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Screening and Quantification of Selected Tetracycline (TET, OTC) and Sulphonamide (SMX, SDI) Group Antibiotics and their Resistant Bacteria in Solid Waste Dump Leachates

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Abstract

Antibiotics are newly emerging contaminants (ECs) in landfill leachate that have led to antibiotic-resistant bacteria (ARB) which has become an immense threat to public health globally. Tetracyclines and sulphonamides are broad-spectrum antibiotics used in the healthcare sector, aquaculture, and veterinary medicine in Sri Lanka. The objectives of this research were to characterize the leachate samples, analyse the antibiotic residues, and isolate ARB against Tetracycline (TET), Oxytetracycline (OTC), Sulfamethoxazole (SMX), and Sulfadiazine (SDI) antibiotics. Leachates were collected into 2L glass bottles from Karadiyana and Deldorawatta open dumpsites and were characterized by adhering to APHA guidelines. Antibiotic residues were quantified using High-Performance Liquid Chromatography (HPLC). Resistant bacteria were isolated using the standard pour-plate method, with plate count agar, 48 hours after incubation at 28°C. Minimum Inhibition Concentrations (MIC) were determined from 60 to 360µg/mL concentrations of TET, OTC, SMX, and SDI using the 96-well plate method following CLSI guidelines. Recorded leachate quality parameters indicated that the leachate samples did not meet the standards for effluent discharge to inland surface waters specified by the Central Environmental Authority of Sri Lanka. The study showed that the selected antibiotic residues were not detected in the tested leachates, suggesting that they were below the detection limit (0.05 ppm) of the HPLC. However, ARB were isolated and 83.33% of the isolates from the Karadiyana open dumpsite leachate had MIC values greater than 360µg/mL against OTC. The study also found that 19.23%, 7.69%, and 26.92% of the isolates from the Deldorawatta open dumpsite leachate had MIC values exceeding 360 µg/mL for TET, OTC, and SDI, respectively. The isolates exhibited a Multiple Antibiotic Resistance (MAR) index ranging from 0.75 to 1. This study reveals that the intrinsic nature of antibiotic resistance in bacteria may allow ARB to spread even in the absence of antibiotic residues or at concentrations below detectable levels.

Keywords: Tetracycline, Oxytetracycline, Sulfamethoxazole, Sulfadiazine, Antibiotic Resistance, Leachate

1. Introduction

Due to its low cost and maintenance, landfilling is the favoured method of solid waste disposal and is used in many industrialized and developing countries.[1] Landfill leachate is the liquid leaching out of landfills due to rainwater infiltration through solid waste in dumpsites and it carries all the water-soluble and suspended fractions of waste and by-products of waste degradation.[2] Most studies have found this complex organic effluent very harmful, raising concerns for the surrounding environment[3] as landfills are a major source of water pollution,[4] and leachate may seep into ground and surface waterbodies, endangering aquatic habitats.[2] Karadiyana dumpsite is situated near major river systems and wetlands in Sri Lanka.[2] As a developing country,

protecting our groundwater resources is necessary.

Antibiotics are newly emerging contaminants (ECs) found in landfill leachate due to the unregulated disposal of municipal solid waste in landfills.[5] The landfill receives unused antibiotics through household waste,[6] antibiotic residues from hospital effluent, and used antibiotics in aquaculture, and veterinary medicine.[7] Antibiotics are natural substances produced by microbes that can inhibit/kill competing species and have been used to treat and prevent severe infections in surgical patients, cancer patients undergoing chemotherapy, and immune-compromised individuals.[8] Antibiotics have also considerably improved the health and well-being of animals[9] and have been approved to treat bacterial diseases in aquaculture.[10]

Huge selective pressures are placed on microbial communities due to heavy antibiotic use[11] giving rise to ARB which is a serious health challenge that requires early intervention.[12] Pathogenic bacteria resistant to antibiotics is a global problem linked to increased rates of morbidity and mortality.[13] Gram-positive and negative bacteria that show multidrug resistance have led to infections that are challenging and in certain instances impossible to cure with traditional antimicrobials.[13] It is estimated that by 2050, antimicrobial resistance will cause over 10 million deaths annually.[14]

The presence of high levels of ARB and Antibiotic-Resistant Genes (ARGs) in leachate has been observed.[3] When water bodies get contaminated by landfill leachate, these ARGs can spread among other bacteria in the aquatic environment, and eventually infect fish as pathogens.[15] Given its nutritional value and health benefits, fish is commonly consumed in several Asian countries, including Sri Lanka.[15] Humans are therefore at risk of being exposed to ARB and ARGs through the consumption of contaminated aquaculture food and water.[15] Tetracycline ARB and ARGs (*tetA*) has been found in fish and shellfish that restaurants and supermarkets distribute for direct or indirect consumption, such as sushi.[16]

Tetracyclines and sulphonamides are broad-spectrum antibiotics used to treat human and animal bacterial infections and are very effective against many gram-positive and gram-negative bacteria.[17,18] TET and OTC are antibiotics that fall under the Tetracycline class of antibiotics, and they inhibit protein synthesis (Figure 1A) by selectively blocking the 30S ribosomal subunit, preventing the binding of aminoacyl-tRNA to the A-site on the mRNA-ribosome complex, causing the inability of a bacterium to sustain normal functioning and proliferation.[19]

Tetracycline resistance occurs through ribosome protection, efflux pumps, modification of drug target, and enzymatic

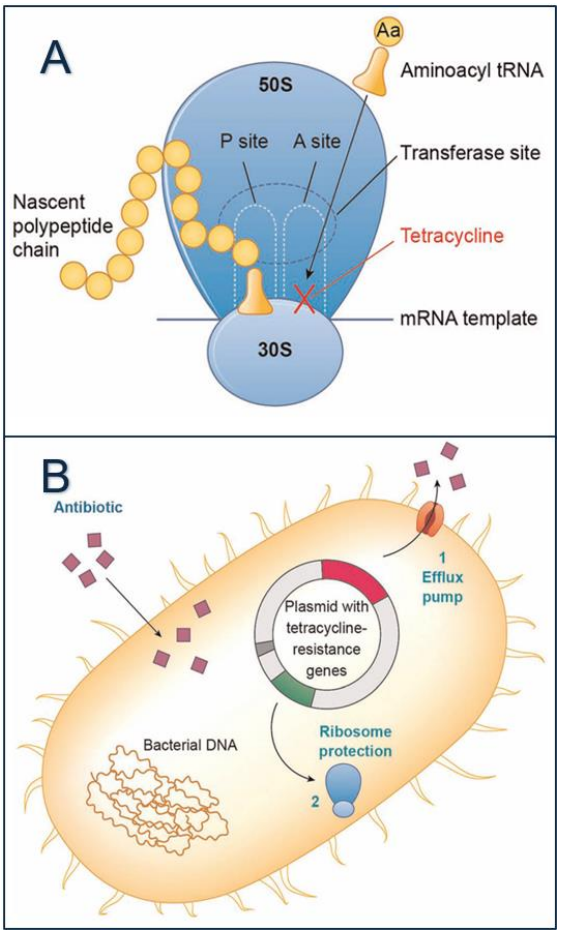

Figure 1. A-Mechanism of action of tetracyclines by inhibiting protein synthesis, B-resistant mechanisms of bacteria against tetracyclines.[20]

alteration (Figure 1B).[20] Ribosomal protection is one of the significant mechanisms of tetracycline resistance, in which ribosomal protection proteins (RPPs) bind to the 30S ribosomal subunit and displace tetracycline from the A-site. Tetracycline was the first to be found to have an efflux pump mechanism.

These specialised protein pumps actively pump out antibiotics reducing the concentration inside the cell.[21] Another type of resistance mechanism is drug target modification, which reduces the binding affinity of tetracycline to the ribosome.[22]

Sulphonamides (SDI and SMX) are a synthetic class of antibiotics.[23] They function as structural analogs and competitive antagonists (Figure 2) of p-aminobenzoic acid (PABA) that is used to synthesise folic acid, which is necessary to continue producing DNA in bacteria. Structural similarity between sulphonamide and PABA, allows sulphonamide to inhibit and replace PABA. Eventually, it can also prevent the formation of dihydrofolate and tetrahydrofolate, which is required for bacterial DNA synthesis and cell division.[18]

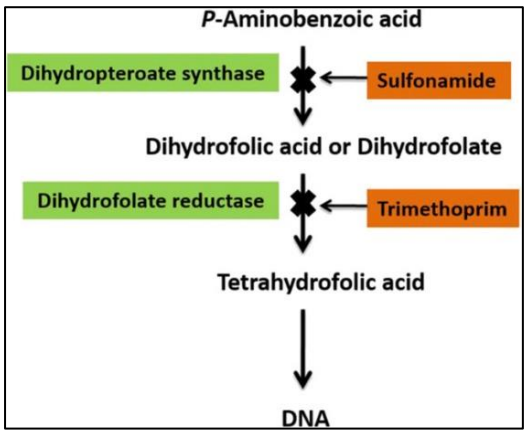

Figure 2. Mechanism of sulphonamide action by preventing the formation of dihydrofolate.[18]

The acquisition of sulphonamide resistant bacterial genes (sul) results in antibiotic resistance to sulphonamides. Two methods by which bacteria might develop resistance to sulphonamides include: intrinsic vertical gene transfer (VGT) and extrinsic horizontal gene transfer (HGT). While HGT involves the transfer of resistance genes between unrelated bacteria, VGT refers to the acquisition of resistance through spontaneous mutation within the bacterial genome that subsequently transmits to its offspring.[24]

In Sri Lanka, the contamination levels of tetracyclines and sulphonamides in different environmental samples exceeded the maximum permissible level recommended by the World Health Organization (WHO).[25-26] However, studies about antibiotic concentrations, antibiotic resistance, and ARB in solid waste dump leachate in Sri Lanka are limited. More than 260 small and large-scale landfills are found in Sri Lanka and most of them are unregulated open dumpsites.[27] The purpose of this study is to evaluate the contamination levels of tetracyclines (TET, OTC) and sulphonamides (SMX, SDI), and isolate resistant bacteria in leachate. The results of this research will provide evidence-based data to policymakers, helping them to formulate necessary environmental policies and regulations to safeguard public health and the environment.

2. Methodology

2.1 Study area and sample collection. The leachate samples were collected into 2L sterilized glass bottles from Karadiyana (6.814388, 79.902023), and Deldorawatta (6.669438, 80.022813) open dump sites (Figure 3).

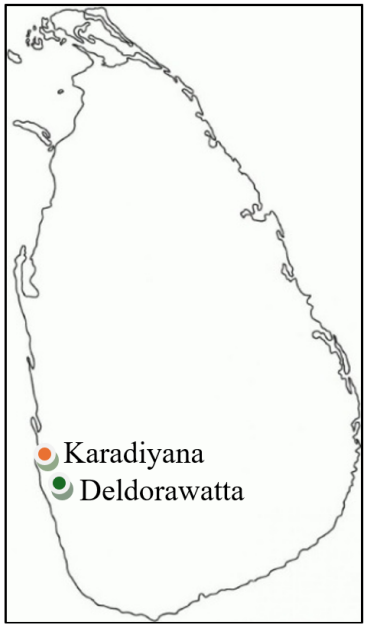

Figure 3. Sample collection points (Karadiyana and Deldorawatta open dumpsites)

2.2 Leachate Characterization. Electrical conductivity (EC) and pH were measured using a conductivity meter (340A-Set 1) and a pH meter (330 I/Set, WTW Co., Weilheim, Germany) respectively. Temperature and dissolved oxygen (DO) were measured using a multi-parameter probe. Total phosphate, ammonia (NH_3), nitrate (NO^{3-}), and nitrite (NO^{2-}) concentrations were assessed according to standard procedures specified by APHA for the Examination of Water and Wastewater.[28] The Chemical Oxygen Demand (COD) of the leachates was measured using the closed reflux method.[28]

2.3 Extraction of antibiotics from leachate samples

Antibiotics were extracted from leachates using the solid-phase extraction method by mixing 100 mL of distilled water with 100 mL of each leachate sample. Duplicates were made. The samples were sonicated for 15 minutes. A few drops of HCl were added to lower the pH down to 3. The samples were allowed to settle for 24 hours, and the supernatant was poured into centrifuge tubes and centrifuged for 10 minutes at 6800 rpm.[29]

The C18 cartridges were preconditioned with 10 mL of 100% methanol and then with 10 mL of milli-Q water. 50 mL of the centrifuged samples were passed through the cartridges. The extracted antibiotics were eluted with 3 mL of 100% methanol into HPLC vials.[15,30]

2.4 Identification and Quantification of Antibiotics in Samples

The antibiotics were quantified using Agilent 1200 series High-Performance Liquid Chromatography (HPLC) equipped with a diode array (DAD) and fluorescence detector.[29] 20µL was injected and chromatography was performed at 30ºC. 100% methanol (polar protic solvent) was pumped in the beginning at a flow rate of 4 mL/min. The column effluent was monitored by a DAD detector in the range of 200 - 450 nm. Then the identification and quantification were done by DAD. Wavelength and retention times for each antibiotic were selected.[29] Table 1 shows conditions and retention times employed for antibiotic analysis.

Table 1. Conditions and retention times employed for antibiotic analysis

Analyte	γ absorption (nm)	Retention time (min)
TET	272	14.001
OTC	280	15.851
SMX	250	4.836
SDI	238	3.034

2.5 Isolation of Antibiotic-Resistant Bacteria (ARB)

2.5.1. Total Viable Counts (TVC) of bacteria and resistant bacteria in leachate samples. TVC was measured using the standard pour plate method with plate count agar.[15] The colony forming units (CFU/mL) were counted 2 days after incubation at 28°C. Antibiotics (TET, OTC, SMX, SDI) at a final concentration of 60 µg/mL were added to each medium to take the TVC,[15] and ARB were isolated into slant bottles and incubated at 37°C and refrigerated after 24 hours.

2.6 Determination of the Minimum Inhibition Concentration (MIC). Determination of MIC was carried out using the broth dilution method following CLSI guidelines.[31] A nutrient broth culture was prepared for each isolate by inoculating a loop of bacteria. The broth cultures were incubated at 37°C for 24 hours. Cell densities were equalized with McFarland No.0.5.[29] The broth dilution method was done on 96 well plates with different antibiotic concentrations (60 to 360µg/mL). Positive and negative controls were carried out. Plates were incubated at 28°C for 24 - 48 hours. The absorbance of the wells was recorded at 595nm using an ELISA reader.[32]

2.7 Determination of Multiple Antibiotic Resistance (MAR). Liquid bacteria cultures were prepared and equalized with McFarland No. 0.5. MAR against TET, OTC, SMX, and SDI was determined at a final concentration (60µg/mL) of each antibiotic using the 96 well-plate method.[33] MAR index was calculated.

Results and Data Analysis

3.1 Leachate Characterization

Table 2. Leachate parameters (temperature, pH, EC, DO, COD, nitrate, nitrite, ammonia, phosphate levels) of Karadiyana and Deldorawatta open dumpsite leachates.

Parameter	Karadiyana sample	Deldorawatta sample
Temperature (°C)	27.0	25.8
pH	8.32	3.93
Electrical conductivity (EC) (mS/cm)	32.24	27.07
Dissolved Oxygen (DO) (mg/L)	1.13	1.23
Chemical Oxygen Demand (COD) (mg O_2/L)	2,000	36,000
Nitrate (mg/L)	99.3788	136.646
Nitrite (mg/L)	3.44	26.0521
Ammonia (mg/L)	794.4828	<Minimum Detection Limit
Phosphate (mg/L)	493.6842	6,418.947

Table 2 indicates the calculated leachate quality parameters. Temperature, pH, EC, and DO vary from 25.8°C to 27.0°C, 3.93 to 8.32, 27.07 to 32.24 mS/cm, and 1.13 to 1.23 mg/L respectively. COD values ranged from 2,000 to 36,000 mg/L. Further, nitrate, nitrite, ammonia, and total phosphate levels varied from 99.38 to 136.65 mg/L, 3.44 to 26.05 mg/L, 794.48 mg/L in Karadiyana, and 493.68 to 6,418.95 mg/L respectively.

3.2 Total Viable Counts (TVC) of bacteria and resistant bacteria in leachates. Figures 4-6 show TVC in samples. As depicted in Figure 5, the environment sample (Env.) of the Karadiyana leachate had a bacterial concentration of $2.2*10^5$ CFU/mL. No resistant bacteria were isolated against TET, OTC, SMX, and SDI. In the Deldorawatta leachate sample, a bacterial concentration of $4.2*10^5$ CFU/mL was present in Env. sample, while only $3*10^4$ CFU/mL were resistant to OTC, and $6*10^4$ CFU/mL were resistant to SMX (at 60µg/mL). No bacterial growth was present in plates with TET and SDI.

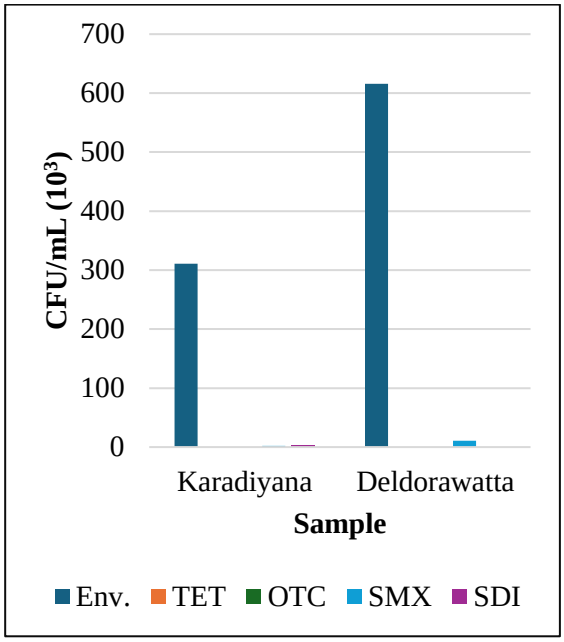

Figure 4. Concentrations of bacteria and resistant bacteria in environment sample (10^{-3})

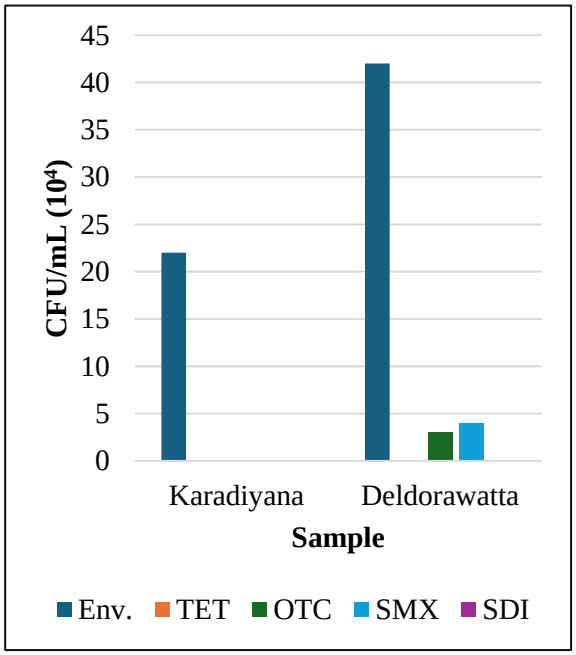

Figure 5. Concentrations of bacteria and resistant bacteria in environment sample (10^{-4})

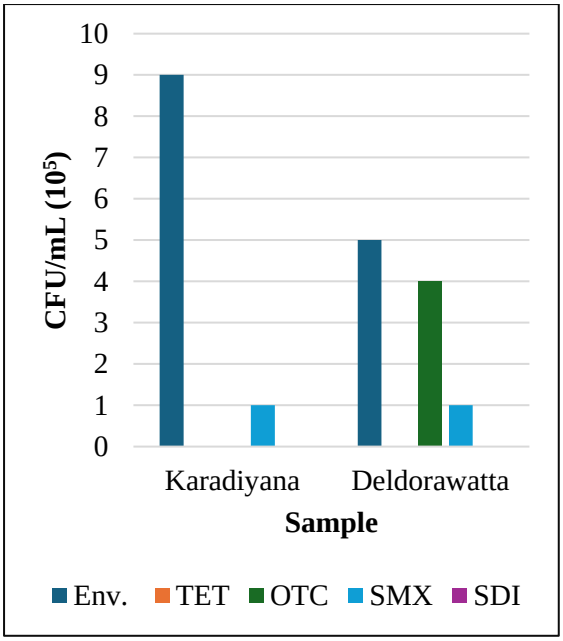

Figure 6. Concentrations of bacteria and resistant bacteria in environment sample (10^{-5})

3.3 Minimum Inhibitory Concentration (MIC) of the isolates. 1-26 were isolated from the Deldorawatta leachate sample, and 27-32 were isolated from the Karadiyana leachate sample.

As denoted in Table 3, of the isolates,

40.625% had 60 ≤ MIC >120µg/mL, 31.25% had 120 ≤ MIC >180µg/mL, 9.375% had 300 ≤ MIC >360µg/mL, and 15.625% had MIC ≥360µg/mL against TET.

12.5% had 60 ≤ MIC >120µg/mL, 18.75% had 180 ≤ MIC >240µg/mL, 25% had 240 ≤ MIC >300µg/mL, 21.875% had 300 ≤ MIC >360µg/mL, and 21.875% had MIC ≥360µg/mL against OTC.

43.75% had 60 ≤ MIC >120µg/mL, 6.25% had 120 ≤ MIC >180µg/mL, 25% had 180 ≤ MIC >240µg/mL, and 25% had 240 ≤ MIC >300µg/mL against SMX.

62.5% of the isolates had 60 ≤ MIC >120µg/mL, 15.625% had 120 ≤ MIC >180µg/mL, and 21.875% had MIC ≥360µg/mL against SDI.

3.4 Multiple Antibiotic Resistance (MAR). The resistance of all isolated bacteria to TET, OTC, SMX, and SDI antibiotics was checked at a concentration of 60µg/mL. Table 4 shows the calculated MAR indexes.

MAR index range varies from 0.75 to 1 for the isolates. 3.125% of the isolates had a MAR index of 0.75 while 96.875% had a MAR index of 1 which indicates resistance to TET, OTC, SMX, and SDI antibiotics at 60µg/mL.

Table 4. MAR index for isolates

Reference Number	MAR index
1	1
2	1
3	1
4	1
5	0.75
6	1
7	1
8	1
9	1
10	1
11	1
12	1
13	1
14	1
15	1
16	1
17	1
18	1
19	1
20	1
21	1
22	1
23	1
24	1
25	1
26	1
27	1
28	1
29	1
30	1
31	1
32	1

Table 3. MIC of isolates against TET, OTC, SMX, and SDI

Antibiotics			Reference No.																														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Tetracycline group antibiotics	TET (μg/mL)	60	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		120	+	+	+	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+
		180	-	-	+	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		240	-	-	+	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		300	-	-	+	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		360	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	OTC (μg/mL)	60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		120	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
		180	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
		240	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
		300	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
		360	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Sulphonamide group antibiotics	SMX (μg/mL)	60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		120	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
		180	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
		240	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-
		300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		360	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	SDI (μg/mL)	60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		120	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
		180	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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		300	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		360	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4. Discussion

Appropriate disposal of leachate requires a proper understanding of its physiochemical properties to prevent ecological harm and ecotoxicity.[3] Some of the basic leachate parameters measured were pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Nitrate, Nitrite, Ammonia, and Phosphate concentrations.

A young landfill (that has been in operation for less than 10 years) has a lower pH and a higher content of volatile fatty acids. pH of Deldorawatta leachate was 3.93, indicating that it might be at the beginning of the acetogenic phase in waste degradation, where the pH is often <6.6.[34] pH of Karadiyana leachate was 8.32, indicating that it might be in the methanogenic phase where pH values are >7.5, as volatile fatty acids are converted to CO_2 and CH_4 by methane-producing bacteria and are seen in mature landfills (in operation over 10 years).[34] According to a study, the Karadiyana dumpsite has been in operation for more than 20 years.[2] The pH of leachate gradually increases as the landfill gets older and more stabilized.[35]

EC is the mineralization of the analysed sample, and it increases with high biowaste fractions, inorganic components, ions, soluble salts,[36] minerals, and dissolved.[37] EC is high in young landfills.[38] However, Karadiyana leachate had the highest EC, even though it is an old dumpsite.[2]

COD measures the amount of oxygen required to chemically oxidize the organic and inorganic material present in a sample. COD is low in old landfill leachates and high in young landfill leachates due to unstable waste decomposition.[38] Deldorawatta sample recorded the highest COD, showing characteristics of a young landfill. COD increases with higher biowaste fractions, thereby, increasing the solubility of many compounds.[38]

Both leachates recorded high phosphate concentrations.[39] Phosphorus is released by organic matter during biodegradation. Agricultural fertilizers, detergents, and household and industrial waste in the landfill are a few sources of phosphates.[36] Old landfills have nitrate values <100 mg/L as seen in Karadiyana leachate.[2] Nitrate is the most oxidized form of nitrogen found in natural systems when ammonium is oxidized to nitrite and then, later, to nitrates by denitrifying bacteria.[40] The highest nitrate levels were recorded in Deldorawatta leachate (136.65 mg/L). Ammonia, a water-soluble gas, is the main reducing agent, and a significant long-term pollutant in leachates.[37] Higher NH_3 levels have been linked to eutrophication and a reduction in DO, which is evident in the results of this study.[35]

In Sri Lanka, leachate discharge is regulated under the National Environmental Act.[39] The regulations specify that the discharge of pollutants into inland surface waters must adhere to the Ambient Water Quality Standards. However, recorded pH (3.93 in Deldorawatta leachate), COD (2,000 to 36,000 mg/L), EC (27.07 to 32.24 mS/cm), DO (1.13 to 1.23 mg/L), nitrate (99.38 to 136.65 mg/L), ammonia (794.48 mg/L in Karadiyana), and dissolved phosphates (493.68 to 6,418.95 mg/L), were beyond the tolerance limits specified by these standards. Therefore, leachates must be treated before discharging them into inland waters.

Antibiotics from the leachates were extracted using the solid phase extraction (SPE) method and were quantified using HPLC. However, no TET, OTC, SMX, or SDI antibiotic residues were detected. This may indicate that the selected antibiotics were below the detection limit (0.05 ppm) of the HPLC. However, resistant bacteria were isolated from plates that contained OTC, SMX, and SDI at 60µg/mL.

OTC is used to treat respiratory, and urinary tract infections (UTIs), and in veterinary medicine, it is used to treat bovine respiratory disease (BRD) in cattle, and respiratory infections in horses.[41] TET and OTC are utilized as growth promoters in animal feed at sub-therapeutic concentrations. SMX is used to treat UTIs, chronic bronchitis, traveller's diarrhoea, and shigellosis.[42] SDI is used to treat pneumococcal, staphylococcal, and streptococcal infections as well as gonorrhea.[43]

The Minimum Inhibition Concentration (MIC) was checked for the isolates against TET, OTC, SMX, and SDI from

60 to 360µg/mL. MIC is the lowest concentration of an antibiotic that completely prevents visible growth of the isolate.[38] 15.625%, 21.875%, and 21.875% had MIC ≥ 360µg/mL against TET, OTC, and SDI respectively. It should be noted that isolates 14 and 15 had a MIC ≥ 360µg/mL for TET, OTC, and SDI.

Multiple Antibiotic Resistance (MAR) is when an organism shows resistance to two or more classes of antibiotics. The MAR index is useful when locating sources of ARB. Antibiotic usage sites are considered high-risk sources of contamination if their MAR > 0.2.[45] Calculated MAR index values ranged from 0.75-1. MAR index of 96.875% of isolates was 1, indicating resistance to all 4 selected antibiotics at 60 µg/mL.

There is an increasing interest in how sub-inhibitory concentrations of antibiotics can increase mutation rates, and HGT and continue to exert selection pressure through resistant mechanisms.[46] This may be why the isolates were resistant to certain concentrations of the tested antibiotics, even though negligible concentrations of antibiotic residues were present in the leachates.

Bacterial resistance mechanisms can be classified as intrinsic, acquired, or adaptive.[47] Resistance due to the natural abilities of the bacterium is known as intrinsic resistance. Acquired resistance is when a previously susceptible bacterium develops a resistance mechanism through a mutation or the acquisition of new genetic material from an external source.[48]

Adaptive resistance is the resistance to one or more antibiotics that arises through particular environmental conditions, such as stress, growth state, pH, ion concentrations, nutritional circumstances, or sub-inhibitory antibiotic levels. It is temporary, compared to inherent and acquired resistance. This enables bacteria to react to antibiotic challenges rapidly. When the stimulus is eliminated, adaptive resistance usually returns to its initial state.[47]

Due to the overuse and misuse of antibiotics, this natural genetic evolution of microbes to resist antibiotics has reached absurd levels in the 21st century, affecting the effectiveness of pathogen control and leading to significant medical consequences.[49] Antibiotic resistance compromises the ability to cure common infections such as the flu and typhoid, challenges the treatment of many microbial infections, and can result in treatment failure, extended sickness, permanent disability, or even death.[50] Individuals with antibiotic resistance require extended treatments and expensive medications which is a burden for low-income and developing countries.[51]

5. Conclusion

It is necessary to treat landfill leachates by adhering to Central Environment Authority guidelines before discharging them into the environment as they pose risks to surface and groundwaters. Antibiotics are newly emerging contaminants found in landfill leachate. Millions of lives have been saved by antibiotics, rendering them effective in preventing and treating microbial illnesses. However, bacteria have and will gradually evolve resistance to these antibiotics through a variety of innate and acquired mechanisms.

The study finds that although no antibiotic residues were present in the leachates, isolated bacteria showed resistance to TET, OTC, SMX, and SDI from 60 to 360 µg/mL. This suggests that the inherent nature of antibiotic resistance in bacteria may allow ARB to spread even in the absence of antibiotic residues or at undetectable levels, creating a serious risk of spread of antibiotic resistance, leading to reduced efficacy in pathogen control. Should these circumstances persist, a "post-antibiotic era" may arise in which common infections and minor injuries are the predominant causes of death.

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