Journal of Water & Health

© 2023 The Authors Journal of Water and Health Vol 21 No 8, 981 doi: 10.2166/wh.2023.283

Non-potable water reuse and the public health risks from protozoa and helminths: a case study from a city with a semi-arid climate

Ayushi Chaudhary[a], Shubham Rana[a], Rajveer Singh[b], Patrick L. Gurian[b], Walter Betancourt[c], Arun Kumar[d] and Amit Kumar[a,*]

[a] Department of Civil Engineering, MNIT Jaipur, Jaipur, India
[b] Department of Civil, Architectural and Environmental Engineering, Drexel University, Philadelphia, PA 19104, USA
[c] Water and Energy Sustainable Technology Centre, University of Arizona, Tucson, AZ, USA
[d] Department of Civil Engineering, IIT Delhi, Delhi, India
*Corresponding author. E-mail: amitrathi.ucf@gmail.com; amitkumar.ce@mnit.ac.in

AK, 0000-0002-0817-9004

ABSTRACT

The study estimated the risk due to *Cryptosporidium, Giardia*, and *Ascaris*, associated with non-potable water reuse in the city of Jaipur, India. The study first determined the exposure dose of *Cryptosporidium*, *Giardia*, and *Ascaris* based on various wastewater treatment technologies for various scenarios of reuse for six wastewater treatment plants (WWTPs) in the city. The exposure scenarios considered were (1) garden irrigation; (2) working and lounging in the garden; and (3) consumption of crops irrigated with recycled water. The estimated annual risk of infection varied between 8.57×10^{-7} and 1.0 for protozoa and helminths, respectively. The order of treatment processes, in decreasing order of annual risk of infection, was found to be: moving-bed bioreactor (MBBR) technology > activated sludge process (ASP) technology > sequencing batch reactor (SBR) technology. The estimated annual risk was found to be in this order: *Ascaris* > *Giardia* > *Cryptosporidium*. The study also estimated the maximum allowable concentration ($C_{\max}$) of pathogen in the effluent for a benchmark value of annual infection of risk equal to 1:10,000, the acceptable level of risk used for drinking water. The estimated $C_{\max}$ values were found to be 6.54×10^{-5}, 1.37×10^{-5}, and 2.89×10^{-6} (oo) cysts/mL for *Cryptosporidium, Giardia*, and *Ascaris*, respectively.

Key words: *Ascaris*, *Cryptosporidium*, *Giardia*, microbial risk assessment, water quality, water reuse

HIGHLIGHTS

- Use of the Sketcher tool for modelling concentrations of *Cryptosporidium*, *Giardia*, and *Ascaris* in treated wastewater.
- Estimation of annual risk of infection due to *Cryptosporidium*, *Giardia*, and *Ascaris* during reuse of treated wastewater.
- Estimation of concentrations of *Cryptosporidium*, *Giardia*, and *Ascaris* corresponding to annual risk of infection value (i.e., 1:10,000).

1. INTRODUCTION

Water reuse is an option for adapting to diminishing water supplies and achieving sustainable development goals, specifically in water-stressed regions. Nevertheless, treated wastewater may contain pathogenic microorganisms, as some of these organisms including Norovirus, Adenovirus, Rotavirus, *Cryptosporidium*, and *Giardia* have demonstrated persistence in tertiary treated wastewater (Quintero-Betancourt *et al.* 2003; Hewitt *et al.* 2011; Schmitz *et al.* 2016).

The removal of pathogens in municipal wastewater treatment plants (WWTPs) depends on hydraulic retention time, influent characteristics, and treatment design (Hajare *et al.* 2021a). In underdeveloped regions of the world, where there is a lack of reliable wastewater treatment processes, municipal effluents that carry harmful pathogens might be released into surface waters. These pathogens include *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Entamoeba hystolitica*, and helminths such as *Ascaris*. The World Health Organization (WHO) recommends the monitoring of indicator microorganisms (i.e., coliform bacteria, *E. coli*, faecal streptococci, *Clostridium perfringens*, and enterococci) in wastewater before discharge (Al-Gheethi *et al.* 2013). Likewise, regulatory guidelines in different countries include the monitoring of indicator microorganisms in treated water before discharge or reuse (Al-Gheethi *et al.* 2013). Additional bacterial pathogens with

---

documented environmental persistence, such as *E. coli O157:H7*, *Streptococcus faecalis*, *Salmonella* spp. (*S. typhi*, *S. typhimurium*), *Shigella* spp., *K. pneumonia*, *E. aerogenes*, have been reported in treated wastewater (Al-Gheethi *et al.* 2013). Extensive literature is available on the health concerns associated with these waterborne pathogens (Al-Gheethi *et al.* 2013; Hajare *et al.* 2021a, 2021b; Chowdhari *et al.* 2022). As a result, improperly treated reclaimed water may expose people to pathogenic microorganisms that may result in the risk of infection (Ueki *et al.* 2005; Ito *et al.* 2016, 2017; Sano *et al.* 2016; Gerba *et al.* 2017, 2018; Khalid *et al.* 2018; Ofori *et al.* 2021).

Quantitative microbial risk assessment (QMRA) is an established framework for estimating the probability of infection/illness from exposure to pathogens in multiple environmental scenarios. In general, risk assessment is described as the process of determining the likelihood of an event occurring as well as the likely scale of its negative consequences – economic, health/safety-related, or ecological – over a certain time period (Gerba 2000; Quintero-Betancourt *et al.* 2003; Ueki *et al.* 2005; Blatchley *et al.* 2007; Ito *et al.* 2016, 2017; Sano *et al.* 2016; Chaudhry *et al.* 2017; Gerba *et al.* 2017, 2018; Nappier *et al.* 2018; Soller *et al.* 2018).

For non-potable reuse options, deterministic QMRA has been used to estimate the risk of infection/illness from crop irrigation, toilet flushing, and recreational use (Jolis *et al.* 1999; Quintero-Betancourt *et al.* 2003; Ueki *et al.* 2005; Ryu *et al.* 2007; Amha *et al.* 2015; Ito *et al.* 2016; Sano *et al.* 2016; Chaudhry *et al.* 2017; Moazeni *et al.* 2017; Ronco *et al.* 2017; Gerba *et al.* 2018; Soller *et al.* 2018; Rock *et al.* 2019; Ezzat 2020; Alegbeleye & Sant'Ana 2021; Emilse *et al.* 2021). The estimation of the risk of infection or illness due to waterborne pathogens is often under-reported due to the limited data on the concentrations of pathogenic bacteria and protozoa in the treated effluent. The under-reporting may further subvert the efforts to prevent infections, especially in developing countries. The challenge is to identify correct exposure routes, analyse microbial loads accurately and define appropriate model constants to avoid over- or under-estimating the risks associated with water reuse. These factors may have significant temporal and geographical variations (Hajare *et al.* 2021b).

In India, the trend of water reuse has been increasing, and therefore understanding the risk of microbial infection during water reuse has become increasingly important. This study investigates the microbial risks associated with current practices of non-potable water reuse in Jaipur city (India). Jaipur is the capital city of the state of Rajasthan in India and the home to approximately 3 million people as per Census 2011. It has a semi-arid climate with limited and highly exploited water resources. Moreover, being a UNESCO world heritage site, the city has a very high influx of tourists every year.

For non-potable reuse, the risks associated with bacteria and viruses have been estimated (Moazeni *et al.* 2017; Marques *et al.* 2021). However, the risks from protozoa and helminths have received less attention (Soller *et al.* 2018; Hajare *et al.* 2021b). Furthermore, there is a lack of comprehensive characterization regarding how the risks of infection may vary based on different wastewater treatment technologies. In this study, the risk of infection is calculated for protozoa and helminths using estimated removal efficiencies for six WWTPs with different treatment technologies, i.e., activated sludge process (ASP), moving-bed bioreactor (MBBR) and sequencing batch reactor (SBR) (see Figure 1 for locations).

The present study was undertaken in order to fulfil the following objectives: (i) to determine the exposure concentration of selected pathogens for various water reuse scenarios under different wastewater treatment technologies; (ii) to estimate value of annual risk of infection for each scenario; (iii) to calculate the permissible pathogen concentration corresponding to typical risk targets for water reuse applications. The findings of this work are expected to be useful for policymakers formulating regulatory guidelines regarding wastewater reuse in India; for wastewater operators to understand the need to upgrade their treatment systems and, for the general public to understand exposure scenarios when visiting public parks for leisure.

2. METHODOLOGY

This study relies on the QMRA framework to identify public health risks associated with water reuse applications from six municipal WWTPs in operation in the city of Jaipur, India (Table 1). The selected treatment plants employ three different wastewater treatment technologies including ASP, MBBR, and SBR.

The reuse of treated wastewater is practiced for irrigation in two ways: either onsite (before dilution) or offsite (after dilution) irrigation. In case of onsite irrigation, the treated wastewater is sent directly from the WWTP to irrigate the surrounding landscaping. However, for offsite irrigation, the treated wastewater is first discharged into a surface water body, i.e., Dravyavati River in Jaipur and then only utilized for irrigation purposes. The risk of infection was estimated for two protozoan parasites (*Cryptosporidium* and *Giardia*) and helminths (*Ascaris*). These pathogens were selected by the following criteria: (a) the pathogens should not be a part of routine monitoring and, (b) the pathogens have been found to be resistant

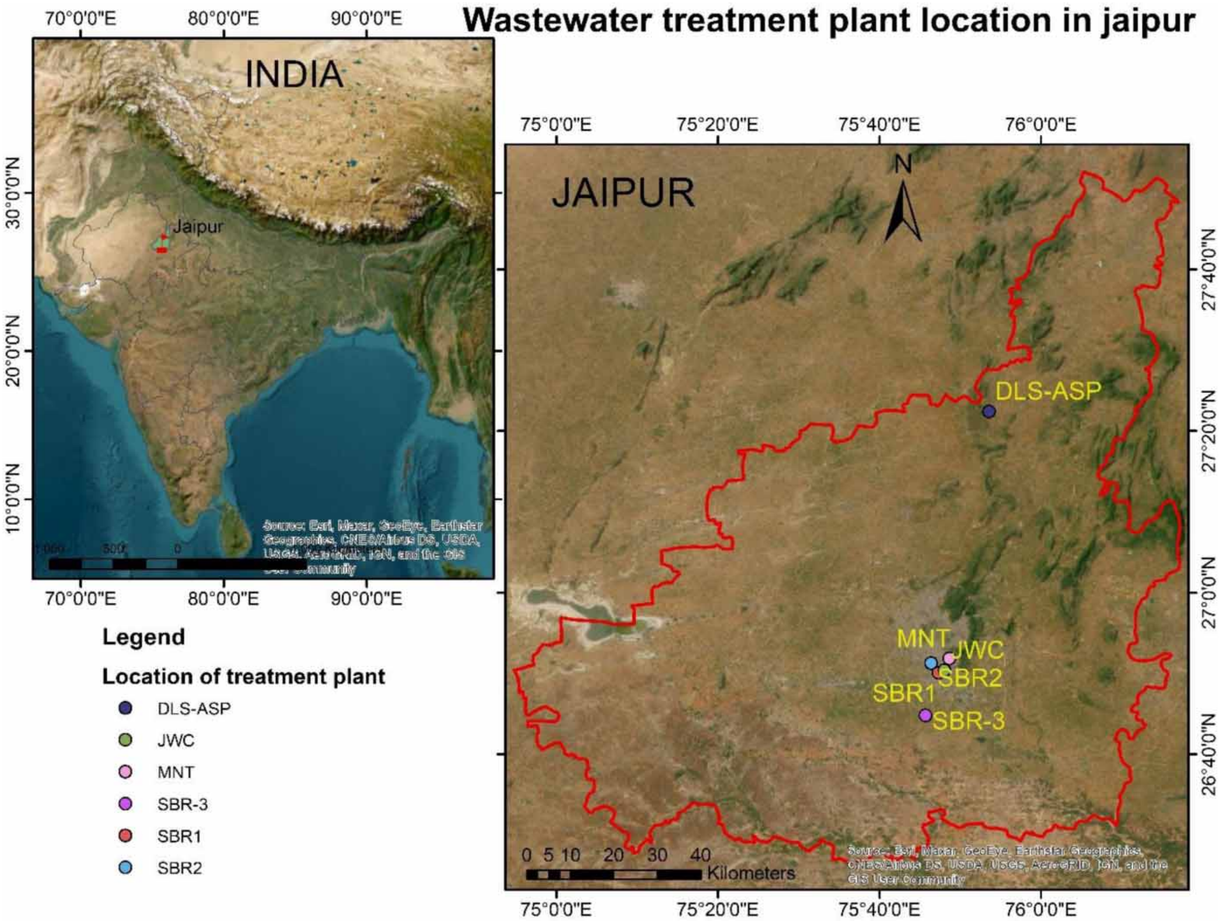

Figure 1 | Wastewater treatment plant locations in Jaipur (*source*: Google maps). (SBR-1: sequencing batch reactor of 100 (million litres per day) MLD municipal WWTP at RIICO industrial area; SBR-2: 15 MLD municipal WWTP at Shipra path; SBR-3: 20 MLD municipal WWTP at Bassi; DLS-ASP: 62.5 MLD activated sludge process municipal WWTP at Delawas; MNT: 1 MLD moving-bed bioreactor institutional WWTP at Malaviya National Institute of Technology; JWC: 1 MLD moving-bed bioreactor municipal WWTP at Jawahar circle).

to chlorine or combined forms (e.g., chloramines). The hazard characterization and exposure assessment steps were carried out for the selected pathogens taking into consideration the concentration values from the literature; their removal in WWTPs and the reuse conditions for the treated wastewater. Each of the QMRA steps are described below in detail.

2.1. Hazard identification

For the risk evaluation, the most common protozoan parasites associated with gastrointestinal illness (i.e., *Giardia* and *Cryptosporidium*) and the most common intestinal worm associated with helminth infection (i.e., *Ascaris*) were included (Westrell *et al.* 2004). *Ascaris* ova can persist in harsh environments for months to years, making it a good candidate for QMRAs in developing countries (Seidu *et al.* 2008).

Protozoa go through a number of stages in their life cycle, each with unique activities and structure. Oocysts are a thick-walled, environmentally resistant pathogenic stage that coccidian parasites, such as *Cryptosporidium* generate during their life cycle. *Cryptosporidium* can cause gastrointestinal infections that are potentially lethal in infants and in immune-compromised individuals. Cysts, which are dormant phases of Giardia, have a protective membrane or thicker wall that allows them to live in unfavourable environmental conditions (Jain *et al.* 2019). *Giardia intestinalis*, which is also referred to as *G. duodenalis* or *G. lambia*, is the most common species infecting humans. Giardiasis develops after ingestion of the cyst that is spread through contaminated food, water, or human-to-human contact (faecal–oral pathway). It causes symptoms similar to diarrhoea in 90% of symptomatic patients, which often resolve within 2–6 weeks in healthy people (Jain *et al.* 2019).

Table 1 | Characteristic of selected WWTPs in Jaipur city

S. No.	WWTP location (short name)	Technology	Treatment scheme	Capacity (MLD)	Average daily flow (MLD)	HRT (h)	SRT (days)	Chlorine dosage	Effluent reuse (as obtained by personal communication with WWTP officials)
1	SBR-1	SBR + Cl_2	Screening – Settling -SBR; Sludge Drying beds; Chlorination	100	60	3	13	90–150 CT	Treated effluent is discharged into Dravyavati river
2	SBR-2	SBR + Cl_2	Screening – Settling-SBR; Sludge Drying beds; Chlorination	15	13	3	13	60 CT	Treated effluent is discharged into Dravyavati river
3	DLS-ASP	ASP	Screening-Settling-ASP; Sludge Drying beds	62.5	62.5	6	8	NA	Treated effluent is discharged into Dravyavati river
4	MNT	MBBR + Cl_2	Screening – MBBR-Settling; Sludge holding tanks; Chlorination	1	1	4.3		150–180 CT	Plantation, Irrigation
5	JWC	MBBR	Screening – MBBR -Settling; Chlorination	1	1	6		NA	Plantation
6	SBR-3	SBR + Cl_2	Screening – Settling-SBR; Sludge Drying beds; Chlorination	20	15	3	13	90 CT	Treated effluent is discharged into Dravyavati river

SBR-1, sequencing batch reactor; DLS-ASP, Delawas activated sludge process; MNT, Malaviya Institute of technology; JWC Jawahar circle; SBR + Cl_2; sequencing batch reactor with chlorination for disinfection; MBBR + $Cl_{2;}$, moving-bed bio reactor with chlorination for disinfection; HRT, hydraulic retention time; SRT, solid retention time.

2.2. Exposure assessment

The treated wastewater from the selected treatment plants is mainly used for irrigation (onsite and offsite) as per the information obtained in personal communication with WWTP officials (Table 1). Accordingly, three scenarios were considered: (i) Scenario 1 considers direct ingestion of treated wastewater and ingestion of aerosols; (ii) Scenario 2 considers exposure during working and lounging in a garden irrigated with treated wastewater and is mainly concerned with the general public visiting for leisure; (iii) Scenario 3 is related to the consumption of produce from a field irrigated with treated wastewater (Chhipi-Shrestha *et al.* 2017; Busgang *et al.* 2018; Hajare *et al.* 2021a, 2021b). In each of these scenarios, there may be two cases: (i) onsite irrigation and (ii) offsite irrigation (Table 2). The exposed population consists of mainly workers in the gardens or on the farms, and children visiting the garden for leisure.

2.2.1. Pathogen concentration in raw wastewater

A compilation of the influent concentrations of the selected pathogens in municipal wastewater from studies all over the world are shown in the supplementary information (Table S1). The concentration of protozoan parasites (i.e., *Cryptosporidium* and *Giardia*) in raw wastewater has been taken from a number of studies spanning various countries including Sweden, Ireland, USA, UK, Spain, Malaysia, and China (Ottoson *et al.* 2006; Cheng *et al.* 2009; Fu *et al.* 2010). For helminths (i.e., *Ascaris*), the concentration in raw wastewater has been taken from France, Germany, Great Britain, the United States, and other countries (Navarro & Jiménez 2011) (Table S1). As for India, there are limited studies on the concentration of these

Table 2 | Ingestion volume of treated wastewater per event for different scenarios

Scenario number	Scenario name	Ingestion volume (mL)	Reference
Scenario 1	Garden irrigation	0.1	Natural Resources Management Ministerial Council (2006)
Scenario 2	Garden lounging	1	Busgang *et al.* (2018)
Scenario 3	Food crop consumption (ingestion of crops)	5	Chhipi-Shrestha *et al.* (2017)

pathogens in treated effluent (Hajare *et al.* 2021b) and none in raw wastewater. The maximum values of the pathogen's concentrations, obtained from the literature, have been taken to derive a conservative or health protective risk estimate. (Table 3).

2.2.1.1. Estimation of pathogens concentrations after treatment, and after dilution in surface water. The $\log_{10}$ reduction values (LRV) for pathogens in WWTPs vary from 0.44 (Cheng *et al.* 2009) to 3.60 (Sidhu *et al.* 2017). The organism-specific LRV vary between 0.44 and 2.15 for *Cryptosporidium* and between 1.70 and 2.61 for *Giardia* (Ottoson *et al.* 2006; Cheng *et al.* 2009) (Table 4). One study in India, Hajare *et al.* (2021b) reported the presence of protozoan parasites in treated effluent; however, this study did not determine the protozoan removal efficiency of the treatment process. The study considered 13 treatment plants in Delhi having the following treatment train: grid chamber and screens followed by equalization tanks, pre-chlorination tanks, tube settlers, and dual media and activated carbon filters. For Jaipur city, there has been one study which considered the removal of *E.coliO157:H7*, *Salmonella* spp, and *Pseudomonas* spp in two municipal WWTPs, i.e., MNT and DLS-ASP (Bhatt *et al.* 2020).

Although these studies discuss the removal of pathogens in WWTPs (Ottoson *et al.* 2006; Cheng *et al.* 2009), the technologies used in the treatment plants were not discussed. There are some other studies also on pathogen detection and wastewater treatment (Tan 1993; Kobayashi *et al.* 2017; Moazeni *et al.* 2017; Saidulu *et al.* 2021; Gupta *et al.* 2022). Howe studies (Tan 1993; Kobayashi *et al.* 2017) did not study the removal of protozoa and helminth in full-scale treatment plants. Moazeni *et al.* (2017) studied only the influent concentrations of *Enterovirus,* fecal coliform, and total coliform and did not determine the pathogen removal. Although Gupta *et al.* (2022) and Saidulu *et al.* (2021) reviewed MBBR technology for removal of parameters such as chemical oxygen demand (COD), total nitrogen, phosphorus, and emerging contaminants, they did not consider any pathogens. In addition, researchers have also investigated the inactivation of these pathogens by using different kinds of disinfectants (Campbell *et al.* 1995; Betancourt & Rose 2004; Craun *et al.* 2010; Esther *et al.* 2019). *Cryptosporidium* oocysts and *Giardia* cysts are well known to be resistant to chlorination. As the effect of specific processes on the pathgoen removal was not in the scope of the study, it was not investigated further.

In the present study, the pathogen removal in WWTPs was determined using the Sketcher tool (Musaazi 2020; Tumwebaze *et al.* 2021) available at https://www.waterpathogens.org/tools/treatment-plant-sketcher-tool. The Sketcher tool can predict the proportion of pathogens attenuated by a treatment system and allows users to view the fraction of pathogens ending up in the liquid effluent using statistical models based on data from scientific publications. It can be used to build a customized 'sketch' of a treatment system, including information regarding treatment reactors. It predicts pathogen removal by group (like viruses, protozoa, bacteria) so all the pathogens belonging to a specific group are modelled as one pathogen. For the modelling of treatment technologies, the only secondary treatment processes available in the model are ASP, trickling

Table 3 | Concentrations of selected pathogens in raw wastewater from literature and the values assumed for Jaipur city for all WWTPs

Pathogen type	Concentration	Reference
Cryptosporidium (oocysts/l)	1,000	Fu *et al.* (2010)
Giardia (cysts/l)	13,600	Fu *et al.* (2010)
Ascaris (eggs/l)	3,000	Navarro & Jiménez (2011)

Table 4 | Pathogen removal information in terms of log reduction value (LRV) in WWTPs from literature

Location (remark)	WWTP name (if identified)	Pathogen	Raw influent	LRV	Reference
Different cities in Sweden (average values of four WWTPs analysed from different parts of Sweden)	WWTP	*Cryptosporidium* (oocysts/L)	20	1.18	Ottoson *et al.* (2006)
	WWTP	*Giardia cysts* (cysts/L)	2,042	2.61	
North-western Ireland	Plant A	*Cryptosporidium* (oocysts/L)	592	2.15	Cheng *et al.* (2009)
	Plant A	*Giardia* (cysts/L)	320	2.52	
	Plant B	*Cryptosporidium* (oocysts/L)	280	1.60	
	Plant B	*Giardia* (cysts/L)	123	1.70	
	Plant C	*Cryptosporidium* (oocysts/L)	11	0.44	

filter and waste stabilization pond. For the WWTPs based on SBR and MBBR technologies, this study modelled them as both, i.e., ASP and trickling filter and, the log-reduction values on the conservative side were employed for dose calculations.

Water ingestion concentration (N_{ie}) of *Cryptosporidium*, *Giardia*, and *Ascaris* in the effluent of WWTP was calculated by the following equation:

$$N_{ie} = N_{i0}(1 - f_{i2})(1 - f_{id}) \tag{1}$$

where N_{io} denotes the concentration of specific pathogen (*Cryptosporidium, Giardia*, and *Ascaris*) in the raw wastewater; f_{i2} and f_{id} are the fractions of microorganisms removed during combined primary and secondary treatment, and during disinfection, respectively (in the absence of disinfection, f_{id} is taken as 0). The log-reduction values obtained from the Sketcher tool ranged from 0.92 to 1.24 $\log_{10}$ for both protozoa and helminths (Table 5). For the helminths, the Sketcher tool modelled the log reduction values near a single value of 1. Based on these log-reduction values, the values of f_{i2} were determined by converting LRV value to percentage value (Table 5). As the Sketcher tool models all the protozoa in a similar way, the log-reduction values for both *Cryptosporidium* and *Giardia* are the same.

The reduction of the microbes in the surface water body occurs by decay and dilution. Following assumptions were made based on the (Haas 1983) study: (i) uniform, plug-flow conditions at a steady state in the river, (ii) same value of decay constant ($k_i = 0.69$/day) in the river length considered and (iii) instant dilution of the WWTP effluent in the river.

The pathogen concentration after dilution in surface water is estimated by Equation (2) (Haas 1983; Tyagi *et al.* 2022). The attenuation of microbes and the resulting concentration during travel in a surface water body, for time '*t*' from the discharge was calculated using the following equation:

$$N_{it} = \frac{N_{ie} * \exp(-kt)}{1 + D} \tag{2}$$

Where N_{ie} is pathogen concentration in WWTP (oocysts/ml), N_{ii} is pathogen concentration in downstream river after discharge from WWTP (oocysts/ml), k is the decay coefficient (=0.69/day), t (=2 days) is the travel time, and D is the dilution factor (100:1) (Tyagi *et al.* 2022).

Table 5 | Log- reduction values calculated for selected pathogens with the help of the Sketcher tool and pathogens concentration in wastewater effluent

						$C_{effluent}$	
Pathogen	**WWTP**	**Capacity (MLD)**	**Modelling in Sketcher tool**	**LRV obtained from the Sketcher tool**	**Calculated f_{i2}**	***Cryptosporidium* (oocysts/mL)**	***Giardia* (cysts/mL)**
Protozoa	SBR-1	100	As ASP	1.22	0.94	6×10^{-02}	8.16×10^{-01}
	SBR-2	15	As ASP	1.21	0.94	6×10^{-02}	8.16×10^{-01}
	DLS-ASP	62.5	As ASP	1.02	0.9	0.1	1.36
	MNT	1	As Trickling filter	0.94	0.89	0.11	1.5
		1	As ASP	1.24	–		
	JWC	1	As Trickling filter	0.92	0.88	0.12	1.63
		1	As ASP	1.21	–		
	SBR-3	20	As Tickling filter	1.21	0.94	6×10^{-02}	8.61×10^{-01}
Helminth							*Ascaris* (eggs/mL)
	SBR-1	100	As ASP	1	0.9	3×10^{-01}	
	SBR-2	15	As ASP	1.01	0.9	3×10^{-01}	
	DLS-ASP	62.5	As ASP	1	0.9	3×10^{-01}	
	MNT	1	As Trickling filter	1.01	0.9	3×10^{-01}	
			As ASP	1.01	–		
	JWC	1	As Trickling filter	1.01	0.9	3×10^{-01}	
			As ASP	1.01	–		
	SBR-3	20	As ASP	1.01	0.9	3×10^{-01}	

SBR, sequencing batch reactor; DLS-ASP; Delawas activated sludge process; MNT, Malaviya national institute of technology; JWC, Jawahar circle.

Aerosol ingestion in scenario 1, i.e., the number of organisms ingested per exposure (N) was calculated using the following equation:

$$N = (ec)(pc)(br)(t)(ag) \tag{3}$$

where ec is the pathogen concentration in treated wastewater before or after dilution, pc is the partitioning coefficient ($=1.07 \times 10^{-5}$ L/m^3), br ($=0.61$ m^3/h) is the breathing rate, t ($=8$ h) is the time of exposure and ag is the aerosol ingestion rate ($=0.1$) (Brooks *et al.* 2005; Dungan 2014; Chattopadhyay *et al.* 2017).

2.3. Dose–response assessment

To calculate the probability of infection from *Ascaris*, beta-possion dose–response modelled (Mara & Sleigh 2010) as per the following equation.

$$P(N) = 1 - \left[1 + \left(\frac{N}{N_{50}}\right)\left(2\frac{1}{\alpha} - 1\right)\right]^{-\alpha} \tag{4}$$

In Equation (4), P (N) is the risk of infection due to ingestion of *Ascaris* eggs on one occasion; N_{50} is the *Ascaris* median infective dose; and α is an *Ascaris* 'infectivity constant'. The values of N_{50} and α are 859 and 0.104, respectively.

Similarly, an exponential model was used for estimating the risk of infection due to exposures to *Cryptosporidium* and *Giardia* (Gerba 2000; Chhipi-Shrestha *et al.* 2017)

$$P(N) = 1 - \exp\,(-r\,N) \tag{5}$$

where 'r' is the dose–response parameter whose values are taken as 0.004191 and 0.02 for *Cryptosporidium* and *Giardia,* respectively (Gerba 2000).

For estimating annual risk of infection values, the following equation was used (Rose *et al.* 1990; Gerba 2000; Haas *et al.* 2014):

$$P_a = 1 - (1 - P)^n \tag{6}$$

where n is the number of days (260) per year for workers.

2.4. Risk management

After the estimation of risk from treated wastewater for various scenarios, it becomes important to understand the concentration of pathogens in treated wastewater above which the risk becomes higher than the widely used threshold of 1:10,000. As the value for acceptable risk is not available for non-potable applications, the benchmark level of risk for drinking (i.e., 1:10,000) has been used for this study (U.S. Environmental Protection Agency 1989; Regli *et al.* 1991; Gerba 2000). As the estimated annual risk values would be increasing from scenarios 1 to 3 on the account of an increase in ingestion volume, the maximum allowable concentration ($C_{\max}$) values of different pathogens may be calculated for scenario 3 by equating annual risk of infection values to 1:10,000 as per the following equation.

$$C_{\max} = \frac{\ln(1 - P)}{(-r)} \tag{7}$$

In Equation (7), the risk of infection (P) value was taken from the Equation (6). For calculating $C_{\max}$ for helminths, the following equation is used:

$$C_{\max} = \frac{(1 - P)^{-1/\alpha} - 1}{2^{1/\alpha} - 1} \times N_{50} \tag{8}$$

where the risk of infection (P) value was taken from Equation (4).

3. RESULTS

The final effluent concentrations of *Cryptosporidium* and *Giardia* ranged between 1.33×10^{-2} and 2.65×10^{-2} cysts/mL. For the helminths, there was no change in final effluent concentration for different WWTPs, yielding 3.00×10^{-1} eggs/mL of wastewater (Table 5).

The estimated annual risk of infection from selected pathogens in treated wastewater (Table 6) was found to exceed the target value of 1:10,000 (being represented as 10^{-4}) in most of the scenarios. In terms of the technology employed by WWTPs, the annual risk of infection values from pathogens in treated wastewater have been found to be following this order: SBR-based WWTPs < ASP-based WWTP < MBBR-based WWTPs. The minimum values of annual risk of infection for all three pathogens have been found in case of WWTPs based on SBR technology, i.e., SBR-1, 2, and 3. On the other hand, the maximum value of the annual risk of infection is always found for JWC, a WWTP based on MBBR technology. The risks posed by the effluent from the WWTP based on ASP fall in the middle for all three scenarios.

For the scenarios, the values for estimated annual risk of infection were increased from scenario 1 to scenario 3. The estimated annual risk of infection value for scenario 3 (i.e., food crop consumption involving irrigation by treated wastewater) was found to be the highest. For all the organisms, treatment technologies, and dilution cases, the annual risk of infection value was found to be higher than the target value for scenarios 2 and 3. However, for scenario 1, the annual risk of infection was not found to be always higher than the target value. For *Cryptosporidium*, the annual risk of infection was found to be below the benchmark while irrigating with treated wastewater after dilution resulted in the value of the annual risk of infection ranging between 8.50×10^{-7} and 3.26×10^{-5} (aerosol/routine ingestion). Similar trends were observed for *Giardia* and *Ascaris* in the case of scenario 1 with dilution.

For pathogens in treated wastewater before dilution, the scenario involving ingestion of aerosols poses minimum risk. The minimum values of risk for *Cryptosporidium*, *Giardia*, and *Ascaris* are obtained in this scenario for WWTPs based on SBR technology. However, within scenario 1, the risk from routine ingestion is always higher than the threshold value for the case of treatment plants considered except for the case of *Cryptosporidium*. In the case of scenario 2 again, all the estimated risk

Table 6 | Estimated annual risk of infection for general population for all the WWTPs

Pathogen	Scenarios		Sample type	SBR 1,2,3 $f_{i2} = 0.94$	DLS-ASP $f_{i2} = 0.90$	MNT $f_{i2} = 0.89$	JWC $f_{i2} = 0.88$
Cryptosporidium	Scenario 1	Aerosol ingestion	Treated wastewater	3.41×10^{-04}	5.69×10^{-04}	6.24×10^{-04}	6.83×10^{-04}
			Water after dilution	*8.50×10^{-07}*	*1.42×10^{-06}*	*1.56×10^{-06}*	*1.70×10^{-06}*
		Routine ingestion	Treated wastewater	6.52×10^{-03}	1.08×10^{-02}	1.19×10^{-02}	1.30×10^{-02}
			Water after dilution	*1.63×10^{-05}*	*2.71×10^{-05}*	*2.99×10^{-05}*	*3.26×10^{-05}*
	Scenario 2	Garden work and lounging	Treated wastewater	6.33×10^{-02}	1.03×10^{-01}	1.13×10^{-01}	1.23×10^{-01}
			Water after dilution	1.63×10^{-04}	2.71×10^{-04}	2.99×10^{-04}	3.26×10^{-04}
	Scenario 3	Food crop consumption	Treated wastewater	2.79×10^{-01}	4.20×10^{-01}	4.51×10^{-01}	4.80×10^{-01}
			Water after dilution	8.14×10^{-04}	1.36×10^{-03}	1.49×10^{-03}	1.63×10^{-03}
Giardia	Scenario 1	Aerosol ingestion	Treated wastewater	2.19×10^{-02}	3.63×10^{-02}	3.98×10^{-02}	4.33×10^{-02}
			Water after dilution	*5.52×10^{-05}*	*9.20×10^{-05}*	1.01×10^{-04}	1.10×10^{-04}
		Routine ingestion	Treated wastewater	3.46×10^{-01}	5.07×10^{-01}	5.41×10^{-01}	5.72×10^{-01}
			Water after dilution	1.06×10^{-03}	1.76×10^{-03}	1.94×10^{-03}	2.11×10^{-03}
	Scenario 2	Garden work and lounging	Treated wastewater	9.86×10^{-01}	9.99×10^{-01}	1	1
			Water after dilution	1.05×10^{-02}	1.75×10^{-02}	1.92×10^{-02}	2.09×10^{-02}
	Scenario 3	Food crop consumption	Treated wastewater	1	1	1	1
			Water after dilution	5.15×10^{-02}	8.43×10^{-02}	9.23×10^{-02}	1.00×10^{-01}
Ascaris	Scenario 1	Aerosol ingestion	Treated wastewater	3.79×10^{-02}	3.79×10^{-02}	3.79×10^{-02}	3.79×10^{-02}
			Water after dilution	*9.62×10^{-05}*	*9.62×10^{-05}*	*9.62×10^{-05}*	*9.62×10^{-05}*
		Routine ingestion	Treated wastewater	5.18×10^{-01}	5.18×10^{-01}	5.18×10^{-01}	5.18×10^{-01}
			Water after dilution	1.84×10^{-03}	1.84×10^{-03}	1.84×10^{-03}	1.84×10^{-03}
	Scenario 2	Garden work and lounging	Treated wastewater	9.99×10^{-01}	9.99×10^{-01}	9.99×10^{-01}	9.99×10^{-01}
			Water after dilution	1.83×10^{-02}	1.83×10^{-02}	1.83×10^{-02}	1.83×10^{-02}
	Scenario 3	Food crop consumption	Treated wastewater	1	1	1	1
			Water after dilution	8.79×10^{-02}	8.79×10^{-02}	8.79×10^{-02}	8.79×10^{-02}

Notes: Number in italics represents risk values less than the target value of 1:10,000 (equivalent to 10^{-4}).

values are found to be above the threshold value. The maximum values of 1.00×10^{0} are observed in this scenario for *Giardia* and *Ascaris* with treated wastewater before dilution. Again, the estimated risk in scenario 3 for *Giardia* and *Ascaris* (for treated wastewater before dilution) has the same maximum value of 1.00×10^{0} for all the six WWTPs. Furthermore, all the other estimated values for both the cases (i.e., treated wastewater before and after dilution) in scenario 3 are found to be higher than the threshold value (Kandiah 1991; Blumenthal *et al.* 2000).

For *Cryptosporidium*, the values of the estimated annual risk of infection vary from 8.50×10^{-7} to 4.80×10^{-1}. For *Giardia*, these values vary from 5.52×10^{-5} to 1.00×10^{0}. When comparing the scenario-wise annual risk of infection values from *Cryptosporidium* and *Giardia*, the risk from *Giardia* was always found to be higher. As far as *Ascaris* is concerned, the estimated risk is in the range of 9.62×10^{-5} and 1.00×10^{0}. As there was no difference in the removal of *Ascaris* for all the treatment plants, the estimated risk values only differ among the scenarios. In the case of *Ascaris*, the only estimated values that are below the 1:10,000 threshold belong to Scenario 1 (aerosol ingestion and treated wastewater after dilution). For all the other scenarios and cases, the risk posed is more than 10^{-4}, the benchmark used for drinking water.

3.1. Maximum allowable concentration of pathogen per technology

This section discusses the value of maximum allowable concentration of a pathogen in the treated wastewater ($C_{\max}$) for an estimated annual risk of infection equal to 1:10,000. An annual risk of infection of 1:10,000 means that in a population of 10,000 people, one person is expected to contract the infection in question each year (Regli *et al.* 1991) (Table 7). These concentrations ($C_{\max}$) were calculated to be in the range of 8.15×10^{-7} to 1.84×10^{-5} which is lower than the calculated concentrations of various pathogens in treated wastewater using the Sketcher tool. The value of $C_{\max}$ was found to be lowest for *Ascaris* while the maximum value was obtained for *Cryptosporidium*. This emphasises the need for enhanced removal of pathogens in WWTPs. The additional treatment measures should achieve higher removal of helminths in particular. The additional removals required for all the pathogens with different types of technologies are shown in Table 7. For protozoa, i.e., *Cryptosporidium* and *Giardia*, maximum additional removal is required in the case of JWC, an MBBR-based WWTP, whereas the minimum additional removal is required in the case of SBR based WWTPs. For *Ascaris*, additional removal required is 5-log removal and this value is the same for all the WWTPs.

3.2. Discussion

This study performs theoretical risk characterization for exposure of pathogens during water reuse associated with WWTPs. In India, there is one other study (Hajare *et al.* 2021b) which has estimated the risk of infection with the reuse of treated wastewater of 11 effluent treatment plants in Delhi, considering four representative pathogens (pathogenic *Escherichia coli* spp., *Salmonella* spp., *Cryptosporidium* spp., and *Giardia* spp.) In Delhi, the estimated annual risk of infection ranged between 2.00×10^{-4} and 5.74×10^{-4} and between 4.63×10^{-7} and 1.22×10^{-6} for *Cryptosporidium* and *Giardia*, respectively. In the present study, these values are 8.50×10^{-7} to 4.80×10^{-1} and 5.52×10^{-5} to 1.00×10^{0} for *Cryptosporidium* and *Giardia*, respectively. The study in Delhi did not estimate risk from *Ascaris*. In terms of parameters, the present study assumes exposure of 270 days per year while the study in Delhi considered exposure for fewer days per year.

Table 7 | Maximum permissible concentration of pathogens in treated wastewater

	$C_{\max}$ (maximum permissible concentration of pathogens in treated wastewater)			**Additional removal required (LRV) at WWTP to meet $C_{\max}$ limit**		
WWTP	***Cryptosporidium* (oocysts/mL)**	***Giardia* (cysts/mL)**	***Ascaris* (eggs/mL)**	***Cryptosporidium***	***Giardia***	***Ascaris***
SBR-1	6.54×10^{-05}	1.37×10^{-05}	2.89×10^{-06}	2.96	4.77	5.02
SBR-2	6.54×10^{-05}	1.37×10^{-05}	2.89×10^{-06}	2.96	4.77	5.02
DLS-ASP	6.54×10^{-05}	1.37×10^{-05}	2.89×10^{-06}	3.18	5.00	5.02
MNT	6.54×10^{-05}	1.37×10^{-05}	2.89×10^{-06}	3.23	5.04	5.02
JWC	6.54×10^{-05}	1.37×10^{-05}	2.89×10^{-06}	3.26	5.08	5.02
SBR-3	6.54×10^{-05}	1.37×10^{-05}	2.89×10^{-06}	2.96	4.77	5.02

SBR, sequencing batch reactor; DLS-ASP, Delawas activated sludge process; MNT, Malaviya national institute of technology; JWC, Jawahar circle WWTP.

The present study also estimates the maximum permissible concentration ($C_{\max}$). The $C_{\max}$ values corresponded to 6.54×10^{-5} (oocysts/mL), 1.37×10^{-5} (cysts/mL), and 2.89×10^{-6} (eggs/mL) for *Cryptosporidium, Giardia*, and *Ascaris*, respectively. The $C_{\max}$ value for treated effluent depends on the framework employed for determining the estimated annual risk of infection and, does not depend on the treatment technology. However, the additional removal of pathogens required to match the pathogen concentrations in treated wastewater with that of $C_{\max}$ depends on the technology employed in a treatment plant.

As pathogen removal is a function of the technology being used in a WWTP, the estimated annual risk of infection to the workers and general public varies with the technology being employed in a WWTP. This fact also introduces uncertainty in our results as the pathogen removal was modelled by a statistics-based tool, known as the Sketcher tool. Presently, the Sketcher tool is able to estimate the pathogen removal only for a limited number of secondary treatment technologies, i.e., ASP, trickling filter and waste stabilization pond. LRVs calculated by the Sketcher tool for *Cryptosporidium* and *Giardia* are the same as the tool that gives LRV for pathogen groups like viruses, protozoa, bacteria, etc.

The study may assist in selecting the process which should be installed for treating wastewater in Jaipur, as the results show that the risk of infection is lowest for SBR WWTP. Another study (Hajare *et al.* 2021a) estimated the probability of infection for effluent treatment plants (ETPs) but did not focus on the technology of the treatment plants.

The main limitation of the study lies in modelling of pathogen removal in WWTPs. The pathogen removal in MBBR-based WWTPs was calculated by modelling these units as ASP and trickling filter technologies. The results obtained for the trickling filter were employed finally in the risk assessment model in order to keep the values on the conservative side. This study also did not consider the removal of pathogens resulting from specific processes such as sedimentation or disinfection in the treatment plants being studied. Further work is necessary in this area, especially for accurate estimation of the removal of different types of pathogens in WWTPs. This study also does not estimate the cumulative risk of infection and the Disability Adjusted Life Year approach (DALYS), which may be dealt with in future studies. Also, the study has compared the estimated theoretical risk with the threshold of 1:10,000, the allowable risk value for drinking water. Hence these estimated values can again be revisited when an acceptable risk value for non-potable applications is available in the literature.

Overall, risk estimation for the case of protozoa and helminths has been done for the first time in India, as per the authors' knowledge. It also provided an approach for selecting wastewater treatment technologies capable of producing water safe for reuse applications which had not been addressed before. These aspects are important for effective design decision-making on predicting pathogen concentration at exposure points during reuse practices.

4. CONCLUSIONS

This study estimated the theoretical risk of the reuse of treated wastewater from six WWTPs in Jaipur, India. The important conclusions of the study are as follows:

1. In most of the scenarios being considered in the study, the estimated annual risk of infection from selected pathogens in treated wastewater was found to exceed the target value of 1:10,000. When comparing the technology employed by WWTPs, the estimated risks associated with the pathogens followed this order: SBR based WWTPs < ASP-based WWTP < MBBR-based WWTPs. The minimum values of estimated annual risk for all three pathogens have been found in the case of pathogens in treated wastewater from WWTPs based on SBR technology, i.e., SBR-1, 2 and 3. On the other hand, the maximum value of the estimated annual risk is always posed by pathogens in treated wastewater from JWC, a WWTP based on MBBR technology. The estimated risks posed by the effluent from the ASP-based WWTP fall in the middle for all three scenarios.
2. For the scenarios evaluated, the estimated annual risk of infection increased from scenario 1 to scenario 3. For scenarios 2 and 3, the estimated annual risk of infection was always higher than the benchmark (i.e., 10^{-4}) irrespective of pathogen types, treatment technologies and dilution cases. However, for scenario 1, the estimated annual risk of infection was not always higher. For C*ryptosporidium*, the infection risk was found to be below the benchmark while irrigating with treated wastewater after dilution. Similar trends were observed for *Giardia* and *Ascaris* in the case of scenario 1 with dilution.
3. For *Cryptosporidium*, the values of the estimated annual risk of infection vary from 8.50×10^{-7} to 4.80×10^{-1}. For *Giardia*, the values of the estimated annual risk vary from 5.52×10^{-5} to 1.00×10^{0}, higher than that for the case of *Cryptosporidium*. As far as *Ascaris* is concerned, the estimated risk is in the range of 9.62×10^{-5} and 1.00×10^{0}.
4. At the selected benchmark level of annual risk of infection (i.e., 10^{-4}), the acceptable concentrations of pathogens in the treated wastewater ranged from 2.89×10^{-6} to 6.54×10^{-5}. The lowest value was observed for *Ascaris*, while the highest

value was found for *Cryptosporidium*. These findings highlight the necessity for improved removal of helminths from wastewater prior to its reuse.

A number of studies have performed risk estimation from water reuse, but results have not been clearly compared by the biological treatment method and the complete wastewater treatment scheme. This study presents this information which can be used by wastewater treatment plant designers in selecting appropriate treatment schemes for achieving the desired water quality. The stakeholders in developing countries and locations where treatment plants are being upgraded may use this information for making corrective measures if needed.

FUNDING

This research was supported through PARTNERSHIP2020 program, a collaborative agreement between University of Nebraska at Omaha (UNO) and US Department of State (DOS) with Centre for Strategic and International Studies (CSIS) playing a key advisory role. This project was funded through an agreement between the University of Nebraska, Omaha and Drexel University, Philadelphia under Federal Award# SIN65018CA0034.

AUTHOR CONTRIBUTIONS

Amit Kumar, Arun Kumar, Walter Batencourt, Rajveer Singh and Patrick L. Gurian contributed to the study conception and design. Material preparation and data collection were performed by Ayushi Chaudhary, Shubham Rana and Amit Kumar. Ayushi Chaudhary, Arun Kumar and Amit Kumar performed the data analysis. The first draft of the manuscript was written by Ayushi Chaudhary, Arun Kumar and Amit Kumar and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL

The research did not involve human or animal subjects.

CONSENT TO PARTICIPATE

The research did not involve human subjects.

CONSENT TO PUBLISH

The manuscript does not contain any individual person's data in any form.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Alegbeleye, O. O. & Sant'Ana, A. S. 2021 Risks associated with the consumption of irrigation water contaminated produce: on the role of quantitative microbial risk assessment. *Current Opinion in Food Science* **41**, 88–98.

AL-Gheethi, A. A., Ismail, N., Lalung, J., Talib, A. & Kadir, M. O. A. 2013 Reduction of faecal indicators and elimination of pathogens from sewage treated effluents by heat treatment. *Caspian Journal of Applied Sciences Research* **2** (2), 39–55. Available from: http://www.cjasr.com.

Amha, Y. M., Kumaraswamy, R. & Ahmad, F. 2015 A probabilistic QMRA of *Salmonella* in direct agricultural reuse of treated municipal wastewater. *Water Science and Technology* **71** (8), 1203–1211.

Betancourt, W. Q. & Rose, J. B. 2004 Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. *Veterinary parasitology* **126**, 219–234.

Bhatt, P., Mathur, N., Singh, A., Pareek, H. & Bhatnagar, P. 2020 Evaluation of factors influencing the environmental spread of pathogens by wastewater treatment plants. *Water, Air, and Soil Pollution* **231** (8), 1–14.

Blatchley III, E. R., Gong, W. L., Alleman, J. E., Rose, J. B., Huffman, D. E., Otaki, M. & Lisle, J. T. 2007 Effects of wastewater disinfection on waterborne bacteria and viruses. *Water Environment Research* **79** (1), 81–92.

Blumenthal, U. J., Mara, D. D., Peasey, A., Ruiz-Palacios, G. & Stott, R. 2000 Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. *Bulletin of the World Health Organization* **78** (9), 1104–1116.

Brooks, J. P., Tanner, B. D., Gerba, C. P., Haas, C. N. & Pepper, I. L. 2005 Estimation of bioaerosol risk of infection to residents adjacent to a land applied biosolids site using an empirically derived transport model. *Journal of Applied Microbiology* **98** (2), 397–405.

Busgang, A., Friedler, E., Gilboa, Y. & Gross, A. 2018 Quantitative microbial risk analysis for various bacterial exposure scenarios involving greywater reuse for irrigation. *Water (Switzerland)* **10** (4), 1–15.

Campbell, A. T., Robertson, L. J., Snowball, M. R. & Smith, H. V. 1995 Pergamon inactivation of oocysts of cryptosporidium. *Water Research* **29** (95), 2583–2586.

Chattopadhyay, S., Perkins, S. D., Shaw, M. & Nichols, T. L. 2017 Evaluation of exposure to *Brevundimonas diminuta* and *Pseudomonas aeruginosa* during showering. *Journal of Aerosol Science* **114**, 77–93. http://dx.doi.org/10.1016/j.jaerosci.2017.08.008.

Chaudhry, R. M., Hamilton, K. A., Haas, C. N. & Nelson, K. L. 2017 Drivers of microbial risk for direct potable reuse and de facto reuse treatment schemes: the impacts of source water quality and blending. *International Journal of Environmental Research and Public Health* **14** (6), 1–20.

Cheng, H. W. A., Lucy, F. E., Graczyk, T. K., Broaders, M. A., Tamang, L. & Connolly, M. 2009 Fate of *Cryptosporidium parvum* and *Cryptosporidium hominis* oocysts and *Giardia duodenalis* cysts during secondary wastewater treatments. *Parasitology Research* **105** (3), 689–696.

Chhipi-Shrestha, G., Hewage, K. & Sadiq, R. 2017 Microbial quality of reclaimed water for urban reuses: probabilistic risk-based investigation and recommendations. *Science of the Total Environment* **576**, 738–751. http://dx.doi.org/10.1016/j.scitotenv.2016.10.105.

Chowdhari, S., Rana, S., Rana, S., Morrison, C., Abney, S., Singh, R., Gurian, P., Kumar, A., Kumar, A., Betancourt, W. & Perumal, V. 2022 Quantitative assessment of microbial pathogens and indicators of wastewater treatment performance for safe and sustainable water reuse in India. microbiology spectrum. *Microbiology Spectrum* **10** (6), e01720–e01722

Craun, G. F., Brunkard, J. M., Yoder, J. S., Roberts, V. A., Carpenter, J., Wade, T., Calderon, R.L., Roberts, J. M., Beach, M. J. & Roy, S. L. 2010 Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. *Clinical Microbiology Reviews* **23** (3), 507–528.

Dungan, R. S. 2014 Estimation of infectious risks in residential populations exposed to airborne pathogens during center pivot irrigation of dairy wastewaters. *Environmental Science and Technology* **48** (9), 5033–5042.

Emilse, P. V., Matías, V., Cecilia, M. L., Oscar, G. M., Gisela, M., Guadalupe, D., Elizabeth, R. V., Victorio, P. J., Rodney, C., Viviana, N. S., & Angélica, B. P. 2021 Enteric virus presence in green vegetables and associated irrigation waters in a rural area from Argentina: a quantitative microbial risk assessment. *Lwt* **144**, 111201.

Esther, F., Singh, G., Reddy, P., Bux, F. & Stenström, T. A. 2019 Efficiency of chlorine and UV in the inactivation of *Cryptosporidium* and *Giardia* in wastewater. *PLoS One* **14** (5), e0216040.

Ezzat, S. M. 2020 Applying quantitative microbial risk assessment model in developing appropriate standards for irrigation water. *Integrated Environmental Assessment and Management* **16**, 353–361.

Fu, C. Y., Xie, X., Huang, J. J., Zhang, T., Wu, Q. Y., Chen, J. N. & Hu, H. Y. 2010 Monitoring and evaluation of removal of pathogens at municipal wastewater treatment plants. *Water Science and Technology* **61** (6), 1589–1599.

Gerba, C. P. 2000 Risk assessment. In: *Environmental Microbiology* (Maier, R.M., Pepper, I.L. & Gerba, C.P., eds.). Academic Press, Cambridge, MA, pp. 557–571.

Gerba, C. P., Betancourt, W. Q. & Kitajima, M. 2017 How much reduction of virus is needed for recycled water: a continuous changing need for assessment? *Water Research* **108**, 25–31. http://dx.doi.org/10.1016/j.watres.2016.11.020.

Gerba, C. P., Betancourt, W. Q., Kitajima, M. & Rock, C. M. 2018 Reducing uncertainty in estimating virus reduction by advanced water treatment processes. *Water Research* **133**, 282–288. https://doi.org/10.1016/j.watres.2018.01.044.

Gupta, B., Kumar, A., Sarathi, P. & Lal, S. 2022 Journal of environmental chemical engineering recent advances in application of moving bed biofilm reactor for wastewater treatment : insights into critical operational parameters, modifications, field-scale performance, and sustainable aspects. *Journal of Environmental Chemical Engineering* **10** (3), 107742.

Haas, C. N. 1983 Effect of effluent disinfection on risks of viral disease transmission via recreational water exposure. *Journal of the Water Pollution Control Federation* **55** (8), 1111–1116.

Haas, C. N., Rose, J. B. & Gerba, C. P. 2014 *Quantitative Microbial Risk Assessment*, John Wiley & Sons, New York.

Hajare, R., Labhasetwar, P. & Nagarnaik, P. 2021a A critical review of applications of QMRA for healthy and safe reclaimed water management. *Environmental Modeling and Assessment* **26** (3), 339–354.

Hajare, R., Labhasetwar, P. & Nagarnaik, P. 2021b Evaluation of pathogen risks using QMRA to explore wastewater reuse options: a case study from New Delhi in India. *Water Science and Technology* **83** (3), 543–555.

Hewitt, J., Leonard, M., Greening, G. E. & Lewis, G. D. 2011 Influence of wastewater treatment process and the population size on human virus profiles in wastewater. *Water Research* **45** (18), 6267–6276. http://dx.doi.org/10.1016/j.watres.2011.09.029.

Ito, T., Kato, T., Hasegawa, M., Katayama, H., Ishii, S., Okabe, S. & Sano, D. 2016 Evaluation of virus reduction efficiency in wastewater treatment unit processes as a credit value in the multiplebarrier system for wastewater reclamation and reuse. *Journal of Water and Health* **14** (6), 879–889.

Ito, T., Kitajima, M., Kato, T., Ishii, S., Segawa, T., Okabe, S. & Sano, D. 2017 Target virus log10 reduction values determined for two reclaimed wastewater irrigation scenarios in Japan based on tolerable annual disease burden. *Water Research* **125**, 438–448. https://doi.org/10.1016/j.watres.2017.08.057.

Jain, S., Melo, T. G. C., Dolabella, S. S. & Liu, J. 2019 Current and emerging tools for detecting protozoan cysts and oocysts in water. *TrAC – Trends in Analytical Chemistry* **121**, 115695. https://doi.org/10.1016/j.trac.2019.115695.

Jolis, D., Pitt, P. & Hirano, R. 1999 Risk assessment for *Cryptosporidium parvum* in reclaimed water. *Water Research* **33** (13), 3051–3055.

Kandiah, A. 1991 Guidelines for the safe use of wastewater and excreta in agriculture and aquaculture. *Food Control* **2** (4), 241.

Khalid, S., Shahid, M., Natasha, Bibi, I., Sarwar, T., Shah, A. H. & & Niazi, N. K. 2018 A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-Income countries. *International Journal of Environmental Research and Public Health* **15** (5), 1–36.

Kobayashi, N., Oshiki, M., Ito, T. & Segawa, T. 2017 Removal of human pathogenic viruses in a down-flow hanging sponge (DHS) reactor treating municipal wastewater and health risks associated with utilization of the Effluent for agricultural irrigation. *Water Research* **110**, 389–398.

Mara, D. & Sleigh, A. 2010 Estimation of *Ascaris* infection risks in children under 15 from the consumption of wastewater-irrigated carrots. *Journal of Water and Health* **8** (1), 35–38.

Marques, F. R., Magri, M. E., Amoah, I. D., Stenström, T. A. & Paulo, P. 2021 Development of a semi-quantitative approach for the assessment of microbial health risk associated with wastewater reuse: a case study at the household level. *Environmental Challenges* **4**, 100182. https://doi.org/10.1016/j.envc.2021.100182.

Moazeni, M., Nikaeen, M., Hadi, M., Moghim, S., Mouhebat, L., Hatamzadeh, M. & Hassanzadeh, A. 2017 Estimation of health risks caused by exposure to enteroviruses from agricultural application of wastewater effluents. *Water Research* **125**, 104–113. https://doi.org/10.1016/j.watres.2017.08.028.

Musaazi, I. G. 2020 *Predicting Pathogen Fate and Transport Throughout The Sanitation Service Chain: Knowledge to Practice With the Global Water Pathogens Project*. San Diego State University.

Nappier, S. P., Soller, J. A. & Eftim, S. E. 2018 Potable water reuse: what are the microbiological risks? *Current Environmental Health Reports* **5** (2), 283–292.

Natural Resources Management Ministerial Council. 2006 *Australia Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1)*. National Water Quality Management Strategy, Canberra. p. 415.

Navarro, I. & Jiménez, B. 2011 Evaluation of the WHO helminth eggs criteria using a QMRA approach for the safe reuse of wastewater and sludge in developing countries. *Water Science and Technology* **63** (7), 1499–1505.

Ofori, S., Puškáčová, A., Růžičková, I. & Wanner, J. 2021 Treated wastewater reuse for irrigation: pros and cons. *Science of the Total Environment* **760**, 144026.

Ottoson, J., Hansen, A., Westrell, T., Johansen, K., Norder, H. & Stenström, T. A. 2006 Removal of noro- and enteroviruses, giardia cysts, cryptosporidium oocysts, and fecal indicators at four secondary wastewater treatment plants in Sweden. *Water Environment Research* **78** (8), 828–834.

Quintero-Betancourt, W., Gennaccaro, A. L., Scott, T. M. & Rose, J. B. 2003 Assessment of methods for detection of infectious *Cryptosporidium* oocysts and *Giardia* cysts in reclaimed effluents. *Applied and Environmental Microbiology* **69** (9), 5380–5388.

Regli, S., Rose, J. B., Haas, C. N. & Gerba, C. P. 1991 Modeling the risk from giardia and viruses in drinking water. *Journal/American Water Works Association* **83** (11), 76–84.

Rock, C. M., Brassill, N., Dery, J. L., Carr, D., McLain, J. E., Bright, K. R. & Gerba, C. P. 2019 Review of water quality criteria for water reuse and risk-based implications for irrigated produce under the FDA Food Safety Modernization Act, produce safety rule. *Environmental Research* **172**, 616–629. https://doi.org/10.1016/j.envres.2018.12.050.

Ronco, P., Zennaro, F., Torresan, S. I. L. V. I. A., Critto, A., Santini, M., Trabucco, A. & Marcomini, A. 2017 A risk assessment framework for irrigated agriculture under climate change. *Advances in Water Resources* **110**, 562–578. https://doi.org/10.1016/j.advwatres.2017.08.003.

Rose, J. B., Haas, C. N. & Regli, S. 1990 Risk assessment and control of waterborne giardiasis. *American Journal of Public Health* **81** (6), 709–713.

Ryu, H., Alum, A., Mena, K. D. & Abbaszadegan, M. 2007 Assessment of the risk of infection by *Cryptosporidium* and *Giardia* in non-potable reclaimed water. *Water Science and Technology* **55** (1–2), 283–290.

Saidulu, D., Majumder, A. & Gupta, A. K. 2021 A systematic review of moving bed biofilm reactor, membrane bioreactor, and moving bed membrane bioreactor for wastewater treatment: comparison of research trends, removal mechanisms, and performance. *Journal of Environmental Chemical Engineering* **9** (5), 106112.

Sano, D., Amarasiri, M., Hata, A., Watanabe, T. & Katayama, H. 2016 Risk management of viral infectious diseases in wastewater reclamation and reuse: review. *Environment International* **91**, 220–229. http://dx.doi.org/10.1016/j.envint.2016.03.001.

Schmitz, B. W., Kitajima, M., Campillo, M. E., Gerba, C. P. & Pepper, I. L. 2016 Virus reduction during advanced bardenpho and conventional wastewater treatment processes. *Environmental Science and Technology* **50** (17), 9524–9532.

Seidu, R., Heistad, A., Amoah, P., Drechsel, P., Jenssen, P. D. & Stenström, T. A. 2008 Quantification of the health risk associated with wastewater reuse in Accra, Ghana: a contribution toward local guidelines. *Journal of Water and Health* **6** (4), 461–471.

Sidhu, J. P. S., Sena, K., Hodgers, L., Palmer, A. & Toze, S. 2017 Optimization of sampling strategy to determine pathogen removal efficacy of activated sludge treatment plant. *Environmental Science and Pollution Research* **24** (23), 19001–19010.

Soller, J. A., Eftim, S. E. & Nappier, S. P. 2018 Direct potable reuse microbial risk assessment methodology: sensitivity analysis and application to state log credit allocations. *Water Research* **128**, 286–292. https://doi.org/10.1016/j.watres.2017.10.034.

Tan, K. N. 1993 Efficiency of sequencing batch reactor (SBR) in the removal of selected microorganisms from domestic sewage. *Water Research* **27** (10), 1591–1600.

Tumwebaze, I. K., Rose, J. B., Hofstra, N., Verbyla, M. E., Okaali, D. A., Katsivelis, P. & Murphy, H. M. 2021 Bridging science and practice-importance of stakeholders in the development of decision support: lessons learned. *Sustainability (Switzerland)* **13** (10), 5744.

Tyagi, N., Gurian, P. L. & Kumar, A. 2022 Using QMRA to understand possible exposure risks of SARS-CoV-2 from the water environment. *Environmental Science and Pollution Research* **29** (5), 7240–7253.

Ueki, Y., Sano, D., Watanabe, T., Akiyama, K. & Omura, T. 2005 Norovirus pathway in water environment estimated by genetic analysis of strains from patients of gastroenteritis, sewage, treated wastewater, river water and oysters. *Water Research* **39** (18), 4271–4280.

U.S. Environmental Protection Agency. 1989 *National Primary Drinking Water Regulations: Filtration, Disinfection; Turbidity, Giardia Lamblia, Viruses, Legionella and Heterotrophic Bacteria; Final Rule*.

Westrell, T., Schönning, C., Stenström, T. A. & Ashbolt, N. J. 2004 QMRA (Quantitative microbial risk assessment) and HACCP (Hazard analysis and critical points) for management of pathogens in wastewater and sewage sludge treatment and reuse. *Water Science and Technology* **50** (2), 23–30.

First received 31 October 2022; accepted in revised form 26 June 2023. Available online 5 July 2023