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Research Article

Evaluating Genotoxicity of Treated Wastewater from Health Centers with Special Reference to their Mutagenicity

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Abstract

Context: Health centers waste consists of both contagious and non-contagious substances including pathogenic microorganisms. The major sources of medical waste are released from healthcare premises, such as hospitals, clinics, veterinary hospitals and diagnostic labs.

Objective: To check the effectiveness of the wastewater treatment plants (WWTP) functional in the health care establishments and evaluate the genotoxic potential of waste effluents that are discharged from the hospitals.

Methods: *Salmonella* Ames test, SOS chromotest microtiter plate and Chromosome aberration's assays used for monitoring the genotoxic activities present in the wastewater of health institutes during various seasons.

Results and Discussions: In the present study, genotoxicity of waste waters of two health centers at different stages of treatment was evaluated by the SOS chromotest (on *E. coli* PQ37) without metabolic activation, *Salmonella* mutagenicity test (on TA98, TA100 and TA102) with and without metabolic activation and by Chromosome aberrations. The untreated hospital liquid waste from SDMH and Fortis hospitals which proved to be potent genotoxic have been significantly correlated using both the assays. While liquid wastewater of health centers are the potential source of genotoxic pollutant release in the environment, assessment of effectiveness of WWTP in terms of removal of genotoxicity and toxicity determination using the aforementioned assays helped us to find out the effectiveness of both treatment process in reducing the genotoxicity.

Conclusions: Liquid wastewater of health centers are the potential source of genotoxic pollutant release in the environment. An Advanced and Efficient on-site treatment plants for hospital effluents is necessary reducing the risk exposed to the environment and human health by these toxic substances.

Keywords: *Hospital Effluents; Mutagenicity; Ames Assay; DNA Damage*

Introduction

Medical Science is advancing rapidly with ever increasing momentum providing best facilities to patients, but the waste generating from health care establishments has become a critical issue for the environmental protection and public safety, due to its infectious and hazardous character. Irregular disposal of health centers wastewater directly into the city sewerage system without pre-treatment, contaminate the surface and underground water table which may cause serious epidemic diseases like cholera, typhoid and enteric illness in the population [1]. Waste water discharged from health care establishments needs an urgent attention, because it is a complex matrix containing different types of toxins, which can pose serious threat to the human life, if directly dispensed into the environment. Health centers discharge a significant amount of water in a day, ranging from 400 to 1200 L/day/bed [2,3] which contains various genotoxic

potent toxins, suspended solids, biodegradable organics, hazardous pollutants, anti-neoplasm agents, anesthetic gases and other cytotoxic agents which can cause crucial damage to the natural environment. From last decades, various toxic potents are released from these discharging effluents reckon to be cancer causing agent [4-6].

In this study, we attempted to measure and compare the genotoxicity and cytogenetic potential of untreated, filtrate and treated wastewater of health centers. It is probably the first study conducted to compare the efficacy of two onsite health care establishments wastewater treatment plants with special reference to genotoxins. In the present research, a battery of short-term microbial assays for screening the possible genotoxicity of effluent released by health centers has been used. Three bioassays viz. The *Salmonella* Ames test, SOS chromotest and Chromosome aberration's are viable *in vitro* test systems for the assessment of genotoxicity on environmental samples. Since Ames and SOS chromotest have been shown to be effective as a test system for bacteria, the SOS chromotest, a new assay for mutagenicity and carcinogenicity which is sensitive, rapid and practical, and successfully is used to detect primary DNA damaging agents [7]. The genotoxic effects detected by the *Salmonella* Ames test include three different molecular mechanisms: base-pair substitution mutation (TA 100 positive), Transition mutation (TA 102 positive) and frameshift mutation caused by nucleotide insertion or deletion (TA 98 positive). Cytogenic assay viz chromosome aberrations provides a direct and effective way of identifying chemical agents that induce genetic damage in mammals [8].

The *Salmonella* microsome mutagenicity test and the SOS chromotest, are widespread bacterial short term tests used as screening techniques for identifying hazardous chemicals as they are rapid and inexpensive has been reported by Ames., *et al*. [9] and Mersch-Sundermann., *et al* [10]. Hence, these two *in vitro* genotoxicity tests are not equal but are complement to each other [11]. Hence, these genotoxic bioassays are jointly selected to investigate the efficacy of health center treatment plants and to study the genotoxicity risk present in the health care establishments' waste water. To overcome these problems major hospitals of Jaipur city in Rajasthan, India have installed WWTPs, after treatment they release the wastewater in the municipal city sewage system. The present study was aimed at comparing the mutagenic potential of treated wastewater of two major hospitals; Santokba Durlabhji Memorial Hospital (SDMH) and Fortis hospital located at Jaipur city have treatment plants. Further, the genotoxicity of wastewater was monitored at different stages of treatment process. Since, the treated waters of these two major health centers is used for gardening, from where it may contaminate underground water and it is therefore important to know the effectiveness of treatment plants in terms of mutagenicity.

Materials and Methods

Sampling of Health centers wastewater

The samples from all the hospital sites were taken twice in 6 months. First sampling was done during May 2013 (summer season) while the second in November 2013 (winter season). The samples were taken during the maximal hospital activity period (8:00 a.m. to 6:00 p.m.). The samples were stored at 4ºC until tested. Samples were collected from three different stages of treatment plant.

Site 1. Santokba Durlabhji Memorial Hospital: Santokba Durlabhji Memorial Hospital (SDMH) is the largest multi-specialty hospital in private sector in Jaipur with the bed strength of 400 and with 12 operation theaters, with latest technology. The hospital has a fully functional Effluent Treatment Plant (ETP). The treatment plant generates approximately 55,000 litres of treated water daily.

Site 2. Fortis Hospital: Fortis is a Multi Super-Specialty Hospital with major focus on super specialties of Cardiology, Neurology, Nephrology and astro-Intestinal diseases backed up by a wide range of specialties. It's having 7 operation theatre and 210 operation theaters with total bed strength of 350. The hospital has the treatment plant for the hospital liquid waste. Fortis has a fully functional effluent treatment plant. The treatment plant generates approximately 64,000 liters of treated water daily.

Samples were taken from different stages of treatment plant of the both hospitals as under (Figure 1):

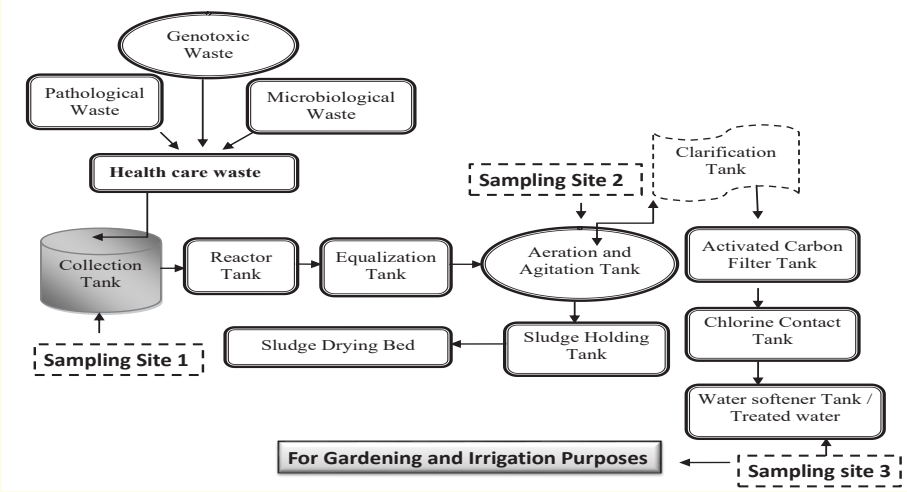

Figure 1: *Collection of wastewater from WTP of Health care Establishments (SDMH and Fortis) (Urvashi.V, 2014).*

- Untreated sample- From the main sewer of the hospital, where the entire water from the premises is collected.
- Sample after aeration and filtration – During this process, wastewater from the main sewer goes to the ETP. The effluent is stored before passing to Activated carbon filter. This sample was collected after the initial steps of the treatment process after filtration and aeration chamber.
- Finally the treated sample after Chlorination - Chlorine is added continuously to inactivate the microbial population. This sample was collected from the outlet from where the treated water comes out of the ETP and use for gardening.

Compounds

Benzo(a)pyrene(B[a]P), 2-Nitroflourene (2-NF), 2-Aminoflourine (2-AF) and Sodium Ammonium Phosphate were procured from Sigma-Aldrich. Ampicillin trihydrate, Tetracycline, D-biotin, L-Histidine. HCL (monohydrate), D-Glucose-6-Phosphate (monosodium salt) and NADP (Sodium salt) were purchased from Himedia. Citric Monohydrate and Potassium phosphate dibasic and Sodium azide were procured from Merck. The solvent used were Dimethylsulfoxide (DMSO) were supplied by Merck and distilled water for all injectable preparation was prepared from Sartorious stidem biotech instrument.

Genotoxicity assay with bacteria

***Salmonella* microsome mutagenicity test**

The tester strains TA98, TA100 and TA102 were obtained from Microbial Type Culture Collection and the genebank at Institute of Microbial Technology (IMTECH), Chandigarh (India). The genotype of *Salmonella typhimurium* was described by Ames., *et al.* [9] and revised by Maron and Ames [12]. The genetic analyses of strains were regularly checked for genetic markers. The tester strain genotypes are check by Histidine and Biotin dependence, *rfa* mutation, *uvr*A/B and R-factor plasmid pKM101 (Ampicillin resistance) or pAQ1 (Tetracycline resistance for TA 102) were confirmed immediately after receiving the cultures and every time a new set of frozen permanents were used for experiment using the protocol given by Mortelmans and Zeiger [13]. All samples were tested in their crude natural state and in unconcentrated form with assay carried out both in the absence or presence of 10% hepatic S9 mix fraction of liver homogenate from uninduced Swiss–Albino mice, which incorporates an important aspect of mammalian metabolism into *in vitro* test. Based on the recommendations of Prival and Mitchell [14] uninduced Swiss-Albino mouse liver was used to prepare the 10% S9 mixture. Five dose levels of individual samples were tested (2, 5, 10, 50 and 100 µl). The cultures were grown in 20 ml of oxoid nutrient broth no. 2, and 0.5

ml of ampicillin (1 mg/ml) for TA98, TA100 and 0.5 ml of tetracycline (1 mg/ml) for the strain TA102. The cultures were incubated for 10 - 12h at 37ºC in a Shaker incubator in order to insure adequate aeration of 10^9 bacterial cells; 0.1 ml of this fresh culture was mixed with 0.2 ml of His/Bio solution, 0.1 ml of sample, 0.5 ml of sodium phosphate buffer, or 0.5 ml of S9 mix and total volume was made up to 1.0 ml by autoclaved distilled water. This mixture was then shaken and poured on plates containing 25 ml of minimal glucose agar medium. The positive controls used were 1 mg/plate Sodium azide for TA100, 2 mg/plate 2-NF for TA98 and 0.125 mg/plate mitomycin C for TA102. When the assays were performed in the presence of S9 mix, 2 mg/plate 2-AF was used for TA98, TA100 and TA102. Without metabolic activation Spontaneous Revertants for TA 98, TA 100, TA102 were 44, 52, 143 respectively) and with metabolic activation spontaneous revertants per plate for the strains TA98, TA100, TA102 were 96, 153, 224 respectively). Sterile distilled water was used as negative control. All plates were run in duplicate. The wastewater samples were tested twice further to predict downstream mutagenicity. All tester strains were maintained and stored according to standard methods [9-12].

SOS Chromotest

The SOS chromotest is a colorimetric assay of the enzymatic activities that occur after incubating the test strain of bacteria in the presence of various amounts of experimental sample has been miniaturized by Fish., *et al.* [15] to run in 96 microtiter plates [16,17]. The test utilizes a genetically engineered bacterium, *E. coli* PQ37, to detect DNA-damaging agents [4]. In this study, the SOS chromotest was performed, without metabolic activation, as described by Quillardet and Hofnung [18]. The *E. coli* PQ37 tester strain was kindly provided by Environmental Bio-Detection Products Inc. (Brampton, Ontario, Canada) and we followed the protocol and the results were analysed according to EBPI [19]. In this assay, the β-galactosidase (β-gal) gene (lacZ) of the *E. coli* PQ37 tester strain is fused to the bacterial *sfiA* SOS operon. Thus, lacZ is concomitantly expressed during the bacterial SOS response, and this gene expression can be photometrically determined by the induction of β-gal. The amount of β-gal induction is indicative of the extent of SOS induction and bacterial genotoxicity. The samples (20 µL) were tested pure and concentrated 14 two-fold serial dilutions were done in remaining wells for tested compounds. Bacterial Alkaline phosphatase (AP) activity was used to determine the range of bacterial viability/cytotoxicity at the adsorption was measured at 405 nm with reference solution no bacteria and read absorbance at 615 nm to measure the genotoxicity activity of β-galactosidase. The measurement of enzymatic activities was performed using microtiter plates. The amount of enzymatic activity was determined by an automatic enzyme linked immunosorbent assay (Elisa; Biorad instrument) microtiter plate reader. To ensure the validity of the assay, a positive control was included in each experiment. For the direct assay, the negative control was composed of a 1 µg/ml p-nitro phenyl phosphate (pNPP) diluents and the positive control was (4-NQO) 4-nitro-quinolineoxide (1 µg/ml). A compound is considered as an SOS repair system inducer in *E. coli* if the 4 following conditions were fulfilled: (1) the IF is higher than 1.5; (2) the β-galactosidase activity is significantly increased compared to the solvent control; (3) the IF vs. concentration graph shows a dose–effect relationship; and (4). the result is reproducible suggested by most of the previously published studies [10,16,20].

Chromosome Aberrations

This cytogenetic assay was studied to determine the extent of DNA damage in peripheral blood lymphocytes of Swiss albino mice by exposing them orally different concentration of wastewater at different durations. Chromosomal study was performed by the technique suggested by Preston., *et al* [21]. The randomly five-six weeks old Swiss albino mice (25 ± 2 body weight) were selected. They were fed on standard mice feed procured from Medical Institute, Chandigarh. The waste water was provided for the three different duration: 24, 48, 72 hours. The animals of control group were given distilled water. The animals of positive group are treated with Cyclophosphamide, a well-known clastogene and mutagen [22]. Before two hours of sacrificing, Colchicine of 1.0 ml was administered intraperitoneally to test animals at the dose level of three mg/kg body weight. The Swiss Albino mice were sacrificed by cervical dislocation and their femur were surgically removed. The femurs were immediately put in normal saline. The epiphyses are cut off by sterile scissor and the bone marrow was aspirated using 0.56% (w/v) KCL solution. The harvested cells are incubated at 37ºC for 30 - 35 minutes and then centrifuged for 10 minutes at 2000 rpm. After the decantation of supernatant, cells are replaced with freshly prepared cold solution, i.e. Carnoy's fixative (methanol: glacial acetic acid, 3:1 v/v). The suspension was allowed to stand for 10 minutes after which it was centrifuged for 10 minutes

at 2000 rpm. Again after the decantation of supernatant, cells were replaced with fresh fixative. The process of fixing and centrifuging was done thrice. Slides were prepared by dropping the fixed cells from a height of 30 - 40 cmat an angle of 45º on clean, cold, dry, grease-free slides. Finally, the slides were air-dried and stained with 5% Giemsa (v/v, stock Giemsa stain/distilled water) for 10 - 20 minutes. Slides were then transferred to a jar containing water for 2 to 5 minutes for washing. Keep the slides in titled position for drying and examined under light microscope using immersion oil 100X.

Interpretation and reporting

Data analysis for *Salmonella* Mutagenicity Test

The result are generally expressed by using "two–fold rule" for *Salmonella* Assay which is not only sufficient to allow an objective evaluation of the data or the effectiveness of the experiment [13]. This rule shows that if there is a two- fold increase in spontaneous revertants of sample test, then the change in the average plate count by one revertant out of one or few hundred can be the calling difference between mutagenic and non-mutagenic. A non-statistical interpretation of data has been done by Zieger., *et al* [23]. Using this procedure the following criteria were used to interpret results:

Positive: A compound is considered a mutagen if it produces a reproducible, dose-related increase in the number of revertant colonies in one or more strains of *Salmonella typhimurium*. A compound is considered a weak mutagen if it produces a reproducible dose-related increase in the number of revertant colonies in one or more strains but the number of revertants is not double the background number of colonies.

Negative: A compound is considered a non-mutagen if no dose-related increase in the number of revertant colonies is observed in at least two independent experiments.

Inconclusive: If a compound cannot be identified clearly as a mutagen or a non-mutagen, the results are classified as inconclusive (e.g. if there is one elevated count). For this analysis the dose related increases in the number of revertant colonies were observed for the test compounds and mutagenicity ratios were calculated. Mutagenicity ratio is the ratio of average induced revertants on test plates (spontaneous revertants plus induced revertants) to average spontaneous revertants on negative control plates (spontaneous revertants) Mathur., *et al* [24]. Mutagenicity ratio of 2.0 or more is regarded as a significant indication of mutagenicity.

Data Interpretation for SOS chromotest

The SOS chromotest results were expressed in the ratio Rc = β/p where, β represents β-galactosidase activity in (mUI) and p, alkaline phosphatase activity in (mUI). The Induction Factor (IF) for a compound at concentration is defined as IF = Rc/Ro, in which Ro is the spontaneous ratio measured in the blank test (solvent control). The results are compiled and expressed as Induction Factor (IF ± SD).

Data analysis of Chromosome aberration

Chromosomal aberrations such as gaps, chromatid breaks, etc. are scored and represented as % chromosomal aberrations (Tripathi., *et al*. 2011). All the results are compiled and expressed as mean of all three hours experiments (± S.D).

Result

The results emphasized the importance of the determination of health centers effluents genotoxicity with an aim of evaluating the efficiency of wastewater treatment plants (WTPs). The data on different stages of treatment plant inducing genotoxic activity in the SOS chromotest are reported in table 2 and the results of *Salmonella* mutagenicity assay activity for six different sampling sites are expressed in table 1.

Health Centers	Sites	Sample Aliquots (µl)	Mutagenicity Ratio TA 98				Mutagenicity Ratio TA 100				Mutagenicity Ratio TA 102			
			June 2013		Dec 2013		June 2013		Dec 2013		June 2013		Dec 2013	
			-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
SDMH	Main Sewage (Untreated)	2	+	+	+	+	+	+	+	+	+	+	+	+
		5	+	+	+	+	+	+	+	+	+	+	+	+
		10	+	+	+	+	+	+	+	+	+	+	+	+
		50	+	+	+	+	+	+	+	+	+	+	+	+
		100	+	+	+	+	+	+	+	+	+	+	+	+
	Filtrate and Aeration	2	-	-	-	-	-	-	-	-	-	-	-	-
		5	+	+	+	-	-	-	-	-	-	-	-	-
		10	+	+	+	+	+	-	-	-	+	-	-	-
		50	+	+	+	+	+	+	+	+	+	+	+	+
		100	+	+	+	+	+	+	+	+	+	+	+	+
	Treated Influents	2	-	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-	-
		10	-	-	-	-	-	-	-	-	-	-	-	-
		50	-	-	+	-	-	-	-	-	-	+	+	-
		100	-	-	-	-	-	-	+	-	-	-	-	-
Fortis	Main sewage (Untreated)	2	+	+	+	+	+	+	+	+	+	+	+	+
		5	+	+	+	+	+	+	+	+	+	+	+	+
		10	+	+	+	+	+	+	+	+	+	+	+	+
		50	+	+	+	+	+	+	+	+	+	+	+	+
		100	+	+	+	+	+	+	+	+	+	+	+	+
	Filtrate and Aeration	2	-	-	-	-	-	-	-	-	-	-	-	-
		5	+	+	+	-	-	-	-	-	+	+	-	-
		10	+	+	+	+	+	-	-	+	+	+	-	-
		50	+	+	+	+	+	+	+	+	+	+	+	+
		100	+	+	+	+	+	+	+	+	+	+	+	+
	Treated Influents	2	-	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-	-	-
		10	-	-	-	-	-	-	-	-	-	-	-	-
		50	-	-	-	-	-	-	-	+	+	-	-	-
		100	-	-	-	+	-	-	-	-	-	-	-	-

Table 1: *Mutagenicity ratio of Salmonella strains TA 98, TA 100 and TA 102 in Ames test on wastewater effluent from Health care Establishments.*

+: Ratio greater than 2.0 indicating possible mutagenicity; -: Ratio less than 2.0 indicating non-mutagenicity

Untreated Samples

During both season sampling, all the untreated wastewater samples from the two hospitals have showed significant mutagenic activity, with comparable dose response profile concluding mutagenicity ratios much higher than 2.0.

The detailed observations were made with dose response curves in the water samples from different stages of treatment plant are shown in figure 2-4. Different concentrations were evaluated when the assay is carried out in *Salmonella*, both in the presence and absence of metabolic activation, depending on the results obtained at standard dose range are described in table 1. The specific mutagenic activity of untreated effluents of Fortis and SDMH hospitals with strain TA98 were positively found in all doses with S9 metabolic activation (Figure 2) while with strain TA100 the number of induced revertants with 10µl, 50µl and 100µl of sample have shown positive, dose response relationship in the absence (Figure 3) and with strain TA102 weak increase in the mutagenicity was observed in induced revertants per 50 µl and 100 µl of sample, presence of S9 hepatic fraction (Figure 4) shown in table 1 indicates that the untreated waste water from both hospitals under study was strongly genotoxic. Thus, in all positive assays there was a dose-related increase in the number of mutant colonies with all three strains. These results are in agreement with mutagenicity rankings by Jolibois and Guerbet [4-6] and Gupta., *et al*. [25] who placed hospital waste effluents in the category of strong mutagenicity.

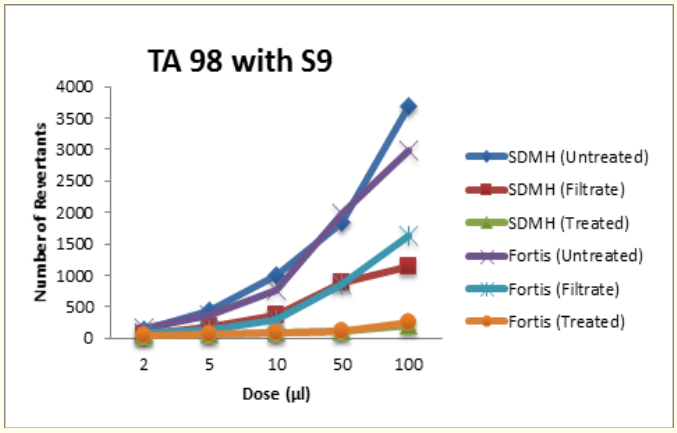

Figure 2: *Concentration response curves for health care establishments wastewater with strain TA 98 with S9 metabolic activation.*

Filtrate from WTPs

The filtered samples collected from ETP of Fortis and SDMH hospitals showed positive significant, with mutagenicity ratios higher than 2.0, mostly at higher doses of the sample. The results were almost similar with all the three strains viz.TA98, TA100 and TA102. With strain TA98, the filtrate sample showed positive mutagenicity at lower dose in SDMH hospitals further the Fortis hospital do not show mutagenicity at lower concentration of dose (Table 1). The specific mutagenic activity of filtrate effluents of Fortis and SDMH hospitals with strain TA100 number of induced revertants (642 - 887 and 947 - 1589) per 100 µl of sample, in the absence of the S9 hepatic fraction (Figure 3) and with strain TA102 number of induced revertants (856-1078 and 962 - 1384) per 100 µl of sample, in the absence and presence of the S9 hepatic fraction (Figure 4) indicates that the filtrate waste water after aeration and filtration from Fortis and SDMH hospital are moderately genotoxic (Table 1). This indicates that mutagenicity is slightly reduced after the process of filtration and aeration during the functioning of ETP. During both season sampling, there is no significant difference was observed in mutagenic activity in May and November, suggesting no seasonal influence on the genotoxicity of samples.

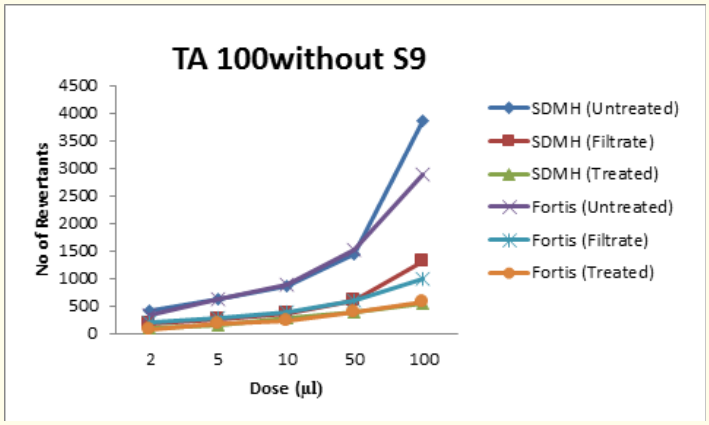

Figure 3: *Concentration response curves for health care establishments wastewater with strain TA100 without S9 metabolic activation.*

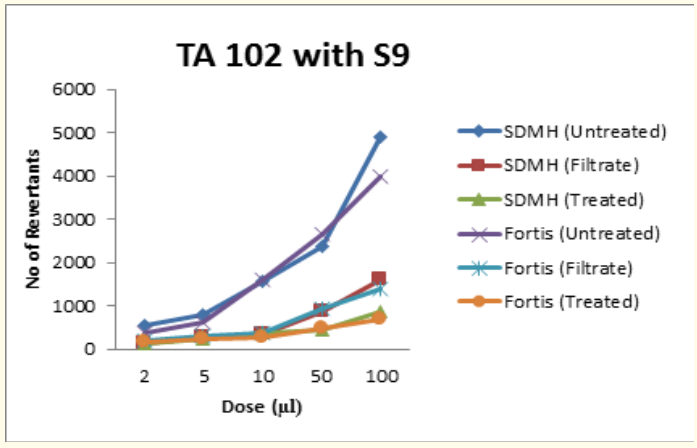

Figure 4: *Concentration response curves for health care establishments wastewater with strain TA102 with S9 metabolic activation.*

Treated wastewaters from WTPs

Finally treated samples collected from ETP of Fortis and SDMH showed very weak mutagenicity activity with all the three strains viz. TA98, TA100 and TA102. Thus, a dose - effect relationship with and without metabolic activation, observed less mutagenicity (mutagenicity ratio being less than 2) while the spontaneous number of revertants observed in the control. The finally treated waste water from the ETP of Fortis and SDMH was found to be slightly genotoxic with strain TA98, 100 and 102 number of induced revertants (119 - 130 and 271 - 287; 316 - 1067 and 547 - 1299; 387 - 424 and 612 - 665) induced revertants per 100µl of sample, in the absence and presence of the S9 hepatic fraction. The detail observation in mutagenic activity was expressed in Table 1. Therefore, from the dose response curve suggested that usage of ETP plant at the Fortis and SDMH hospital is significantly reduced the number of colonies. Similarly observations have been reported by Jolibois and Guerbet [4-6] and Gupta., *et al*. [25] proved that the waste water treatment plant have been able to remove the genotoxicity. For SDMH hospitals, the data were found to be significantly different in the months of May comparing with Fortis hospital. We conclude that the hospitals studied have correct and refined WTP and disposal systems, since the efficiency of treatment and removal of the pollutants is decreased after each treatment process, which further suggests that WTPs are efficient in removal of genotoxins from the hospital waste.

SOS Chromtest

The result of SOS chromotest indicate the genotoxic effect on health centers waste water on *E. coli* PQ37 without metabolic activation. Based on, the IF obtained, untreated sample of both the hospital have IF value > 1.5 hence the result obtained with untreated sample while in filtrate and treated sample of Fortis and SDMH hospital exhibited no significant effect on *E. coli*, the activities of two enzymes and calculated IF values are less than 1.5 showing in less genotoxic response (Figure 5; Table 2). The alkaline phosphatase activity is slightly increased with increasing the concentration while similarly the β-galactosidase activity and Induction Factor (IF) also increased with increasing the concentration, it may be concluded that treatment plant is able to detoxify the genotoxins present in the health care establishment's waste water.

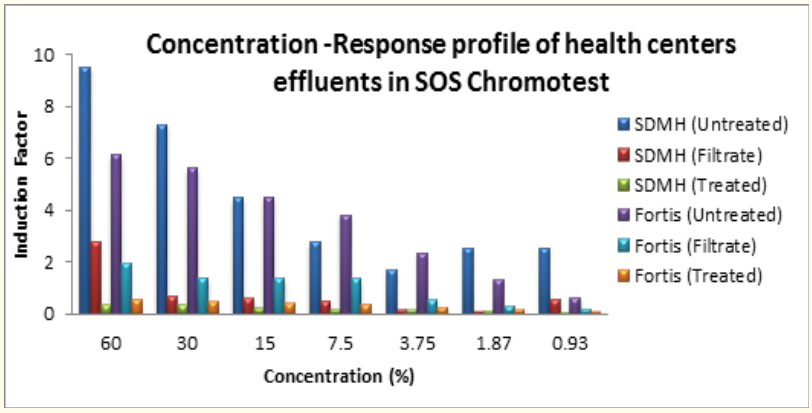

Figure 5: *Concentration – response profile of health centers effluents in SOS Chromotest.*

Concentration (%)	SDMH (Untreated)	SDMH (Filtrate)	SDMH (Treated)	Fortis (Untreated)	Fortis (Filtrate)	Fortis (Treated)
	IF ± S.D	IF ± S.D	IF ± S.D	IF ± S.D	IF ± S.D	IF ± S.D
60	9.5 ± 1.03	2.97 ± 0.67	0.89 ± 0.35	6.1 ± 1.11	1.97 ± 1.07	0.70 ± 0.95
30	7.3 ± 1.43	0.70 ± 0.02	0.54 ± 0.47	5.6 ± 1.34	0.56 ± 1.22	0.56 ± 0.92
15	4.46 ± 1.0	0.61 ± 0.76	0.039 ± 0.08	4.5 ± 1.77	1.61 ± 1.34	0.47 ± 0.89
7.5	2.7 ± 1.42	0.51 ± 0.22	0.021 ± 0.30	3.8 ± 1.44	0.51 ± 1.15	0.323 ± 0.05
3.75	1.2 ± 1.37	0.54 ± 0.21	-0.02 ± 0.10	2.8 ± 1.23	0.54 ± 1.12	0.22 ± 0.06
1.87	0.3 ± 1.02	0.49 ± 0.13	-0.02 ± 0.04	1.3 ± 1.52	0.49 ± 0.27	0.14 ± 0.18
0.93	0.21 ± 0.09	0.43 ± 0.01	-0.01 ± 0.01	0.63 ± 1.56	0.431 ± 0.28	0.10 ± 0.02

Table 2: *Induction Factor (IF)*observed in SOS Chromotest in the effluents of SDMH and Fortis Health centers.*

Values in bold are showing significant SOS inducing activity IF >1.5

Chromosome Aberrations

The results of chromosome aberration bioassay are studies as hundred cells were scored per slide. Types of aberration observed were chromosome break, gap and fragmentation. The wastewater was provided ad libitum for three different duration: 24, 48 and 72 hours. Untreated effluents of both Fortis and SDMH shows maximum chromosome aberration, i.e. fragmentation. Maximum structural chromosome aberrations were observed after exposure of untreated wastewater at the duration of 48 hours and 72 hours are were compiled for further analysis and are expressed as (± SD) for SDMH and Fortis as 2.21 ± 0.4, 1.1 ± 0.03 for gap%, 2.1 ± 0.54, 0.0 ± 0.0 for break% and

3.0 ± 1.41, 2.10 ± 1.4 for fragmentation (Tables 3). The filtrate and treated sample of both the health centers have shown less significant aberration as compared to untreated samples. A significant increase ($p \leq 0.01$) in the chromosomal aberration in untreated effluents indicated mutagenicity of health centers effluent.

S. No	Health center's	No of cell scored	Gap (%)	Break (%)	Fragment (%)	Metaphase (%)
1	Cyclophosphamide (Positive control) (48 hrs)	100	3.21 ± 1.8	1.7 ± 0.5	1.4 ± 1.1	45
2.	SDMH (Untreated)	100	2.21 ± 0.4	2.1 ± 0.54	3.0 ± 1.41	26
3	SDMH (Filtrate)	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10
4	SDMH (Treated)	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	11
5	Fortis (Untreated)	100	1.1 ± 0.03	0.0 ± 0.0	2.10 ± 1.4	9
6	Fortis (Filtrate)	100	1.20 ± 0.1	0.0 ± 0.0	0.4 ± 0.0	12
7	Fortis (Treated)	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	14
8	Distilled water (Negative Control) 48 hours	100	0.1 ± 0.0	0.0 ± 0.0	0.8 ± 0.2	15

Table 3: *Chromosome aberration cause by the effluents of health care establishments in Swiss albino mice.*

Value in bold having significant p > 0.05 chromosome aberration

Discussion

Waste water released by the health care establishments needs an urgent attention, because it is a complex matrix containing different types of toxins, which may pose serious threat to the life, if released in the environment without proper treatment. It was probably first study conducted on the comparison at the efficacy of two onsite Hospital treatment plants with reference to genotoxins. In the present research, we used short term assay to screen the possible genotoxicity of hospital treatment plant. The genotoxic effects of bacterial population in health care waste water was evaluated by *Salmonella* micro some mutagenicity test system and SOS chromotest. The mutagenic effect of untreated effluent of Fortis and SDMH hospital in TA98 at doses of 50 and 100 µl number of induced revertants (824 - 3865) without metabolic activation. The highest number of revertants (3865) was obtained when assay was performed on untreated effluents of SDMH hospitals at 100µl dose. Since, in TA98 strain, untreated waste water was found to be the most mutagenic effluents (Table 1).

In a TA 100 strain, the data obtained from different stages of treatment plant exhibit positively mutagenic activity in untreated and treated effluents of Fortis and SDMH with S9 metabolic activation, may be due to the complex nature of the S9 mix that indicating that mammalian enzymes can convert some of the promutagenic compounds into active mutagenic metabolites or its protein binding. In TA102 strain, becomes strong genotoxic effect significant effect without S9 to the medium, which concise that liver detoxification enzymes probably detoxified some of the mutagenic compounds.

Thus, treated health centers waste water showed significant genotoxic activity in all three strains of *Salmonella* test. It also showed increased mutagenic effect with and without metabolic activation (Figure 2-4). Since, the variability of the obtained response (strain sensitivity, proportion, and intensity of positive response) tended to show a large variety of the compounds responsible for the genotoxicity. Hence, the data obtained from the present study proved that the process such as Filtration, Aeration, Activated carbon filter and Chlorination used in the hospital waste water treatment plants has been effective in removing the genotoxicity detected in the waste water treatment plant influents in both the assay Ames and SOS chromotest. Hartmann., *et al*. [26] identified this compound as the source of genotoxins in the wastewater from a German hospital. Ciprofloxacin, the antibiotic and popular antihistamine cetirizine had the higher level in hospitals waste water tested. Both drug measured for below a human dose but the results are still alarming.

Alabi and Shokunbi., *et al*. [8] noted the impact of genotoxic wastewater on the environment and the significant to human health are difficult to predict because wastewaters are complex mixtures of chemical substances. IF of health centers waste water at various concentrations without, S9 addition are expressed in table 2. With the increased in alkaline phosphatase activity, IF was also high in the higher concentration, and at lower concentration of filtrate and treated effluents (which was constant from 3.75 to 0 µg/ml). At 3.75 µg/ml, β-galactosidase activity started to decline. When the SOS chromotest was performed, alkaline phosphatase was nearly constant throughout the tested dose range, while, β-galactosidase activity increased with increasing concentrations in the absence S9 mix. β -galactosidase activity began to decrease due to the toxicity of effluents from the different stages of treatment plant (Table 2). The maximum IF of untreated effluents of Fortis hospital was 6.1 to 2.80 and SDMH hospital was 9.5 to 2.7, respectively. Our results indicate that untreated waste water was found to be more genotoxic on strain PQ37 of *E. coli*.

Thus, the Hospitals WTPs treated effluent were effective at removing genotoxins detected by the TA 98 - S9 but its effectiveness was potential on the TA100 +S9 and TA102 +S9. These two genotoxicity tests are sensitive to different genotoxic effects such as base pair substitution mutation (TA100 and TA 102 positive), frameshift mutation (TA98 positive) or primary damage and variability of the result obtained proofs that many toxic compounds responsible for genotoxicity.

Chromosome Aberration was used to study the DNA damage in peripheral blood lymphocytes of Swiss albino mice. Maximum structural chromosome aberrations have been observed after exposure of untreated wastewater of both the health centers at the duration of 48 hours and 72 hours. A significant increase ($P \leq 0.01$) in untreated effluents of Fortis and SDMH are observed as compared to control animals indicating mutagenic behavior of health centers wastewater (Tables 3). While the filtrate, treated effluent did not cause any significant change in chromosomes. The findings of present study revealed that health centers wastewater is both mutagenic and cytogenetic. Occurrence of chromosome aberration indicated the distortion of microtubule, kinetochore and formation of several macromolecules attached with centromere. Lagging of chromosomes is caused by the dysfunctioning of the mitotic apparatus. It might be due to the aneugenic activity of wastewater. These results are in agreement with study conducted by Alabi and Shokunbi [8], who performed several bioassays like sperm count, sperm morphology, and micronucleus assay on Swiss albino mice by feeding hospital wastewater at different concentrations. The study revealed out that the hospital wastewater possess a significant effect on mice at cellular level, causing chromosome aberration, micronucleus and effecting sperm morphology at different concentration. It was only a single study conducted on eukaryotic organism for evaluating the genotoxicological evaluation of hospital wastewater.

Mutagenic potential of treated effluents depends on the various factors such as nature of effluents and the treatment process we found that unprocessed effluents of hospitals were showing significant mutagenic potential (characteristics) and capacities. These WTP efficacy was noted in a systematic way, both on slightly or strongly genotoxins influents. Similar study performed by other workers in different hospitals also showed similar results while in some instances potent mutagenic activity was found in treated water. These results confirm that, due to their micropollutant content Hospital waste water require more specific management and treatment in order to protect and safeguard the environment, in particular surface water body will receive the final treated effluents from the WTPs. Further it can be conclude that treated water can be used for gardening. Studies could be undertaken in WTPs to determine which step of the treatment process removes the genotoxins. In India, underground tube well water forms the major form of drinking water source while some times hospitals do not having treatment plant directly drained into community sewer system. Therefore, it would be interesting to know if the genotoxic compounds release in concentrated form and whether it poses possible genotoxic contamination for humans and the environment. With several zoonotic infections, there is a scope for studying the biology of mutagenic strains at systems level [27] and the intense requirement in health centers for the imperative assessment towards the critical discharge of the wastewater directly into the environment both in developing and under-developing countries. In view of the above mentioned features, it is clear that health centers wastewater is consist of a complex matrix which requires effectual treatment before discharging into the environment. Following are the perspective points to treat the health centers wastewater.

1. On-site establishment of wastewater treatment plant in health centers,
2. Chlorination of treated health center wastewater before discharging directly into the environment,
3. Health center and Municipal waste should be treated separately [28-32].

Conclusion

The *in vitro* Ames assay, SOS chromotest and chromosome aberrations help us for evaluating the efficacy of both health care establishments/treatment plants in removing or reducing the genotoxicity. The untreated hospital liquid waste from SDMH and Fortis hospitals are potent genotoxic have been significantly proved in both the assays. The treatment processes such as Filtration, Aeration, Activated carbon filter and chlorination helps in reducing the mutagenicity of hospital liquid waste. The hospital waste containing a bulk of genotoxic, virus, radioactive markers, disinfectants, heavy metals and their metabolites could be a source of mutagenic for humans and environment. An advanced and efficient on-site treatment plants for hospital effluents is necessary in reducing the risk exposed to the environment and human health by these toxic substances.

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Bibliography

1. Urvashi V. "Physico-Chemical Characterization and Toxicological Evaluation of Liquid Effluents Generated By Health Care Establishments of Jaipur". Department of Life science. PhD Thesis, The IIS University, Jaipur (2014).

2. Deloffre-Bonnamour N. "Wastes rejects from health establishments: liquid effluents to solid wastes". Master's Thesis, University Claude Bernard-Lyon 1, Institute Universitaire Professionnalise, Department of Environment and Ecodevelopment, Lyon (1995): 75.

3. CCLIN, Paris-Nord. Elimination of liquid effluents hospitals establishments - Recommendations. Institute Biomedical des Cordeliers, Paris (1999): 74.

4. Jolibois B and Guerbet M. «Hospital wastewater genotoxicity". *Annals of Occupational Hygiene* 50.2 (2005a): 89-196.

5. Jolibois B and Guerbet M. "Evaluation of industrial, hospital and domestic wastewater genotoxicity with the Salmonella fluctuation test and the SOS chromotest". *Mutation Research* 565.2 (2005b): 51-62.

6. Jolibois B and Guerbet M. "Efficiency of two waste water treatment plant in removing genotoxicity". *Archives of Environmental Contamination and Toxicology* 48.3 (2005c): 289-295.

7. Quillardet P., *et al*. "SOS chromotest, a direct assay of induction of an SOS function in Escherichia coli K12 to measure genotoxicity". *Proceeding of National Academics Science USA* 79.19 (1982): 5971-5975.

8. Alabi O and Shokunbi OS. "Toxicological effects of hospital wastewater using animal bioassays". *Annals of Biological Research* 2.2 (2011): 265-275.

9. Ames BN., *et al*. "Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test". *Mutation Research* 31.6 (1975): 347-364.

10. Mersch-Sundermann VU., *et al*. "SOS induction in Escherichia coli and Salmonella mutagenicity: a comparison using 330 compounds". *Mutagenesis* 9.3 (1994): 205-224.

11. Rosenkranz HS., *et al*. "SOS chromotest and mutagenicity in Salmonella: evidence for mechanistic difference". *Mutation Research* 431.1 (1999): 31-38.

12. Maron M and Ames BN. "Revised methods for the Salmonella mutagenicity test". *Mutation Research* 113.3-4 (1983): 173-215.

13. Mortelmans K and Zeiger E. "The Ames Salmonella /microsome mutagenicity assay". *Mutation Research* 455.1-2 (2000): 29-60.

14. Prival MJ., *et al*. "Mutagenicity of benzidine and benzidine congener dyes and selected monoazo dyes in a modified Salmonella assay". *Mutation Research* 136.1 (1982): 33-47.

15. Fish F., *et al*. "The SOS chromotest kit: a rapid method for the detection of genotoxicity". *Toxicology Assessment* 2.2 (1987): 135-147.

16. Ruiz MJ and Marzin D. "Genotoxicity of six pesticides by Salmonella mutagenicity test and SOS chromotest". *Mutation Research* 390.3 (1997): 245-255.

17. Kocak E., *et al*. "A statistical evaluation of the potential genotoxic activity in the surface waters of the Golden Horn Estuary". *Marine Pollution Bulletin* 60.10 (2010): 1708-1717.

18. Quillardet P and Hofnung M. "The SOS chromotest: a review". *Mutation Research* 297 (1993): 235-279.

19. Environmental Bio. Detection Products Inc. (EBPI). The SOS-Chromotest Version 6.3, Instruction for Use, Canada (2011).

20. Margulis AB., *et al*. "Induction of SOS response by autoregulatory factors of microorganisms". *Russian Journal of Genetics* 39.9 (2003): 993-996.

21. Preston RJ., *et al*. "Mammalian in vivo cytogenetic assay: analysis of chromosome aberrations in bone marrow cells". *Mutation Research* 198.2 (1987): 157-165.

22. Anderson D., *et al*. "Cyclophosphamide: review of its mutagenicity for an assessment of potential germ cell risk". *Mutation Research* 330.1-2 (1995): 115-118.

23. Zeiger E., *et al*. "Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals". *Environmental and Molecular Mutagenesis* 19.21 (1992): 2-141.

24. Mathur N., *et al*. "Assessing mutagenicity of textile dyes from Pali (Rajasthan) using Ames Bioassay". *Applied* E*cology and Environmental Research* 4.1 (2005): 111-118.

25. Gupta P., *et al*. "Genotoxicity evaluation of hospital waste water". *Ecotoxicology and Environmental Safety* 72.7 (2009): 1925-1932.

26. Hartmann A., *et al*. "Primary DNA damage but not mutagenicity correlates with ciprofloxacin concentrations in German hospital wastewaters". *Archives of Environmental Contamination and Toxicology* 36.2 (1999): 115-119.

27. Chiliveru S., *et al*. "On Brucella pathogenesis: looking for the unified challenge in systems and synthetic biology". *Systems and Synthetic Biology* 9.1-2 (2015): 73-75.

28. Akter N., *et al*. "Medical waste disposal at BRAC Health Centre: An environmental Study". DRAC Research and Evaluation Division, Dhaka (1998): 688.

29. Nemerow NL. "Major Industrial Wastes. InIndustrial Waste Pollution". ReadingMassachusetts. Addison-Wesley (1978): 305-310.

30. Shaner H. "Becoming a mercury free facility: A priority to be achieved by the 2000". *Professional Development Series* (1997): 1-30.

31. Sakamoto H and Hayatsu H. "A simple method for monitoring mutagenicity of river water. Mutagens in Yodo river system, Kyoto-Osaka". *Bulletin of Environmental Contamination and Toxicology* 44 (1990): 521-528.

32. WHO. "Guidelines for safe disposal of unwanted pharmaceuticals in and after emergencies". Essential drugs and other Medicines Department. World Health Organization, Geneva, Switzerland (1999): 180.

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