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ORIGINAL PAPER

Assessment of Virulence Potential and Antibiotic Resistance Profiles in *E. coli* Isolates from Selected Ground Water Samples Around the Control Open Dump Sites in Sri Lanka

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Abstract

Urbanization and accelerated industrialization have led to significant waste generation following the accumulation of massive amounts of solid waste in open dump sites. Groundwater contamination is one of the critical ecological concerns associated with the percolation of leachate from dump sites. *E.coli* O157 is a particular virulent serotype which produces Intimin and Shiga toxins, that cause severe diseases including Hemorrhagic Colitis, Hemolytic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura in humans. The focus of the present study is to study the virulence potential and antibiotic resistance profiles in *E. coli* isolates from selected Karadiyana, Methotamulla and Kerawalapitiya control open dump sites in Sri Lanka. The *E.coli* was isolated following the standard MPN method and 5 randomly selected (n = 5) colonies from each location were subjected to the virulence potential tests and antibiotic resistivity study. The results showed the total coliform count was ranged from 0–120 MPN/mL around the Kardiyana dump site whereas 0–75 MPN/mL and 3–115 MPN/mL recorded in the Methotamulla and the Kerawalapitiya dump sites. Overall, resistance in isolated *E.coli* against AMX, AMP, SUF/ TRI, SDI, CLOX, TET and ERM was high (> 70%) compared with the other tested antibiotics namely CIP, GEN and AZY (< 40%). According to the results, the Enteropathogenic *E. coli* pathotype was identified in 17 samples, whereas the Enterohaemorrhagic *E. coli* pathotype was found in only 3 samples.

Keywords Municipal solid waste · Leachate · *Escherichia coli* (*E. coli*) · Antibiotic resistance · Groundwater contamination

Introduction

In most developed countries, the technology for the treatment of landfill leachate has been maintained well and monitored strictly. However, for developing countries, waste classification and sealed management systems have not yet been perfectly established due to the lack of waste recycling legislation, technique equipment, and public awareness [18, 23]. In landfills, a significant volume of human waste, animal waste, and industrial waste with high antibiotic levels have been discarded [2, 11, 14]. Antibiotic-resistant bacteria (ARB) may become the predominant communities in landfills as a result of the presence and ongoing input of such antibiotics [4, 15]. Additionally, the presence of mobile genetic elements would encourage the frequency of horizontal gene transfer between pathogenic bacteria and ARB, making the landfill a hotspot for pathogenic bacteria and ARGs [7]. This is especially true for landfills without proper seepage control facilities [16]. A serious threat to public health and environmental safety exists at these sites because mixed pollutants (ARB and ARGs) migrate with

Statement of Novelty This is the first study conducted to investigate open dump sites in Sri Lanka as potential environmental reservoirs of antibiotic-resistant pathogenic *E. coli* and their virulence potential. These organisms could represent a potential health threat through the contamination of groundwater.

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landfill leachate and contaminate surface or underground water [7, 16]. Therefore, it is critical to create regulating technologies to lessen the prevalence of dangerous bacteria and ARG spread.

One of the most prevalent facultative pathogens in human health, *Escherichia coli* (*E. coli*), is also a crucial component of the study of water quality, particularly concerning faecal contamination [3, 21]. According to studies, a significant amount of the *E. coli* population detected in surface water already has at least one acquired resistance, and multi-resistant isolates are no longer unusual.

However, some strains of *E. coli* have developed virulence factors and genes for antibiotic resistance, which can cause serious infections and represent a serious risk to the general public's health [5, 20]. Due to the risk of environmental contamination and subsequent spread of antibiotic resistance to other ecological niches, the prevalence and antimicrobial resistance patterns of *E. coli* in landfill leachates are of the utmost importance [5, 20].

The management of garbage, including the eradication of municipal solid waste, is a major concern in Sri Lanka, a developing country that is experiencing fast urbanization and industrialization. Landfills are frequently used to dispose of garbage, and the leachates they produce frequently find their way into the surface and groundwater in the area, endangering ecosystems and human health [19] Since they may serve as reservoirs for antimicrobial resistance genes that can spread to other bacteria in the environment, the existence of antimicrobial-resistant *E. coli* strains in landfill leachates can exacerbate this issue.

To evaluate the possible dangers connected with these waste disposal sites, it is essential to understand the prevalence and antibiotic resistance profiles of *E. coli* isolated from landfill leachates. We can learn a lot about the prevalence and antibiotic resistance trends of E. coli strains, which will help us understand how landfill leachates affect the propagation of antimicrobial resistance and the risk of human exposure to contaminated water sources.

This study aims to determine the prevalence and antimicrobial resistance profiles of *E. coli* strains isolated from landfill leachate sites in Sri Lanka. The objectives include: (1) identifying the prevalence and distribution of *E. coli* in landfill leachates, (2) characterizing the antimicrobial resistance patterns of the isolated *E. coli* strains, and (3) exploring potential correlations between the occurrence of antimicrobial resistance and the physicochemical parameters of the leachate.

The results of this study will be crucial for the creation of efficient waste management plans and for reducing any potential health risks brought on by landfill leachate contamination in Sri Lanka.

Methodology

Study Area

The Karadiyana, Meethotamulla and Kerawalapitiya dumping sites were selected as the study area for the present study (Fig. 1). Those sites are identified as major solid waste disposal sites in the Colombo area. The number of groundwater sampling locations depended on the availability of groundwater sources in each area.

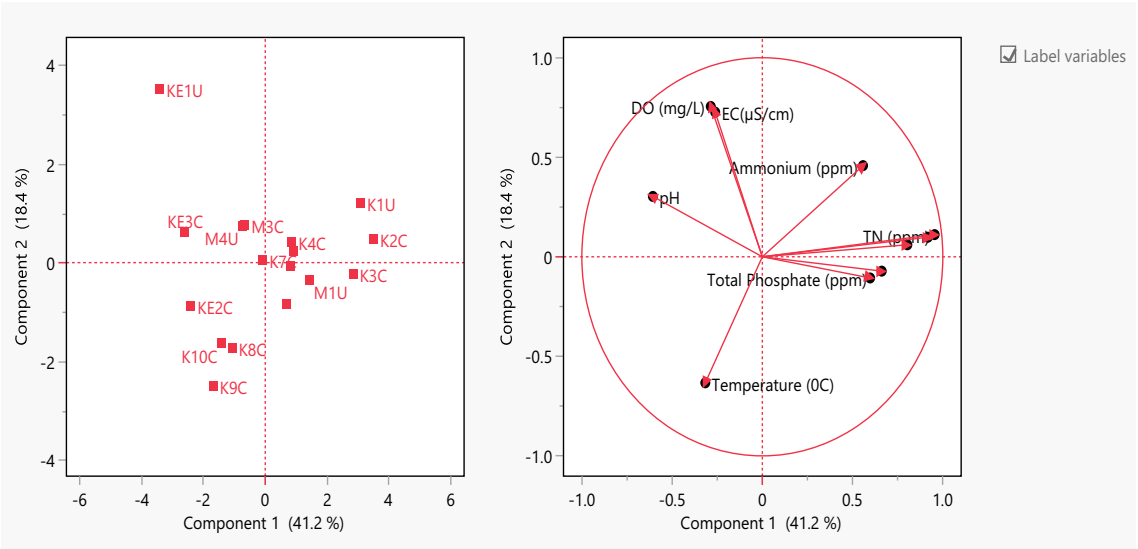

Fig. 1 Pearson Coefficient Analysis (PCA) for water quality parameters, distance of sampling site from the dumping site and covered and uncovered wells [water quality parameters and distance ($p > 0.05$, $R^2 = 0.776$); water quality parameters and covered and uncovered wells ($p > 0.05$, $R.^2 = 0.776$)]

Water Samples Collection

For the study, 17 dug-well water samples were collected from the Karadiyana (10), Meethotamulla (4) and Kerawalapitiya (3) open dump sites respectively on 15th and 16th August, 2023 (Table 1). Pre-cleaned polypropylene bottles and amber-coloured sterile glass bottles were used to collect water for chemical and microbial analysis respectively. Water samples were transported to the laboratory in a refrigerated condition within 24 h and stored in a cold room. Microbiological and chemical analyses were performed within 24 h after the collection of samples. The GPS coordinates were recorded via a hand-held GPS receiver (Model -etrex® 22x) at the site.

Physico Chemical Analysis in Water Samples

Water quality parameters; water temperature, pH, Dissolved Oxygen (DO), and Electrical Conductivity (EC) were measured using a thermometer (Immersion, Philip Harris, and England), pH meter (330 I/ Set, WTW Co., Weilheim, Germany), DO meter (HQD portable multimeter -HACH—HQ 40D) and a conductivity meter (340A-Set 1) respectively at the site itself. Chemical parameters such as N- Nitrate (as NO_3^-), N-nitrite (as NO_2^-), N-Ammonia (as NH_3), total inorganic nitrogen and total phosphorous were measured in the laboratory using Standard Methods for the Examination of Water and Wastewater published by American Public Health Association (APHA 2012). The Chemical Oxygen Demand (COD) of the water was measured following the closed reflux method [17, 22].

Microbiological Analysis

Total and Faecal Coliform Bacteria (Most Probable Number (MPN) Method)

The Most Probable Number method was performed to determine the Total coliform (TC) and *E. coli* count per 0.1 dm^3 of the water samples. Presumptive test, confirmed test and completed test were carried out to isolate and identify *E.coli* and Total coliform in the samples [Manjula et al., 2011 and WHO,2012, SLSI 2013, [22]].

Colonies developed on EMB agar, were further identified as coliforms or faecal coliforms (*Escherichia coli*) using colony characteristics, morphology and biochemical tests. For faecal coliforms, cell smears prepared from green metallic sheen were Gram stained and the IMVIC test was carried out to identify the colony as *E.coli* colonies. The MPN per 100 mL water was determined using the completed test.

Isolation and Confirmation of Pure Cultures of *E.coli*

Five colonies were randomly selected from each plate. For plates with ≤ 5 colonies, all the isolates were selected for further purification. Following incubation, a single colony was selected and streaked further on EMB agar to obtain pure isolates, which were then used for virulence and antibiotic susceptibility profiling.

Screening of Antibiotic Resistance in Isolated *E.coli*

Following incubation in nutrient broth, the turbidity of the broth culture was adjusted to a 0.5 McFarland standard before inoculating onto a pre-prepared nutrient agar medium. Filter sterilized (0.2 µm) antibiotics; Tetracycline (TET), Amphicillin (AMP), Amoxicillin (AMX), Cloxacillin (CLOX) and Ciprofloxacin (CIP) at a final concentration of 60 µg/mL were spiked to each molting nutrient agar media (40 ^{0}C) before inoculating bacteria [9, 12]. Then equalized bacterial samples were inoculated on the prepared nutrient agar medium according to CLSI guidelines and screened for antibiotic resistance in each bacterium [11].

Determination of Virulence Potentials of Isolates

Extraction of DNA

Following [8], the genomic DNA of isolated bacteria was extracted. Resuspended purified DNA was kept at -20 ^{0}C in 50 µL of TE buffer.

Detection of the Virulence Genes by PCR

The extracted DNA was used as the template DNA in different real-time PCR assays for the identification of genes associated with virulence in two (02) *E. coli* pathotypes. The various genes tested and the associated pathotypes are shown in Table 2.

The primers and PCR conditions used for the various genes were previously described by [1]. All controls were obtained from the Medical Research Laboratory in Sri Lanka. Reaction mixtures without DNA, which were used as negative controls, were also included in each PCR assay. All PCR assays were performed on a BIORAD PCR machine (Qiagen, Hilden, Germany). From Integrated DNA Technologies (IDT), primer sets were acquired. For each, PCR master mixture was prepared as follows (Table 3).

PCR amplification was performed to screen for *eaeA, stx1 and stx2* (Table 2) genes. Initial denaturation at 94 °C for 3 min was followed by 30 cycles at 94 °C for 10 s, 50 °C for 20 s, and 72 °C for 60 s in the PCR for *eaeA*. Initial denaturation at 94 °C for 10 min was followed by 30 cycles at 94 °C for 30 s, 45 °C for 30 s, and 72 °C for 60 s for *stx1 and stx2*.

Table 1 Description of groundwater sampling locations in the selected dumping site

No	Dumping site	Sample Number	Usage	Description of the dug well	Physical appearance of the water
01	Karadiyana	K 1	Irrigation	Uncovered well, 2 m deep, 50 m away from the dump site, Surface water percolates into the well	Dark-coloured water with an unpleasant smell
02		K 2	Irrigation and washing	Covered well, concrete lining 4 m deep, 100 m away from the dump site, Surface water does not percolate into the well	A bit muddy coloured with an unpleasant odour
03		K 3	Irrigation, washing and cooking	Covered well with a concrete lining 5 m deep,75 m away from the dump site, Surface water does not percolate to the well	Low coloured water Odourless
04		K4	Irrigation, washing and cooking	Covered well with a concrete lining 4 m deep, 250 m away from the dump site, Surface water does not percolate to the well	Colourless Odourless
05		K5	Irrigation, washing and cooking	Covered well with a concrete lining 4 m deep, 200 m away from the dump site, Surface water does not percolate to the well	Colourless Odourless
06		K6	Irrigation, washing and cooking	Uncovered well with 3 m deep, 300 m away from the dump site, Surface water percolates into the well	Low coloured water Odourless
07		K7	Irrigation, washing and cooking	Covered well with a concrete lining 4 m deep, 320 m away from the dump site, Surface water does not percolate to the well	Colourless Odourless
08		K8	Irrigation, washing and cooking	Covered well with a concrete lining 4 m deep, 350 m away from the dump site, Surface water does not percolate to the well	Pale coloured water Odourless
09		K9	Irrigation, washing and cooking	Covered well with a concrete lining 6 m deep, 520 m away from the dump site, Surface water does not percolate to the well	Colourless Odourless
10		K10	Irrigation, washing and cooking	Covered well with a concrete lining 6 m deep, 400 m away from the dump site, Surface water does not percolate to the well	Colourless Odourless

Table 1 (continued)

No	Dumping site	Sample Number	Usage	Description of the dug well	Physical appearance of the water
11	Meethotamulla	M1	Abundant	Uncovered well with 2 m deep, 20 m away from the dump site, Surface water percolates into the well	Black coloured Unpleasant odour
12		M2	Washing, cooking and drinking	Covered well with a concrete lining 3 m deep, 30 m away from the dump site, Surface water does not percolate to the well	Pale muddy coloured muddy odour
13		M3	Washing and cooking	Covered well with a concrete lining 3 m deep, 190 m away from the dump site, Surface water does not percolate to the well	Colourless Odourless
14		M4	Washing and cooking	Uncovered well with 2 m deep, 110 m away from the dump site, Surface water percolates into the well	Muddy Coloured Odourless
15	Kerawalapitiya	KE 1	Irrigation	Uncovered well with 3 m deep, 200 m away from the dump site, Surface water percolates into the well	Muddy Coloured Odourless
16		KE2	Abundant	Covered, 4 m deep, 180 m away from the dump site, Surface water does not percolate to the well	Muddy Coloured Unpleasant Odours
17		KE3	Irrigation and washing	Covered, 4 m deep, 350 m away from the dump site, Surface water does not percolate to the well	Colourless Odourless

Table 2 Virulence genes investigated and associated *E. coli* pathotypes

Pathotype	Screened Genes
Enteropathogenic *E.coli*	*eae A*
Enterohaemorrhagic *E.coli*	*eae A, stx 1, stx 2*

Table 3 Composition of PCR mixture

PCR ingredient	Volume per sample/ µL
PCR water (PROMEGA, Cat No: MC1191)	9.5 to15.0
Go Taq Polymerase (5U/µl) (PROMEGA, Madison, USA, Ref- M829B),	0.5
$MgCl_2$ (50 mM) (PROMEGA, Madison, USA, Ref- M891A)	0.5 to 1.0
dNTP mix (10 µM) (PROMEGA, Madison, USA, Cat No: PAU1515)	0.5
10 µM Forward primer	0.5
10 µM Reverse primer	0.5
5×reaction buffer (PROMEGA, Madison, USA, Ref- M829B)	2.5
DNA template	5.0 -10.0
Total volume 25.0	

Results

Physico-Chemical Water Quality Parameters in Dug Well Water Samples

Tables 4 and 5 describe the recorded physicochemical parameters of the well water in the dug wells of the Kardiyana, Meethotamulla and Kerawalapitiya dump sites. According to the recorded values, the pH was recorded from 4.25 ± 0.52 to 7.8 ± 0.42 in all the dumping sites which indicates the water pH was within the standards for inland water (SLS- 6.5—8.5, WHO- 6.5–8.5). The DO ranged from 0.5 mg/L to 7.64 mg/L in all selected wells around dump sites. The concentrations of NO_3^-, NO_2^- and NH_4^+ were ranged from 1.05 – 15.25 mg/L, 1.02 – 5.04 mg/L, and 0.05 >—0.58 mg/L respectively. The total phosphate concentrations ranged from 0.28 – 1.65 mg/L around the Karadiyana dump site and it

Table 4 Water quality parameters of the dug wells around the Karadiyana dump site

Sample Number	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	SLS (614:2013)	Range
pH	4.25 ±0.01	5.56 ±0.01	4.88 ±0.01	5.41 ±0.01	6.64 ±0.01	6.57 ±0.01	7.44 ±0.01	5.78 ±0.01	5.62 ±0.01	5.16 ±0.01	6.0 – 8.5	4.25–7.44
DO (mg/L)	4.27 ±0.01	4.02 ±0.02	2.81 ±0.01	6.51 ±0.01	0.50 ±0.03	2.71 ±0.01	3.37 ±0.04	3.02 ±0.05	2.28 ±0.01	3.35 ±0.03	3.0	0.5–6.51
Temperature (^{0}C)	27.2	28.1	28.6	30.3	28.9	28.5	28.9	31.3	31.3	30.0	-	27.2–31.3
EC(µS/cm)	2462	195.9	166.8	209.3	748.9	561.9	667.6	190.9	226.4	181.8	750	166.8–2462
Nitrate (ppm)	15.25 ±0.21	15.22 ±0.21	10.52 ±0.21	8.52 ±0.21	6.05 ±0.21	8.52 ±0.21	10.52 ±0.21	6.52 ±0.21	4.25 ±0.00	5.85 ±0.07	50	4.25 – 15.25
Nitrite (ppm)	4.03 ±0.00	4.02 ±0.00	5.04 ±0.00	5.02 ±0.00	3.15 ±0.07	4.02 ±0.00	2.02 ±0.00	1.03 ±0.00	1.02 ±0.00	1.02 ±0.01	3	1.02 – 5.04
Ammonium (ppm)	0.25 ±0.05	0.52 ±0.05	0.58 ±0.05	0.55 ±0.05	0.45 ±0.05	0.35 ±0.05	0.25 ±0.05	0.25 ±0.05	ND	ND	0.06	0 – 0.58
TN (ppm)	19.53	19.76	16.14	14.09	9.65	12.89	12.79	7.8	5.27	6.87	-	5.27 – 19.76
Total Phosphate (ppm)	1.55 ±0.05	1.65 ±0.05	1.52 ±0.05	0.52 ±0.05	0.53 ±0.05	1.52 ±0.05	1.29 ±0.84	0.28 ±0.05	1.50 ±0.05	0.52 ±0.05	2.0	0.28 – 1.65
COD (ppm)	460 ±1.21	596 ±2.34	452 ±1.67	224 ±2.01	596 ±2.05	264 ±1.89	256 ±1.09	352 ±2.87	224 ±1.26	224 ±3.08	40	224—596

ranged from 0.25 – 3.52.mg/L (Table 4) in the Meethotamiulla dump site respectively (Table 5). All the recorded total phosphate concentrations were within the SLS standard level. Importantly the the COD values ranged from 224 -596 mg/L, 224 – 488 mg/L and 150 – 428 mg/L in dug wells around Karadiyana, Meethotamulla and Kerawalapitiya dump sites respectively. Further, the recorded COD of all the dug well water exceeded the maximum permissible tolerance level given by SLS Sri Lanka.

A correlation analysis was performed to elucidate a possible relationship between the water quality parameters, distance of the sampling site from the dumping site and covered and uncovered wells. There were no significant variations detected between water quality parameters and covered and uncovered wells even from distance (Fig. 1). The correlation analysis revealed that K1U, K2U, K3C, M1U, and K4C showed the highest total nitrogen, total coliform, *E.coli* and total phosphate.

Total and Fecal Coliform Bacteria

Figures 2 and 3 represent the distribution pattern of faecal and total coliform levels in groundwater around the dump sites. According to the figures, the total coliform counts were greater in the nearby wells around the dump site and comparatively lower faecal coliform value was recorded in the distance wells. The total coliform count ranged from 0—120 MPN/mL around the Kardiyana dump site. The contamination pattern of fecal coliform also was similar to the total coliform distribution pattern around the Kardiyana dump site which ranged from 0 – 75 MPN/mL. The well water around the Meethotamulla dump site recorded the faecal and total coliform counts ranging from 0–94 MPN/mL and 3–115 MPN/mL respectively. Moreover, compared to the other two dump sites the well water around the Kerawalpitiya dump site recorded a lower value of total and fecal coliform count which ranged from 3–11 MPN/mL and 7–60 MPN/mL respectively (Table 6).

Antibiotic Resistance

Overall bacteria isolates showed that the highest resistance against AMX (100%) and AMP (100%), following descending order SUF (Karadiyana: 95%, Meethotamulla; 100%; Kerawalapitiya; 80%), SDI (Karadiyana: 95%, Meethotamulla; 90%; Kerawalapitiya; 80), ERM (Karadiyana: 85%, Meethotamulla; 60%; Kerawalapitiya; 90%), TET (Karadiyana: 80%, Meethotamulla; 85%; Kerawalapitiya; 70), CLOX (Karadiyana: 45%, Meethotamulla; 40%; Kerawalapitiya; 0), GEN (Karadiyana: 10%), and AZY (0) respectively. Resistance against AMX, AMP, SUF/ TRI, SDI, CLOX, TET and ERM was high (>70%) compared with the other tested antibiotics namely CIP, GEN and AZY (<40%) (Table 7).

Table 5 Water quality parameters of the dug wells around the Meethotamulla and Kerawalapitiya open dumpsites

	Meethotamulla					Kerawalapitiya				SLS (614: 2013)
Sample Number	M1	M2	M3	M4	Range	KE1	KE2	KE3	Range	
pH	6.84 ±0.01	6.18 ±0.01	6.17 ±0.01	6.75 ±0.01	6.17 – 6.84	7.80 ±0.01	7.19 ±0.01	7.23 ±0.01	7.19 – 7.8	6.5–8.5
DO (mg/L)	2.58	4.68	5.81	4.72	2.58 – 5.81	7.64	2.74	6.03	2.74 – 7.64	3.0
Temperature (^{0}C)	28.9	28.8	28.5	28.5	28.5 – 28.9	28.8	29.0	27.4	27.4 – 29.0	-
EC (µS/cm)	407.7	315.8	163.9	446.3	163.9 – 446.3	5248	548.0	375.9	375.9–5248	700
Nitrate (ppm)	8.85 ±0.07	8.52 ±0.00	6.25 ±0.00	7.75 ±0.11	6.25 – 8.85	2.52 ±0.00	2.52 ±0.00	1.05 ±0.01	1.05 – 2.25	50
Nitrite (ppm)	2.02 ±0.00	1.25	1.52 ±0.00	1.05 ±0.01	1.05 – 2.02	0.52 ±0.00	1.45 ±0.04	1.01 ±0.00	0.52 – 1.45	3
Ammonium (ppm)	0.52 ±0.00	0.55 ±0.00	0.50 ±0.00	0.52 ±0.00	0.5 – 0.55	0.45 ±0.00	ND	ND	0 – 0.35	0.06
TN (ppm)	11.39	10.32	8.27	9.32	8.27 – 11.39	3.49	3.97	2.06	2.06 – 3.97	
Total Phosphate (ppm)	3.52 ±0.00	2.55 ±0.00	0.25 ±0.00	0.52 ±0.00	0.25 – 3.52	0.28 ±0.00	0.35 ±0.00	ND	0 – 0.35	2.0
COD (ppm)	488	484	320	224	224 – 488	150	256	428	150—428	40

Explanation: ND = Not detected, TN = Total Nitrogen, EC = Electrical Conductivity, COD = Chemical Oxygen Demand, DO = Dissolved Oxygen. M1,M2,M3,M4:Sample locations in Meethotamulla; KE1,KE2, KE3:Sample Locations in Kerawalapitiya

SLS (614: 2013)—SRI LANKA STANDARD 614: 2013 UDC 663.6

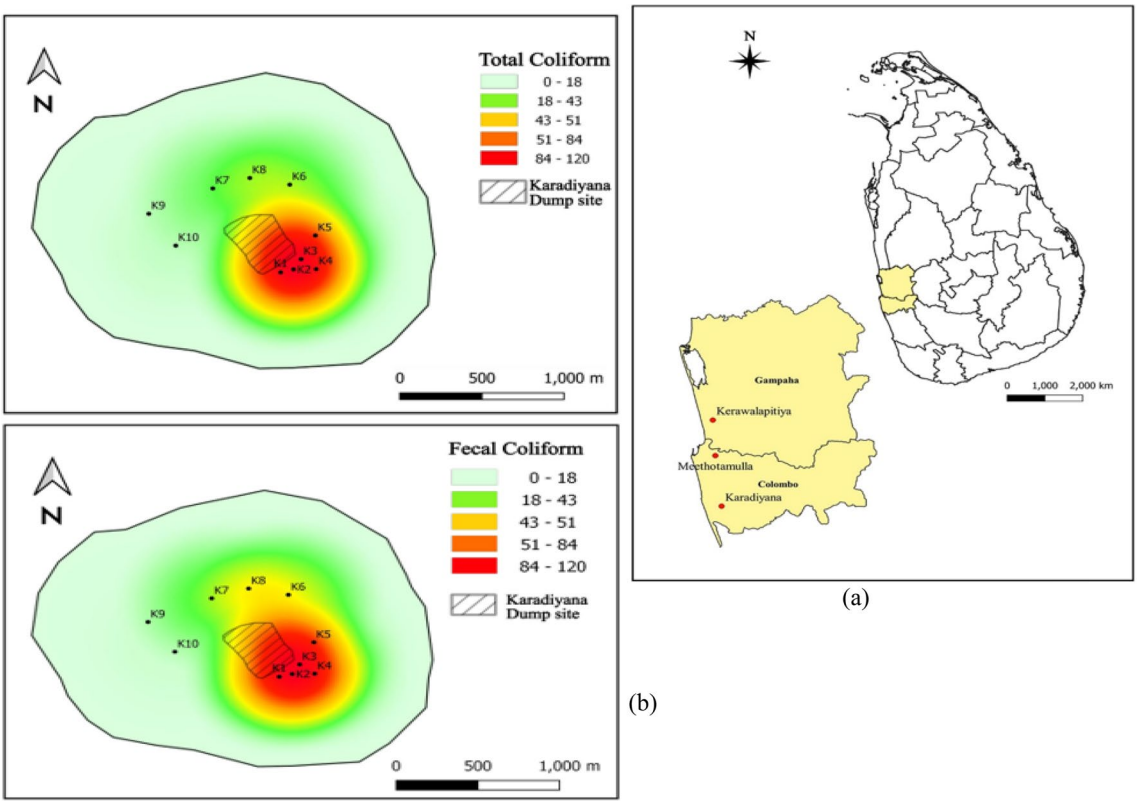

Fig. 2 Total coliform (**a**) and faecal coliform (**b**) distribution pattern of groundwater around Karadiyana control open dump site

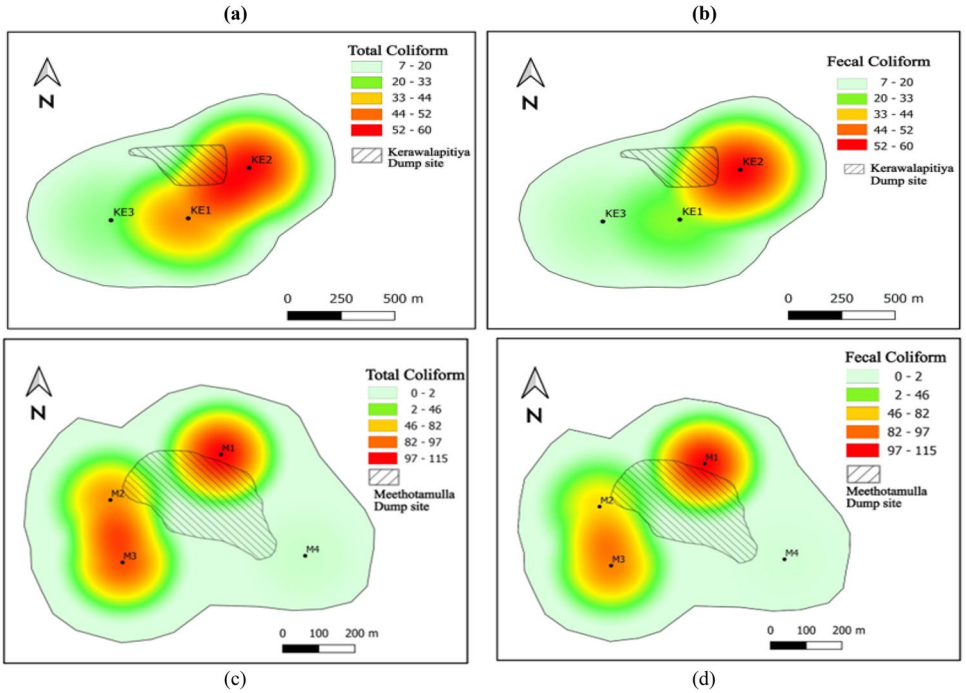

Fig. 3 Fecal and total coliform distribution pattern of ground water around Meethotamulla and Kerawalapitiya open dump sites

Table 6 Total and faecal coliform numbers in different sampling locations of the open solid waste dump sites in the study

Sampling location	Sample Number	Fecal Coliform MPN/mL	Total coliform (MPN/mL)
Karadiyana	K 1	75	120
	K 2	64	120
	K 3	20	64
	K4	43	43
	K5	20	43
	K6	64	75
	K7	40	43
	K8	11	20
	K9	ND	ND
	K10	3	11
Meethotamulla	M1	93	115
	M2	43	75
	M3	64	93
	M4	ND	3
Kerawalapitiya	KE 1	11	40
	KE2	43	60
	KE3	3	7

Detection of the Virulence Genes by PCR

E. coli isolated from three different dumpsites were screened for virulence genes eae A, *stx* 1, *stx* 2 by direct PCR. PCR running conditions for these virulence markers were optimized for any deviation from the earlier reported conditions to suit the reagents and thermal cycler used in the present experiment (Table 3) [1].

Among the 70 isolates of *E. coli* samples analyzed, 17 samples tested positive for the eaeA gene, while 10 and 4 samples were positive for the stx1 and stx2 genes, respectively. According to the results, the Enteropathogenic *E. coli* pathotype was identified in 17 samples, whereas the Enterohaemorrhagic *E. coli* pathotype was found in only 3 samples.

The percentage of *E. coli* isolates positive for the *eae*A gene, ranging from 20% to 40%, as compared to the positive percentages for stx1 (12.5% to 20%) and stx2 (2.5% to 10%) in isolated *E. coli* strains (Fig. 4). Among the *E. coli* isolates the highest number of positive samples was recorded for the *eae*A gene in Kerawalapitiya (40%), followed by Meethotamulla (25%) and Karadiyana (20%) in descending order (Fig. 4). For stx1, the highest number of positive isolates

Table 7 Recorded antibiotic resistance *E.coli* isolates

Location	No of *E. coli*	AMX	AMP	CLOX	CIP	TET	SUF	SDI	GEN	AZY	ERM
Karadiyana	40	40	40	18	2	32	38	38	4	0	34
Meethotamulla	20	20	20	8	0	17	20	18	0	0	12
Kerawalapitiya	10	10	10	0	0	7	8	8	0	0	9

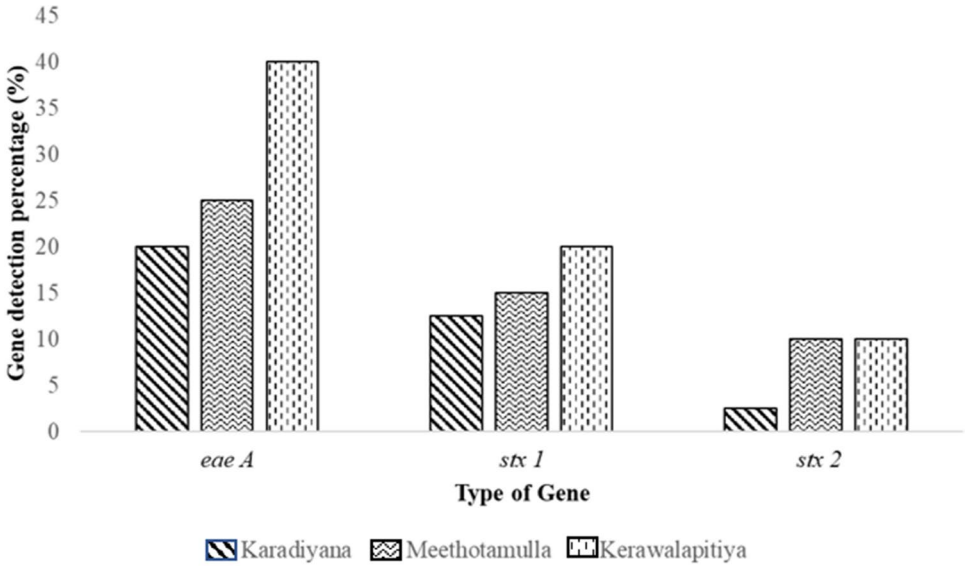

Fig. 4 Gene detection percentage of isolated *E.coli* from open dumpsites

was observed in Kerawalapitiya (20%), while the highest detection of stx2 was found in isolates from Meethotamulla and Kerawalapitiya (10% each).

Discussion

Escherichia coli (*E. coli*) stands as a dependable biological indicator of faecal contamination in water sources, notably accounting for causing various waterborne infections in humans, particularly gastrointestinal diseases [5]. Furthermore, the presence of antimicrobial-resistant pathogenic strains of *E. coli* in water sources can potentially facilitate the transfer of antimicrobial resistance and virulence genes to other environmental bacteria [19]. In addition, the release of leachate from landfills has significant implications for the physical, chemical, biological, and groundwater attributes associated with agriculture and human well-being [6, 23].

Excessive levels of nitrates and nitrites in groundwater can lead to serious health risks, including methemoglobinemia, blue baby syndrome, cancer, and central nervous system disorders in humans [2, 23]. According to the ambient water quality guidelines set by the Sri Lanka Standard Institute (SLSI), certain wells around the Karadiyana dump site have surpassed the maximum acceptable nitrate concentration, raising concerns about potential health risks for the general public [22]. Moreover, the Chemical Oxygen Demand (COD) levels in all the selected wells exceeded the SLSI standard concentration significantly [2]. COD is a crucial parameter for assessing groundwater contamination by a wide range of organic and inorganic pollutants [1, 22]. The results of the present studies showed, that the COD levels around the Karadiyana dump site were greater than those at the other two sites, likely due to the continuous disposal of a substantial amount of municipal solid waste from the Western Province in Karadiyana. There are three main types of soil: sand, silt and clay. Particle size and distribution will affect a soil's capacity for holding water and therefore the movement of pollutants [1].

The contamination of well water around open dump sites is a global concern due to the potential spread of pathogenic microorganisms within the groundwater system [1]. Most of the wells studied in the present study were contaminated with faecal and total coliform, indicating severe groundwater pollution in the vicinity of the open solid waste dump area. Landfills often containing expired medications, used diapers, and sanitary products from households and healthcare facilities were observed during the sampling. When these waste items are mixed with general refuse, they can be exposed to various medications, including antibiotics. *E. coli* can acquire antibiotic resistance traits during prolonged incubation within landfills [1, 5]. Antibiotic resistance genes (ARGs) contribute to bacterial antibiotic resistance development and can be transferred via conjugation, transformation, and transduction to pathogenic or environmental bacteria in the environment through horizontal gene transfer [5, 11, 20]. The presence of *E. coli* is a significant risk factor for human infectious diseases caused by these microorganisms, as coliform pathogens can acquire resistance genes from bacterial populations in aquatic environments through horizontal gene transfer, neutralizing various classes of antibiotics [10, 14]. The findings of this study suggest that landfills could contribute to the proliferation of antibiotic-resistant bacteria in the environment, which has implications for human health.

The battle against antibiotic resistance remains unresolved, with bacteria increasingly developing resistance even to newly developed antibiotics [13]. In the present study, 70

E. coli isolates were tested for resistance to 10 antibiotics from seven different classes. All of these isolates (100%) exhibited resistance to at least one of the tested antibiotics. Resistance was particularly high (> 70%) against AMX, AMP, SUF/TRI, SDI, CLOX, TET, and ERM, in contrast to CIP, GEN, and AZY, which showed lower resistance levels (< 40%).

To the best of our knowledge, this is the first documented report on antibiotic resistance in *E. coli* isolated from open controlled solid waste dump sites. While the precise reasons for this phenomenon are not easily explained, it is suggested that, in addition to exposure to antibiotics in the environment, other stressors such as exposure to heavy metals may contribute to increased antibiotic resistance in environmental strains [16].

Some pathogenic strains of *E. coli*, such as Enteropathogenic *E. coli* and Enterohaemorrhagic *E. coli,* have been recognized as emerging bacterial pathogens [15]. Various pathogenic *E. coli* strains have been isolated from diverse aquatic environments worldwide, including rivers [4], lakes, seas, and groundwater resources [15]. In the present study, 8% of the pure isolates carried at least one of the tested virulence genes. It is worth noting that the majority of the isolates (approximately 90%) were negative for the genes examined, which could be attributed to the specific selection of genes for testing.

Conclusion

The assessment of virulence factors in the *E. coli* isolates indicated that a proportion of these bacteria carried genes associated with pathogenicity. This finding raises concerns about the potential for these isolates to cause diseases in humans and other organism. The study also demonstrated a concerning level of antibiotic resistance among the *E. coli* isolates. This implies that these bacteria have developed resistance mechanisms against commonly used antibiotics, which can complicate the treatment of infections caused by these strains. The study opens avenues for further research into the specific sources and routes of contamination in open dump sites, as well as the potential impact on nearby water sources and communities.

Author's Contribution P. A. K. C. Wijerathna- samples collection, microbiological analysis, water quality analysis manuscript writing and editing.

G. Y. Liyanage – Molecular analysis, manuscript writing editing and proofreading.

S.M.T.V Bandara- sample collection, microbiological analysis.

P. M. Manage conceptualization, sample collection, manuscript writing, editing and proofreading.

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Data Availability The datasets generated during and analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest or personal relationships that could have appeared to influence the work reported in this paper.

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