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BACTERIOLOGICAL EVALUATION IN EXPERIMENTAL LANDFILL CELLS: CO-DISPOSAL OF HOUSEHOLD SOLID WASTE AND HEALTHCARE SOLID WASTE

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ABSTRACT

This work aimed to study the effects of co-disposal of household solid waste (HHSW) and healthcare solid waste (HCW) on the microbiota of the leachate generated in experimental landfill cells. To this end, three waste containment units were built, differentiated by the percentage of HHSW and HCW: HHSW cell (100% household solid waste), HCW cell (100% healthcare solid waste) and COD cell (cell simulating co-disposal containing 98% HHSW and 2% HCW). Volume, temperature, pH and evaluation of the leachate's microbiota from the cells were monitored over 810 days of confinement. Culture-dependent methods (quantitative analysis of environmental contamination indicators and investigation in enterobacteria and *Staphylococcus aureus*), antimicrobial susceptibility patterns, and inhibitory activity were evaluated for microbiota monitoring. The microbiological characterization of the leachates from the cells showed no statistically significant differences in total coliforms, *Escherichia coli*, enterococci and *Pseudomonas aeruginosa* densities during the period evaluated. Enterobacteria of medical importance were identified in the leachates from the three cells: *E. coli, Klebsiella pneumoniaea, Enterobacter* sp and *Proteus mirabilis*. After 810 days of confinement, the leachates showed no antimicrobial activity against *S. aureus, P. aeruginosa* and *Salmonella enterica* serovar Choleraesuis. Statistical analysis of antimicrobial resistance patterns revealed significant similarities among the three cells. The results suggest no significant differences in the behavior of experimental landfills containing HHSW and HCW concerning the presence of pathogenic microbiota.

1. INTRODUCTION

All waste produced by medical facilities, labs, and research facilities is referred to as healthcare waste (HCW). Most HCW is non-hazardous, and 85% in weight is similar to household waste; the remaining 15%, which may be radioactive, toxic, or pathogenic, is considered dangerous (Aziz et al., 2022). The HCW management includes the stages of minimization, generation, segregation, packaging, identification, collection, storage, transportation, treatment and environmentally appropriate final disposal (Santos et al., 2022).

There are increasing concerns about the negative impacts of healthcare waste generated in hospitals, especially in low- and middle-income countries. According to Mol et al. (2022), median values indicated that HCW generation was the highest in North and South America (4.42 and 1.64 kg HCW/bed/day, respectively); Africa was the lowest HCW producer (0.19 kg HCW/bed/day).

Several types of hazardous waste include infectious, sharp, chemical, pharmaceutical, and non-hazardous. Therefore, proper methods for handling HCW must be considered, and segregation must be implemented (Lee et al., 2024).

The treatment of HCW in Brazil is an important public and environmental health issue regulated by various standards and legislation. The main regulation is Resolution

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222 from the National Health Surveillance Agency (ANVISA, 2018), which provides for managing waste from healthcare services. The resolution classifies the HCW into different groups according to its risk potential (Table S1 – Supplementary Material). In Brazil, some state or municipal regulations require the thermal treatment (autoclaving) of part of Group A waste (subgroup A1), such as cultures and stocks of microorganisms, waste from manufacturing biological products, culture media and instruments, and genetic manipulation laboratories. They are treated directly at the source, usually by autoclaving, and are treated again (incineration or autoclave processes) after leaving the healthcare facility. After the second treatment, they become considered common waste. In some cities, the same occurs with some biological waste (subgroup A3, for example, human anatomical parts) that, by law, does not need to be treated. This causes a burden for municipalities, especially low-income ones, as thermal treatment systems are much more expensive than sending them to landfills.

Some researchers consider HHSW a potential agent for spreading pathogenic microorganisms to humans and other animals and a vehicle for spreading antimicrobial drug resistance markers (Nascimento et al., 2009; Chagas et al., 2011; Zhang et al., 2012). On the contrary, studies on the bacterial content of hospital waste indicate that it is not a very good culture medium for pathogens and disease transmission (Mühlich et al., 2003).

While it is true that the treatment of HCW is mandatory in the United States and Europe and similarly required in Brazil, it is important to consider the economic realities developing countries face. In many regions, the high cost of advanced waste treatment technologies presents a significant barrier to implementation. Advanced technologies, such as incineration, autoclaving and microwave ovens, may be inaccessible or limited in scope due to a lack of technical support or investment (Blenkharn, 2006). Around 891 tonnes of solid healthcare waste in Brazil are collected daily (Theophilo, 2018). Although regulations mandate healthcare waste treatment in Brazil, the financial burden associated with these treatments is often prohibitive for many municipalities. These costs are not always sustainable for cities in developing countries, where resources are limited and competing priorities are numerous. Therefore, it is crucial to explore alternative solutions that are both effective and economically feasible for ensuring public health and environmental standards are met without imposing undue financial strain on developing regions.

In this context, the ability to release microorganisms and substances that compromise the environment is not inherent to waste originating from healthcare establishments. Waste generated at home, in commerce and industry, as well as effluents from water treatment plants, are known for their contribution to the load of antimicrobial drugs and sanitizers in wastewater and microorganisms from different sources of contamination, such as diapers, condoms, sanitary pads and paper (Threedeach et al., 2012; Theophilo et al., 2018), as well as piercing and scarifying materials (Cussiol, 2005; Theophilo et al., 2017).In contrast, not all waste classified as HCW results from procedures applied to patients with infectious diseases, but from plastic surgery, victims of various accidents (car accidents, falls, cuts) and diseases non-infectious, like heart disease. Recently, SARS-CoV-2 was detected on HHSW fresh truck leachate in a study to investigate the potential of SARS-CoV-2 surveillance in these samples (Lanzarini et al., 2023).

The co-disposal method can be used to treat HCW. However, this method is only allowed during emergencies in some countries (Lee et al., 2024).

Because of the disposal of solid waste in landfills, leachate is currently considered the most impactful emission from an environmental point of view. Exposure of the environment to leachate can occur in different ways: through excessive rainfall, uncontrolled overflow from equalization ponds, infiltration or failures in treating leachate discharged into water bodies. The impact of landfill leachate on microflora and microfauna is determined by factors such as high concentrations of organic matter and ammonia nitrogen, as well as other contaminants carried by the mass of waste (Liu et al., 2011; Costa et al., 2019). In this sense, the landfill is a complex heterogeneous system in terms of its physical, chemical and biological characteristics, where different microorganisms coexist and interact (Szyłak-Szydłowski and Kornilłowicz-Kowalska, 2011).

The characteristics of the leachate generated from the disposal of solid healthcare waste in landfills can be similar to those of hospital wastewater (Novo and Manaia, 2010). The entry of hospital-origin bacteria into an environment can represent a significant source of the spread of antimicrobial resistance (Prado et al., 2008). In particular, the occurrence of antibacterial agents in the environment raises concerns about environmental risks and the spread of antimicrobial resistance among microorganisms (Chagas et al., 2011). Whether products with antimicrobial activity are transported into the environment from human or animal sources, select antimicrobial-resistant strains of bacteria, or cause deleterious effects on water quality are controversial but unresolved issues (Chang et al., 2010).

The confinement of waste in landfills can provide microbial communities with the right conditions for their interactions and successions, making leachate a vehicle for multidrug-resistant microorganisms and producers of pathogenicity mechanisms (Liu et al., 2019).

This study aimed to investigate whether healthcare solid waste and household solid waste differ significantly in terms of their microbiota and their pathogenic potential when confined in a landfill. For this purpose, the presence and the diversity of pathogenic microorganisms and phenotypic characteristics were analyzed using experimental landfills for the confinement of household solid waste and healthcare solid waste.The novelty of this work lies in comparing the microbiota and pathogenic potential of healthcare solid waste and household solid waste when confined in a landfill. This study investigated potential differences by analyzing the diversity of pathogenic microorganisms and phenotypic characteristics in experimental landfills dedicated to each waste type.

2. MATERIALS AND METHODS

2.1 Landfill experimental cells

The study was carried out by monitoring leachates generated from three landfill experimental cells. The site chosen to construct the experimental cells was the Recycling and Composting Plant of Jacarepaguá, which belongs to COMLURB (Municipal Urban Cleaning Company – Rio de Janeiro Municipality, Brazil). Three experimental cells were built, arranged side by side, separated by a distance of 2.0 m each other (top of slope) in a total plane area of 129 m^2 model as shown in Figures S1a, b and c (Supplementary Material).

Implementation steps followed the essential criteria for the construction of landfills (Brazilian Association of Technical Standards- ABNT NBR 15849, 2010), such as liner system, waste compaction, drainage of the leachate, rainwater and gases, input control waste (weighing), solid waste disposal, coatings with a layer of soil and environmental monitoring.

The cells were identified as the composition of their waste. The HHSW cell comprised 100% household solid waste and 43.350 tonnes of mass. The HCW cell comprised 100% infectious healthcare solid waste and a mass of 13.680 tonnes. The third cell, the COD cell comprised 98% of HHSW and 2% of HCW, with a mass of 43.207 tonnes. This composition corresponds to the mass percentage of urban solid waste sent to the final disposal units of the public system of the city of Rio de Janeiro (PMGIRS, 2024).

HCW was deposited in the cell as they were collected from the generating units and belonged to groups A, D and E (Table S1, Supplementary Material). According to Brazilian legislation (ANVISA, 2018), subgroup A1 waste cannot leave the generating unit without prior treatment. This waste is autoclaved but still leaves the units belonging to subgroup A1. The other groups, for which no prior treatment was required, were disposed of as they were collected at the generating unit. HCW from Group E was disposed of in cardboard boxes at the generating unit and received no treatment before final disposal.

Each cell had the following dimensions: 4.5 m lower base, 6.0 m higher base, 5.0 m high and 6.0 m side length, totaling 157.5 m^3 volume capacity. For forming embankments and bases of cells (berms), construction wastes were used. The base and sides of the cells were lined with a high-density polyethylene (HDPE) geomembrane liner, with 1.0 mm thickness. The drainage gutters were excavated manually, and the center of the cell had a depth of 30 cm wide and 4.0 m long. After HDPE (high-density polyethylene) geomembrane, drainage trenches are filled with a layer of geotextile (Bidim 400.5 x 2.5 m). In sequence, the drainage system was installed, consisting of a tube canaflex type, connected to a PVC tube receiver coupled to a 50 mm PVC-registration union 50 mm and a tap with a 90° curve exit for collection samples. The tubes containing the trenches were filled with crushed n°. 3, starting at a height of 10 cm (back of the cell) and deepening 30 cm (front cell). The Bidim was then folded over the gravel bed, wrapping it completely. Besides filtering the effluent, this device facilitates its flow, avoiding clogging. Vertical drains were built in a circular section with PVC pipe DN 100 mm perforated (3/8 mm holes), 2.3 m long, and a wire mesh of 1 m wide and 2 m long. The system extended from the base of the waste mass to 20 cm above the surface layer of clay. The tube was disposed of at the center of the cell, on the drainage leachate. Then, the wire screen is arranged around the pipe, 10 cm, and its side was stitched with wire to wrap around it. The space between the tube and the screen was filled with crushed stone n° 3. After filling the cells and surface waterproofing, galvanized iron burners were attached to the PVC pipe because there was no intention to collect gas generated during the experiment.

2.2 Gravimetric composition of waste

The gravimetric composition consisted of determining the percentage fractions of different types of waste obtained by sampling the collections made in the following neighborhoods in the municipality of Rio de Janeiro. One 240L container of waste was taken from the contents of each truck. The waste was homogenized using a mechanical shovel. HCW was collected on 2 consecutive days from the route of various healthcare establishments.

Samples were collected using the quartering method (ASTM, 2003). After homogenization, the waste was divided into four equal parts. From each part, two samples were taken from diametrically opposite positions. Each sample was equivalent to a 200L container. The total amount sampled at this stage was approximately 1600L. The quartering procedure was repeated thrice on the split samples, reducing the total sampled to 800L, 400L and 200L (final sample). From the 200L sample, the materials were separated and differentiated into 11 components: Paper, Cardboard, Plastic (polyethylene, polypropylene, polystyrene), Glass (colorless/colored), Metal (ferrous/non-ferrous), Textiles, Putrescible Organic Matter, Wood, Electronics, Inert materials and Others.

The components separated by the methodology proposed in this project to determine the gravimetric composition of household waste were assessed for the presence of hazardous waste. They were characterized by previously determined elements, as shown in Table 1.

2.3 Leachate Collection

Leachate were drained daily through the tap until the cell was empty. The samples were preserved by packaging at a temperature below 4°C for later carrying out the physicochemical and microbiological analyses.

2.3.1 Physicochemical analyses

The pH and temperature were measured. For temperature measure, a mercury glass thermometer with a scale of -10 to 100°C was inserted into the volume of liquid used to measure the temperature. The temperature in the external environment was also measured.

2.3.2 Microbiota analyses- culture-dependent methods

- Total coliforms, *Escherichia coli* and enterococci
  The analyses were carried out following the APHA (2012). The densities of total coliforms, *Escherichia coli* and enterococci were expressed as the Most Probable

TABLE 1: Potentially hazardous components of HHSW.

Component	Potentially infectious or chemical constituents
Paper	Toilet paper; Paper towel
Plastic	Disposable syringe; Shaving razor; Flexible rods; Serum bottles; Absorbent; Disposable diaper; Hair dye gloves; Vaginal applicator; Bandages; Chemical product bottles
Glass	Shards; Ampoule; Lamp; Medicine bottles
Putrescible organic matter	Animal feces, Carcasses, animal parts
Metal	Needle; Pin; Open can; Nail, screw, tack; Staple; Cutlery; Razor blades; Rebar; Chemical bottles; Pill bottle
Rubber	Procedure gloves
Textile material	Bandage; gauze; cotton; adhesive plaster; burlap
Other	Cat sand; ceramic shards, wood splinters

Numbers per 100 milliliters of sample (MPN 100 mL^{-1}) and determined using the Multiple Tube Technique.

- *Pseudomonas aeruginosa*
  The multiple tube technique determined the analysis according to ISO 16266 (2006). The method consisted of inoculating serial dilutions in asparagine broth (5 tubes per dilution) followed by incubation at 36°C ± 0.5/24-48h.
- Total aerobic mesophilic heterotrophic bacteria
  The deep plating technique was used with Tryptone Glucose Yeast Extract Agar ("Plate Count Agar"), starting with dilutions 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. The samples were analyzed in duplicate for each dilution and incubated at 36°C ± 0.5/24-48h. Heterotrophic bacterial populations were expressed as Colony Forming Units per milliliter sample (CFU mL^{-1}).
- Enterobacteria testing
  For the isolation of enterobacteria, an inoculum taken from the leachate sample was transferred to eosin methylene blue agar (EMB agar), and plated in duplicate, followed by incubation at 35-37°C for 24 hours.
  Suspicious colonies were selected, and the identification of the bacterial species was based on the phenotypic characteristics provided by conventional biochemical tests following the ANVISA (2013) for the detection and identification of bacteria of medical importance, which indicates the following tests as the main ones for identifying enterobacteria: oxidase, lactose fermentation, glucose fermentation, L-lysine decarboxylation, gas production, use of citrate as the sole carbon source, indole production, hydrogen sulfide (H_2S) gas production, urease production, phenylalanine deaminase production and motility. The complementary tests were used for the beta-galactosidase (ONPG), ornithine decarboxylation, arabinose fermentation, and tryptophan deamination tests.
  Confirmation of the identification was made using the API (Analytical Profile Index) System program for detecting similarities between species.
  Salmonella testing was based on ISO 6579 (2017). The test consisted of the selective enrichment of 1 mL of the crude sample in 10 mL of Kauffmann's tetrathionate broth, added to an aqueous iodine solution and a 0.1% brilliant green solution. After incubation, an inoculum of the broth was transferred to plates with the selective-indicator media Salmonella-Shigella agar (SS agar) and xylose lysine deoxycholate agar (XLD), followed by incubation at 35-37°C for 24 hours. Slide agglutination tests using polyvalent serum "O" (Probac do Brasil Produtos Bacteriológicos Ltda.) confirmed colonies indicative of Salmonella.
- *Staphylococcus aureus* testing
  The occurrence of *Staphylococcus aureus* was recorded as presence or absence. As a selective enrichment method, 10 mL of the raw leachate was transferred to salt mannitol broth, followed by incubation at 36°C ± 0.5 / 24 hours. After this period, the culture was sown on salt mannitol agar with egg yolk (20g/L) in triplicate, followed by incubation at 36°C ± 0.5 for 24 hours (McFaddin, 1977). Colonies that were translucent yellow surrounded by a precipitation halo were subjected to the catalase and coagulase tests (Nascimento et al., 2009). *Staphylococcus aureus* (ATCC 6538) strains were used as a positive control, and *Enterococcus faecalis* (ATCC 11700) as a negative control.
- Susceptibility of the isolated strains to antimicrobials
  According to the CLSI (2023), the agar diffusion method was used for this analysis. Pure colonies of recently cultured bacteria (18-24 hours) isolated from non-selective culture medium were suspended in sterile saline solution (0.85% NaCl) until turbidity compatible with 0.5 on the Mac Farland scale (106 CFU/mL) was obtained. A sterile swab was soaked in the bacterial suspension and then sown gently in all directions on the plate (five directions), trying to cover the entire surface. The disks were deposited on the surface of the inoculated medium. The plates with the disks were incubated in a bacteriological oven at 36°C for 18 to 24 hours.
  Based on the diameters of the inhibition halos, the samples were defined as susceptible or resistant to each of the antimicrobials tested, using standards established by CLSI (2023). Standard strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27953) and *S. aureus* (ATCC 25923) were used as a quality control. In this study, the intermediate susceptibility profile was considered resistant. For the strains of enterobacteria isolated in the leachate, the following antimicrobials were selected according to CLSI (2013): Ampicillin (10µg); Amoxicillin-clavulanic acid (20/10µg); Cefoxitin (30µg); Cefazolin (30µg); Cefotaxime (30µg); Ceftazidime (30µg); Cefepime (30µg); Amikacin (30µg);

Gentamicin (10µg); Meropenem (10µg); Imipenem (10µg); Ciprofloxacin (5µg); Aztreonam (30µg) and Sulfamethoxazole-Trimethoprim (25µg). For the enterococci strains isolated in the leachate, the following antimicrobials were selected according to CLSI (2013): Ampicillin (10µg); Oxacillin (10µg); Gentamicin (10µg); Gentamicin (120µg); Streptomycin (10µg); Streptomycin (300µg); Cefoxitin (30µg); Ciprofloxacin (5µg); Teicoplanin (10µg); Sulfamethoxazole-Trimethoprim (25µg) and Vancomycin (30µg). For the *Pseudomonas aeruginosa* strains isolated in the leachate, the following antimicrobials were selected according to CLSI (2013): Amikacin (30µg); Gentamicin (10µg); Meropenem (10 µg); Imipenem (10µg); Ciprofloxacin (5µg); Aztreonam (30µg); Cefotaxime (30µg); Ceftazidime (30µg); Cefepime (30µg); Piperacillin/Tazobactam (100/10µg).

- Search for ESBL-producing enterobacteria (extended-spectrum beta-lactamase enzymes)

  The procedure recommended by CLSI (2023) included an initial screening test using disk diffusion by analyzing the inhibition halos obtained using disks of third-generation cephalosporins and aztreonam The presence of inhibition halos smaller than 22 mm for the ceftazidime disk (30 µg), < 27 mm for the cefotaxime disk (30µg), < 25 mm for the ceftriaxone disk (30µg) or < 27 mm for the aztreonam disk (30µg) should raise the suspicion of ESBL production (CLSI, 2013). To confirm the phenotype, the disk approximation test proposed by Jarlier et al. (1988) was used (Zemelman et al., 2002; Dalmarco et al., 2006). This method placed a disc containing amoxicillin and clavulanic acid in the center of a Mueller-Hinton agar plate. Around this, the marker antimicrobials (Aztreonan, Cefepime, Ceftazidime and Cefotaxime) were positioned at 20 mm from the amoxicillin-clavulanate disk. The appearance of a "ghost zone" or the widening of the cephalosporin inhibition halo confirms the production of ESBL. The standard strain *Klebsiella pneumoniae* (ATCC 700603) was used as a positive control for the test. In contrast, *Escherichia coli* (ATCC 25922) was used as a negative control.

2.4 Statistical analysis

The data obtained from determining the volume of leachate and rainfall at the site was analyzed using Pearson's correlation coefficient. Pearson correlation is a statistical technique used to measure the degree of relationship between two variables. Pearson correlation coefficient takes values between -1 and 1, where 1, 0, and -1 indicate a perfect match, no correlation, and perfect negative correlation, respectively (Berman, 2016).

The data obtained from determining microbiological parameters was applied to Box Plot graphs and subjected to statistical analysis using non-parametric tests.

The Kruskal-Wallis test was used to compare the medians (the null hypothesis in Kruskal-Wallis testing was Ho) of the population densities of total coliforms, *E. coli*, *Enterococci* and *P. aeruginosa* and pH, with a confidence level of 95%. When the value of the Kruskal-Wallis test statistic indicated that at least one pair of sample groups was different from the others, the multiple comparisons test should be applied, as suggested by Siegal and Castellan (1988).

Fisher's exact probability test was applied to check the similarities between antimicrobial susceptibility profiles. This test requires the samples to be random and independent, where the classes are mutually exclusive, and the measurement levels are on a nominal scale (Siegel and Castellan, 2008). An advantage of this test is that there are no restrictions on the sample size of each cell.

Statistical tests were performed on Statistica version 7.

3. RESULTS AND DISCUSSION

3.1 Cell content and waste composition

The mass of waste needed to fill the HHSW and COD cells was approximately 3 times greater than that of HCW needed to fill HCW. The differences in the specific weight of HCW and HHSW explain this fact. This characteristic is related to the compression or compaction properties of the waste. The HHSW deposited in the HHSW and COD cells had a specific weight of 320 kg/m³, while the waste disposed of in the HCW cell had a specific mass of 101 kg/m³.

Figure 1 (a and b) shows the gravimetric composition of the HHSW destined for the cells. The highest percentage found among the components was the putrescible organic matter, represented by the remains of processed food, expired food, fruit and vegetable peelings and feces. As for the recyclable components of HHSW, plastic had the highest percentage among potentially recyclable materials (19%w/w), followed by paper (16% w/w). The waste composition that filled the cells (HHSW and COD) is similar to the mean composition of Brazilian solid waste (SNIR, 2024). Figure 1 (c and d) show the percentages of the components identified as HCW. Of the total recyclable materials, plastic products accounted for the most considerable fraction (38% w/w), followed by "Other" components (31% w/w). In contrast to HHSW, the "organic matter" component in HCW, represented by anatomical parts, blood, viscera, and food scraps, is found in a lower percentage concerning recyclable materials, contributing 13%.

Common solid waste was also found with the HCW, such as leftover food, yogurt pots, cookie wrappers and soda bottles, revealing the poor management of this waste in hospital environments, mainly due to the lack of preparation of healthcare professionals.

In the context of this study, it was essential to highlight the presence of materials with infectious piercing or scarifying potential originating in the home. The infectious portion of HHSW was characterized by 859 kg of waste collected from 22 residential districts in Rio de Janeiro municipality. Voudrias et al. (2012) determined the composition and production rates of pharmaceutical and chemical waste produced by a Hospital in Greece and found that the total production of chemical waste comprised 1.8% w/w of the total hazardous medical waste produced by the hospital.

Around 17% of the mass of HHSW collected corresponded to hazardous waste. Plastic was

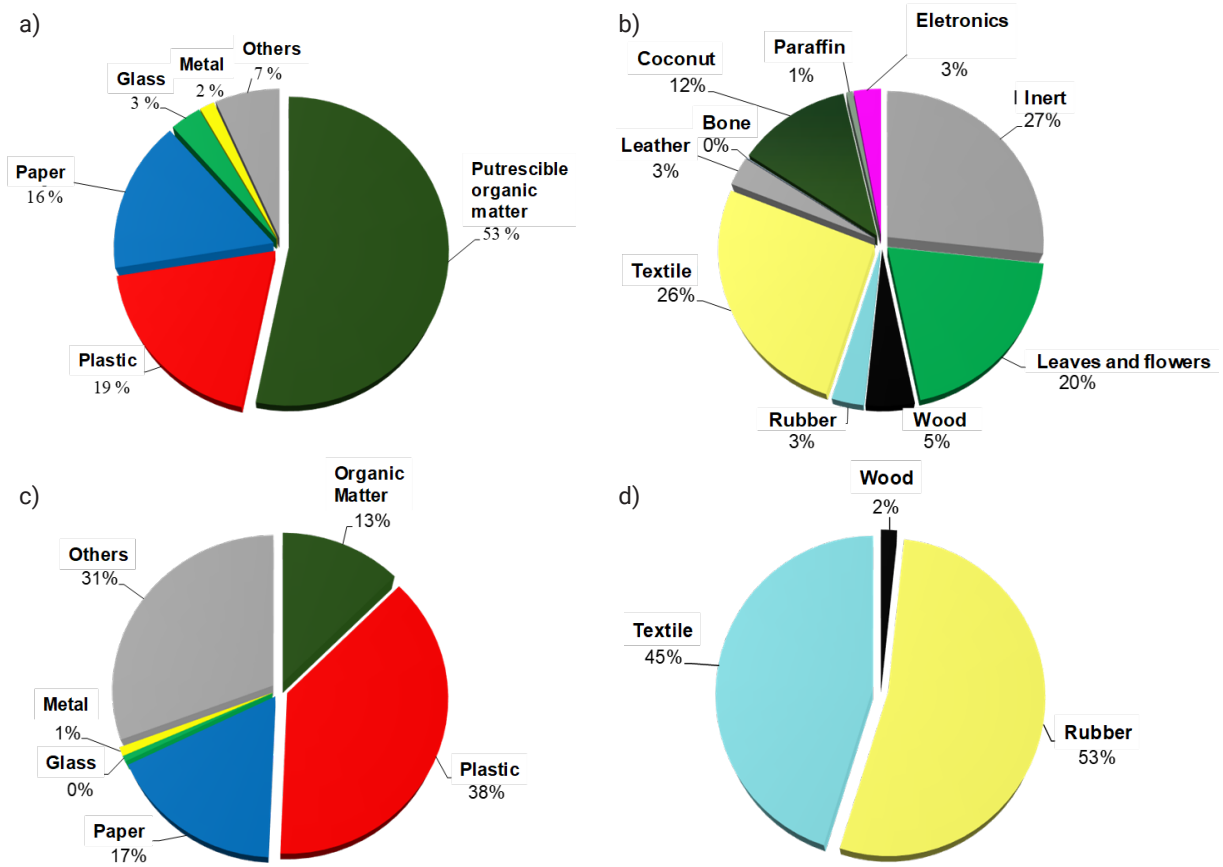

FIGURE 1: Gravimetric composition of the (a) HHSW destined to fill the HHSW and COD Cells; (b) fraction "Other"; (c) HCW destined to fill the HCW and COD Cells; and (d) fraction "Other".

predominantly used as a potentially infectious or chemical material. Figures S2, S3 and S4 (Supplementary Material) show the identification percentages of these materials according to the main component. Around 25% of the sample's paper/cardboard and plastic components were identified as potentially infectious (hazardous waste) (Figures S2 and S3 – Supplementary Material). Regarding the organic fraction, around 1.53% showed infectious characteristics (Figure S4 – Supplementary Material).

Materials such as needles, razors, vaginal applicators, disposable syringes and gloves, flexible rods, dye gloves, ceramic shards and condoms were detected. However, their percentage was less than 0.01% in HHSW. Based on the information from the analysis of the composition of HHSW and HCW, both had in their composition medicines, drugs, used diapers, sanitary napkins, condoms, disposable clothing, food waste, sanitary paper, disinfectant bottles, and other chemical substances such as cresols and ammonia, solvents, pigments, batteries and sharp materials (hypodermic needles) and scarifying materials (razor blades).

3.2 The volume of leachate produced

In this study, leachate generation was constant, and the efficiency of the drainage and collection system installed in the cells was proved. The mean volumes of leachate generated in the cells each month and the rainfall in mm measured at the pluviometry station are shown in Table 2. When comparing the mean volumes of leachate generated during the month with the pluviometry using Pearson correlation analysis, a perfect positive association was found between the values (>0.8). A Pearson coefficient greater than 0.7 indicates a strong correlation between two variables (Berman, 2016). Therefore, the results indicate a positive and strong correlation between the rainfall and leachate generation.

During the driest season (winter), a lower volume was generated in the systems of the 3 cells (maximum volume values of 28.3, 47 and 55.2 L for HHSW, HCW and COD cells, respectively). During the rainiest periods of the year (summer) in the municipality of Rio de Janeiro, the volumes reached maximum values (254, 327.5 and 450 L for HHSW, HCW and COD cells, respectively).The presence of water in the waste due to the initial humidity and the rainfall that infiltrates are important mainly for the first step of anaerobic degradation (hydrolysis), promoting the dilution of inhibitory agents and facilitating the distribution of microorganisms and nutrients in the MSW mass.

The volume of percolate produced in sanitary landfills depends on the following factors: precipitation and evapotranspiration in the landfill area; surface run-off; underground infiltration; natural humidity of the waste mass; degree of compaction of the waste; moisture retention capacity of the waste mass (Costa et al., 2019). In addition, the quality of a landfill's operation affects the amount of leachate produced. Operational care with adequate cover

TABLE 2: Average values of leachate generation and rainfall per monitoring month. HHSW - Household Solid Waste; HCW - Healthcare Waste and COD - Co-disposal of HHSW and HCW.

Season/ Year	Average leachate volume (L) generated in each cell per month of monitoring			Accumulated rainfall per month of monitoring (mm)
	HSW	HCW	COD	
Spring – Year 1	115	233	159.5	179.2
Summer - Year 1	152.5	327.5	398	82.8
	26	84	80.5	45.2
	3	6.2	5.8	123.6
Autumm - Year 1	44.7	80.4	139.9	154.6
	27.4	58.9	88.6	113.6
	8.9	21.6	20.4	34
Winter - Year 1	17	28.6	55.2	49.4
	7.4	11.6	14.3	51.6
	28.3	47	27.8	32
Spring - Year 2	20.7	100.9	140.5	160.4
	26	26.9	19	88.2
	40.2	35.9	39.6	204
Summer - Year 2	48.9	48.9	87	155.4
	16.5	23.9	24.3	48.6
	6	8.1	9.9	80.6
Autumm - Year 2	5.1	4.9	6	119.4
	113	109.2	167	148.8
	117.4	100.5	162.5	156
Winter - Year 2	15.8	22	24.3	54.6
	19.9	27.2	36.7	16.8
	22	11.2	30.3	88.4
Spring - Year 3	39.6	31.3	47.8	44.4
	77	61.5	141	94.2
	45	65.5	88.9	28.4
Summer - Year 3	126	91	245	125
	254	225	450	128.6

can reduce the amount of leachate generated, even in conditions of heavy rainfall (Gomes and Silva, 2005).

In this study, some factors were equivalents among the cells, such as environmental conditions (local rainfall, external temperature, and wind regime), constant cover layer material, absence of a vegetation cover layer and construction aspects (occupied area, slope inclination, drainage system and base waterproofing). Other factors were different, such as the percentage of recyclable and organic material in each cell and the degree of compaction of the waste and the cover layer.

3.3 pH and temperature

It was expected that during this first decomposition stage, there would be an increase in temperature because the oxygen present in the spaces between the newly buried waste is quickly consumed by aerobic microorganisms, producing carbon dioxide, water and an increase in temperature. However, the values indicate no variations in the 342 temperature measurements taken directly on the liquids leached from the cells (low standard deviation). Among the cells, the mean values were not statistically different (HSW cell=29.1±0.8°C; HCW cell=28.4±0.5°C; COD cell=28.6±0.6°C). The mean leachate temperature was in the mesophilic range (between 25 and 45°C), which is suitable for the growth of most pathogenic microorganisms, as well as indicators of environmental contamination.

The pH monitoring results are shown in Figure S5 (Supplementary Material). The pH values showed statistically significant differences. The average values and ranges for the HHSW, HCW and COD cells were, respectively, 7.0 (5.8-7.7), 6.8 (5.6-7.4) and 7.2 (6.7 - 7.7). Only one observation with a pH of 5.8 was obtained in the HHSW cell after 15 days of confinement and one with a pH of 5.6 in the HCW cell after 45 days of confinement, which can be explained by the acidic characteristic of the leachate in the initial stages of biological degradation of the organic matter present in the solid waste (Kjeldsen et al., 2002; Costa et al., 2019). The fact that the pH of the COD cell does not reduce to weak acid at the beginning of confinement can be explained by the heterogeneous nature of the waste. Alkaline materials, such as construction

waste, can increase the leachate pH. However, the pH results of the leachate generated in the 3 cells point to the optimal range for bacterial growth, between 6.5 and 7.5 (Kjeldsen et al., 2002) in all the observations after 45 days of confinement.

Other physicochemical parameters and ecotoxicity (organisms *Danio rerio* and *Vibrio fischeri*) of the HHSW and HCW cells at different monitoring times were statistically evaluated by Costa et al. (2019b). This study concluded that leachate from healthcare solid waste presented less or equal polluting potential compared with household solid waste: Values for organic matter parameters (COD-Chemical Oxygen Demand, TOC-Total Organic Carbon, BOD-Biochemical Oxygen Demand and Absorbance at 254nm), color, hardness, conductivity, sulfate, alkalinity, chloride, total solids were significantly higher from HHSW cell than HCW cell. Phosphorus, ammonia nitrogen and metals showed results statistically similar for both cells. Comparing EC50-50% Effective Concentration (for *V. fischeri*) and LC50-50% Lethal Concentration (for *Danio rerio*), the results showed that the HCW cell generated leachate that presented less or equal toxicity than the HHSW cell.

3.4 Microbiota analyses- culture-dependent methods

Figure 2 (a, b, c and d) shows the boxplot of the log of for the total coliform*, E. coli*, Enterococci and *P. aeruginosa* densities in the 3 cells. Table S2 (Supplementary Material) shows the mean values and variations for these measures.

The comparative evaluation of the cells showed similarities between the values observed, highlighting the biochemical versatility of this microorganism and its ability to survive in different environments. The bacterial populations of total coliforms, *E. coli* and *P. aeruginosa* in the three cells showed no statistically significant difference ($p>0.05$) over 810 days of monitoring (54 observations) when subjected to the Kruskal-Wallis test. Enterococci density from HCW presented a significant difference compared to other cells presenting lower values.

By dividing the monitoring time into two phases, the first year and the second year, the results were different: the statistical evaluation showed different results for each year of analysis: 25 observations referring to the first year of monitoring and 29 observations referring to the second year of monitoring. In the first period evaluated, the bacterial populations of total coliforms, *E. coli,* enterococci and *P. aeruginosa* in the three cells showed no statistically significant difference ($p>0.05$) when subjected to the Kruskal-Wallis test. However, in the second year evaluated, the data show that only for the *P. aeruginosa* population, the values are not statistically different for the three cells. The leachates from the cells showed different values for the total coliforms, *E. coli* and enterococci densities. There were no differences between the values of the HHSW and COD cells for any bacterial populations evaluated. However, when comparing HHSW and HCW cells, there were differences in the densities of these bacterial populations. Analysis of the average of the 29 observations in each cell indicated that the density of total coliforms and E. *coli* was higher in the HHSW cell than in the HCW cell. When comparing the HCW and COD cells, there were differences in the values obtained for the total coliform and enterococcus populations, with the lowest value for the HCW cell, while for the *E. coli* parameter, the values were significantly the same.

It was not possible to establish a correlation between the values observed and the time of confinement. Populations of total coliforms and *E. coli* were detected at 15 days of confinement (first observation) and 810 days of confinement (last observation) in the 3 cells. The results point to understanding the solid waste landfill as a dynamic biological system in which different microbial growth phases occur simultaneously.

Smaller enterococci density was obtained in the HCW cell in most observations. This result may be associated with the absence of fresh feces in the HCW, as opposed to the HHSW, whose gravimetric composition includes human feces and, above all, the mammals and birds feces, as well as materials from domestic animals (sand and absorbents for hygiene). The increase in the population of animals in households has been considered a public health problem due to the presence of pathogenic microorganisms and because they are reservoirs of antimicrobial-resistant bacteria, such as vancomycin-resistant enterococci (VRE) (Cinquepalmi et al., 2013).

Another point to note is the presence of *Escherichia coli* in most of the observations, which has the highest population density among the microorganisms in the coliform group. This result indicates that the solid waste confined in landfills provides the conditions for *E. coli* to survive and develop, even outside the intestinal tract. The intestinal tract is the primary habitat of this microorganism. For some authors, *Escherichia coli* did not demonstrate the ability to survive well in the environment, i.e., in secondary habitats such as surface water, sediments and soil (Ishii et al., 2007). However, it has been shown that *E. coli* can survive in these environments for long periods and grow, even in temperate climates (Ibekwe et al., 2011).

As a strict aerobic microorganism, detecting *P. aeruginosa* throughout the experiment indicates that the landfill has aerobic points among the various accumulated materials. The oxygen dissolved in the rainwater supplies its growth needs. This strengthens the hypothesis that such specialized degraders are favored in the lack of easily degradable organic matter and the initiation of oxygen penetration (Remmas et al., 2017). Song et al. (2015), using 454 pyrosequencing, showed that Pseudomonas dominated in landfill leachate in China and in the solid phase of landfill refuse (2017).

Saini et al. (2004) confirmed that non-hospital waste contains more pathogens than HCW and that the microbiota present in HCW and non-hospital waste are similar. For Souza (2003), with a few exceptions, pathogenic microorganisms do not resist environmental conditions external to the human body and other animals, which makes environmental contamination in the medium- and long-term equivalent to that of HCW.

Comparisons were made between the average bacterial population densities of the waste that filled the

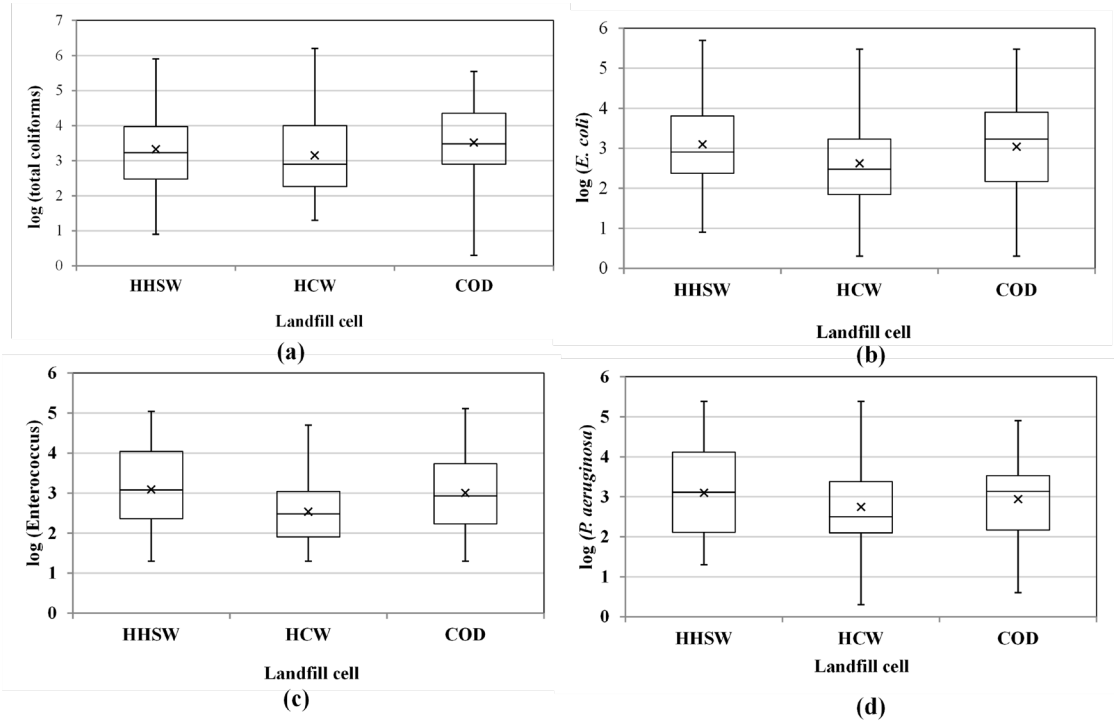

FIGURE 2: Boxplot of results of microorganism densities. (a) total coliforms (b) *E. coli* (c) Enterococci (d) *P. aeruginosa*. Results 810 days of confinement (n=54). All results (y-axis) are reported as the logarithm of the most probable number (MPN per 100mL).

cells and the average values observed in the last 90 days of monitoring. Before confinement, samples were taken of the accumulated liquid from the basins of the trucks that transported the waste. Figure 3 shows that confining the waste reduced the bacterial density in HHSW and HCW cells. As described by Mangimbulude et al. (2009), it is possible to observe a decrease in the absolute load of parameters such as microorganisms, ammonia and heavy metals in landfill leachate.

Based on the data obtained from the microbiota characterization using cultivation-dependent methods, the leachate generated in the HCW cell showed lower values for total coliforms, *Escherichia* coli and enterococci compared to the other cells. The values for the same parameters were significantly similar for HHSW and COD. To this end, the multiple comparison test was used, suggested by Siegal and Castellan (1988). Analysis of the median indicated that the population of enterococci was lower in the RSS Cell than in the others.

Table 3 shows the densities of heterotrophic bacteria detected in the leachate from the three landfill cells. These bacteria were detected in all observations and had compatible average densities considering the standard deviations. The maximum values were observed in the 3 cells after 165 days of confinement, the minimum values after 300 days for the HHSW cell, and 480 days of confinement for the others.

The diversity and concentration of heterotrophic bacteria determined by an analytical method will depend on the variables of that method, which include the composition of the medium, the incubation time and temperature, and the inoculation technique. Only a tiny proportion of the metabolically active microorganisms present in the sample will grow and be detected depending on these variables. These results can also vary significantly among different sites, periods of year and even between consecutive samples taken at the same site (Gòmez et al., 2012).

Figure 4 shows the number of strains isolated in the cell leachate in 54 observations collected over 810 days of monitoring, highlighting the prevalence of 3 strains: *E. coli*, *K. pneumoniae* and *Enterobacter* sp. According to Adeyeba and Akimbo (2002), landfill leachate is generally considered free of pathogenic organisms due to its inactivation under adverse conditions during waste degradation. However, in the present study, 192 strains of enterobacteria of medical importance were isolated in the leachate samples from the 3 cells.

In order to evaluate the profiles of bacteria identified in the cells in association with the time of confinement, isolation patterns were generated in two periods: the first year and the second year of confinement, as seen in Table S3 (Supplementary Material). The patterns observed in the second year showed greater diversity than in the previous year for the 3 cells. *Enterobacter* sp, *K. pneumoniae*, *E. coli*, and *P. mirabilis* were identified in all samples. The detection of strains of *Salmonella* spp, *Providencia rettgeri*, *K. oxytoca*, *C. freündii* and *Pantoae* sp occurred in the second monitoring year in the HHSW and COD cells and of *Serratia* sp and *K. oxytoca* in the HCW cell.

The occurrence of pathogenic microorganisms in HCW landfill leachate was reported by Nascimento et al. (2010), who identified strains of *Citrobacter* sp, *Providencia* sp, *Klebsiella* sp, *Proteus* sp, *E. coli*, *Hafnia* sp, *Morganella* sp,

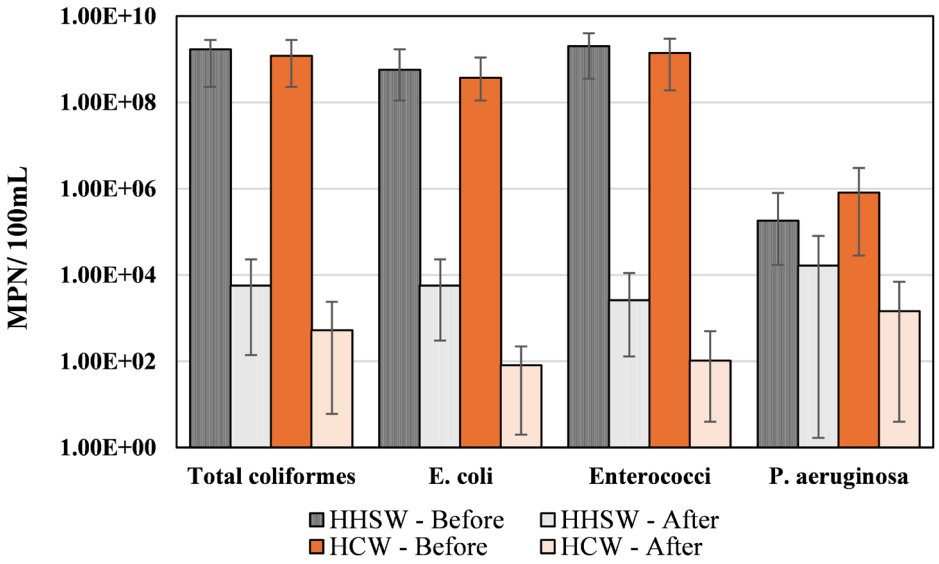

FIGURE 3: Results of microorganisms' density before and after (last 90 days) confinement for HHSW and HCW cells.

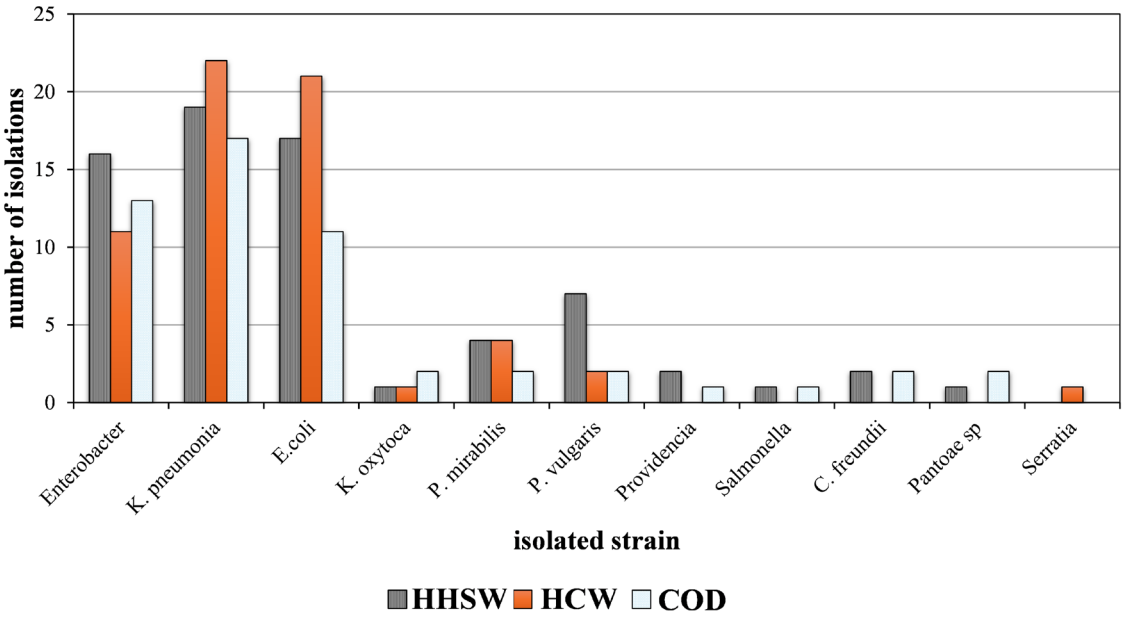

FIGURE 4: Number of enterobacteria strains isolated in cell leachate over 810 days of monitoring.

Salmonella sp, *Shigella* sp and *Serratia* sp.

3.4.1 Search for Staphylococcus aureus

No *Staphylococcus aureus* was isolated from the 54 leachate samples generated in each cell. This result suggests that it is difficult for this bacterial strain to recover or survive in the leachate due to adverse factors such as microbial competition, metabolism products, the antibiotic activity of the microbiota, pH, conductivity, heavy metals, medicines, disinfectants and other sanitizers, paints and solvents. This result is consistent with that Grisey et al. (2010) obtained when assessing the transit and survival of pathogenic bacterial populations in an aquifer under the Etueffont landfill site in France. Cussiol (2005) described landfill leachate as not offering adequate and sufficient nutrients for developing *S. aureus*.

Therefore, it can be considered, by the culture-dependent methods, that the co-disposal of hospital waste and household waste does not differ from the disposal of only household waste and that the fraction of hospital waste collected in the municipality of Rio de Janeiro does not interfere with the microbiota of landfill leachate.

TABLE 3: Mean, maximum and minimum (in brackets) density of heterotrophic bacteria of leachate generated in waste disposal cells (n=43).

Microorganism/ Landfill Cell	HHSW	HCW	COD
Heterotrophic bacteria (CFU/ mL)	1.3×10^{6} ($20\text{-}2.6\times10^{7}$)	1.0×10^{6} ($30\text{-}2.5\times10^{7}$)	9.6×10^{5} ($50\text{-}2.8\times10^{7}$)

3.4.2 Susceptibility of the isolated strains to antimicrobials

Table 4 shows the overall results of the susceptibility of the isolated strain to antimicrobials. Regarding to enterobacteria strains isolated in the HHSW cells, all samples exhibited sensitivity to gentamicin (GEN) and ciprofloxacin (CIP). In contrast, in the HCW cell, sensitivity was observed to amikacin (AMI), aztreonam (ATM), GEN, cefepime (CPM), and CIP. Conversely, complete sensitivity was observed for AMI, CPM, and CIP in the COD cell. Across all cells, the antimicrobial cefazolin (CFZ) displayed the highest resistance percentage.

Furthermore, among the 192 enterobacterial isolates, various phenotypes, including sensitive (S), resistance to a single antimicrobial (R1), resistance to two antimicrobials (R2), and multidrug resistance (MDR), were identified in the three cells. The prevalence of multidrug resistance was notably high in all cells, with the highest frequency observed. Additionally, strains sensitive to the tested antimicrobials were lower in the COD cell.

Concerning enterococci, this group of microorganisms demonstrates diverse resistance mechanisms to antimicrobials, some intrinsic to the group. The resistance profile of *Enterococcus* sp. strains to various antimicrobials was compared among the three cells. It was found that 100% of the strains isolated in the 3 cells were sensitive to the following antimicrobials: CIP, TEC and VAN. In the HHSW cell, 9.4% of the strains showed a resistance profile to AMP; 7.1% of the strains isolated in the HCW cell were resistant to this antimicrobial, and 7.9% in the COD cell. Murray (1997) states that vancomycin resistance in enterococci (VRE) associated with ampicillin resistance is a combination of devastating therapeutic consequences. Enterococci have been recognized as a reservoir of antimicrobial resistance genes with the potential to transfer them to other bacteria in food-containing environments (Aslam et al., 2012). Enterococci with resistance to high concentrations of aminoglycosides (HLAR) have been described in strains isolated from humans and animals (Han et al., 2011). No HLAR-producing strains were detected in cell leachates.

The percentage of phenotypes identified for *P. aeruginosa* (Figure S6) showed the absence of multidrug resistance and a prevalent sensitivity to all tested antimicrobials for the three cells. Strains isolated from hospital wastewater displayed resistance to carbapenem antimicrobials (Fuentefria et al., 2011), as well as in the leachates of the 3 cells in this study, emphasizing the importance of monitoring antimicrobial resistance in *P. aeruginosa* within environmental settings.

3.4.3 Detection of ESBL-producing enterobacteria

Screening for the ESBL phenotype (Table S4) indicated that 32% of the Enterobacteria isolated in the HHSW cell were classified as potential ESBL producers as per CLSI (2023) criteria, 30% in the HCW cell, and 44% in the COD cell. The disk approximation method substantiated the suspected presence of ESBL-producing Enterobacteria in 15.6% of the strains from HHSW, 10.7% from HCW, and 13.6% from COD.

Figure S7 (Supplementary Material) illustrates the positive outcomes of the disk approximation method, demonstrating an increase in the zone of inhibition for oxyimino-cephalosporins (CTX and CAZ) and monobactam (ATM) due to synergy with clavulanic acid present in the amoxicillin-clavulanic acid conjugate disk.

The emergence of extended-spectrum beta-lactamase enzymes represents a critical resistance mechanism against third-generation cephalosporins within the Enterobacteriaceae family, particularly noteworthy in Klebsiella pneumoniae (Vespero et al., 2007). Although ESBL-producing strains were identified in all three cells, 81% of the 68 suspected Enterobacteria strains tested were negative. In a related study, Chagas et al. (2011) investigated antimicrobial resistance patterns in gram-negative bacteria isolated from hospital sewage treatment facilities, identifying 44% of the isolated bacteria as ESBL producers, with *K. pneumoniae* and *Enterobacter cloacae* prominently featured.

3.4.4 Detection of carbapenemase-producing enterobacteria

Screening for carbapenemase production revealed that 22% of the isolated bacteria were probable carbapenemase producers in the HHSW cell, 23% in the HCW cell and 51% in the COD Cell. The modified Hodge test detected the carbapenemase-producing strains (Table S5 -Supplementary Material).

3.4.5 Detection of AmpC-producing enterobacteria (ESBLS), AMBLER Class C

To detect the production of these enzymes in other bacteria that do not show this phenomenon constitutively, as is the case with *K. pneumoniae*, the best way is to visualize resistance to cephamycin, such as cefoxitin (CFO), as this is the best inducer of group C beta-lactamase production. At the phenotypic level, this phenomenon was observed in *K. pneumoniae* strains that screened positive for ESBL using the modified disk approximation methodology. Figure S8 (Supplementary Material) shows the positive result for the presumption of Ambler class C ESBLs. In 15% of the *K. pneumoniae* strains isolated from the leachate of the HHSW cell, in 7.5% of the HCW cell and in 10% of the COD cell, a junction zone appeared between CFO and ATM (Aztreonam, a beta-lactam drug), to which the bacteria were initially sensitive, demonstrating the decrease in sensitivity caused by the bacteria's exposure to the inducing antibiotic (CFO).

4. CONCLUSIONS

This study simulated three landfill cells for disposal of HHSW and HCW and co-disposal of both wastes. The results suggest no significant differences in the behavior of experimental landfills containing HHSW and HCW concerning the presence of pathogenic microbiota. This finding indicates that pathogenic microbiota patterns are similar in HCW and HHSW. It does not influence the leachate characteristics in these landfill models under the conditions tested.

TABLE 4: Overall results of antimicrobial activity in cell leachates.

Isolated	Total number	Experimental landfill cells	Antibiotic resistance pattern	Antibiotic resistance results	
E. coli	17	HSW	82%S 18%R1	AMC - 11.7% CFZ -11.7% ESBL- 0% Carbapenemase - 0% ESBL Amp - 0%	
	22	HCW	86.5%S 9%R1 4.5% R2	SUT - 9% CFZ - 4.5%; CTX - 4.5% 4.5% ESBL Carbapenemase - 0% ESBL Amp - 0%	
	14	COD	86%S 7%R1 7% R2	AMC - 14% CFZ - 29% IPM - 7% ESBL - 21% Carbapenemase - 0% ESBL Amp - 0%	
Enterobacter sp	21	HSW	10%S 52%MDR 14%R1 24%R2	AMP - 62% CAZ - 4.7% COM - 9.5% CFO - 87,5% Carbapenemase-0%	AMC - 67%0% CFZ - 76% IMP - 4.7% ESBL - 14% ESBL Amp
	10	HCW	0%S 80%MDR 0%R1 20%R2	AMC - 80% CFO - 90% CFZ - 100% ESBL- 20% Carbapenemase - 0% ESBL Amp - 0%	
	12	COD	8.3%S 50%MDR 33.3%R1 8.3%R2	AMP - 8% AMC - 50% SUT - 8% CAZ - 33% Carbapenemase - 0%	COM - 8% CFO - 50% CFZ - 67% ESBL - 16.7% ESBL Amp - 0%
Klebsiella sp	21	HSW	14%S 48%MDR 29%R1 9%R2	AMP - 71% AMI - 4.7% AMC - 33% ATM - 14% SUT - 14% CAZ - 14% Carbapenemase - 19.4%	CPM - 4.7% CFO - 33% CFZ - 52% CTX - 10% MER - 4.7% ESBL - 28.6% ESBL Amp - 15%
	16	HCW	12.5%S 50%MDR 0%R1 12.5%R2	AMP - 44% AMC - 50% SUT - 12.5% CFO - 81.2% Carbapenemase - 23.2%	CFZ - 81.2% IMP - 18.75% ESBL18.75%; ESBL Amp - 7.5%
	20	COD	18%S 53%MDR 29%R1 0%R2	AMP - 47% AMC - 47% CFO - 41% CFZ - 65% ESBL - 17.6% Carbapenemase - 27.1% ESBL Amp - 10%	
Proteus sp	11	HSW	9%S 46%MDR 27%R1 18%R2	AMP - 11% CFO - 11% CFZ - 11% ESBL - 0% Carbapenemase - 0% ESBL Amp - 0%	
	9	HCW	67%S 22%MDR 11%R1 0%R2	AMP - 11% CFO - 11% CFZ - 11% ESBL-33% Carbapenemase - 0% ESBL Amp - 0%	
	6	COD	25%S 50%MDR 25%R1 0%R2	AMP - 73% AMI - 9% CFZ - 91% CTX - 36% ESBL-0% Carbapenemase-0%	ATM - 18% CAZ - 9% CPM - 18% CFO - 27% ESBL Amp - 0%

AMP- Ampicilin; AMI – Amikacin; AMC – Amoxicillin+ clavulanic acid; ATM – Aztreonam; GEN – Gentamicin; SUT – Sulfametoxazol-trimetoprim; CAZ – Ceftazidime; CPM – Cefepime; CFO – Cefoxitine; CFZ – Cefazolin; CTX – Cefotaxime; CIP – Cipfrofloxacin; IMP – Imipenem; MER – Meropenem

The following considerations stand out:

- The cells constructed proved to be a good study model for the biological parameters of landfill leachate.
- The values found for the environmental contamination indicators of the 3 cells suggest that pathogens persist in the leachate after 810 days of confinement.
- The landfill system reduced the population densities of microorganisms that are indicators of fecal pollution from the start of confinement.
- The leached liquids from the HHSW and COD experimental cells showed no significant difference in microbiota throughout the study. In contrast, results from the HCW cell showed lower values for total coliforms, *Escherichia coli* and enterococci than the other cells.
- The co-disposal of HHSW and HCW did not differ from the exclusive disposal of HHSW regarding antimicrobial resistance.
- Microorganisms with a hospital multidrug resistance profile were similarly isolated in the 3 cells.

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