



Hospital wastewater genotoxicity: A comparison study between an urban and rural university hospital with and without metabolic activation

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Abstract

Background: Hospital wastewater is a major source of micropollutants and toxic chemicals. It ends up draining into many aquatic bodies, causing environmental hazards. The aim of the study was to perform genotoxic tests on two major Lebanese University hospitals: Hospital A (300 beds) situated in the city and Hospital B (180 beds) in a rural area.

Methods: Samples from Hospitals A and B were taken from different pits on a 5-day period. Two genotoxic tests were performed: the SOS Chromotest (*Escherichia coli* PQ37) and the Ames Fluctuation Test (*Salmonella Typhimurium* TA98) with and without metabolic activation (S9 addition).

Results and conclusions: In Hospital A, 20% of the samples tested by the SOS Chromotest were genotoxic without metabolic activation, whereas around 46% of the same samples were genotoxic after metabolic activation. Similarly, an increase of 25% of genotoxic activity was observed in Hospital A samples using the Ames Fluctuation Test after metabolic activation. On the other hand, wastewater samples from Hospital B tested with the SOS Chromotest showed no or low genotoxicity at the concentrations tested both with and without metabolic activation. Using the Ames Fluctuation test, samples from Hospital B showed slight genotoxicity only after activation. Hospital B implemented a policy of collecting all the excess pharmaceuticals to limit the disposal of harmful chemicals into the drain.

Keywords: Genotoxicity, wastewater, SOS chromotest, ames fluctuation test, metabolic activation

Introduction

Genotoxicity is the study of genotoxins, which are substances that may have the potential to engender damage in the DNA [1]. Over the past few decades, interest in the genotoxic assessment of various sources has been growing, especially that there has been evidence of various unlisted genotoxins present on land, water, and air [2]. Hospital wastewater presents an ecotoxicological hazard on the environment because it eventually drains into aquatic surfaces and is composed of various substances that contribute to water pollution [3]. The reason behind hospital wastewater toxicity is partially due to the fact that some non-metabolized pharmaceuticals are excreted into the sewage of the hospital [4]. The first occurrence of pharmaceuticals in nature was in the 1970s in rivers and lakes nearby hospitals, and their concentrations ranged between ng/L and µg/L [4]. Even if some of these compounds

are biodegradable, the fact that they are in constant flow into the sewage system will lead to a persistent toxic effect on the environment [5].

In the current study, the SOS Chromotest and the Ames Fluctuation Test were used for the assessment of hospital wastewater genotoxicity. The SOS Chromotest is a colorimetric assay, developed by Quillardet and Hofnung [6]. The test relies on the capabilities of a modified strain of *E. coli* PQ37 to detect the SOS responses activated by DNA damage. The Ames Fluctuation Test is the liquid microplate version of the classical Ames test that was developed by Ames et al [7]. It depends on the genetically modified strains of *Salmonella typhimurium*. TA98, TA100 and TA102 are customized to be deficient in the production of the essential amino acid histidine [8]. The test allows mutagens to cause a back-mutation permitting the bacteria to produce histidine and thus survive in a histidine-deficient medium [9].

Moreover, both tests were studied with and without metabolic activation. Metabolic activation was achieved through the addition of S9 liver enzymes [10]. Consisting of both liver Phases I and II enzymes, S9 fractions bear suitable parameters for mimicking the reactions occurring within hepatocytes [11]. Metabolic activation has a role in the complementation of the prokaryotic tests, since it would provide an estimation of the genotoxicity of promutagens that manifest an effect only after being metabolized by the liver.

The present study compares the wastewater genotoxic profiles with and without metabolic activation of two Lebanese University hospitals. These hospitals were selected due to their importance in their regions and due to the similarity in the medical procedures performed. Hospital A, situated in the city, holds around 300 beds and Hospital B, in a rural area, holds around 180 beds. Hospital B minimized its disposal of chemicals into the drain by storing excess drugs or pharmaceuticals.

Materials and methods

Test bacterial strains

The two genetically engineered strains, *Escherichia coli* PQ37 and *Salmonella typhimurium* TA98 were purchased from EBPI (Environmental Biodetection Products Inc.). They were stored at -80°C and thawed before the assay.

Sampling of hospital wastewater

Hospital wastewater samples were collected and placed in two one-liter polyethylene bottles. The samples were taken from five different pits from Hospital A and four different pits from Hospital B, which are derived from different floors (Table 1). The samples were collected over a 5-day period, twice per day (once in the morning and once in the afternoon). The samples were filtered with 1 mm Whatmann filter papers, followed by vacuum filtration using 0.45 mm cellulose acetate filter papers (Sartorius Minisart, Germany). The samples were stored at -20°C.

SOS chromotest

The SOS chromotest was performed with and without metabolic activation for each sample as described by Quillardet & Hofnung [12] with modifications [13]. The assay was performed by mixing 600 µL of *E. coli* PQ37 (2×10^8 cfu/mL) with 20 µL sample of different dilutions. In the case of metabolic activation, a solution consisting of centrifuged S9 liver homogenates treated with Arochlor-1254 along with the S9 mix were added to the tubes. The negative controls were water, Dimethyl Sulfoxide (DMSO), and Luria broth, while the positive controls were 4-nitroquinoline 1-oxide (4NQO) (2.5 µg/ml) for the assays without metabolic activation, and 2-aminoanthracene (2-AA) (200 µg/ml) for those with metabolic activation. The samples were tested at 5 concentrations: $\times 2$, $\times 1$, $\times 3/4$, $\times 1/2$, and $\times 1/4$ concentrated samples. The ($\times 2$) sample was concentrated by means of a Speedvac concentrator. The tubes were then incubated at 37°C with shaking. After 120 min, the tubes were then assayed for their β -galactosidase (β -GAL) and

Table 1. Source of Wastewater Drainage from Different Pits in Hospitals A and B.

| Pit | Hospital A | Hospital B |
|-------|--|---|
| Pit 1 | Anatomy-pathology lab | Operation Room, Cardiac Support Unit, Offices and Nurses toilets |
| Pit 2 | Lower floors (B2 to B5) Practice division, Housekeeping, Operating rooms, Catheter labs, Laundry, One day surgery | The catheter labs, toilets (Pit Not accessible) |
| Pit 3 | Mechanical equipment | Preparation room of nurses Dialysis, Neonatal Intensive Care Unit Chemotherapy, Radiotherapy, Emergency Room |
| Pit 4 | Pharmacy lab | Radiotherapy, Pharmacy, Morgue |
| Pit 5 | 9 th floor till the first basement, Patient floors, Doctors' clinics, Emergency rooms, Kitchen, Outpatient department, Blood bank, Laboratory, Radiology | Drainage of all the previous pits |

phosphatase alkaline (PAL) activities using O-nitrophenyl- β -D-galactopyranoside (ONPG) and p-nitrophenyl phosphate disodium (PNPP) as substrate. β -GAL and PAL assays were terminated with Na_2CO_3 and HCl followed by 1 mL of Tris buffer after 5 minutes, respectively. Absorbance was then measured at 420 nm. Enzyme Units, Genotoxic Activity, and Induction Factor were calculated as suggested by Quillardet & Hofnung [12].

For a sample to be considered genotoxic, the induction factor value has to be higher than 1.5 and a dose-dependent response between the samples of different concentrations has to be observed. The results are the mean of three experiments each.

Ames Fluctuation Test

Genotoxicity of the samples was also tested using the Ames Fluctuation Test with and without metabolic activation, as described by Legault et al [14]. The fluctuation medium consists of Davis-Mingioli salts (5.5 \times), D-glucose (400 mg/ml), D-biotin (0.1 mg/ml), L-histidine (1 mg/ml), and bromocresol purple (2 mg/ml). *Salmonella* TA98 (20 µl) was added to 2.5 mL of the fluctuation mixture along with the sample tested. Samples were tested as ($\times 1$) or diluted in half ($\times 1/2$). For tubes with metabolic activation, S9 liver fractions suspended in S9 mix replaced the same volume of water. Samples of 200 µl were added to 96-well plates, and left in an incubator for 4 days.

The wells are considered positive if they become yellow, partially yellow or turbid. The numerical results are expressed in the form of a Mutagenicity Ratio which is the ratio of positive wells in the treated plates over the positive wells in the corresponding background or negative control plates [15]. The level of toxicity of the pits is evaluated using *Chi*-square analysis.

Metabolic activation

The S9 mix was prepared according to Quillardet et al [12]. It consists of S9 liver fraction added to Luria broth, salt solution (KCl (1.65 M) and MgCl₂·6H₂O (0.4 M)), Tris buffer (0.4 M), G6P (1 M), and NADP (0.1 M).

Results

SOS chromotest

SOS chromotest for wastewater from Hospital A and Hospital B, both with and without activation, were performed. Induction factors (IF) for wastewater samples tested at different concentrations, negative controls (water and media), and positive controls (4NQO without metabolic activation and 2AA with metabolic activation) are summarized in **Tables 2** and **3**. In Hospital A, 20% of the samples were genotoxic (without metabolic activation) as they exhibit a dose-dependent IF above 1.5 (**Table 2**) with a stable increase in beta-galactosidase activity (data not shown). A 26% increase in genotoxic activity was observed after metabolic activation (**Table 2**).

However, in Hospital B, the samples tested did not appear to have any genotoxic activity without metabolic activation (**Table 3**). When assayed with metabolic activation, these samples showed a slight increase in their induction factor (**Table 3**), without a dose-dependent beta-galactosidase activity (data not shown). Samples from Hospital B were not genotoxic at concentrations tested, even after metabolic activation.

Ames Fluctuation Test

Ames Fluctuation Test was performed on wastewater from samples collected from Hospitals A and B with and without metabolic activation. Mutagenicity Ratios (MR) for samples from Hospital A at concentrations ($\times 1/2$) and ($\times 1$) were calculated (**Table 4**). Pit 1 from Hospital A could not be tested with Ames Fluctuation Test due to its high acidity. A sample was considered genotoxic as evaluated by *Chi-square* test with significances of $p < 0.05$, $p < 0.01$, or $p < 0.001$. The highest MR obtained was the undiluted sample ($\times 1$) from pit 4 with metabolic activation (MR=11.57) (**Table 4**). Most samples from all four pits were highly genotoxic at ($\times 1$) concentration and showed increased mutagenic activity after metabolic activation.

As for Hospital B, samples were tested at ($\times 1$) concentration only; since they showed limited genotoxicity (**Table 4**). An increase in genotoxicity was generally observed after metabolic activation (**Table 4**).

Discussion

The SOS chromotest and the Ames Fluctuation Test were performed on samples from different pits taken from Hospitals A and B and tested with and without metabolic activation. The results generally reflect a higher genotoxic activity for Hospital A. The results of wastewater from Hospital A are consistent with a study performed by Jolibois and Guerbet [15] on a University hospital using the SOS Chromotest and the Ames Fluctuation Test, where most of the samples were

positive with at least one of the assays.

Hospital B's results are more consistent with the study performed by Jolibois and Guerbet [3] on hospital wastewater treatment plants in Rouen, France. In that study, the SOS chromotest did not show any genotoxicity. However, the Ames Fluctuation Test was genotoxic for the influent samples (71%) whereas the effluents samples were not genotoxic [3]. Hospital B's policy of collecting the excess pharmaceuticals and decreasing disposal of pharmaceuticals may have led to a positive temporary effect (decrease in genotoxicity) similar to that of effluents in the Rouen study.

The highest IF observed in this study was for Hospital A's pit 1 sample. It represents the drainage of the anatomy-pathology lab. The high levels of genotoxicity in this pit may be attributed to the presence of formaldehyde, which is one of the substances that are abundantly used in this lab. Formaldehyde was shown to be highly genotoxic as tested by *in vitro* micronucleus tests (MN), sister chromatid exchange tests (SCE), and Comet assays [16]. This among other substances such as cytostatic drugs, and iodinated X-ray contrast media may all contribute to the high genotoxicity of pit 1 [4,17]. These chemicals are also expected to be found in Hospital B, and most probably in the samples collected from pit 5. However, high levels of genotoxicity were not observed, probably due to the fact that pit 5 is a collection of all the drainage pits, which might have caused the dilution of these substances and a subsequent reduction in the pit's overall genotoxicity. This may also be attributed to the policy administrated by the hospital to collect all of the excess pharmaceuticals.

Samples were incubated with S9 liver enzymes to study the genotoxicity of these samples after activation. This is indicative of the presence of pro-mutagens, such as antibiotics, drugs, dyes, or others. In this study, an increase of around 26% was observed after metabolic activation of Hospital A samples with SOS treatment. A slight increase in induction factor was observed after metabolic activation of Hospital B samples, but it still exhibited low genotoxicity. On the other hand, with the Ames Fluctuation Test, all the samples showed an increase in genotoxicity after metabolic activation. This may be due to the fact that the metabolic activation increased the sensitivity of TA98 [3] and/or increased the genotoxicity of wastewater samples.

Samples from Hospital B showed no genotoxicity at concentrations tested with SOS Chromotest. However, low genotoxicity was observed with the Ames Fluctuation Test after metabolic activation. The sensitivity of the Ames Fluctuation Test maybe higher than that of the SOS chromotest [14]. The Ames Fluctuation Test and SOS chromotest test are complementary and sensitive to different genotoxic effects (frameshift mutation, point mutation, deletion mutation...). Both hospitals are central hospitals in their areas and are involved in similar operations and activities. However, different factors may contribute to the difference in their genotoxicity. Hospital A is a larger hospital (300 beds), situated in an

Table 2. Hospital A Wastewater Genotoxicity as tested by the SOS chromotest (with and without metabolic activation) at different concentrations. Induction Factors for samples collected at Day 1 (D1) and Day 5 (D5) were calculated; genotoxic samples (GS) are in bold.

| Without Metabolic Activation | | | | | | | | | | | | | With Metabolic Activation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|------------------------------|-------|------|------|------|------|-------|------|------|------|-------|-------|-------|---------------------------|------|------|------|------|-------|------|-------|-------|------|------|-------|------|------|------|------|------|-------|-------|------|-----|------|------|------|------|------|------|------|------|------|------|------|---|
| Pit 1 | | | | | | Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | |
| Water | Media | DMSO | 1/4 | 1/2 | 3/4 | x1 | x2 | 4NQO | GS | Water | Media | DMSO | 1/4 | 1/2 | 3/4 | x1 | x2 | 4NQO | GS | Water | Media | DMSO | 1/4 | 1/2 | 3/4 | x1 | x2 | 4NQO | GS | Water | Media | DMSO | 1/4 | 1/2 | 3/4 | x1 | x2 | 4NQO | GS | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 1.71 | 2.41 | 3.02 | 3.27 | 4.38 | 3.36 | + | D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.73 | 0.79 | 0.70 | 0.80 | 0.83 | 2.82 | - | D 1 | 0.00 | 0.00 | 1.00 | 1.55 | 1.61 | 1.86 | 1.97 | 2.20 | 1.65 | 4.67 | + | |
| D 5 | 0.00 | 0.00 | 1.00 | 3.38 | 3.58 | 4.59 | 4.97 | 5.80 | 5.47 | + | D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.68 | 0.89 | 0.97 | 1.15 | 1.31 | 2.54 | 3.15 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |
| D 5 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.73 | 0.66 | 1.00 | 1.11 | 1.33 | 4.12 | - | D 5 | 0.00 | 0.00 | 1.00 | 0.58 | 0.86 | 0.73 | 1.19 | 1.42 | 2.55 | 3.86 | - | |
| Pit 2 | | | | | | Pit 3 | | | | | | Pit 4 | | | | | | Pit 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D 1 | 0.00 | 0.00 | 1.00 | 0.99 | 0.87 | 0.83 | 0.79 | 1.02 | 4.12 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.52 | 0.46 | 0.69 | 0.52 | 0.62 | 4.37 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.68 | 0.97 | 0.80 | 0.69 | 1.33 | 2.75 | 3.25 | - | D 1 | 0.00 | 0.00 | 1.00 | 0.75 | 0.96 | 1.16 | 1.06 | 1.04 | 2.54 | 3.15 | - |

Table 4. Ames Fluctuation Test results for Hospital A and B.

| Hospital A | Pit 2 | | Pit 3 | | Pit 4 | | Pit 5 | |
|--------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|
| Sample dilution | ×1/2 | ×1 | ×1/2 | ×1 | ×1/2 | ×1 | ×1/2 | ×1 |
| Without activation | 1.70 | 2.50^b | 2.50^b | 5.20^c | 6.80^c | 9.40^c | 2.20 | 4.00^c |
| With activation | 2.00^a | 3.55^c | 4.00^c | 7.55^c | 6.43^c | 11.57^c | 2.14^a | 3.43^c |
| Hospital B | Pit 1 | | Pit 3 | | Pit 4 | | Pit 5 | |
| Sample dilution | ×1 | | ×1 | | ×1 | | ×1 | |
| Without activation | 0.42 | | 0.71 | | 1.40 | | 0.61 | |
| With activation | 0.72^a | | 1.41^a | | 1.76^b | | 1.20^a | |

Mutagenicity Ratios (MR) were calculated and their significance was evaluated by Chi-Square Analysis (^ap<0.05; ^bp<0.01; ^cp<0.001). Genotoxic samples are indicated in bold.

urban, industrial and highly populated area. On the other hand, Hospital B is a smaller hospital (180 beds) situated in a rural, and less populated area. Furthermore, Hospital A only applies a simple wastewater treatment plan that consists of throwing calcified rocks into the sewage so the toxins would adhere to it. A prevention policy is endorsed in Hospital B which involves collecting all the excess pharmaceuticals and storing them for future disposal.

Conclusion

The SOS chromotest and the Ames Fluctuation Test revealed that various levels of genotoxicity were present in samples from both Lebanese University hospitals. Metabolic activation generally caused an increase in the genotoxic activity of hospital wastewater samples. This implies that the treatments used in neutralizing the mutagenic effects of substances found in the wastewater are not adequate. These methods may be effective at relieving some of the hazardous effects of chemicals, but nonetheless they are not enough to reduce their genotoxic effects. Even though dilution with non-genotoxic sources of water can minimize the bioavailability of genotoxins in genotoxic pits [18], it would not be a permanent solution or an environmental-friendly one. Solving the issue may begin with monitoring the contents dumped in the pits (as performed in Hospital B). Eventually, a treatment plant is indispensable in minimizing the harmful impacts on the environment.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

| Authors' contributions | JJ | JF | RMA |
|------------------------------------|----|----|-----|
| Research concept and design | -- | -- | ✓ |
| Collection and/or assembly of data | ✓ | ✓ | -- |
| Data analysis and interpretation | ✓ | ✓ | ✓ |
| Writing the article | ✓ | ✓ | ✓ |
| Critical revision of the article | -- | -- | ✓ |
| Final approval of article | ✓ | ✓ | ✓ |
| Statistical analysis | ✓ | ✓ | -- |

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