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RESEARCH ARTICLE

Microbiology

Recreational water quality in the river Mahaweli, Sri Lanka: Enumeration and antibiotic sensitivity of *Escherichia coli* and sociological aspects

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Abstract: Although recreational water quality affects health, in Sri Lanka, recreational water quality is hardly assessed. Faecal contamination of water poses health risks as faeces are a source of pathogens. The current study aimed to investigate *Escherichia coli* in recreational water by enumerating and assessing antibiotic sensitivity, and to conduct a sociological survey. Water samples (n = 72) were collected from six bathing locations along the river Mahaweli during rainy and dry periods, and subjected to membrane filtration. Typical blue colonies on m-FC agar were enumerated. An antibiotic sensitivity test was performed on *E. coli* isolates using seven antibiotics commonly used in Sri Lanka. *Escherichia coli* counts in 95.0% of water samples exceeded the permissible limit (235 CFU/100 mL). Mean *E. coli* counts were significantly higher ($p < 0.05$) during the rainy period compared to the dry period. In both periods, mean counts were significantly higher ($p < 0.05$) in more populated locations than less populated locations. Antibiotic-resistant *E. coli* were present in varying degrees in all locations. Isolates were resistant to ciprofloxacin (56.5%), cefotaxime (4.8%), amoxicillin-clavulanate (4.2%), amikacin (3.0%), and ticarcillin-clavulanate (0.6%). None were resistant to imipenem or meropenem. The percentages of isolates resistant to amikacin, amoxicillin-clavulanate, and ciprofloxacin were significantly higher in less populated locations compared to more populated locations ($p < 0.05$), while the percentage for cefotaxime was higher in more populated locations. According to the sociological survey (n = 60), inadequate sewage facilities and urban runoff are likely reasons of pollution. Elevated *E. coli* counts and the presence of antibiotic-resistant *E. coli* render the water unsuitable for recreational purposes.

Keywords: Antibiotic resistance, bathing water, faecal contamination, faecal indicator organisms, river water.

INTRODUCTION

In terms of water quality, recreational water includes water of rivers, lakes, reservoirs etc., that is used for bathing, swimming, fishing, boating, whitewater rafting, and surfing (US EPA, 2022). The use of recreational water has significant benefits for health and well-being, including relaxation, rest, cultural and religious practices, exercise, and aesthetic pleasure (Crouse *et al*., 2018; White *et al*., 2020). However, pathogen-contaminated recreational water environments can pose a significant risk to the health of water users. Routes of exposure to infectious agents in water depend on the degree of water contact. The probability of ingestion of water is greater for whole-body contact activities including swimming, surfing, diving etc. Microbial pathogens are the most common public health hazard in recreational water (WHO, 2021). Faecal contamination of water may introduce pathogenic microorganisms that are mainly derived from human sewage or excreta from other warm-blooded animals (WHO, 2021).

The sources of faecal contamination include point sources and non-point or diffuse sources. Point sources

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are contaminants that enter a waterway from a single, identifiable source. Non-point sources refer to diffuse contamination that does not originate from a single discrete source (Soni, 2019). Point sources include effluents from wastewater treatment plants, discharges of raw wastewater, discharges of industrial effluents, direct contamination from recreational water users, discharges of urban stormwater, and combined sewer overflow which contains a mixture of urban runoff water and wastewater (Brauwere *et al*., 2014; WHO, 2021). Urban runoff is a key diffuse source that contaminates river water (Müller *et al*., 2020).

Recreational water use can lead to a number of health problems, including gastrointestinal illness, and also non-enteric diseases caused by respiratory, ear, eye, and skin infections (Fleisher *et al*., 1993; Kay *et al*., 1994; Fewtrell & Kay, 2015). The waterborne disease outbreaks associated with enteric illness are attributed to microbial agents including bacteria (*Escherichia coli, Shigella* spp., *Vibrio cholerae*, *Salmonella* spp., *Yersinia enterocolitica* and *Campylobacter jejuni*), viruses (Enterovirus, Rotavirus and Adenovirus), and protozoa (*Giardia lamblia, Cryptosporidium parvum,* and *Entamoeba histolytica*) (Fewtrell & Kay, 2015; Neil *et al*., 2018; Lugo *et al*., 2021). Worldwide, waterborne gastrointestinal illnesses account for an estimated 16.4 million cases annually. The number of enteric disease outbreaks associated with recreational water that is caused by pathogens transmitted by ingesting, or by having contact with contaminated water, has considerably increased (Neil *et al*., 2018). Therefore, it is necessary to assess the microbiological quality of recreational water to ensure that no health risk due to pathogenic microorganisms is associated with its use. Since it is impossible to measure the abundance of all possible pathogenic microorganisms, the general practice is to quantify the abundance of only one or a few faecal indicator bacteria (FIB), *i.e*., organisms that are selected to be indicative of faecal pollution and can therefore serve as general indicators of microbiological water quality (Brauwere *et al*., 2014). Total coliforms, faecal coliforms, Enterococci, and *E. coli* are used as faecal indicators (Ishii & Sadowsky, 2008; Odonkor & Ampofo, 2013).

An ideal indicator bacterium should be present in the intestinal tracts of warm-blooded animals. Besides, indicators should be present in faeces, sewage, and sewage-polluted waters whenever the pathogens are also present, and absent in waters without faecal pollution. The levels of indicators should have some direct relationship to the density of pathogens in the environment and be proportional to the extent of the pollution. Furthermore, an indicator organism should be non-pathogenic and present in greater numbers than pathogens. The survival rate in water must be at least similar to that of pathogens and it should be unable to grow and multiply in the environment. Further, an indicator bacterium should be detected and quantified by rapid, easy, and inexpensive methods (Borrego & Figueras, 1997; Havelaar *et al*., 2001; Ishii & Sadowsky, 2008; Lugo *et al*., 2021). *Escherichia coli* meets most of these criteria and is widely used as an indicator organism for monitoring faecal contamination of recreational freshwater ecosystems (Odonkor & Ampofo, 2013; Rossi *et al*., 2020). For recreational activities, *E. coli* levels should not exceed 235 CFU/100 mL of water for full-body contact (US EPA, 2012).

Antibiotics are compounds that are intended to treat bacterial infections (Calhoun *et al*., 2022). Antibiotics can be classified in several ways based on their mode of action, molecular structures and spectrum of activity, *i.e.*, β-lactams, quinolones, sulphonamides, macrolides, and tetracyclines (Kümmerer, 2009; Etebu & Arikekpar, 2016).

Antibiotic resistance is the ability of bacteria to resist the effects of an antibiotic to which they were previously sensitive. Infections with bacteria that express antibiotic resistance genes can be difficult or even impossible to treat, posing a serious threat to public health (Amarasiri *et al*., 2020). High mortality rates are associated with multidrug-resistant bacterial infections (Wu *et al*., 2021). After using antibiotics, a large proportion of the antibiotics that are absorbed are excreted into the environment by faeces or urine in non-metabolized forms or coupled with polar molecules. Therefore, antibiotics and their degradation products are considered emergent contaminants of water (Oberlé *et al*., 2012), and may lead to the development of resistance in target and non-target organisms in the environment (Kümmerer, 2009). The leftover antibiotics are disposed of and become part of urban waste. These also can end up in river water through urban runoff or leachate from solid waste dump sites (Wimalasuriya *et al*., 2011).

In veterinary clinical practice, antibiotics are widely used to treat a variety of bacterial infections. However, the use and improper use of antibiotics in animal husbandry, and agriculture has led to the emergence of several drug-resistant bacterial communities (Economou & Gousia, 2015; Ranasinghe *et al*., 2022). The runoff from livestock fields into the river may lead to the spread of antibiotic-resistant bacteria in the river water.

Regardless of the multi-faceted health hazards, unlike developed countries, in Sri Lanka, people are generally ignorant about faecal contamination of recreational water. The current study aimed to enumerate *E. coli* in water at selected bathing locations of the river Mahaweli and assess the sensitivity of isolates to selected antibiotics that are of common clinical use in Sri Lanka. As a part of this study, a sociological survey was conducted to gather information on water quality and implications of contamination at the selected bathing locations.

MATERIALS AND METHODS

Study area and sample collection

Water samples were collected from six bathing locations in the river Mahaweli from Peradeniya to Katugastota, Sri Lanka, during rainy and dry periods (Figure 1). Each sample was 200 mL and was collected from the surface of the river, at each bathing location. Six replicate water samples were collected per location per period. In total, 72 water samples were collected.

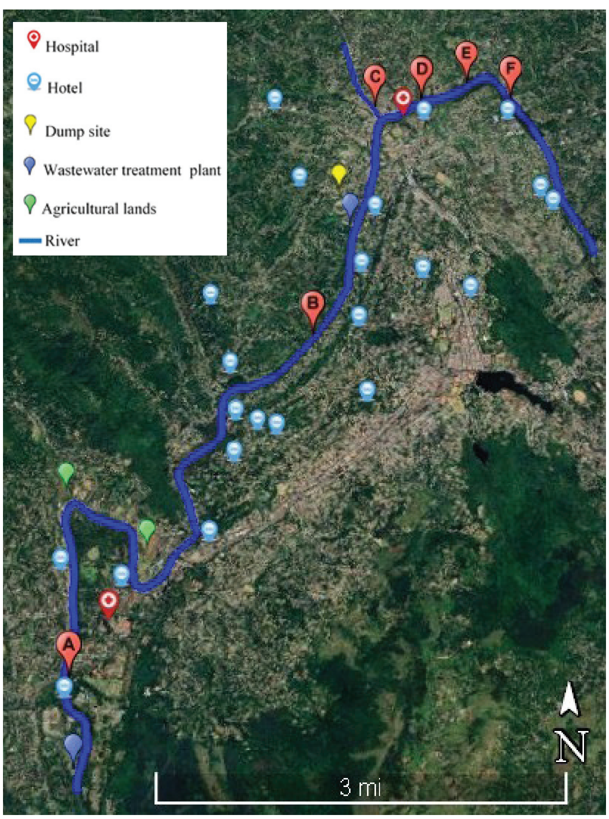

Figure 1: Selected bathing locations along the river Mahaweli between Peradeniya and Katugastota, and possible sources of water contamination. A - Devil's Bridge, B - Dodamwala, C- Ranawana Road, D - Madawala Road 1, E- Madawala Road 2, F - Madawala Road 3.

The six bathing locations were classified according to proximity to a town area, as defined by a population density of ≥ 2000, which was considered as the level of urbanization. Accordingly, locations A and B were in less-populated areas whereas locations C, D, E and F were in more-populated areas.

Isolation and enumeration of *Escherichia coli*

Water samples were analyzed using the membrane filtration apparatus (Rocker, Taiwan). The samples were diluted where necessary, to ensure the colonies were within the countable range. A 100 mL aliquot of each water sample was separately filtered through a sterile, 0.45 µm pore-sized, gridded membrane filter paper (Aisimo Corporation) using a vacuum filtration system. After each filtration, the membrane filter paper was placed on m-FC agar (HIMEDIA) and incubated for 24 - 48 h at 44.5 °C. An elevated temperature of 44.5 °C was used to inhibit the growth of other fecal coliforms and promote the growth specifically of *E. coli*. After incubation, the typical blue colonies grown on the plate were counted and reported as colony forming units (CFU). The colonies that had the typical blue colour indicative of *E. coli* were taken as *E. coli* and were confirmed by using eosin methylene blue (EMB) agar (HIMEDIA) with which *E. coli* produces dark colonies with a metallic green sheen, MacConkey agar (HIMEDIA) with which *E. coli*, being lactose-positive, produces pink colonies, and Simmons citrate agar (HIMEDIA) where the colour of the medium remains green as *E. coli* is citrate-negative.

Escherichia coli colonies had the characteristic metallic green sheen on EMB agar and colonies on MacConkey agar appeared pink. When inoculated with *E. coli*, the green color of Simmons citrate agar remained green, producing no color change. The *E. coli* colonies were used to prepare stock cultures, which were preserved at -80 °C, to be used for antibiotic sensitivity testing.

Antibiotic sensitivity test

Quality control test

Quality control tests were performed according to the guidelines specified by the Clinical and Laboratory Standards Institute (CLSI, 2020). Disks impregnated with seven antibiotics which are clinically used in Sri Lanka were employed; amikacin (30 µg), amoxicillin-clavulanate (20/10 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), and ticarcillin-clavulanate (75/10 µg). A five-day quality control test was performed continuously, for two reference strains of *E. coli* (ATCC 35218 and ATCC 25922), to ensure that the disks function well. *Escherichia coli* ATCC 35218 was used for quality control of amoxicillin-clavulanate (20/10 µg) and ticarcillin-clavulanate (85 µg). *Escherichia coli* ATCC 25922 was used for quality control of amikacin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), and meropenem (10 µg). The diameters of zones of inhibition obtained were checked against the standard quality control ranges (CLSI, 2020).

Antibiotic sensitivity test for Escherichia coli isolates obtained from water

Isolated, pure colonies of *E. coli* obtained by sub-culturing from the previously prepared freezer cultures were transferred separately to sterile normal saline (0.85% NaCl). The inoculum density was standardized by making the turbidity of the suspensions similar to that of 0.5 McFarland standard. The inocula thus prepared were used to inoculate Mueller Hinton agar (MHA) (HIMEDIA) plates.

Antibiotic disks were equidistantly placed on MHA plates. Two replicates were prepared for each *E. coli* isolate. The plates were incubated at 35 ^{o}C for 18 h and the diameter of the zone of inhibition was measured for each antibiotic. Two perpendicular measurements were taken for each zone of inhibition, and the values were averaged. Among the 72 water samples (n = 36 in dry period, n = 36 for rainy period), one *E. coli* isolate per sample was subjected to antibiotic testing. Accordingly, 72 *E. coli* isolates were subjected to antibiotic sensitivity testing, in duplicate and antibiotic susceptibility / resistance was ascertained in comparison with the zone diameter breakpoints given under the CLSI guidelines (CLSI, 2020).

Sociological survey

A questionnaire comprising multiple choice questions and closed questions (yes/no) was designed to gather information on the water quality of the six selected bathing locations. The questionnaire was prepared in Sinhala, Tamil, and English, to cater to the proficiency of the respondents. A Google form-based pre-test was done. The questionnaire was distributed among the target group (n = 60 in total; n = 10 per location), among the general public near the selected bathing locations.

Statistical analysis

Minitab version 21.2 was used for analyzing the data. The *E. coli* counts obtained for the bathing water samples collected in the dry and rainy periods were analyzed using parametric tests. A previous set of data collected during the year 2020-2021 was analyzed to explore any changes in the trends of *E. coli* counts with time. Differences at $p \leq 0.05$ (95% significance level) were considered statistically significant, whereas differences at $p > 0.05$ were considered statistically not significant. The one-way analysis of variance (one-way ANOVA) test was used in determining differences in mean counts between selected locations. The two-way analysis of variance (two-way ANOVA) test was used to study the interaction effect in mean counts due to the location and the period. The Mann-Whitney test was used to determine the differences in median counts between the dry and rainy periods in selected locations, while the two sample t-test was used in determining the differences in mean counts between more populated and less populated locations.

The two-proportion test was used in determining the differences in the percentage of resistant *E. coli* isolates for each antibiotic between the rainy and dry periods and also to determine the differences in the percentage of resistant *E. coli* isolates for each antibiotic between the more populated and less populated locations.

RESULTS AND DISCUSSION

Enumeration of *Escherichia coli*

The *E. coli* counts in 95.0% of the bathing water samples exceeded the permissible limit of 235 CFU/100 mL (US EPA, 2012) accepted for recreational water. Counts of *E. coli* were higher in the rainy period compared to those in the dry period in locations A, B, C, D, and E, while counts were higher during the dry period in location F. An overall increase in *E. coli* counts was observed in both the dry and rainy periods of the year 2022, compared to 2020-2021, in selected locations (Figures 2 and 3). It could be due to increased effluents from wastewater treatment plants, discharges of industrial effluents and urban runoff compared to the past. Due to the COVID-19 pandemic in Sri Lanka, people were under a lockdown at home during the period from 2020 – 2021, reducing the amount of urban waste production and resultant contamination of water, compared to 2022, when community activities were restored.

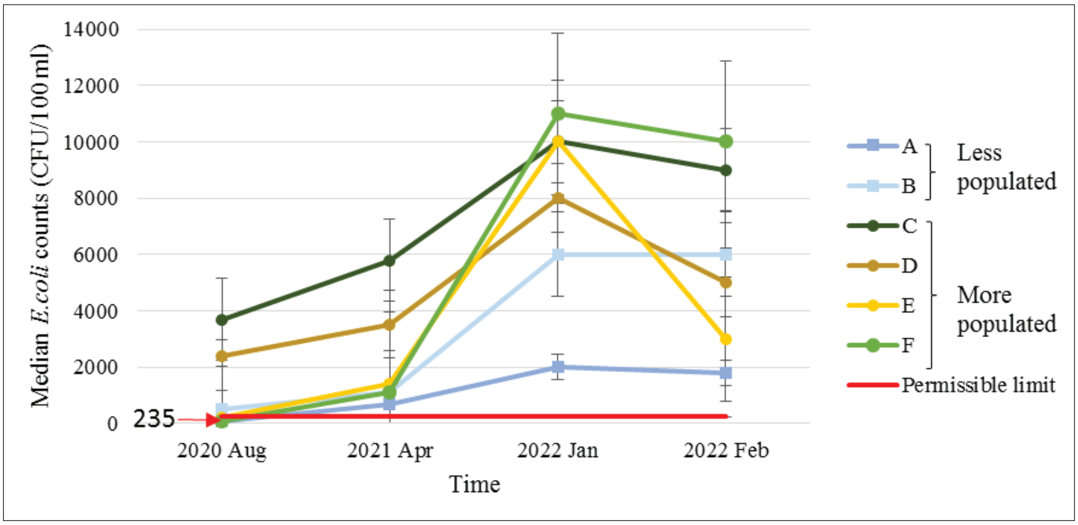

Figure 2: *Escherichia coli* counts in the dry period from the year 2020 - 2022.

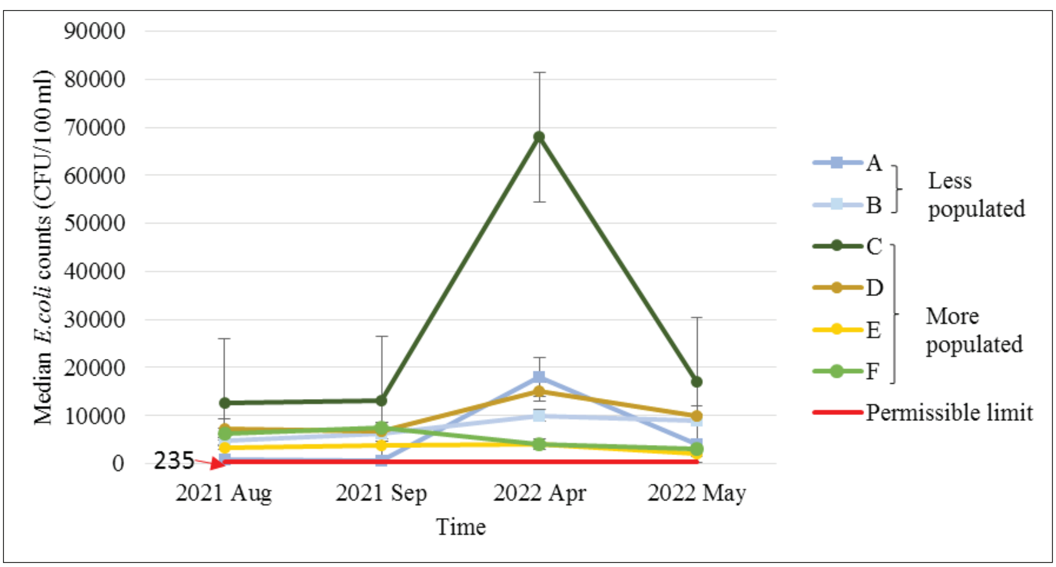

Figure 3: *Escherichia coli* counts in the rainy period from the year 2021 - 2022.

Two of the more populated locations (C and D) had higher counts during the rainy period. It can be speculated that urban runoff may be high during rain. The lower counts in locations E and F in the rainy period may be due to water being diluted by increased volume and flow rate of water during rain. Water tends to stagnate during the dry period and this might be accountable for the higher counts observed in the dry period for location F. Location B is of particular interest as it is located close to a municipal solid waste dump site. The *E. coli* counts observed in this location could be attributed to the leachate from the dump site.

According to the mean data (Figure 4), location C showed significantly higher values (p = 0.001) for the rainy period compared to all other locations, while having the highest value for the dry period as well.

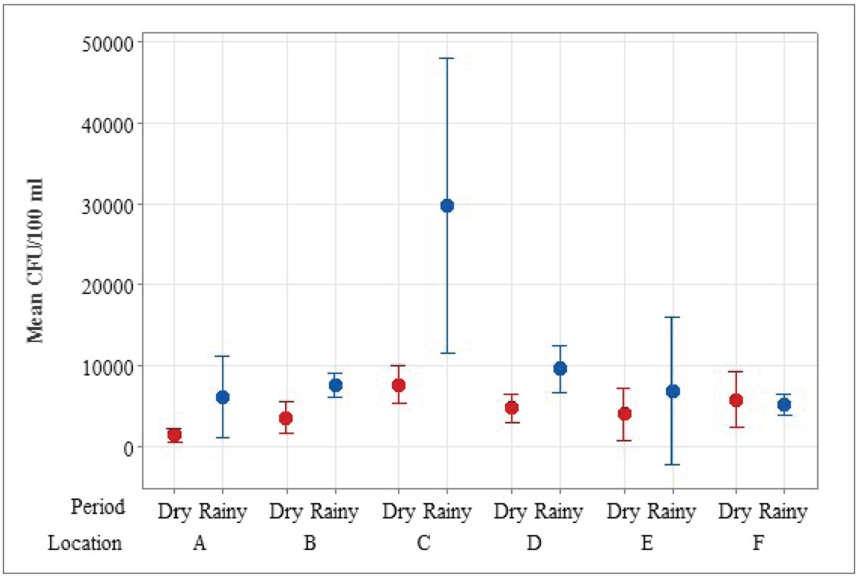

Figure 4: *Escherichia coli* counts in dry and rainy periods in different locations.

Location C lies in close proximity to a hospital. According to the survey, hospital waste is a possible source of river water contamination in this location. Further, it was revealed that toilet pits are directly drained into the river, at or near location C. These may be the reasons for elevated *E. coli* counts in location C. Mean *E. coli* counts in both more populated and less populated locations showed significantly higher values (more populated: $p = 0.012$; less populated: $p = 0.002$) for the rainy period compared to the dry period. In turn, when locations are compared, in both the rainy and dry periods, mean counts were significantly higher in more populated locations than less populated locations (rainy period: $p = 0.047$; dry period: $p < 0.001$). All in all, there was a significant difference in mean counts due to location ($p = 0.001$) and there was a significant difference in mean counts due to period ($p = 0.001$). There was a significant interaction effect between the location and sampling period ($p = 0.002$) (Figure 4).

Both point and non-point sources are greatly variable in space (*i.e.*, depending on the area considered) and in time (depending on hydrological and meteorological conditions, or accidental events). In rural areas, the sources of human origin are often negligible compared to animal sources (cattle, birds, and wildlife). Some animals may defecate directly in the water (birds or land animals at drinking points). In densely populated areas, the faecal contaminants enter the natural water through both point and non-point sources such as discharges of raw wastewater and sewage, discharges of industrial effluents, direct contamination from recreational water users and urban runoff (Servais *et al*., 2007; Ouattara *et al*., 2011; Brauwere *et al*., 2014).

According to the results, the microbiological quality of river water was subject to seasonal variations. During the rainy period, pollutants can enter river water through runoff. The rain washes the particles which have been deposited on nearby surfaces into the water source through leaching processes. This may depend on the type of soil and physical forms of pollution. In dry periods the water table becomes very low and the evaporation rate increases. Potgieter *et al*. (2006) report that this affects the oxygen content which decreases the multiplication of the bacteria in water, and that low temperatures could reduce the amount of available oxygen and disturb the bacterial metabolic processes. Sunlight can be an important factor responsible for the death or loss of culturability of microorganisms of faecal origin in aquatic systems (Brauwere *et al*., 2014). As demonstrated by several studies, temperature has a significant effect on the decay rates of faecal coliforms in fresh, saline, and estuarine waters (Barcina *et al*., 1986; Flint, 1987; Evison, 1988; Craig *et al*., 2004). Thus, the level of contamination of water will depend on the seasonal variation.

Antibiotic sensitivity testing

Quality control test

After a five-day quality control test, the inhibition zone diameters produced by all seven antibiotics tested [amoxicillin-clavulanate (20/10 µg, augmentin), ticarcillin-clavulanate (75/10 µg, timentin), amikacin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), and meropenem (10 µg)], were found to be within the quality control range (CLSI, 2020).

Antibiotic sensitivity testing for Escherichia coli isolates

Antibiotic-resistant *E. coli* isolates were present in all six sampling locations, during both rainy and dry periods. *Escherichia coli* isolates were resistant to ciprofloxacin (56.5%), cefotaxime (4.8%), augmentin (4.2%), amikacin (3.0%), and timentin (0.6%). None of the isolates was resistant to imipenem or meropenem (0%) (Figure 5). ciprofloxacin is the most frequently prescribed empirical antimicrobial for the treatment of urinary tract infections in Sri Lanka (Wijekoon *et al*., 2014). Further, the consumption of beta-lactam antibacterials (augmentin, timentin, cefotaxime) and quinolones (ciprofloxacin) is high in Sri Lanka (Sri Ranganathan *et al*., 2021). Improper use of antibiotics for humans and in animal husbandry has led to the emergence of antibiotic-resistant bacteria (Serwecińska, 2020; Ranasinghe *et al*., 2022). This could be the reason behind the high incidence of antibiotic-resistant *E. coli* in the water samples.

While certain isolates showed intermediate sensitivity to selected antibiotics, a considerable number of isolates were susceptible to the antibiotics (Figure 5). The proportions of isolates that were susceptible to each antibiotic were as follows; meropenem 99.4%, imipenem 98.8%, amikacin 94.0%, cefotaxime 85.1%, ticarcillin-clavulanate (timentin) 64.9%, amoxicillin-clavulanate (augmentin) 58.3%, and ciprofloxacin 22.0%. Thus, it can be argued that the selected antibiotics still exhibit potency against *E. coli*, though this varies with the isolate.

Multi-drug resistance (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Ibrahim *et al*., 2012). In a previous study carried out by Liyanage *et al.,* (2021) at the same locations, 1.0% of the *E. coli* isolates was multi-drug resistant (year 2021). No MDR isolates were reported in the samples collected in 2022 (current study).

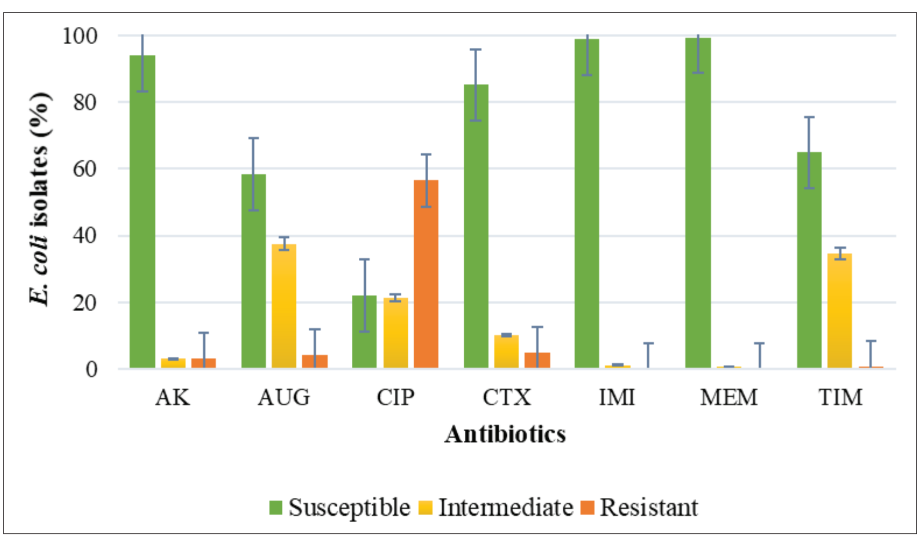

Figure 5: Percentages of *E. coli* isolates that were resistant, intermediate and susceptible to the antibiotics from the year 2020 to 2022. AK – Amikacin, AUG – Augmentin (Amoxicillin-clavulanate), CIP – Ciprofloxacin, CTX – Cefotaxime, IMI – Imipenem, MEM – Meropenem, TIM – Timentin (Ticarcillin-clavulanate).
Breakpoint values were taken from CLSI (2020).

The data was analysed to infer differences between more populated versus less populated locations and between rainy and dry periods. The percentage of isolates that were resistant to amikacin ($p = 0.007$), augmentin ($p = 0.023$) and ciprofloxacin ($p = 0.026$) were significantly higher in less populated locations compared to the more populated locations. No significant differences were observed in the percentages of isolates resistant to imipenem, meropenem, and ticarcillin-clavulanate (Timentin) between more populated and less populated locations ($p > 0.05$).

Antibiotics, antibiotic residues (with functional groups), antibiotic-resistant isolates and a myriad other bacteria can be present in hospital waste (Mackuľak *et al*., 2021). The runoff of hospital waste into the river may lead to the spread of antibiotic-resistant bacteria in the river water. Prolonged exposure to pharmaceutically active compounds in water can lead to changes in the bacterial genomes leading to antibiotic resistance. The resistant genes can horizontally transfer to susceptible strains, contributing to the development and spread of resistance (Mackuľak *et al*., 2021). Also, due to urban runoff and residues from industrial processes, a significant amount of antibiotic-resistant faecal bacteria and antibiotic residues can reach the river. These could be the reasons behind the occurrence of antibiotic-resistant bacteria in more populated locations. Antibiotic-resistant bacteria were also present in less populated locations. A less populated sampling site (B) along the river is located close to the municipal solid waste dump site at Gohagoda. The daily collection of solid waste in the Kandy city is disposed of to this dump site. Due to migration of leachate, the river water can be contaminated with antibiotic-containing wastes including pharmaceuticals (Wimalasuriya *et al*., 2011; Athukorala *et al*., 2021). This could be a reason for the high percentage of antibiotic-resistant isolates in location B, particularly in the rainy period, when more leachate is produced. Lack of sanitary facilities, excessive use and exposure to antimicrobial agents, and detergents may be further reasons for the buildup of resistant strains as reported earlier (Huijbers *et al*., 2015; Leonard *et al*., 2015). Unsupervised self-medication, inappropriate prescriptions, improper use (misuse) of antibiotics, poor hygiene, and inadequacy of duration of use are some reasons behind the emergence and spread of antibiotic resistance in Sri Lanka (Abhayasinghe *et al*., 2021; Sri Ranganathan *et al*., 2021).

It is likely that the water in most of the selected bathing locations in the river Mahaweli is highly contaminated with faecal matter, and it indicates the possible presence of pathogens in the water**.** Thus, the results of the current study emphasize the need for proper monitoring of recreational water quality in Sri Lanka. Depending on the observations made through monitoring, regulatory actions including closure of water bodies for public access, should be taken, especially if the level of contamination is high. Such regulatory action is taken in developed countries, where recreational water quality is much heeded (Power *et al*., 2005; Janezic *et al*., 2013). Action needs to be taken to control the disposal of waste at these locations with the support of the government, in conjunction with increased public awareness. Awareness programs would definitely serve to ensure public health and safety.

Sociological survey

As revealed by the survey, compared to the past, the use of river water for daily living activities has decreased, with increased access to pipe-borne water at these locations. Yet, some people continue to use the water of the river Mahaweli at the selected locations for daily living activities, since they have no other sources of water. All respondents had observed a notable difference in the clarity of the water between the dry and rainy periods. The use of river water in the rainy period is lower than that in the dry period. Other than recreational activities including bathing, some people use the river water for their daily living activities including washing clothes, gardening, and animal bathing. Inadequate municipal sewage facilities, industrial effluent discharge, and urban runoff are likely reasons for the contamination of river water at the selected bathing locations. Urban runoff appears to have a significant impact on water quality, especially at locations C and D (more populated), while it does play a role in contamination of water at the other locations as well. In addition to the above reasons, hospital waste is a possible source of water contamination in location C.

CONCLUSION

The water quality in the sampling locations undergoes seasonal variations. In 95% of the bathing water samples, the *E. coli* counts exceeded the permissible limit of 235 CFU/100 mL of water, and the counts vary in the rainy and dry periods. Antibiotic-resistant *E. coli* isolates were present in the water at all selected bathing locations. Compared to the year 2021, the *E. coli* counts observed in the year 2022 were higher, at all selected bathing locations. Compared to the year 2021, some resistant isolates were observed in 2022, for previously effective antibiotics. Elevated *E. coli* counts and the presence of antibiotic-resistant *E. coli* render the water at these locations unsuitable for recreational purposes. The sociological survey revealed that improper management and disposal of waste lead to contamination of water at these locations. This emphasizes the need for proper monitoring and action being taken by authorities to ensure public health and safety.

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