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Unsafe containment: Public health risks of septic tanks discharging to drains in Dhaka Bangladesh

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Abstract

Sanitation approaches in low-income urban areas are predominately on-site sanitation, with septic tanks promoted as an improved sanitation solution. While a septic tank system is designed to contain sludge in the tank and discharge effluent to a soil infiltration system, in many urban contexts effluent from tanks discharge directly to open drains or surface waters. This research addresses the paucity of data on pathogen removal and discharge from septic tanks as operated in low-income contexts and the significance of this public health hazard. This study assessed the performance and risks of "septic tanks" discharging to open drains in a low-income neighbourhood in Dhaka, Bangladesh, considering the influence of usage and tank operation on concentrations of Norovirus GII, Salmonella Typhi, Vibrio cholerae, Giardia, Shigella and E. coli in the effluent and receiving drains. While 66% of septic tanks were functioning within design limits, multiple pathogens were detected in all effluent samples, with a mean concentration of 7.6 log₁₀ MPN/100mL for E. coli and 4.2–5.6 log₁₀ genome copies/100mL for pathogens, excluding S. Typhi which was not detected. Children's exposure to septic tank discharge in drains could result in an 18% risk of illness from Norovirus GII and 3% from Giardia annually. The pathogen reduction between the estimated septic tank inflows and measured effluent concentration ranged from 1.3 log10 MPN reduction for E. coli to 2.2 log₁₀ genome copies reduction for Giardia. Increased coverage of septic tanks was significantly associated with reduced concentrations of Shigella in open drains compared to direct discharge from toilets, with increased reduction for septic tanks operating within design standards. Implementing septic tanks without sub-surface infiltration or treatment of effluent is a major concern. The potential health risk of exposure to septic tank effluent warrants increased attention to appropriate technical design, effluent management and alternatives such as networked sanitation.

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Introduction

Septic tanks are used by 2.1 billion people globally [1]. Between 2000 and 2022, six times as many households gained a septic tank compared to households gaining a new sewer connection [1]. Septic tank systems are promoted as an improved sanitation solution in urban and rural areas, particularly when sewers are unavailable. The citywide inclusive sanitation approach promotes on-site sanitation as part of a mix of technical solutions for urban areas [2]. However, many 'septic tank systems' do not comply with technical standards. A standard septic tank system consists of a two-chamber baffled tank for settling and storing sludge and a subsurface infiltration system (i.e. soak pit or leach field) for effluent treatment and disposal [3]. The tank only provides primary treatment and further effluent treatment, particularly to reduce microorganisms, is achieved through filtration and absorption in unsaturated soil [3, 4]. However, recent data indicates many so-called "septic tanks" lack the critical soli infiltration step and discharge directly to surface drains [5, 6]. Little is known about the public health risks associated with septic tanks as they are used in low- and middle-income countries.

Global monitoring indicates that septic tanks discharging to the surface environment are prevalent in both low- and high-income countries [7]. Analysis of faecal waste flows in 39 cities in Asia and Africa found that 39% of tanks and pits were connected to open drains or water bodies [5]. In India, a survey of 3000 households in 10 cities found that 72% of septic tanks discharged effluent to drains [8]. In Hanoi, Vietnam, a study of 750 households found that 98% of septic tanks discharge to open channels or old sewer pipes not connected to treatment facilities [9]. National inspections in Ireland found that 9–13% of on-site systems discharge directly to streams and drains [10, 11]. Discharge to the surface is also common in rural United States [12]. These findings are now also reflected in global monitoring of the Sustainable Development Goal (SDG) target 6.2.1 of safely managed sanitation services, which requires that on-site sanitation systems contain excreta so they are not discharged to the surface environment [1]. Where local data are unavailable, estimates for safely managed sanitation are based on the assumption that 50% of septic tanks are not contained.

Contaminated open drains are a critical pathway of human exposure to faecal pathogens in low-income areas. While there are multiple pathways for exposure to pathogens in urban areas, several studies have applied quantitative microbial risk assessments (QMRA) or similar approaches (i.e. the SaniPath method) to compare health risks from different pathways and found that direct exposure to pathogens in open drains or gullies was a greater risk than exposure to contaminated drinking water, soils, and other pathways [13–17]. People in low-income areas, particularly children, are more frequently exposed to open drains and pollutants than in high-income communities [14, 18–21]. Drainage networks transport pathogens across cities; therefore, the entire community, not only the households with inadequate sanitation, are at risk of exposure to untreated excreta discharged to the environment [22]. The human right to sanitation implies that people not only have a right to a hygienic toilet but also have a right not to be negatively affected by poorly managed faecal waste. This point is also emphasised in the UN-adopted human right to a clean, healthy, and sustainable environment [7, 23].

The above studies identified the high levels of contamination and exposure to polluted drains but did not point to the sources of contamination. Various sanitation failures contribute to faecal contamination of drains, such as runoff from open defecation, direct discharge from toilets, on-site sanitation directly connected or overflowing to drains, and sludge dumped locally [5]. Environmental sampling in Ghana found *E. coli* concentrations were lower in, or near, clusters of households with high coverage of sanitation facilities, especially contained

facilities [24]. Environment sampling in India and a desk-based model in Uganda found that direct discharge from on-site systems contributed to greater pathogen releases to the environment than dumping of faecal sludge in drains [21, 25]. Furthermore, climate change is predicted to increase the frequency and severity of flooding in many of the same low-income urban areas where septic tanks discharge to drains, likely increasing exposure to contaminated drains [21].

Despite the widespread use of septic tanks, limited data exist on the fate of pathogens in septic effluent and the magnitude of related public health risks [4, 26]. While the impact of pathogens from septic tank effluent has been studied in relation to the groundwater risk from sub-surface infiltration [27–30], few studies have assessed the risks of septic tanks discharging to drains or the environment. A number of studies have investigated pathogen concentrations in dry pit latrines and a recent systematic review of pathogen reduction in on-site systems also only included dry latrines and sludge with no mention of effluent [31–33]. Another recent compilation of data on pathogen reduction within septic tanks only identified two studies with in-situ data from standard two-chamber septic tanks [34, 35], with other data from models, laboratories or advanced on-site treatment systems [36]. Data on pathogen concentrations in septic tank effluent were available from single studies for *Giardia* (twin settling tanks receiving sewer inflows) [37] and *Shigella* (modified septic tank including filter chamber in a laboratory) [38] and from a small number of studies on *E.coli* [11, 39–42].

Many of these studies were conducted in high-income countries, where influent pathogen loads are expected to be lower, and from controlled studies which do not consider that systems in situ may not follow ideal operating conditions. The implication of poor operation has been studied in relation to nutrient releases [9, 43, 44]; however, only two studies, both in India, assessed the influence of septic tank operation on pathogen release. One found a significant reduction in the concentrations of *E. coli* in tank effluent with increased liquid retention time and increased years of use, but no significant association with emptying frequency, sludge depth or user numbers [25]. The other research indicated a reduction in faecal coliforms in drains and rivers over three years following the implementation of regular emptying of septic tanks [45]. There remains a gap in data on the pathogen removal and discharge from standard two-chamber septic tanks discharging to drains in the conditions in which they are implemented and operated in low- and middle-income countries.

Given many so-called "septic tank systems" only include a tank discharging directly to open drains or other surface environments, it is critical to understand the contribution of these tanks to the faecal load and exposure to pathogens in open drains. This research aims to provide insights into the faecal pathogen discharge and risks associated with the current use of "septic tanks" in dense low-income areas. Specifically, the objectives were to: 1) quantify the presence and concentration of different pathogens discharged from identical well-constructed two-chamber tanks; 2) consider the factors that influence operation and treatment performance; and 3) provide insights about the extent to which such systems provide a meaningful public health improvement as compared with direct discharge from toilets to drains.

Methods

Data collection

Ethics statement. The study protocol for this and the broader data collection was approved by the International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr, b) scientific and ethical review committees (protocol number PR-19011, 2019) and also by the University of Technology Sydney (UTS HREC REF NO. ETH18-2599). Icddr,b secured agreement of the community to participate through the community leaders and informed

concent was received from all participants in household surveys and compound inspections. Additional information regarding the ethical, cultural, and scientific considerations specific to inclusivity in global research is included in the Supporting Information (S1 Checklist).

Study site and population. The study site of Mirpur, Dhaka, was selected to represent urban areas in low-income countries with high population density, poor quality sanitation services and human exposure to water in open drains. It also had characteristics necessary for the study purpose, including the presence of a mix of sanitation systems, including toilets discharging directly to drains and to septic tanks, and the hydraulic characteristics that ensure that all drain flows were generated (and contaminated) from within the same community without upstream inflows under normal conditions (i.e. only during significant flood events). The study site consisted of four parallel streets (Fig 1), each with a similar arrangement of residents living in clusters of households, typically single rooms, located within compounds. The compound enclosed facilities shared between the residents, including shared toilets, water supply, bathing, cooking, and cleaning areas. The study area included 172 compounds, housing 4,792 people, with an average of 8.5 households per compound and 3.2 people per household (Table 1).

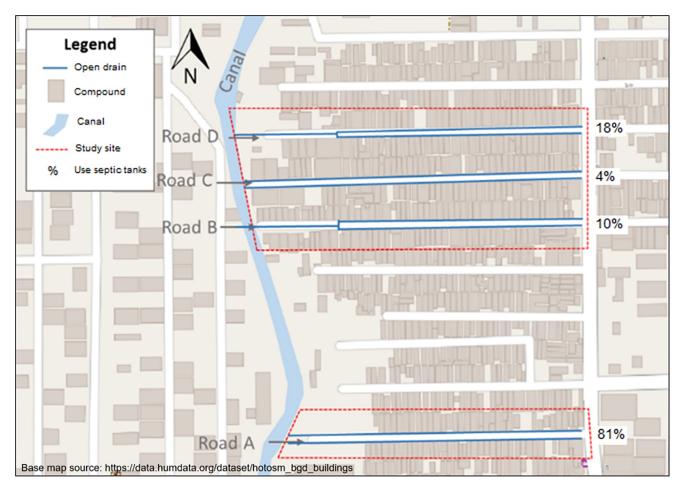


Fig 1. Layout of four roads study site and proportion septic tank use (bounded by dotted red perimeter) (adapted from Foster et al. 2021, source open street map, humanitarian data exchange CCA 4.0).

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Road	Population	Compounds	% population use septic tank	% population use functioning septic tank	Average age of tank
D	1351	51	18%	15%	2.9
С	1277	47	4%	2%	2.2
В	1194	42	10%	13%	1.3
A	970	32	81%	28%	4.8
TOTAL	4792	172	24%	14%	3.4

Table 1. Summary of site characteristics by road.

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Data collection. This research was part of a broader project that developed new methods for sampling and analysis of pathogens in the environment [46], and produced a model of pathogen flows from sanitation systems to assess improvement options [47]. Data collection relevant to this study included household surveys, infrastructure assessments and environmental sampling, which were conducted by trained research staff. Respondents for the household survey were randomly sampled and included 2–3 households per compound resulting in a sample size of 349 households (30% of the study site population). The survey assessed water and sanitation use and frequency of human exposure to open drains. The infrastructure census captured 96% of the survey area (173 compounds) to assess all inflows into the open drain through observation of the sanitation facilities, water meter and drain, and included a survey for each compound to establish the number of users per sanitation facility, emptying practices, and frequency of flooding. The survey and census were conducted the 7–26 May 2019 and 16–22 April 2019, respectively, in Bangla with adult respondents only and formal informed verbal consent provided and recorded, with all data recorded using DoForms, a mobile-based data collection platform.

Environmental sampling was conducted to estimate pathogen concentrations in septic tank effluent and open drains. Faecal pathogen and faecal indicator bacteria (FIB) concentrations were measured from grab samples from the septic tank effluent (n = 18 and four repeats) and at the mid and end point locations of drains in each street (n = 33) in wet and dry seasons. Quantitative polymerase chain reaction (qPCR) was used to detect and measure the concentration of Norovirus GII, Salmonella Typhi, Vibrio cholerae, Giardia and Shigella. The concentration is expressed as \log_{10} EGC per 100mL, with the equivalent genome copies (EGC) estimated in each sample by interpolation of the mean Ct value (averaged from duplicate wells) to the standard curve and the concentration calculated using the dilution factor for each sample type. For quality assurance, one sample processing negative control was included for every 10 samples and one pathogen specific positive control and one negative control (molecular water) were included in each real-time PCR plate (See Liu 2021 for further details) [48]. The IDEXX-Quanti-tray 2000 technique with Colilert-24 media (IDEXX Laboratories, Westbrook, Seattle, WA) was used to quantify the most probable number (MPN) of E. coli per 100 mL of liquid sample. The estimation method from the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM) was adapted to determine the E. coli concentration in the test sample from the combined result of the three dilutions, resulting in a single MPN estimate with 95% confidence limits [49]. The environmental sampling technique and laboratory methods for sample analysis are presented in Amin et al. [46]. These pathogens were chosen due to their reported prevalence of disease in Dhaka [50–54], pre-testing using TaqMan to identify priority pathogens and the availability of sensitive and specific methods for detection in environmental samples (see Foster et al.) [47]. Sludge depths were measured in seven tanks that could feasibly be opened, with samples taken from both chambers using a core sampling device (i.e. sludge checker) which allowed measurement of the depth of sludge and supernatant [55].

Analysis of data

Assessment of septic tank operation. Septic tank operation was assessed based on two widely accepted criteria for septic tank design: the hydraulic retention time (HRT) and the accumulated sludge volume at emptying [56]. Common national design standards suggest tank sizing should be based on maintaining 24-hour minimum design HRT when the tank is around two-thirds full of sludge and ready for emptying [56–59]. For this analysis, a septic tank operating within design standards was defined as having at least 24 hours HRT and less than two-thirds sludge volume. The sludge accumulation rate was calculated using data from complete sludge depth samples for six individual sample systems with the reported number of users and years of operation from the census. The resultant average sludge accumulation rate (28.8L/p/year) was used to estimate sludge volume in the remaining systems (see Tables C and D in S1 Appendix). The HRT was calculated from the reported percentage water used for flushing (6%), median daily water-use from meter readings and water bills (196 L/p/d), the sludge volume and tank hydraulic volume (5.3m³) from the construction drawings provided by the organisation that managed the construction.

Analysis of septic tank effluent and pathogen reduction. Analysis of effluent samples was in IBM SPSS v28. Firstly, we analysed the variability of repeat effluent measurements which were collected two months apart for four tanks, with the second measurement included in analysis. The relative difference between repeats was calculated for repeats with pathogens present in both samples (n = 10 pairs). Next, the association between septic tank operation parameters and the concentration of positive samples of pathogens and E. coli in effluent were assessed using Pearson's correlation coefficients with a state of significance of <0.05. The operational parameters considered were (i) years of operation, (ii) reported numbers of users, (iii) whether sampling occurred in the wet or dry season, (iv) HRT, (v) estimated sludge depth, and (vi) sludge volume as a proportion of total volume (expressed as a binary, with a positive value if sludge volume was less than two-thirds full, i.e. operating within design standards). Lastly, generalised estimating equations (GEEs) were used to analyse the association between the mean concentration in positive drain samples and the population using septic tanks connected to that drain, looking at both general septic tank use and the population using septic tanks operating within design standards. The analysis adjusted for season as an explanatory variable and road as a within-subject variable.

The log reduction of pathogens in septic tanks was assessed by comparing the measured effluent concentration with an estimated influent concentration (see detailed analysis in Tables F-H in S1 Appendix). It was not feasible to capture a representative sample of inflow, so the influent concentrations for Norovirus GII, Giardia and E. coli concentrations were estimated based on reported disease prevalence (literature from Dhaka), burden of disease in Dhaka (local health surveillance data), asymptomatic diarrhoea cases in Dhaka (local data and literature), shedding load (literature), duration of shedding and duration of symptoms (literature), excreta produced (literature) and water volume generated daily per capita (census and questionnaire data). The mean inflow and confidence intervals were estimated from variation in water use (from first to third interquartile range, n = 24) and low and high estimates of prevalence and shedding rates (Tables F-H in S1 Appendix). The influent estimates for Norovirus GII and E. coli were equivalent units to the water quality measurements; however, for Giardia it was necessary to convert the influent estimate in cysts/100mL to genome copies/100mL, assuming 16 genome copies per cyst [60]. A simple conversion was not possible for Shigella, S. Typhi or V. Cholerae; therefore, influent concentration could not be estimated. The log reduction was calculated as the difference between the mean influent concentrations and the arithmetic mean of the measured concentrations of positive effluent samples.

Analysis of septic tank discharge to drains. To compare the influent and effluent concentrations with reports in the literature, the measured blackwater (toilet only) inflows were converted to a combined (blackwater and greywater) equivalent, as literature was only available for combined flows. The combined water flow was calculated by assuming the greywater flows (91% daily water use from survey data) are mixed with the blackwater flows from toilets (6% daily water use), assuming no pathogens in greywater (see Table B in <u>S1 Appendix</u> [3]). This resulted in a median combined flow of 190L/p/d compared with 12L/p/d for blackwater only, and reduced the concentrations by 1.2 log₁₀ genome copies/100mL (see Tables H and M in <u>S1 Appendix</u>).

To demonstrate the potential health risks of pathogens discharged from septic tanks, we conducted a Quantitative Microbial Risk Assessment (QMRA) to assess the probability of illness that exposure to septic tank effluent in open drains poses to children. The probability of illness was calculated for Norovirus GII and Giardia considering drain quality based on three cases: i) a drain with 100% septic tank use (combined discharge of septic tank effluent mixed with greywater to replicate combined flows in drains) ii) a drain with high proportion (81%) of septic tank use (mean concentration of positive samples from the drain in street A), and iii) a drain with a low proportion (4%) of the population using septic tanks (concentration from the drain in street C). The analysis assumed 1mL of water was ingested per drain exposure, which aligned with assumptions used in the Sanipath tool [61], although noting it could be much higher as one study in Dhaka found children ingested 37mL when exposed to flood water [62]. Household survey data indicated that children under five were exposed to drains a median of 14 times per year (from surveys presented by Foster et al. 2021 Fig S3). Given the analysis used the mean positive concentrations yet the pathogens were not present in all samples, the annual exposure frequency was corrected, by multiplying by the occurrence of pathogens in all drain samples (67% Norovirus and 50% Giardia). Dose-response models and probability of illness aligned with the approach described in Foster et al. 2021 [47]. For Norovirus GII we assumed the fractional Poisson dose response model with P = 0.722, $\mu = 1106$ and for *Giardia* the exponential model with $k = 5.72 \times 10^{-2}$ [63, 64]. The probability of illness given infection was assumed to be 55% for Norovirus GII and 40% for Giardia (Table N in S1 Appendix). Note the measured drain concentration for Giardia in GC/100mL was converted into a cyst/100mL concentration for the dose response model, assuming 16 GC/cyst [60].

Results

Study site and septic tank use

The household survey found all households accessed a piped water supply, including 91% piped into the compound and 9% piped into the house. The infrastructure census on sanitation facilities indicated that compounds typically had one toilet facility (i.e. toilet block) with two pour flush toilets (i.e. cubicles/pans), with each toilet facility used by an average of 21 users from 7 households. Toilets predominately discharge directly to the drain (71%), with others discharging to a two-chamber septic tank (24%), a single tank (3%), a concrete ring pit (1%) or an unknown pit (1%) (Table A in <u>S1 Appendix</u>). All tanks and pits had an outlet pipe for effluent to discharge to the drain, and none discharged to subsurface infiltration systems.

Septic tanks were only present in government-owned compounds and were built through externally funded projects. Prevalence of tanks varied between the four streets: in street A 81% of the population used a septic tank, 10% in street B, 4% in street C and 18% in street D (Table 1). Construction of the tanks occurred in stages therefore the tank ages vary with streets, the oldest in street A (4.9 years) and the most recently built in streets B and C (1.5 years). Tanks were all built to the same design standard with two chambers of 5.3m³ total capacity for

50 users. Only 2 of the 40 tanks in the study area had been emptied, and both had been operating for 4.9 years. On average, septic tanks were used by 25 people (range 7–52, SD 9.8) and received blackwater (toilet) flows only. Greywater from the kitchen, washing and cleaning discharged to the open concrete-lined drains. The median estimated inflow to the septic tanks was 12 L/p/d, based on the median water use from the compounds with septic tanks (196 L/p/d) and the reported portion of water used for toilet flushing (6% of daily water use) (Table B in S1 Appendix).

In line with design standards, the assessment of operation considered sludge volume and hydraulic retention time (HRT). From the measured sludge depth in both chambers of six tanks, the mean sludge accumulation rate was 29 L/p/year and ranged from 12 to 49 L/p/yr (Table C in S1 Appendix). The average calculated sludge volume of all tanks (n = 40 tanks) was 50% of the hydraulic tank volume, based on the mean sludge accumulation rate, tank dimensions, the reported number of users, and years of operation per tank. The design limit of two-thirds sludge volume was surpassed in 33% (n = 13) of tanks, all located in street A. The average HRT was 14 days (median 8.5 days) and only two tanks exceeded the design criteria of a minimum one-day HRT. For all tanks in the study area, 68% were estimated to operate within the sludge and HRT design (Table D in S1 Appendix). For the tanks from which effluent samples were taken (n = 18), 61% were assessed as operating within design standards.

Pathogen release from septic tanks

All septic tank effluent samples (n = 18) were positive for one or more pathogens and most samples (56%) were positive for two or more pathogens. The highest occurrence was *Shigella*, with 94% effluent samples testing positive, and the lowest was *S*. Typhi, with no positive samples (Table 2). The arithmetic mean concentration of positive samples (excluding non-detects) ranged from 4.2 to 5.6 log₁₀ genome copies (GC)/100mL and 7.6 MPN/100mL for *E. coli*. The variability of pathogen presence in effluent was evident in repeat measurements collected for four tanks two months apart, of which 10 paired samples (of 26 total) were positive in both repeats, with an average 1.0 log₁₀ /100mL or 27% relative difference (Table E in <u>S1 Appendix</u>). The mean estimated influent concentration (considering blackwater only) was 6.4 and 6.7 log₁₀ GC/100mL for *Giardia* and Norovirus GII, respectively, and 8.9 log₁₀ MPN/100mL for *E. coli* (Table H in <u>S1 Appendix</u>). The reduction in pathogens between the mean estimated influent and mean measured effluent ranged from 1.3 log₁₀ MPN for *E. coli* to 2.2 log₁₀ GC for Giardia (Table 2).

Table 2. Pathogen detection and concentrati	on in septic tank effluent samples.
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Pathogens	% positive (n = 18)	Unit	Mea	sured efflu	ent concentration ^a	Estimated influent concentration ^b	Log reduction
			Mean	Median	Interquartile Range	Mean (Range)	Mean
Norovirus GII	67	Genome copies / 100mL	4.99	4.54	0.49	6.68 (5.3, 7.2)	1.69
V. cholerae	56		4.27	3.20	1.41		
S. Typhi	0		-	-	-		
Giardia	17		4.15	4.21	0.39	6.36 (4.3, 6.9)	2.21
Shigella	94		5.56	2.88	1.49		
FIB (E. coli)	100	MPN/ 100mL	7.61	6.56	0.89	8.92 (7.2, 9.5)	1.31

Notes

^a. Log₁₀ transformed arithmetic mean concentration of positive samples.

^b. Influent concentration and log reduction were only estimated for Norovirus GII, Giardia and E. coli

^c. All concentrations are blackwater only.

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The effluent from septic tanks operating within the design standards had a mean concentration 0.92 log₁₀ GC/100mL lower than those systems exceeding design standards, although the concentration difference was less for V. *cholerae* (0.03 log₁₀GC/100mL) and higher for *Shigella* (2.9 log₁₀GC/100mL) (Table I in S1 Appendix). The concentration of *Shigella* was significantly associated (p < 0.05) with well-operating septic tanks (r = -0.647, p = 0.01), user.years (r = 0.637, p = 0.01), estimated percentage sludge volume (r = 0.635, p = 0.01) and HRT (r =-0.647, p = 0.01) (Table K in S1 Appendix). The concentration of *Giardia* was significantly associated with users (r = 1.00, p = 0.004) and the concentration of *E. coli* was significantly associated with wet conditions during sampling (r = 0.522, p = 0.03). No other significant correlation (p > 0.05) was detected. Compared with unemptied tanks of a similar age and number of users (4.9 years ago and 35 users, n = 6), the concentration of pathogens in the effluent of the one tank that was previously emptied were 2.1 and 3.4 log₁₀ GC/100mL lower for Norovirus GII and *Shigella* and 1.3 log₁₀ MPN/100mL lower for *E. coli*, pointing to the potential for emptying to improve effluent quality (Table L in S1 Appendix).

Comparison of toilet and septic tank discharge to drain

The occurence and concentration of pathogens in open drain samples were higher than from septic tank effluent, as most toilets in the study site (71%) discharged directly to drains without any containment. The presence of pathogens measured in samples from open drains (n = 30) ranged from 27% positive for *S*. Typhi to 100% positive for *V*. *cholerae* and *Shigella*, and half of all samples were positive for at least four pathogens (Table M in S1 Appendix). Drains received a mix of blackwater from toilets and septic tank effluent, and greywater from kitchen, washing, etc. To compare the septic tank effluent concentrations, an equivalent wastewater discharge from compounds with septic tanks was calculated based on the total wastewater flows (190 L/p/d), rather than the blackwater only flows (12 L/p/d). This dilution with greywater reduced the septic tank effluent pathogen concentration by 1.21 log₁₀/100mL for all pathogens. The drain concentration was 1.2 to 2.7 log₁₀ GC/100mL higher than the combined flow discharge from compounds with septic tanks (Fig 2 and Table M in S1 Appendix).

To demonstrate the potential health-related impact of the discharge from septic tanks to open drains, the potential risk of illness was estimated using QMRA found that septic tank discharge (considering the concentration of septic tank effluent diluted with greywater) could result in illness of 1,800 and 300 children per 10,000 per year from Norovirus GII and *Giardia* respectively, assuming 1mL of drain water is ingested by children up to 14 times per year (Table O in S1 Appendix).

Analysis using Generalized Estimating Equations (GEEs) found a 10% increase in the population using septic tanks was significantly correlated (p < 0.05) with a 0.10 log₁₀ GC/100mL reduction in *Shigella*, while a 10% increase in the use of septic tanks operating within design standards was associated with a 0.34 log₁₀ GC/100mL reduction in *Shigella* (Table 3). Wet season was significantly associated with an increase in the concentration of *E. coli*.

Discussion

Septic tanks in Dhaka were found to discharge multiple pathogens in high concentrations into open drains. Well operating systems, less sludge, higher HRT and previous emptying were associated with lower concentrations of pathogens in effluent. Although septic tanks provided some reduction in pathogens and *E. coli* concentrations, and their use was associated with lower concentrations of pathogens in open drains than direct toilet discharge, nonetheless, septic tanks discharging to drains pose a major health risk. This section summarises how these

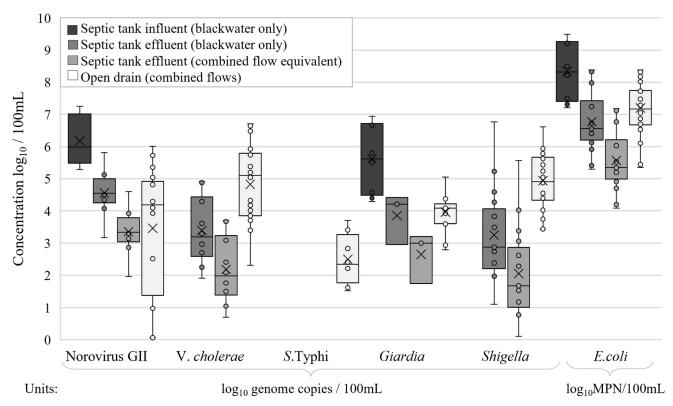


Fig 2. Comparison of pathogen concentrations in septic tank influent (estimated), septic tank effluent (measured blackwater n = 18), septic tank and greywater combined discharge (estimated wastewater equivalent n = 18) and open drains (measured, n = 30) excluding non-detects with error bars indicating the range of concentrations.

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Table 3. Effect of increased septic tank use (typical and well operating) on pathogen concentration in drains, adjusting for season and auto-correlation between	
samples from the same street.	

		All septic tanks		Septic tanks operating within design standards	
		Coefficient (95% CI)	Sig.	Coefficient (95% CI)	Sig.
Norovirus GII	10% increase in use of septic tanks	-0.012 (-0.36,0.34)	0.945	0.22 (-0.85,1.29)	0.688
	Wet Season = 1	0.379 (-1.61, 2.37)	0.709	0.536 (-1.4,2.47)	0.588
V. Cholerae	10% increase in use of septic tanks	0.022 (-0.12, 0.08)	0.657	-0.035 (-0.37,0.3)	0.837
	Wet Season = 1	0.452 (-0.31,1.21)	0.244	0.442 (-0.32,1.2)	0.256
Shigella	10% increase in use of septic tanks	-0.099 (-0.19,-0.01)	0.029*	-0.335 (-0.61,-0.06)	0.018*
	Wet Season = 1	0.53 (-0.03, 1.09)	0.062	0.526 (-0.02,1.08)	0.061
S. Typhi	10% increase in use of septic tanks	0.069 (-0.01,0.15)	0.097	0.378 (-0.02,0.78)	0.062
	Wet Season = 1	0.047 (-1.41,1.5)	0.949	0.128 (-1.35,1.61)	0.865
Giardia	10% increase in use of septic tanks	-0.024 (-0.07,0.02)	0.327	-0.026 (-0.22,0.27)	0.839
	Wet Season = 1	0.544 (-0.07,1.16)	0.081	0.53 (-0.08, 1.14)	0.087
E. coli	10% increase in use of septic tanks	0.032 (-0.02,0.09)	0.255	0.131 (-0.06,0.32)	0.180
	Wet Season = 1	0.961 (0.56, 1.36)	0.000*	0.969 (0.57, 1.36)	0.000*

Note

* indicates a significant association (*p*-value < 0.05 using Wald Chi-square test) between a 10% increase in the population using septic tanks, or a 10% increase in the population using septic tanks operating within design standards and change in the \log_{10} concentration of pathogens in drain samples (n = 30) considering positive samples only and adjusting for season. CI is the 95% confidence interval of the coefficient.

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results compare to the limited available literature on septic tank effluent concentrations, pathogen reductions in standard septic tanks, and concentrations of pathogens in drains. We discuss the high potential risk of illness from septic tank discharge, the difference in pathogen groups and the value of monitoring pathogens, as well as the limitations of this study. Lastly, we reflect on the possible factors influencing the implementation of septic tanks discharging to drains and call for further research to address remaining uncertainties and identify solutions to address this urgent issue facing many low- and middle-income urban areas.

Septic tanks in Dhaka discharged multiple pathogens to open drains with high concentrations that were at the upper end of the limited existing data. Multiple pathogens were present in all effluent samples with a mean concentration ranging from 4.2 log₁₀ GC/100mL for Giardia to 5.6 log₁₀ GC/100mL for Shigella, and 7.6 log₁₀ MPN/100mL for E. coli. Compared to other studies on septic tank effluent, one study in India also monitored effluent from standard septic tanks that received only blackwater and found slightly lower *E.coli* discharge concentrations from private and communal septic tanks that were regularly emptied $(6.0-6.9 \log_{10})$ MPN/100mL) [25]. As the other in-field studies of effluent from standard septic tanks were systems that received combined blackwater and greywater (i.e. kitchen, bathing) inflows, for comparison, the effluent concentrations from this study were converted to an equivalent combined flow based on the estimated greywater volumes (Table B in S1 Appendix). The resultant combined *E.coli* concentration (6.4 \log_{10} MPN/100mL) aligned with the range of effluent concentrations from five studies of standard septic tanks receiving direct household inflows (4.9-7.15 log₁₀ MPN/100mL) [11, 39-42]. The equivalent combined flow of Giardia (1.7 log₁₀ cysts/ 100mL) was lower than the one study from USA on effluent from settling tanks receiving wastewater flows (2.6 log₁₀ cysts/100mL) [37]. Data available on the concentration of Shigella in the effluent from a modified septic tank in India (2.1 log₁₀ CFU/100mL) [38] cannot be directly compared to genome copy units of effluent samples. No literature was found for Norovirus GII, S. Typhi, or V. cholerae concentrations in septic tank effluent.

Septic tanks classified as "operating within design standards", based on measured sludge depth and HRT, performed better than tanks not operating within standards. The assessment of septic tank operation against design criteria found 33% of septic tanks had estimated sludge volumes greater than the design limit of two-thirds full, with only two tanks previously emptied. Overall, the pathogen concentration in effluent samples was 1.0 log₁₀ GC/100mL lower for septic tanks operating within design standards than those beyond standards, although only Shigella effluent concentrations were found to be significantly associated with users years, sludge depth, HRT and the overall indicator of functioning. This result aligned with a study of septic tank effluent in India that found a reduction in E. coli concentrations with increased liquid retention time, but differed with respect to their finding that an increase in E. coli was significantly associated with increased years of use but not with emptying frequency, sludge depth or user numbers [25]. The one sampled tank previously emptied had 1.3, 2.1 and 3.4 log₁₀ lower concentrations for *E. coli*, Norovirus GII and *Shigella*, respectively, than unemptied tanks of the same age, again indicating that less sludge is associated with better quality effluent. The use of septic tanks operating within standards was associated with a three times greater reduction in the concentration of *Shigella* in open drains than use of any septic tanks, yet both were an improvement on direct discharge without storage.

There are very few studies on the removal of specific pathogens by septic tanks in the absence of soil based treatment. The estimated reduction in *E. coli* (1.3 \log_{10} MPN) was within the range reported in studies of in-situ standard septic tanks receiving household flows from Jordan and the USA (0.4 to 2.0 \log_{10} MPN) [41, 65, 66]. The estimated reduction in *Giardia* (1.0 \log_{10} cyst) was higher than found in twin tanks in the USA (0.24 \log_{10} cyst) [37]. Data for *Norovirus GII* reduction was not available for septic tanks but the findings (1.7 \log_{10} GC) were

similar to available data from waste stabilisation ponds in Ghana and USA (1–1.6 \log_{10} GC) [67, 68]. A recent compilation of literature on pathogen removal suggested a much higher log reduction of 4–8 in septic tanks, however, the majority of the data reported in that review were from lab-based studies or more advanced on-site treatment such as package anaerobic filters, MBRs and modified septic tanks, which are expected to have higher removal rates [36].

While septic tanks discharging to drains provided some reduction in pathogens as compared with direct discharge from toilets to drains, the high occurrence and concentration of pathogens released to the environment is concerning. The health risk assessment illustrated that given the high likelihood of exposure to open drains in Dhaka, particularly by children, septic tank effluent released to drains is likely to contribute to multiple illnesses per year. Although it was not possible to calculate the risk of illness, the high occurrence and discharge concentration of V. *cholerae* is particularly concerning given drains are a principal transmission pathway for frequent Cholera outbreaks in Dhaka [62]. While septic tanks were promoted as an upgrade on direct discharge, they continue to create a public health risk, hence, the value of this investment is questionable. Other studies have shown that alternative sanitation solutions could be implemented to reduce health risk. For instance, a pathogen flow systems model comparing improvement options for this neighbourhood identified that piping the septic tank effluent to secondary treatment or shifting to centralised sewerage with off-site treatment would achieve the greatest improvement in terms of local exposure [47].

Monitoring pathogens rather than *E. coli* alone is valuable to understand the health risks posed by septic tank effluent. While septic tank performance is often generalised as "pathogen removal", bacteria, viruses, and protozoa respond differently to environmental conditions and within these groups pathogens vary in infectivity, virulence, and persistence [69-71]. The results align with the expectation that removal of protozoa by sedimentation in septic tanks would be greater than for bacteria and viruses [72]. However, given the low occurrence of Giardia in effluent samples (17%) and the small sample, the difference in removal between pathogen groups requires further validation. Further research would also be valuable to compare the reduction in *E. coli* with other pathogens, given indicators, such as *E. coli*, have been found to not correlate well with pathogens released from on-site sanitation [70, 71, 73, 74]. In some conditions, bacterial pathogens (particularly *E. coli*) can increase between the influent and effluent due to regrowth [36, 73].

Our results indicating poor removal of pathogens in septic tanks is not unexpected, yet this prompts the question of why numerous tanks continue to be built without adequate effluent treatment. One common physical restraint is unfavourable soil conditions for infiltration, which was reported as a reason for direct discharge occurring in Dhaka [75]. Another study in the USA reported that 32% of land areas had unsuitable soil for septic tanks, yet they were built anyway due to a lack of public sewer systems [76]. Inadequate financial resources or space and creating an overflow intentionally to reduce the need for desludging are other possible reasons why septic tanks are installed without leach fields or soak pits. Given that so-called "on-site systems" continue to be built in unsuitable soil conditions, it is possible that the health risks of septic tank effluent are not well understood due to the reported low knowledge on pathogens by many sanitation service providers or environmental health authorities [70]. Low awareness of the need to manage effluent from on-site systems may also be exacerbated by the omission of effluent management (i.e. leach field or soak pits) from most on-site sanitation service chain diagrams [77-80]. The ambiguity of containment terminology also doesn't help, with a variety of wet cesspools, pits, sealed and unsealed tanks without effluent management often classified as "septic tanks" [5, 81]. Literature in high-income countries more often refers to "septic tank system" or "septic tank and soak-away system" [11, 44, 82]. We suggest that the language used for a range of types of tanks with and without soil infiltration systems is in need for review [81].

While this paper provides important new data on and analysis of pathogens in septic tank effluent, we recognise a number of limitations of this study. Due to the difficulty collecting influent samples from septic tanks connected underground to toilets, the analysis relied on estimating pathogen concentrations in the influent. This approach is based on multiple assumptions and does not capture the temporal and spatial variability of enteric infections in the user population or shedding by asymptomatic infections, although extended sampling would also be necessary to capture this variability [14, 36]. However, the estimated combined flow inflows aligned with wastewater concentrations in literature, with concentrations of Norovirus GII mid-range and Giardia and E. coli estimates at the upper end of concentrations in literature (Table H in S1 Appendix). Increasing the number of samples may have provided more information about variability in pathogen occurrence and concentration in effluent from a wider range of septic tanks with different characteristics. However, our analyses were limited due to the cost of analysing effluent samples for pathogens. This will likely also be a constraint for future research, along with the technical capacity and equipment for PCR analysis for pathogen detection in environmental samples, which is not available in all low-income countries. The findings are also expected to be influenced by inherent variations in field conditions and user populations, such as pathogen prevalence, nature of inflows to each tank and possible ingress of flood water. Further research could inform the extent to which septic tank effluent and pathogen removal are influenced by varied pathogen occurrence and concentration in inflows by assessing different populations and larger sample sizes. Lastly, while the data was collected 5 years ago, the situation in Dhaka has not changed and the majority of hosueholds continue to use on-site sanitation or direct discharge, with a large wastewater project only expected to increase sewer connections by 50,000 (0.2% the city population) [83]. Despite these limitations the general finding stands that there is significant flow of pathogens into open drains in dense urban areas which use so-called septic tanks without suitable soil-based treatment and that this is likely to introduce significant health risks.

As septic tanks are often promoted as an improved sanitation solution, the intentional or inadvertent exclusion of the vital soil infiltration treatment step means that these systems may provide a false sense of security, as the tank alone provides minimal health risk reduction compared with direct discharge from toilets. This study raises questions on the value and benefit of continuing to install septic tanks discharging to drains in contexts similar to Dhaka and how to reduce the public health hazard of those that already exist. We call for further research to understand these risks in other contexts and to further investigate the potential for management practices or retrofits (e.g. effluent filters, treatment add-ons, covering drains) to mitigate the significant health risks of existing tanks. More attention is needed to identify and test solutions for septic tank effluent management in dense urban areas where soil, groundwater or density are unsuitable conditions for sub-surface infiltration, and to include these costs when assessing options. To achieve SDG target 6.2 and achieve ongoing health benefits of sanitation many low- and middle-income countries must prioritise safe management of effluent from on-site sanitation, particularly in dense urban areas.

Supporting information

S1 Appendix. Supplementary information. (DOCX)

S1 Checklist. Inclusivity in global research. (PDF)

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