salmonella enterica* subsp.diarizonae str. 61 1v1,5,7
Koththellena 2		*Salmonella enterica* subsp.diarizonae str. 61 1v1,5,7
Ginigathhena	*Campylobacter* spp.	
Badupola		*Salmonella enterica* serovar Typhimurium
Bulathkohupitiya	*Salmonella enterica* serovar Weltevreden	
Kabulumulla		*Salmonella enterica* serovar Corvallis
Kahanavita	*Salmonella enterica serovar* Kentucky	
Kananpella	*Salmonella enterica serovar* Vancouver	
Levent	*Salmonella enterica* serovar Angoda	
Karawanaella	*Salmonella enterica* subsp *indica* str 6,14,25:a:enx/Salmonella enterica subsp. diarizonae Ser.61	
Hingurala	*Salmonella enterica* serovar Poona	*Salmonella enterica* serovar Newport
Vihara kumbura	*Salmonella enterica* serovar Waycross	
Kiridiwela-Delgoda		*Salmonella enterica* serovar Kentucky
Biyagama	*Salmonella enterica* serovar Weltevreden	
Kelaniya	*Campylobacter* spp.	
Moragahahena	*Salmonella enterica* serovar Kentucky	
Arangala	*Salmonella enterica* serovar Mbandaka	
Nawagampura	*Salmonella enterica* serovar Typhimurium	
Bangalawaththa	*Salmonella enterica* subsp indica str 6,14,25:a:enx	

Table 4. Pathogenic bacteria in surface water sampling locations in the Kelani River Basin.

Location	Dry Season	Wet Season
Nallathanniya	*Salmonella enterica* serovar Kentucky	
Kandura (Koththellena)	*Salmonella enterica* subsp. *diarizonae* Ser.61	*Salmonella enterica* subsp. *diarizonae* str. 61 z52z53
Tinsil river	*Salmonella enterica* serovar Javiana	
Bokarabevila river		*Salmonella enterica* subsp *enterica* ser 9, [12]:-:1,5
Vidulipura		*Salmonella enterica* serovar Typhimurium
Gonagamuwa river	*Salmonella enterica* serovar Weltevreden	*Salmonella enterica* serovar Typhimurium
Bulathkohupitiya river	*Campylobacter* spp.	
Ruwanwella	*Salmonella enterica* serovar Bareilly	
Thalduwa		*Salmonella enterica* serovar Kentucky
Aswaththa	*Salmonella enterica* serovar Typhimurium	*Salmonella enterica* serovar Kentucky
Poogoda	*Campylobacter* spp.	
Kaluaggala		*Salmonella enterica* serovar Javiana
Thaligama river		*Salmonella enterica* serovar Paratyphi B Variety Java
Getaheththa river	*Salmonella enterica* serovar Kentucky	
Amthirigala		*Salmonella enterica* serovar Bredeney
Pugada river	*Salmonella enterica* serovar Paratyphi B Variety Java	*Salmonella enterica* serovar Durban
Wewelpanawa		*Salmonella enterica* serovar Angola
Pussella oya		*Salmonella enterica* serovar Paratyphi B Variety Java
Ranala river		*Salmonella enterica* serovar Mount Pleasant

Table 4. *Cont.*

Location	Dry Season	Wet Season
Raggahawaththa ela		*Salmonella enterica* serovar Typhimurium
Kelanithissa	*Salmonella enterica* serovar Stanley	*Salmonella enterica* serovar Agona
Dutch canal		*Salmonella enterica* serovar Enteritidis
Hemilton canal	*Campylobacter* spp.	*Campylobacter* spp.
Meegawaththa-Delgoda	*Salmonella enterica* serovar Newport	*Salmonella enterica* serovar Weltevreden/ *Salmonella enterica* serovar Paratyphi B Variety Java
Thalangama lake	*Salmonella enterica* serovar Manchester	*Salmonella enterica* serovar Typhimurium
Diyawanna oya	*Salmonella enterica* serovar Litchfield	
Mahara	*Salmonella enterica* subsp *enterica* ser 4, [5], 12:b *Salmonella enterica* subsp *enterica* ser 9, [12]:-:1,5	
Wellampitiya	*Salmonella enterica* serovar Kentucky	
Muthuraja ela	*Salmonella enterica* serovar Kentucky	*Salmonella enterica* serovar Kentucky/*Campylobacter* spp.

The occurrence of *Salmonella enterica* serovar Kentucky in the environment is rare [36]. However, in the present study, three groundwater sampling locations were positive for *Salmonella* serovar Kentucky in both dry and wet seasons (Table 3). Barua et al. [37], Fashae and Hendriksen [38] and Afema et al. [39] isolated *Salmonella enterica* serovar Kentucky from farm animals. Therefore, the leachate of farm effluent to the river basin might be a possible point source of such pathogens. In the present study, Moragahahena well, which is situated close to poultry and swine farm positive for *Salmonella enterica* serovar Kentucky. In surface water, four sampling locations namely, Nallathanniya, Getahetthta tributary, Wellampitiya and Kalu oya were positive for *Salmonella enterica* serovar Kentucky during dry season (Table 4). Further, it was found that Nallathanniya location was highly contaminated during the Sri pada season (religious festival season from December to May) due to poor sanitary conditions provided for pilgrims during the Sri-pada season [40]. Wellampitiya (Kiththampahuwa oya) and Kalu oya are the major drainage canals connected with KRB and these sampling locations were located in urban and industrial part of the basin. These drainage canals are open for sewage canals from some areas around. Hellole et al. [41] documented that *Salmonella enterica* serovar Kentucky contamination occurs through septic or direct connect of toilets into the main river. During the wet season, three sampling locations were positive for *Salmonella enterica* serovar Kentucky (Table 4) and out of three locations, Thalduwa (Seethawaka oya) and Eswaththa oya were used for bathing and washing purposes. Therefore, awareness and preventive measures should be taken to avoid pathogenic contamination in the KRB.

In the last two decades, *Salmonella enterica* serovar Weltevreden has appeared as a dominant foodborne pathogen globally; especially in South-East Asian countries, being increasingly isolated from water, vegetables, meat and seafoods [42–44]. In the present study, *Salmonella enterica* serovar Weltevreden was detected in two groundwater sampling locations (Table 3). Dias et al. [45] reported that coliform bacterial contamination was high in surface water of the head region of the Kelani River Basin due to human settlement with lower sanitary facilities. Alagal Oya (Gonagamuwa) location situated in the head region of the river basin which was heavily used for recreational purposes and contamination of *Salmonella* is possible (Table 4).

Ezekwe et al. [46] stated that groundwater sources in Nigeria used for drinking purposes are extremely polluted, especially in urban cities due to on-site sanitation systems such as septic tanks, pits and bucket latrines. The present study recorded *S. enterica* serotype Poona from groundwater sampling location located in Hingurala nearby toilet pits during the dry season (Table 3). Further, it was found that *S. enterica* serovar Newport during the wet season and this serovar has been recorded as a common causative agent for human salmonellosis in the United States and Europe [47]. Some studies

have reported that the development of multidrug-resistant against *Salmonella* serotype Newport is spreading on an epidemic scale in both animals and humans [48] (Table 3).

Salmonella enterica serovar Typhimurium, are host generalists that occur in humans and many other mammalian species [49]. In the present study, Nawagampura and Badupola groundwater sampling locations were positive for pathogenic *Salmonella enterica* serovar Typhimurium (Table 3). Rop [50] documented that the causes of anthropological activities such as bathing and washing mainly contribute to the dispersal of pathogenic bacteria. During the study it was found that public and unprotected wells used for domestic consumption were polluted with *Salmonella* spp. Søborg et al. [51] reported that roadside soil can be contaminated with *Salmonella* spp. and it is possible to leach contaminated water into wells. Badupola groundwater location is being used for drinking purposes which is located close to the roadside and it was possible to get runoff water through the road to contaminate the groundwater. During the dry season of the study, *Salmonella enterica* serovar Typhimurium was isolated from four locations of the surface water (Table 4) and Vidulipura tributary which flows through estate sector is vulnerable to receive polluted water by sewage and toilet pits in the area due to poor sanitation facilities in the area. Further, Polo et al. [52] recorded that contamination of pathogenic bacteria may occur in surface water due to human activities. In the study, it was found that the Alagal Oya and Eswaththa oya are contaminated with *Salmonella enterica* serovar Typhimurium and such tributaries are being used for bathing and washing purposes. Raggahawaththa oya was highly polluted tributary in the river basin which flows through Biyagama industrial zone and the water source was contaminated with *Salmonella enterica* serovar Typhimurium. As per mentioned by Baudart et al. [53] *Salmonella enterica* serovar Typhimurium can tolerate a wide range of physico-chemical ranges in effluent water.

The genus *Salmonella* consists of two species, *S. enterica*, which is divided into six subspecies [54] and *S. enterica* subsp. *diarizonae* is one of the sub species whose occurrence in the environmental samples and outbreaks due to the species was less than other *Salmonella* spp. However, *S. enterica* subsp. *diarizonae* are naturally found in reptiles [55]. The results of the present study showed that some sampling locations were positive for this *Salmonella* spp. (Table 3). It was found that the Kandura (Koththellena) sampling location which was highly used for bathing was positive for *S. enterica* subsp. *diarizonae* during dry and wet seasons (Table 4).

Salmonella enterica serovar Angoda is a new *Salmonella* spp. recorded from Sri Lanka by Gulasekharam [56]. Thereafter, this species was reported in some locations of the world from different outbreaks [57]. In the present study, Levent location situated in the head region was positive for *Salmonella enterica* serovar Angoda (Table 3). Abrahams et al. [58] and Waithaka [59] described that high numbers of *Salmonella* were detected from the intestine of the ruminants and this may be a fact for contamination in Lavent location which was a rubber cultivating area with goats.

Nordmann et al. [60] documented of a *Salmonella enterica* serovar Waycross outbreak from Australia and a patient had a urinary tract infection in several years due to a *Salmonella* species. *Salmonella enterica* serovar Waycross is not a common bacterium for food and waterborne diseases and it was recorded from a Viharakumbura sampling location in the present study (Table 3).

Salmonella enterica subsp. enterica serovar Mbandaka was firstly isolated from human salmonellosis in the Congo in 1948 and it was ranked amongst the 20 most frequent serovars in humans in European countries and continents [61]. In the present study, Arangala location was positive for *Salmonella* Mbandaka (Table 3).

Dolman et al. [62] discovered the *Salmonella enterica serovar* Vancouver from the patient with high fever, vomiting and diarrhea and a few outbreaks were recorded worldwide. In the present study same species were recorded at Kannampella sampling location which was used as a public well and bacterial contamination is possible due to bathing and washing activities (Table 3).

Non-typhoidal *Salmonella* serovars are increasing in importance as significant pathogens of both humans and animals. Ben and AL-Gallas [63] documented that most commonly isolated *Salmonella* in Tunisia was *Salmonella enterica* serovars Corvallis. During the present study, *Salmonella enterica* serovars

Corvallis was recorded from a Kambulumulla groundwater sampling location (Table 3) which is used for drinking purposes.

Moreover, enteric fever remains to be a public health problem in many parts of the world, especially in the developing countries including Sri Lanka. Enteric fever (i.e., Typhoid and paratyphoid fever) is a systemic disease resulting from infection with *Salmonella enterica* subsp. *enterica* serotype Typhi and *Salmonella enterica* subsp. *enterica* serotype Paratyphi A, B or C. Further, enteric fever is typically caused by consumption of food or water that has been contaminated by these pathogens and asymptomatic carriers, particularly food handlers who are the major source of these organisms [64]. Recent reports have documented that increased incidence of *Salmonella enterica* subsp. *enterica* Paratyphi B in Canada—Stratton et al. [65], Germany—Miko et al. [66], Italy—Mammina et al. [67] and Malaysia-Goh et al. [68]. Levings et al. [69] and Hernández et al. [70] recorded that fish and reptiles are a source of contamination of *Salmonella enterica* serovar Paratyphi B. However, *Salmonella enterica* serovar Paratyphi B Variety Java is a non-typhoidal strain of *Salmonella*, which causes gastroenteritis was recorded from four sampling locations (Pugoda river, Kelani River (Thaligama), Pusselli oya and Raggahawatta oya) during the study period.

Salmonella enterica serovar Bareilly was initially identified from India in 1928 [71] and it is known for its wide host range [72]. Some strains are clonal and can be detected in numerous sites throughout Southeast Asia and are given potential reservoirs associated with *Salmonella* Bareilly that include reptiles and other environmental sources [73]. Hoffmann et al. [74] documented that Sri Lanka is contaminated by this particular *Salmonella* serovar from India or Pakistan and imported food from other South Asian countries. The Gurugoda oya location was positive for *Salmonella* Bareilly and it is used for recreational, irrigation and livestock purposes (Table 4). As per mentioned by WHO [75] water bodies used for irrigation and livestock purposes can easily be contaminated with *Salmonella* spp.

Sampling locations of Thalangama Lake and Diyawanna oya where stagnant water bodies directly receive several household drainage systems and agricultural effluent (Table 4). It was found that Diyawanna oya location was positive for *Salmonella* Litchfield during the dry season and Thalangama Lake was positive for *Salmonella* Manchester (Table 4). Polo et al. [52] documented that *Salmonella* is commonly present in sewage effluent that can contaminate recreational waters.

Keselgamu oya (Tientsin) was positive for *Salmonella enterica* subsp. *enterica* serovar Javiana (Table 4) and it is one of the top five most common serotypes of *Salmonella* and is a common food-borne pathogen [76].

Salmonella enterica serovar Stanley (S. Stanley) is a common serovar in Southeast Asia and in contrast, this serovar is relatively uncommon in Europe [77]. Due to international travel, human migration and food and livestock trade, *Salmonella* serovar Stanley was the second most common serovar in Thailand, accounting for 11% of all human salmonellosis cases from 2002 to 2007 [78]. The results of the study revealed that Sebastian canal (Kelanithissa) was positive for S. Stanley during the dry season of river basin (Table 4).

Salmonella enterica subsp indica is usually found in poikilotherms (including reptiles, amphibians and fish) and in the environment. Some of these organisms are occasionally associated with human diseases [79]. Interestingly, limited information is recorded for this *Salmonella* spp. and only Bangalawaththa sampling point was positive for *Salmonella enterica* subsp indica (Table 3).

Raggahawaththa oya (Meegawaththa) was positive for *Salmonella* Newport, *Salmonella enterica* serovar Weltevreden and *Salmonella enterica* serovar Paratyphi B Variety Java (Table 4). Further, Mahara and Sebastian canal sampling locations were positive for *Salmonella* spp. during the study period (Table 4). WHO [75] reported that the industrial and drainage water contains *Salmonella* spp. and it is possible to pollute other water sources of the environment. In the Sri Lankan context, most of the drainage canals were directly connected with some sewage canals from industries and commercial places. Therefore, the occurrence of pathogenic *Salmonella* is possible in surface water sources.

Campylobacter spp. has obtained considerable attention in the recent years as a significant cause of bacterial enteritis in humans. During the last couple of decades, *Campylobacter jejuni* and

Campylobacter coli have been recognized as a common cause of gastroenteritis worldwide. Kelaniya and Ginigathhena groundwater sampling locations were positive for *Campylobacter* spp. during dry season (Table 3). Further, three surface water sampling locations were positive during the dry season and two were positive in wet season, including Hamilton canal which is a major drainage connect with several sewage canals (Table 4). However, Premarathne et al. [80] recorded that the favored environmental place for *Campylobacter* spp. is to be the intestinal tract of most of birds and mammals; Ugarte-Ruiz et al. [81] recorded that survival of *Campylobacter* spp. in surface water for a higher recovery of the organism in wide range of water quality changes. Medema et al. [82] documented similar results on *Campylobacter* spp. contamination in waters and reported contamination may have caused due to animals and faecal contamination of the catchment area with drainage directed into water sources.

However, it should highlight that no *Shigella* spp. positive sampling locations were recorded for both the ground and surface water in the Kelani River Basin during the study period.

According to Pearson correlation, a significant correlation was observed in TC and *E. coli* with *Salmonella* spp. Further, it was revealed that negative correlation of TC and *E. coli* with *Pseudomonas* spp. (Table 5). Two-way ANOVA revealed that there was no significant difference ($p > 0.05$) in two seasons for all microbial parameters and only TC and *E. coli* showed significant difference ($p < 0.05$) with groundwater sampling locations. In addition, Two-way ANOVA for surface water revealed that there was significant difference in two seasons for *E. coli* where *Pseudomonas* spp. *Cam1ylobacter* sp. and *E. coli* showed significant differences with surface water sampling locations.

Table 5. Pearson correlation values for microbial parameters.

	TC	***E. coli***	***Pseudomonas* spp.**	***Salmonella* spp.**
E. coli	0.390			
	0.000			
Pseudomonas spp.	−0.374	−0.460		
	0.000	0.000		
Salmonella spp.	0.146	0.340	−0.111	
	0.025	0.000	0.091	
Cam1ylobacter spp.	0.063	0.075	−0.016	0.082
	0.336	0.256	0.810	0.209

Cell Contents: Pearson correlation. *p*-Value.

4. Conclusions

The entire Kelani River Basin was contaminated with total coliform and *E. coli* bacteria and almost all the sampling locations exceed the standard value 0 CFU/100 mL) given by the SLS guideline for drinking water. High density of human population and poor sanitary conditions were identified as a major reason for contamination of high total coliform and *E. coli* bacteria in surface and groundwater of the head and meandering regions of the river basin. Identification of human pathogenic bacteria showed that seventeen locations of groundwater sources were positive for *Salmonella* spp. and only two locations were positive for *Campylobacter* spp. In surface water, twenty-six sampling locations were positive for *Salmonella* spp. and three sampling locations were positive for *Campylobacter* spp. It was found that, *Salmonella* spp. contamination in surface water was high during the wet season (42%) than the dry season (33%). In groundwater, *Salmonella* spp. contamination was higher during the dry season (17%) than the wet season (8%). Further, 23 types of different serovars were isolated during the study period where *Salmonella enterica* serovar Kentucky was the most common and all the serovars recorded were human pathogenic species which cause salmonellosis, gastroenteritis and typhoid fever. Interestingly, *Shigella* spp. was not recorded in either dry or wet season during the study period in both ground and surface water. The result of the study revealed that people and stakeholders within the premises of the river basin should be aware of both ground and surface water quality of the river basin.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/8/2187/s1, Table S1 Pathogenic bacteria in groundwater sampling locations in the Kelani river basin (Dry season); Table S2 Pathogenic bacteria in groundwater sampling locations in the Kelani river basin (Dry season); Table S3 Pathogenic bacteria in groundwater sampling locations in the Kelani river basin (Wet season); Table S4 Pathogenic bacteria in groundwater sampling locations in the Kelani river basin (Wet season); Table S5 Pathogenic bacteria in surface water sampling locations in the Kelani river basin (Dry season); Table S6 Pathogenic bacteria in surface water sampling locations in the Kelani river basin (wet season).

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