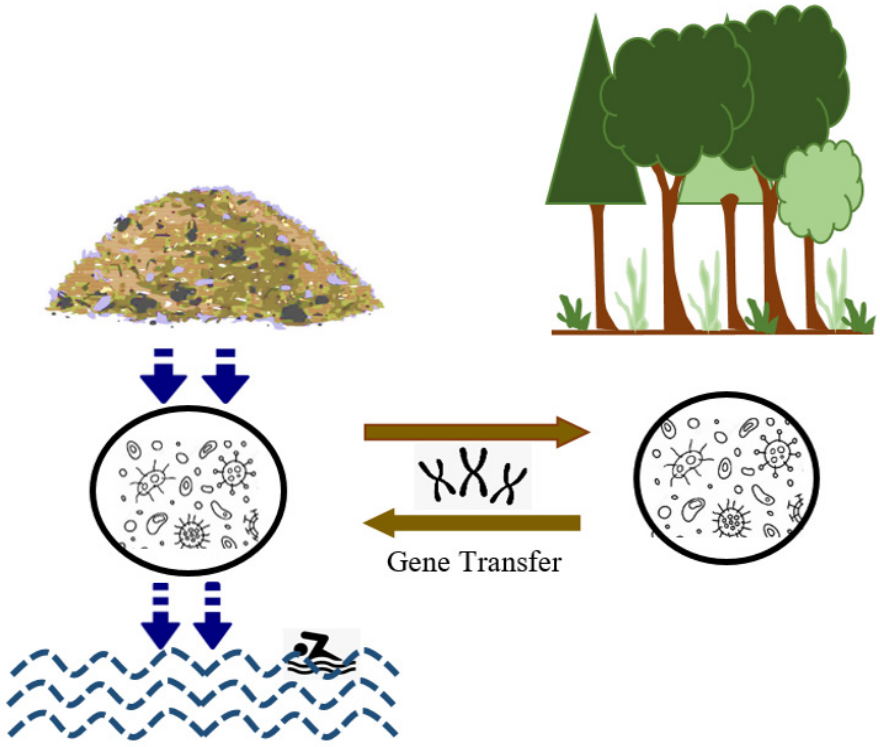
SHORT COMMUNICATION

Solid waste landfill sites: A hotbed for antibiotic-resistant bacteria?

P.A.T.V. Athukorala*, H.T.K. Abeysundara, B.S. Nanayakkara and H.M.S.P. Madawala



Highlights

- Development of antibiotic resistant bacteria (ARB) is a major challenge for human well-being.
- Open landfill sites in developing countries pose a major health and environmental risk.
- Unsorted landfill sites serve as hotbeds for developing antibiotic resistant bacteria.
- Soil leachates emanating from these landfill sites pollute surface and groundwater sources.
- Actions are obligatory to introduce safe waste disposal measures to lessen health risks due to ARB.

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Solid waste landfill site: A hotbed for antibiotic-resistant bacteria?

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Abstract: Open dumping of hospital and livestock waste pollutes soils and water with antibiotics, thus allowing open landfill sites to be lavish breeding grounds for antibiotic-resistant bacteria (ARB). The study investigates the antibiotic sensitivity of soil bacteria isolated from a major municipal solid waste landfill site (Gohagoda) in the Central Province of Sri Lanka, which is located close to the river Mahaweli, the main water source for residents of Kandy, the main city of the Central Province, and its immediate suburbs. Dominant bacterial isolates from soils of the Gohagoda landfill site (polluted, PO) and a nearby wooded area (relatively unpolluted, UP) were tested for antibiotic sensitivity against amikacin, amoxicillin-clavulanate, ciprofloxacin, cefotaxime, imipenem, meropenem and ticarcillin-clavulanate. The results were analyzed using t-test and z-test in Minitab 18.1. Mean diameters of inhibition zones of soil bacterial isolates from UP were significantly higher than those of PO for all antibiotics except for meropenem. The percentage of isolates resistant to all antibiotics was higher in PO compared to UP, except for amikacin and cefotaxime. The findings show a higher prevalence of ARB in the Gohagoda landfill site, emphasizing the importance of identifying alternative measures to dispose municipal solid waste and to introduce proper sanitation practices among landfill workers. Testing soil leachates for ARB is imperative to confirm any threats to the nearby water source.

Keywords: Antibiotic sensitivity; disk diffusion; landfill sites; soil pollution; soil bacteria.

INTRODUCTION

Antibiotic resistance is the loss of susceptibility of bacteria to antibiotics (Davison *et al.,* 2000). The widespread use of antibiotics and unsafe disposal of hospital and livestock waste have led to the development of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARG) causing serious health concerns. This trend has prompted the drug industry to spend more funds on research to invent new drugs (Yoneyama and Katsumata, 2006), though the development of new antibiotics did not match the same momentum as the threats imposed by ARB and ARG (Hutchings *et al*., 2019). The use of antibiotics has gathered its vigor since the 1940s to treat bacterial infections in humans (Andersson and Hughes, 2019). Subsequently, antibiotics were also used in veterinary medicine to prevent the spread of various bacterial infections among farm animals including aquaculture (Lipsitch *et al.,* 2002). The misuse of antibiotics in human and veterinary medicine, sanitizers and disinfectants together with the lack of awareness of its potential consequences eventually resulted in the rapid rise of antibiotic-resistant bacteria, thus rendering some infections untreatable (Singer *et al*., 2003; Prescott, 2014). ARB can transfer their resistance to known human commensal bacteria and clinically known pathogens through gene transmission, thus causing previously non-resistant bacterial species to become antibiotic-resistant bacteria (Adieze *et al.,* 2015).

Antibiotics are considered as soil pollutants (Wang *et al*., 2020). Untreated waste from hospitals, pharmaceutical industries, agriculture, livestock and households can end up in landfill sites facilitating the development of ARB over time. This issue can be a major concern especially in developing countries as they do not always practice safe waste disposal methods. Disposal of municipal solid waste (MSW) is one of the major challenges faced by countries with ever increasing human population in major cities and rapid urbanization. Open dumping of MSW is the most common option of waste disposal in developing countries especially due to lack of resources and facilities. However, open dumping of MSW can cause major environmental, social and health risks through soil pollution, and subsequently even contaminating surface and ground water sources. Agricultural soils may also act as reservoirs of antibiotic-resistant bacteria due to high use of antibiotic-treated animal manures as fertilizers (Xie *et al*., 2018). Open landfill sites seem to act as breeding grounds for ARB and ARG, thus the study of ARB prevalence among soil bacteria is of paramount importance as farmers and landfill workers are exposed to ARB in their day to day activities, making them prone to untreatable bacterial infections (Lee *et al.,* 2018).

Sri Lanka, being a developing country with a growing population, lacks safe waste disposal strategies, thus making the spread of ARB highly likely. Open landfills are the most common way of discharging municipal solid waste. Landfill leachates may also contaminate ground- and surface-water resources as these leachates are enriched with antibiotic-resistant bacteria and genes (Wang *et al.,* 2020). Kandy city is the most populous city in the Central

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Province of Sri Lanka with an approximate population of 160,000 (Department of Census and Statistics, 2012). Kandy Municipal Council (KMC) manages the Gohagoda solid waste landfill site, and dumps an approximately 100 - 130 tons of solid waste of the city daily (Welikannage and Liyanage, 2009). The Gohagoda solid waste landfill site is located close to the river Mahaweli, the main water source for the Kandy city and its suburbs. Therefore, this strategic location enhances the risk of polluting the water source by ARB through leachates from the open landfill site nearby. Accordingly, the current study aimed to investigate the antibiotic sensitivity of soil bacteria isolated from the Gohagoda solid waste landfill site in order to evaluate the direct and indirect risks of this unwarranted practice of waste disposal and potential threats to humans at large.

MATERIALS AND METHODS

Study site

The Gohagoda solid waste landfill site (7° 18' 47.85'' N and 80° 37' 19.02'' E) is the largest landfill site (approximately 2.5 ha in extent) managed by the Municipal Council of the Kandy City (Wijesekara *et al.,* 2014). The daily collection of municipal solid waste in the Kandy city exceeds 130 tons (Welikannage and Liyange, 2009), is mostly un-segregated, and ends up in the Gohagoda solid waste landfill site that is located just 100 m away from the river Mahaweli. The river Mahaweli is the longest river in Sri Lanka and also serves as the main source for pipe-borne water supply in the Kandy city limits and their immediate suburbs.

Sample collection

Five well-composite soil samples (each sample is a composite of 3-4 sub samples) were randomly collected (up to a depth of 20 cm) from the Gohagoda landfill (polluted: PO) site, using a hand-held spade. For comparison, another five samples were collected in a similar manner from a restored pine stand (unpolluted: UP) in the Hantana Mountain Range (7° 17' N and 80° 36' E), which is approximately 5 km away from the Gohagoda landfill site. From each soil sample, a dilution series (10^{-1} to 10^{-6}) was prepared with 1.0 g of soil, and 10^{-2}, 10^{-4} and 10^{-6} dilutions were used to prepare spread plates to culture the soil bacteria. The most dominant morphotypes were selected among all three dilutions of each soil sample for further sub-culturing. Twenty and 10 bacterial isolates were obtained from PO and UP soil samples, respectively.

Antibiotic sensitivity testing

Disk diffusion test was performed on Mueller Hinton Agar (MHA) (HiMedia) for 20 bacterial isolates from PO site and 10 from UP site using antibiotic-impregnated disks. Seven antibiotics commonly used in Sri Lanka, were used in the study *viz*., cefotaxime (30 µg), ticarcillin-clavulanate (75/10 µg), amoxicillin-clavulanate (20/10 µg), imipenem (10 µg), meropenem (10 µg), amikacin (30 µg) and ciprofloxacin (5 µg).

To perform the disk diffusion test, the bacterial inocula were prepared using 24-hour old fresh bacterial cultures in sterile normal saline (0.85% NaCl). The turbidity of each inoculum was made similar to that of the 0.5 MacFarland standard (0.05 mL of 1% $BaCl_2$ mixed with 9.95 mL of 1% H_2SO_4). Mueller Hinton Agar (MHA) plates were inoculated by dipping a sterile cotton swab in the bacterial suspension and spreading on the surface of MHA to obtain an even bacterial lawn. The antibiotic-impregnated disks were then aseptically placed on the surface of the agar plates using sterile forceps. Four antibiotic-impregnated disks were placed in one agar plate and the other three in another plate, equidistantly. Two replicates were carried out per bacterial isolate. The plates were incubated at room temperature for 24 hours. Diameter of the zone of inhibition was measured for each antibiotic at the end of the incubation period.

Statistical analysis

Statistical analysis was carried out with Minitab version 18.1. Data was analyzed using two sample t-test and two proportion Z-test. The t-test was used to compare the mean diameters of inhibition zones of the bacteria isolated from the two soils, PO and UP. Resistance proportion was the ratio between the number of isolates that showed no inhibition zone and the total number of isolates. Antibiotic resistance (as a percentage) was calculated by multiplying the above-mentioned proportion by 100. Two proportion Z-test was conducted to compare resistance proportions of bacterial isolates from the PO and UP soils. All statistical tests were performed at 5% significance level.

RESULTS

For all seven antibiotics, the mean diameters of zones of inhibition obtained for soil bacterial isolates from the UP site were higher compared to those from PO site. This indicates higher antibiotic sensitivity in bacteria isolated from UP site compared to PO site. Compared to the PO site, the mean diameters of zones of inhibition obtained for soil bacterial isolates from the UP site were significantly higher for all antibiotics except for meropenem (Table 1).

The percentage of isolates (as a percentage of the total isolates) that were resistant to antibiotics was higher in PO soils compared to that of UP soils, for all antibiotics. The differences were significant for all antibiotics except for amikacin and cefotaxime (Figure 1).

DISCUSSION

Waste management in developing countries is mostly based on low-cost methods such as open dumping and land-filling of un-sorted waste (Di Maria *et al*., 2018). Therefore, such landfill areas are identified as high-risk areas for generating and spreading antibiotic-resistant bacteria (Ejaz, *et al*., 2010). In favour, the present results also show a higher prevalence of antibiotic-resistant bacteria in soils from the Gohagoda solid waste landfill site compared to the relatively unpolluted pine site. As the landfill leachates may also contain ARB, there is a high chance of polluting the surface and ground-water sources nearby, as this landfill site is located close to the river Mahaweli, a major river in the area. A previous study conducted at the same

Table 1: Means of diameters of inhibition zones obtained from disk diffusion method for bacterial isolates from polluted (PO) and unpolluted (UP) soils with seven antibiotics. The level of significance; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ at significance level 5%.

Antibiotic (strength)	Mean Diameter of Zone of Inhibition (mm)		*p*-value
	Polluted Site (PO)	Unpolluted Site (UP)	
Amoxicillin-clavulanate (20/10 µg)	9.89	17.4	0.029*
Ticarcillin-clavulanate (75/10 µg)	11.4	19.1	0.009**
Imipenem (10 µg)	19.3	28.3	0.003**
Amikacin (30 µg)	14.3	21.6	0.001***
Cefotaxime (30 µg)	15.6	27.1	0.000***
Ciprofloxacin (5 µg)	20.0	25.0	0.000***
Meropenem (10 µg)	23.4	24.2	0.597ns

ns – not significant

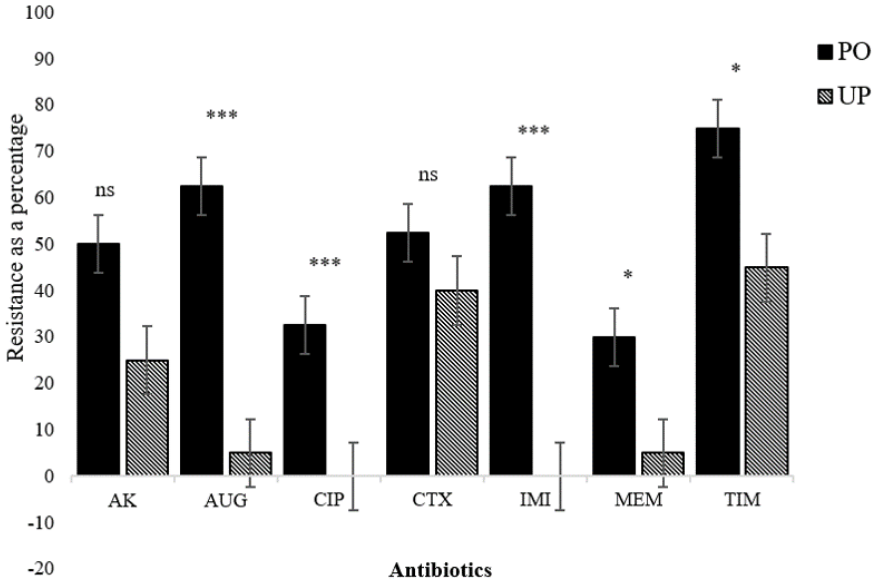

Figure 1: Antibiotic resistance (as a percentage of the total isolates) in bacterial isolates from polluted (PO) and unpolluted (UP) soils tested with seven antibiotics; AK – amikacin ($p = 0.096$), AUG - amoxicillin-clavulanate ($p = 0.000$), CIP – ciprofloxacin ($p = 0.000$), CTX – cefotaxime ($p = 0.355$), IMI – imipenem ($p = 0.000$), MEM – meropenem ($p = 0.043$), TIM - ticarcillin-clavulanate) ($p = 0.000$). Probability values are following the two proportion Z-test.

landfill site revealed that the flow of landfill leachate into the river Mahaweli is facilitated by rainfall and geological features of the area (Wimalasuriya *et al*., 2011). The river Mahaweli flows along the eastern boundary of the Gohagoda solid waste landfill site, thus increasing the chances of contaminating water by leachates from the landfill site. The Greater Kandy Water Treatment Plant's main intake is also located close to the Gohagoda solid waste landfill site, highlighting the gravity of this problem (Balasooriya *et al.,* 2011). The potential contamination of the river Mahaweli with ARB poses a major health risk, as it is not only the main source of drinking but also bathing and recreational activities for communities in and around Kandy (Mayakaduwa *et al*., 2012).

Bacterial infections can be fatal if antibiotics are not effective in treating them (Andersson and Hughes, 2019), thus it is important to avoid development and spread of antibiotic-resistant bacteria. The present results highlight the potential of the Gohagoda solid waste landfill site as becoming a hotbed for ARB. Therefore, it is imperative to introduce healthy waste disposal and management measures to avoid such health catastrophes. Managing suitable sanitation practices among landfill workers and avoiding the establishment of landfill sites close to water sources are also considered as important measures to mitigate this issue (Achudume and Olawale, 2009). The leachates from the Gohagoda solid waste landfill site are known to pollute water of the River Mahaweli (Wijesekara *et al*., 2014). As there is a high likelihood of antibiotics and ARB seeping to the leachate, the analysis of soil leachates is an essential next step in order to confirm potential risks imposed on the nearby water source. In addition, increasing the awareness

among the general public of the health risks of overuse and misuse of antibiotics and introducing regulations to control discharge of unused or expired antibiotics will be helpful in preventing the development and spread of ARB.

CONCLUSION

The study concludes that Gohagoda solid waste landfill site can act as a reservoir of antibiotic-resistant bacteria probably due to the unsafe release of hospital and livestock waste directly into the landfill site, eventually posing a public health risk. Thus, prompt action is necessary to introduce safe disposal methods especially for hospital and livestock waste. Further studies are recommended to explore the chances of polluting the nearby river Mahaweli with ARB.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interests.

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