



## Review

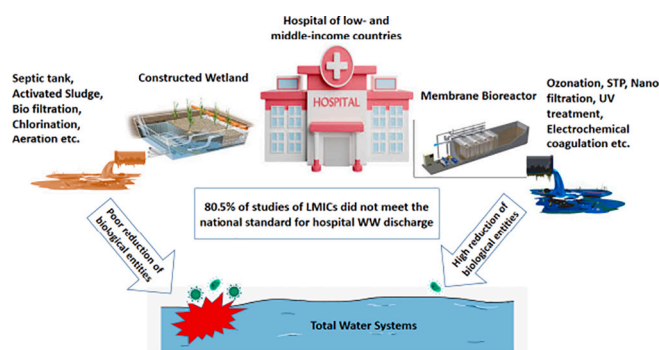
## Hospital wastewater (HWW) treatment in low- and middle-income countries: A systematic review of microbial treatment efficacy

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## HIGHLIGHTS

- First systematic review on hospital wastewater processes and their microbial removal efficacy in LMICs
- Between 2000 and 2022, only 36 articles addressed this area, of which only 17 covered full-scale application
- Several conventional treatment processes are ineffective to treat complex HWW to remove pathogens
- MBRs combined with nano-filtration and/or ozonation provided enhanced removal efficiency of microbes from hospital HWW
- Effluent in 29 ( $N = 36$ ) did not meet the national standard for hospital WW discharge

## GRAPHICAL ABSTRACT



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## ABSTRACT

**Background:** Proper treatment of hospital wastewater (HWW) is crucial to minimize the long-term effects on human health and aquatic ecosystems. However, the majority of HWW generated in low and middle-income countries (LMICs), is discharged without adequate treatment. This systematic review aims to fill the knowledge gap in LMICs by examining the efficacy of HWW treatment and the types of technologies used.

**Methods:** Studies included in the review offered valuable insights into the current state of HWW management in LMICs. Data were extracted on wastewater treatment technologies in hospitals or healthcare settings in LMICs. Data on sampling techniques, effectiveness, microorganisms and risk of bias of included studies were recorded.

**Results:** A total of 36 articles met the eligibility criteria: mentioned about 1) hospitals 2) wastewater treatment 3) LMICs and 4) treatment efficacy. Twenty-two studies were conducted in Asia (22/36), 17 were conducted in countries with high Human Development Index. Constructed wetland, and activated sludge process were the most common technologies used in LMICs. A few studies utilized membrane bioreactors and ozone/UV treatment. Fourteen studies reported the concentration reduction to assess the microbial efficacy of the treatment process, 29/36 studies did not meet the national standards for effluent discharge. Reporting on sampling methods, wastewater treatment processes and efficacy of HWW treatment were at high risk of bias. Extreme heterogeneity in study methods and outcomes reporting precluded meta-analysis.

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**Conclusions:** The existing evidence indicates inadequate microbial treatment in low- and middle-income country hospitals, with this systematic review emphasizing the need for improvement in healthcare waste management. It underscores the importance of long-term studies using innovative treatment methods to better understand waste removal in LMIC hospitals and calls for further research to develop context-specific healthcare waste treatment approaches in these regions.

## 1. Background

Hospital wastewater (HWW) poses significant health and environmental risks due to the presence of infectious agents, pharmaceuticals, and hazardous chemicals (Al-Gheethi et al., 2018; Al Aukidy et al., 2018; Majumder et al., 2021). HWW can contain high levels of bacteria, viruses, and other pathogens, which can cause diseases if they enter the environment or are not properly treated before discharge (Kaur et al., 2020). These pathogens can have long-term effects on human health and aquatic ecosystems (e.g. Antimicrobial-Resistant *Escherichia coli*) (Guruge KSY et al., 2015; Lin and Yang, 2022; Parida et al., 2022).

The wastewater from hospitals comprises resistant bacteria and residues that could inhibit the growth of susceptible bacteria and this may lead to an increase in the prevalence of antibiotic-resistant bacteria in the water bodies where the wastewater is discharged (Kaur et al., 2020). The discharge of these antibiotic-resistant bacteria into environment can function either as carrier, separating transmissible gene or as the reservoirs for antibiotic-resistance genes (ARGs) that could pose a threat to public health (Asfaw et al., 2017). The lack of specific treatment technologies for HWW also increased the concentration of gastroenteric viruses in aquatic bodies (Ibrahim et al., 2018).

The United Nations Water Report 2021 reveals that nearly half (44 %) of the global wastewater generated is not treated properly to ensure its safety (UN-water, 2021). In low-income settings, HWW treatment is particularly challenging, resulting in only 8 % of the total volume of wastewater generated in low and middle-income countries (LMICs) being treated before discharge into the environment (UNESCO, 2017). According to the World Health Organization, HWW management has become an urgent global health priority (WHO/UNICEF, 2023), but the majority of the wastewater generated by hospitals worldwide is discharged without proper treatment (Majumder et al., 2021). This is particularly concerning for LMICs, where hospital wastes are often released into community wastewater systems, and regularly discharged into open water bodies without any treatment measures. (Amin et al., 2023; Pauwels and Verstraete, 2006; Rizzo et al., 2013; Timraz et al., 2017; Verlicchi et al., 2015). In the absence of functional sewerage systems, decentralized approaches to wastewater treatment are necessary, making HWW treatment crucial for many LMICs. Effective on-site treatment systems are needed to reduce the associated health hazards (Majumder et al., 2021; Paulus et al., 2019).

HWW is a complex mixture that poses challenges to its effective treatment. It is therefore important to document the quality of wastewater, treatment technologies used, and treatment efficacy systematically to design appropriate treatment processes. While several systematic reviews have recently been conducted on hospital wastewater management globally (Alam et al., 2021a, 2021b; Frascaroli et al., 2021; Hassoun-Kheir et al., 2020; Majumder et al., 2021; Pariente et al., 2022), there is a no such reviews in LMICs examining the efficacy of HWW treatment plants and types of technologies used. There is a knowledge gap about the healthcare liquid waste management in HCFs in LMICs. This knowledge gap is concerning because low-income countries often lack adequate resources and infrastructure for effective wastewater management, which increases the risk of infectious disease transmission and environmental contamination (Amin et al., 2020; Amin et al., 2019; Foster et al., 2021). Therefore, conducting a systematic review in LMICs could identify the gaps in existing HWW treatment approaches and highlight areas where further research is necessary to overcome the gaps in LMICs.

This research aimed to review the available evidence base on hospital wastewater management in LMICs from 2000 to 2022. The objectives of the study were to identify the number, focus and geographical spread of peer-reviewed papers on this topic, to examine key aspects of their research design (full-scale versus pilot (McMartin, 2017)), approaches to understanding microbial treatment efficacy, and coverage of microorganisms, ARBs and ARGs), and to identify the most common technologies and their performance, including against relevant national standards. Proper treatment of hospital wastewater (HWW) is crucial to minimize the long-term effects on human health and aquatic ecosystems. This study contributes to the emerging field focused on WASH in HCFs, as well as offering insight for researchers focused on wastewater treatment, but who may not yet have given focus to hospitals or HCFs.

## 2. Methods

This systematic review, without meta-analysis, was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations for reporting on systematic reviews (Appendix A: supplement material).

### 2.1. Literature search

A comprehensive search strategy was developed to access all articles published between 01 January 2000 and December 31, 2022. We searched PubMed, Web of Science, Scopus and Embase via Ovid for relevant publications (last search conducted: 01 March 2023). Additionally, we scanned the reference lists of selected studies and relevant review articles that might have been missed during the initial search. Although we did not restrict our inclusion by language or publication status, search terms were restricted to LMICs using a list of country names (Genter et al., 2021). The restriction to LMICs were based on the country in which the study was conducted. We collected and organized the search results using the bibliographic software Endnote™ 20. To ensure comprehensive coverage across various databases, we employed the following search terms:

Waste Water (keyword) OR Sewage (MeSH) OR wastewater (keyword) OR effluent (MeSH) OR sewage (MeSH) OR sewerage (MeSH) OR liquid waste (MeSH)

AND

Hospitals (keyword) OR hospital\* (MeSH) OR health care setting\* (MeSH) OR health care facilit\* (MeSH) OR healthcare facilit\* (MeSH) OR health-care facility (keyword) OR health-care setting\* (MeSH)

AND

Treatment Outcome (MeSH) OR treatment (MeSH) OR treatment outcome (MeSH) OR treatment performance (MeSH)

AND

Efficacy (keyword) OR effectiveness (MeSH) OR onsite (MeSH) OR (on-site) MeSH OR anaerobic baffled reactor (MeSH) OR septic tank\* (MeSH) OR septic-tank\* (MeSH)

AND

Low-and middle-income countries (LMICs) lists adopted from Genter et al., 2021 (Appendix B: Table S1)

### 2.2. Review methods

The screening process was conducted by two authors (NA, NTS), who independently reviewed and evaluated the literature based on the

criteria mentioned earlier. We used Endnote™ 20 software for record identification and removing duplicates. The initial screening of titles and abstracts was carried out using Microsoft Excel, resulting in the selection of 97 studies for full-text review (Fig. 1).

Two review authors (NA and NTS) independently extracted the data from the studies included and performed ROB assessment. Missing data were sought from the preliminary screening. In cases where there were differences in opinions during the preliminary screening, the study was considered for the complete text screening. Following the full-text screening, we excluded 60 articles according to the above-mentioned criteria and considered the remaining 36 studies for analysis. We performed a quality evaluation of the methods, sampling approach and number of samples collected. However, studies were not excluded based on the quality assessment.

### 2.3. Quality assessment

The objective of this systematic review was to understand efficacy of HWW treatment systems in eliminating microorganisms like viruses, bacteria, fungi, and parasites in low- and middle-income nations. We also investigated the kinds of WWTP implemented in hospitals in LMICs. To identify appropriate keywords and MeSH (Medical Subject Headings) terms, one investigator (NA) prepared an initial draft that included research questions, inclusion and exclusion criteria, and key search items. This draft was shared with co-authors iteratively through multiple drafts (JW and TF). Once the search strategies were finalized, they were presented to an internationally recognized sanitation specialist not involved in the review process. The one investigator (NA) incorporated all comments received from the reviewers and ran the key search items/ words in three data sources.

### 2.4. Inclusion and exclusion criteria

The criteria for inclusion of studies in this research were as follows: studies published between 2000 and 2022 that described any type of wastewater treatment technology or process in hospitals or any healthcare settings, studies conducted in LMICs as defined by the World Bank (2023), availability of original peer-reviewed journal articles, retrieval of articles from Medline Indexed journals, and publication in the English language. We included cross sectional studies, non-randomized intervention studies and longitudinal studies with pre-test/post-test designs. We excluded studies that were only conducted in laboratory settings, as well as systematic reviews published in peer-reviewed journals (Appendix B: Table S2).

### 2.5. PICO (population, intervention, comparison, outcome) statement

PICO (Population, Intervention, Comparison, Outcome) statement is presented in Table 1. **Population** was considered as microorganisms, including Antibiotic Resistance Bacteria (ARB), Antimicrobial Resistance Genes (ARGs), Antimicrobial Resistance (AMR), viruses, fungi, parasites, and bacteria. **Interventions** considered as any type of treatment technologies used in hospitals such as full scale, bench scale and pilot scale treatment processes; **comparison** was defined as microbial reduction from influent to effluent and with national standard for wastewater discharge standard; **outcomes** were defined as availability of treatment plant in hospitals and treatment efficacy (Table 1) (Eriksen and Frandsen, 2018).

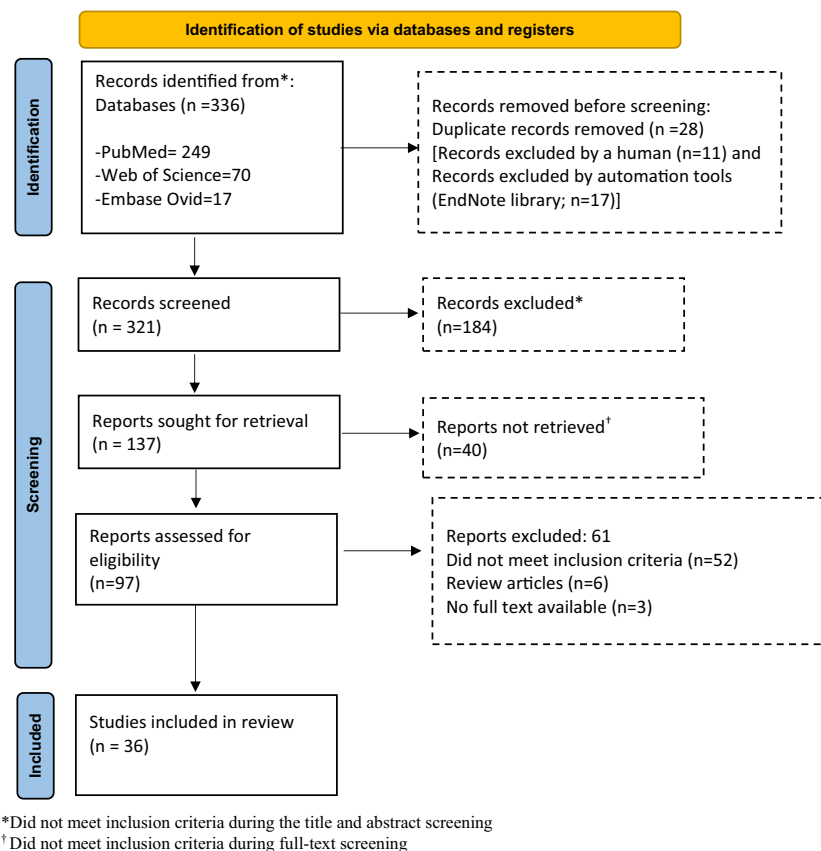


Fig. 1. PRISMA study flowchart.

\*Did not meet inclusion criteria during the title and abstract screening.

† Did not meet inclusion criteria during full-text screening.

**Table 1**  
PICO (population, intervention, comparison, outcome) table.

P	Microorganisms		
	ARB	ARG	AMR
			Bacteria
	Ceftazidime	Amoxicillin/ clavulanic acid	Ampicillin  TVC, TC, FC, <i>Staphylococcus</i> , <i>Salmonella</i> , <i>S. typhi</i> , <i>Shigella</i> , <i>Pseudomonas</i> <i>aeruginosa</i> <i>Klebsiella ornithinoly.</i> , <i>Klebsiella oxytoca</i> , <i>Klebsiella pneumoniae</i> , <i>Klebsiella terrigena</i>
	Gentamycin	cytochrome P450	ciprofloxacin  norfloxacin  Ceftriaxone  Co- trimoxazole  Fosfomycin  Imipenem
		bla <sub>SHV</sub> , bla <sub>KPC</sub> , bla <sub>SPM</sub> , bla <sub>VIM</sub> , bla <sub>SHV</sub> , bla <sub>VIM</sub> , bla <sub>TEM</sub> , bla <sub>KPC</sub> , bla CTX-M-1  bla <sub>TEM</sub> , bla <sub>KPC</sub>  bla <sub>TEM</sub> , bla <sub>VIM</sub> , bla <sub>SHV</sub> , bla <sub>KPC</sub> , bla <sub>SPM</sub>  bla <sub>TEM</sub> , bla <sub>KPC</sub> , bla <sub>VIM</sub> , bla <sub>SPM</sub> aminoglycoside resistance genes, Ib- 01, strB, lphonamide (sul2), and tetracycline re- sistance genes  bla <sub>CTX</sub> , bla <sub>TEM</sub> , bla <sub>VIM</sub> , Sul, qnrs  bla <sub>TEM</sub> , Sul, qnrs	<i>Proteus mirabilis</i>  <i>Serratia marcescens</i> , <i>Serratia rubidaceae</i>  <i>Aeromonadaceae</i>  <i>Kluyvera spp</i>  <i>Citrobacter freundii</i>  <i>Chromobacterium</i> <i>violaceum</i>  <i>Enterococci</i> , <i>Enterobacter spp.</i> , <i>Enterobacter asburiae</i> , <i>Enterobacter cloacae</i> , <i>Pantoea agglomerans</i> <i>Escherichia coli</i> , <i>Escherichia hermannii</i> <i>Bacillus</i>
I	All interventions (treatment technologies) targeting to lower prevalence of target pathogens from the hospital wastewater by using the following treatment processes		
	Full scale	Bench scale	Pilot scale
	Activated sludge (AS)	Biotreatment	Constructed wetland (CW)
	Sludge concentration unit, biological phosphorus unit, aeration tank	Fixed bed system with activated carbon/ potassium permanganate composite	Ozonation (O3)
	Ultraviolet (UV), chlorine	Cassava peel	Sodium hypochlorite solution
	Addition of coffee and brown sugar in wastewater polystyrene filtration and chlorination Filtration, biological, and biochemical mechanisms equalizer tank, aeration tank, sedimentation tank, sand filter, carbon filter	Photo inactivation of bacteria by thiolated iron- doped nanoceria Sponge Membrane bioreactor (MBR)  Nano catalyst	Horizontal surface flow CW, tube settler MBR and Nanofiltration (NF)
	STP	Packed granular carbon (GAC) and smooth activated carbon (SAC)	Duckweed aquaculture
	Affluent, aeration tank, secondary effluent Aeration Aerobic reactor, biological treatment	Electrochemical coagulation	Hydrated lime, hydrochloric acid
C	Microbial reduction from influent to effluent; and effluent versus the national wastewater discharge standard		
		Electro-peroxone and SBR (sequencing batch reactor)	UV, O3 and O3/UV methods

**Table 1 (continued)**

I	All interventions (treatment technologies) targeting to lower prevalence of target pathogens from the hospital wastewater by using the following treatment processes
	Effect of intervention measured by difference of abundance of microorganisms in influent and effluent of WW treatment technologies
O	Effectiveness of treatment methods were evaluated by comparing reduction of microorganisms (post treatment) with the national standard for hospital WW discharge

**2.6. Risk of bias (ROB) assessment**

The assessment of risk of bias (ROB) in environmental reviews is not common. Thus, we included a predetermined set of methodological factors that were considered crucial for evaluating the credibility of studies across three domains: sampling, treatment, and efficacy (Appendix B: Table S3). These factors encompassed elements such as ensuring transparent documentation of the sampling strategy, which involves precise identification of sampling sites, the timing and frequency of sampling, sampling point (pre- and post-sampling), as well as providing comprehensive information on specific WWTP processes and the reported effectiveness of treatments. Additionally, we evaluated the decrease in specific pathogens, antimicrobial resistance (AMR), and antibiotic resistance genes (ARGs) after the treatment process. Furthermore, we evaluated the potential for biased reporting in each study, though it is important to note that the assessment of bias did not lead to the exclusion of any studies.

**2.7. Data extract**

The articles were assessed independently by two reviewers (NA and NTS) based on inclusion and exclusion criteria. Both reviewers later condensed the information, and if any inconsistency existed, the input of two reviewers (JW and TF) was considered. The information extracted from the final set of articles was extracted into an Excel database and shared the output for qualitative approval with co-authors. The checklist included the article tracking number, name of the first author, name of the journal, title of the article, year of publication, the type of study, the country in which the study was conducted, and expected outcomes of the studies. The removal rates of the microbial agents mentioned in the studies were compared with the national standard of each country.

The following data were extracted from included studies: (1) *study characteristics*: year published, treatment options, and geographical distribution of the studies; (2) *sampling characteristics*: dates, frequency, sampling points, locations and number of sample tested; (3) microbial removal and treatment efficacy; and (4) the authors conclusions based on their study on characterization parameters in influent and effluent hospital wastewaters focused on detecting microorganisms, ARBs and ARGs and comparing with national standards.

**2.8. Data analysis**

Descriptive analysis was used to report the frequency and distribution of search outcomes, including the year of publication, countries/regions, and types of treatment technologies used. To estimate the treatment efficacy of the selected studies, we extracted data from those articles that mentioned microbial concentrations in both the influent and effluent of the treatment plant or in the treatment process. We extracted data from the tables and figures from the selected studies. We calculated the microbial reduction rate/percentage by subtracting the effluent (untreated wastewater) concentration from the influent (treated wastewater). If the microbial concentration was reported in log scale first we converted both values (influent and effluent) in to anti-log scale and then subtracted the effluent (untreated wastewater) concentration from the influent (treated wastewater). Effectiveness was determined by comparing post-treatment microorganism reduction against national

wastewater discharge standards.

### 3. Results

#### 3.1. Characteristics of the selected articles

Between 2000 and 2022, only 36 full-text articles that met the eligibility criteria for HWWT in LMICs were found, as presented in Table 3. In LMICs, most of the research related to HWWT process was conducted in Asia (22/36), followed by South America (8/36), North America (1/36) and Africa (5/36). The majority of the articles were published between the years 2016 and 2020 (19/36), and no article was found between 2000 and 2005. Although all 36 studies were conducted in United Nations classified least developed countries (LDCs), most of them (17/36) were retrieved from high HDI (Human Development Index) valued countries, and about 63 % (22/36) studies were conducted in four countries (India = 7, Brazil = 7, Vietnam = 5, and China = 3). Only two studies were conducted in low-income countries (i.e., Ethiopia) based on the GNI (gross national income) classification, while the majority were conducted in middle-income countries (34/35). Universities/academia authors from LMICs led most studies (31/36) (Table 3).

#### 3.2. Treatment options, and geographical distribution of the studies

The choice of the treatment process for HWW varied significantly among the countries included in the studies. Overall, the activated sludge (AS) process (either alone or in combination with other treatment steps) was focused on seven studies, while three studies utilized constructed wetlands (CW). Three studies used membrane bioreactors, and another three studies used either Ozone (O3) and/or ultraviolet (UV) rays to treat the HWW. Based on the review of 36 articles, most of the studies focused on full-scale treatment process (17 articles), with nine articles utilizing bench scale and pilot scale treatment processes. Out of the total treatment options analyzed, the majority were found to be placed on-site (15/36), and 2 out of the 36 treatment plants were located at the community level (Table 2).

Table 4 illustrates how wastewater treatment technologies have been examined in various LMICs and implemented on a country-by-country basis. The articles included in this review were confined to 14 countries. Among them, India and Brazil documented research on full-scale sewage treatment plants (STPs), whereas only Vietnam utilized advanced wastewater treatment plants based on membranes (MBRs). China and Pakistan have recently (years 2018 to 2021) constructed full-scale WWTPs in hospitals, and these countries have utilized advanced methods for treating HWW, including sequencing batch reactors, biological treatment, and photo inactivation treatment process.

#### 3.3. Methods and sampling strategy used

An overview of methods and sampling strategies used in the selected studies is very important for finding out the gap of all the selected studies. The results presented in Table 5 provide information on the objectives, sampling strategies, and number of samples analyzed by the selected studies. Among the 36 studies, 16 collected samples from both the inlet and outlet of the treatment process, while 10 studies collected samples from multiple stages of the treatment process, such as the equalization tank, surface of the sedimentation tank, filtration effluent pipe, and treated water storage. Only 11 studies collected samples at least two time points to explore seasonality, and only three studies used a systematic daily/weekly sampling approach to assess the efficacy of full-scale treatment plants (Lien TQL et al., 2017; Nonfodji et al., 2020; Thanh TTB et al., 2019). Most of the studies did not estimate the sample size required to assess treatment efficacy, and only 17 studies mentioned the number of wastewater samples analyzed, with <100 samples collected in 11 studies. Only five studies (Kalaiselvi, 2016; Lien TQL

**Table 2**  
Characteristics of the selected articles.

Year	Location	Type of Treatment steps	Treatment scale	Reference
2022	Ujjain, India	Constructed wetland (CW)	Pilot	(Parashar et al., 2022)
2021	Tabriz, Iran	Ozonation (O3)	Pilot	(Baghal Asghari, 2021)
2021	Hangzhou, China	Sodium hypochlorite solution	Pilot	(Ge TL et al., 2021)
2021	Lahore, Pakistan	Biotreatment	Bench	(Rashid AM et al., 2021)
2021	Ribeirao Preto, Brazil	Activated sludge (AS)	Full scale	(Zagui GSM et al., 2021)
2020	Istanbul, Turkey	Sludge concentration unit, biological phosphorus unit, aeration tank	Full scale	(Cevik et al., 2020)
2020	New Delhi, India	Horizontal surface flow CW, tube settler	Pilot	(Khan Naem et al., 2020)
2020	NA, Benin	Fixed bed system with activated carbon/potassium permanganate composite	Bench	(Nonfodji et al., 2020)
2020	Harar, Ethiopia	AS	Full scale	(Teshome AA et al., 2020)
2020	Jinan, China	Ultraviolet (UV), chlorine	Full scale	(Wang HW et al., 2020)
2019	Ibadan, Nigeria	Cassava peel	Bench	(Adeolu and Olayinka, 2019)
2019	South Sulawesi, Indonesia	Addition of coffee and brown sugar in wastewater	Full scale	(Fitriany, 2019)
2019	Peshawar, Pakistan	Photo inactivation of bacteria by thiolated iron-doped nanoceria	Bench	(Khan SF et al., 2019)
2019	Jakarta, Indonesia	AS, polystyrene filtration and chlorination	Full scale	(Kristanto and William, 2019)
2019	Ho Chi Minh, Vietnam	Sponge Membrane bioreactor (MBR)	Bench	(Nguyen TTB et al., 2019)
2019	NA, Brazil	Nano catalyst	Bench	(Oliveira AGA et al., 2019)
2019	NA, India	Electrochemical coagulation	Bench	(Singh SM and Sahana, 2019)
2019	Ho Chi Minh City, Vietnam	MBR and Nanofiltration (NF)	Pilot	(Thanh TTB et al., 2019)
2018	Hawassa, Ethiopia	CW	Pilot	(Dires SB et al., 2018)
2018	Kete-Krachi, Ghana	Packed granular carbon (GAC) and smooth activated carbon (SAC)	Bench	(Tulashie SKK et al., 2018)
2018	NA, China	Electro-peroxone and SBR (sequencing batch reactor)	Bench	(Zheng HSG et al., 2018)
2017	Hanoi, Vietnam	Filtration, biological, and biochemical mechanisms	Full scale	(Lien TQL et al., 2017)
2016	India	equalizer tank, aeration tank, sedimentation tank, sand filter, carbon filter	Full scale	(Kalaiselvi, 2016)
2016	Ho Chi Minh, Vietnam	AS	Full scale	(Vo, 2016)
2015	NA, India	AS	Full scale	(Akiba et al., 2015)

(continued on next page)

**Table 2** (continued)

Year	Location	Type of Treatment steps	Treatment scale	Reference
2015	Karnataka, India	STP: equalization, aeration, settling, and outlet	Full scale	(Guruge KSY et al., 2015)
2015	Rio de Janeiro, Brazil	STP: pretreatment, aeration tank, clarifier tank, disinfection	Full scale	(Miranda CCdF et al., 2015)
2015	Rio de Janeiro city, Brazil	STP: Extended aeration, activated sludge system, chlorination	Full scale	(Santoro DOC et al., 2015)
2015	NA, Haiti	Hydrated lime, hydrochloric acid	Pilot	(Sozzi et al., 2015)
2013	NA, Brazil	UV, O3 and O3/UV methods	Pilot	(Kist LTR et al., 2013)
2013	São Paulo, Brazil	Affluent, aeration tank, AS, secondary effluent	Full scale	(Picão, 2013)
2011	Rio de Janeiro, Brazil	Aeration, AS, chlorination	Full scale	(Chagas TPS et al., 2011)
2009	Delhi, India	Filtration, aeration, and chlorination	Multiple treatment plants	(Gupta PM et al., 2009)
2009	Tangail, Bangladesh	STP: Duckweed aquaculture	Full scale	(Rahman MH et al., 2009)
2008	Hanoi, Vietnam	Aerobic reactor, biological treatment	Full scale	(Duong HAP et al., 2008)
2007	Tangail, Bangladesh	Duckweed aquaculture	Pilot	(Rahman et al., 2007)

NA: City not mentioned.

et al., 2017; Nonfodji, 2020; Rahman et al., 2007; Thanh TTB et al., 2019) used a robust sampling approach, and included detailed methodology, sampling strategy and collected >100 samples to assess treatment efficacy.

### 3.4. Microbial removal and treatment efficacy

Microbial removal and treatment efficacy can be analyzed by characterizing parameters in influent and effluent HWW and comparing with national standards. Table 6 contains information about subset of studies evaluated the efficacy of different treatment processes for removing microorganisms from the wastewater. Out of the 36 studies included in this review, three studies focused on the removal of pollutants (Adeolu and Olayinka, 2019; Fitriany, 2019; Khan Naem et al., 2020), while two studies examined the removal of heavy metals (Rashid AM et al., 2021; Zagui GSM et al., 2021). One study investigated mutagenicity and cytotoxicity (Cevik et al., 2020), and another study focused on the removal of ciprofloxacin (Nguyen TTB et al., 2019). Therefore, we excluded these studies from the analysis of pathogen concentrations in wastewater treatment plants. There appears to be heterogeneity among the studies regarding the types of WWTP used to treat HWW, sample collection strategy, and target microorganisms. The heterogeneity among the studies in terms of the types of WWTP used, sample collection strategy, and target pathogens makes it difficult to draw conclusions about the effectiveness of different wastewater treatment technologies for HWW. Therefore, it is important to carefully consider this heterogeneity when interpreting the results of the studies.

The choice of microorganisms used to assess the treatment efficacy varies across the studies. Among the studies included in this review, Antimicrobial Resistant Bacteria (AMR) or Antimicrobial Resistant Genes (ARGs) were the most targeted microorganisms with 14 out of 36 studies focusing on them. Seven studies only (7/36) used indicator bacteria, such as *E. coli*, Total coliform (TC), and Thermo tolerant coliform (TTC), and rest of the studies used other bacteria (i.e., Gram-negative bacilli, *Enterobacteriaceae*, *Aeromonadaceae*, *Staphylococcus*,

**Table 3**

Characteristics of the selected articles between 2000 and 2022: geographical distribution, year published, countries where the studies were conducted.

Year published	Number of articles (N = 36)
2000–2005	0
2006–2010	4
2011–2015	8
2016–2020	19
2021–2022	5
Continents	
Asia	22
Africa	5
North America	1
South America	8
Regions	
Southeast Asia	10
South Asia	3
(n = 21)	
Northeast Asia	1
Southern Asia	8
South America (n = 8)	
Central eastern	7
Southern part	1
North America (1)	1
Africa (n = 5)	
West Africa	3
Northeastern Africa	2
Human Development Index (HDI) value wise position <sup>a</sup>	
Low	7
Medium	10
High <sup>c</sup>	17
Very High <sup>d</sup>	2
Low- and Middle-income Countries Group (gross national income (GNI) per capita) <sup>b</sup>	
Low income	2
Lower middle income	22
Upper middle income	11
High income <sup>e</sup>	1
UN Classification: Least developed countries <sup>f</sup>	36
Lead author institutes (Corresponding or first author)	
University/Academia in low-income countries	31
University/Academia in high-income countries	3
Non-academia in high-income countries	2
Countries where the study conducted	
India	7
Brazil	7
Vietnam	5
China	3
Other	14

<sup>a</sup> A value above 0.800 HDI is classified as very high, between 0.700 and 0.799 HDI as high, 0.550 to 0.699 HDI as medium, and below 0.550 HDI as low (Ref: <https://hdr.undp.org/data-center/human-development-index/#/indicies/HDI>).

<sup>b</sup> Countries with less than \$1035 GNI per capita are classified as low-income countries, those with between \$1036 and \$4085 as lower-middle-income countries, those with between \$4086 and \$12,615 as upper-middle-income countries, and those with incomes of more than \$12,615 as high-income countries.

<sup>c</sup> High HDI countries: Iran, Brazil, China, Indonesia, Vietnam.

<sup>d</sup> Very high HDI countries: Turkey, Chile.

<sup>e</sup> GNI high-income country: Chile.

<sup>f</sup> Least developed countries (LDCs) are low-income countries confronting severe structural impediments to sustainable development (REF: <https://data.worldbank.org/country/XL>).

*Salmonella*, *Shigella*, etc.), and virus (i.e., SARS-CoV-2) to evaluate the treatment effectiveness of the HWWT process (Table 6). Almost all articles that studied AMR bacteria explored the use of antimicrobial-resistant *E. coli*. Four studies assessed antimicrobial susceptibility for different drugs, and two studies focused on multidrug-resistant bacteria.

The efficacy of various wastewater treatment methods in reducing microorganisms and improving wastewater quality was investigated in different countries. Fourteen studies reported the concentration reduction (arithmetic or geometric mean concentration) to assess microbial contamination in the wastewater, while some studies (7 out of 36) only reported the detection or absence of microorganisms. Comparing the

**Table 4**  
Types of treatment technologies studied, and national standard used in different LMICs between 2000 and 2022.

Year	Country	Type of Treatment steps	#of studies	Effluent discharge standard	Reference
2009-2020	India	Constructed wetland (CW) with/without tube statelier	2	pH 6.5–9.0 Suspended solids 100 mg/l	(Parashar et al., 2022), (Khan Naem et al., 2020), (Singh SM and Sahana, 2019), (Kalaiselvi, 2016), (Akiba et al., 2015), (Guruge, 2015), (Gupta PM et al., 2009)
		Electrochemical coagulation (ECC)	1	Oil and grease 10 mg/l	
		STP: Equalizer tank, aeration tank, sedimentation tank, sand filter, carbon filter	2	BOD 30 mg/l COD 250 mg/l	
		Activated Sludge (AS)	1	Bio-assay test 90 % survival of fish after 96 h in 100 % effluent.	
2011-2021	Brazil	Filtration, aeration, and chlorination	1	Ref: (MoEF&CC, 1998) (MoEF, 2016)	(Zagui GSM et al., 2021), (Oliveira AGA et al., 2019), (Miranda CCdF et al., 2015), (Santoro DOC et al., 2015), (Kist LTR et al., 2013), (Picão, 2013), (Chagas TPS et al., 2011)
		AS with or without disinfection	3	BOD5: 60 mg/l	
		STP: pretreatment, aeration, clarifier tank, disinfection	2	COD: 200 mg/l	
		Nano catalyst	1	TSS: 150 mg/l	
		UV, O3 and O3/UV methods	1	Ref: (Da Silva et al., 2011; SEMACE, 2002)	
		Membrane bioreactor (MBR) with or without nitrification	2	pH: 5.5 to 9.0 BOD5: ≤30 mg/l	
2008–2019	Vietnam	Aerobic reactor, biological treatment	1	COD: ≤120 mg/l TSS: ≤100 mg/l	(Nguyen TTB et al., 2019) (Thanh TTB et al., 2019), (Lien TQL et al., 2017), (Vo, 2016), (Duong HAP et al., 2008)
		Filtration, biological, and biochemical mechanisms	1	Oil and Grease: ≤10 mg/l Total Nitrogen (TN): ≤20 mg/l Total Phosphorus (TP): ≤2.0 mg/l	
		AS	1	Total Coliforms: ≤1000 CFU/ml Ref: (World Bank, 2020)	
		NaOCl solution	1	pH 6.5–9.2	
2018–2021	China	Ultraviolet (UV), chlorine	1	SS - 72–150 (71–152) mg/dm3 BOD5 25–50 (20–30) mg/dm3 COD 110–140 mg/dm3 Ammonia nitrogen 14–26 mg/dm3 Fluoride ≤9 mg/dm3 Phosphate 0.4–1.1 mg/dm3 Phosphorus 0.1–0.2 mg/dm3	(Ge TL et al., 2021) (Wang HW et al., 2020) (Zheng HSG et al., 2018)
		Electro-peroxone and SBR (sequencing batch reactor)	1	Fecal coliform [individual/liter] 500 – 1000 and 100–500 Total Chlorine after disinfection <0.4 - > 2.5 (contact time ≥ 1.2 h) and > 5.5 – 7.5 (contact time ≥ 1.6 h) Ref: (MoEE, 2002; MoEE, 2006)	
		Biotreatment	1	Community to water bodies: Temperature: 40C pH: 6 to 9 BOD: 80 mg/l COD: 150 mg/l TSS: 200 mg/l TDS: 2500 mg/l Cl: 1000 mg/l NH3: 40 mg/l Grease & oil: 10 mg/l Total Nitrogen: 40 mg/l Sulphid: 1.0 mg/l Ref: (Pak-EPA, 1996)	
		Photo inactivation by thiolated iron-doped nanoceria	1	N2: 50 mg/l Total Kjeldahl Nitrogen: 250 mg/l CR: 0.5 mg/l Hexavalent Chromium: 0.1 mg/l	
2007–2009	Bangladesh	Duckweed aquaculture	2	pH: 6.5 to 8 BOD5): 50 mg/l COD: 200 mg/l TSS: 100 mg/l Oil& grease: 1o mg/l Ref: (MoE, 1997)	(Rahman MH et al., 2009), (Rahman et al., 2007)
		Addition of coffee and brown sugar in wastewater	1	pH: 6 to 8 BOD5): ≤ 50 mg/l COD: ≤ 100 mg/l	
2019	Indonesia	AS, polystyrene filtration and chlorination	1	TSS: ≤ 50 mg/l Total Coliforms: ≤ 10 <sup>3</sup> CFU/ml Ref: (MoEE, 2006)	(Kristanto and William, 2019)
2018–2020	Ethiopia	AS	1	Temperature: 40C	(Teshome AA et al., 2020),
		CW	1	pH: 6.5 to 8.5 BOD: 60 mg/l COD: 250 mg/l	(Direse SB et al., 2018)

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Table 4 (continued)

Year	Country	Type of Treatment steps	#of studies	Effluent discharge standard	Reference
2021	Iran	Ozonation (O3)	1	TSS: 100 mg/l Total Nitrogen: 40 mg/l NO3-N: 20 mg/l TP: 40 mg/l or 80 % removal Ref: (Haddis et al., 2014) pH 6.5–8.5 TSS 40 mg/l Oil and grease 10 mg/l BOD 30 mg/l COD 60 mg/l Turbidity: 50 mg/l TC: 1000 FC: 400 Ref: (Golbaz, 2016) pH 6–9	(Baghal Asghari, 2021)
2020	Turkey	Sludge concentration, biological phosphorus, aeration tank	1	TSS 30–70 mg/l BOD 45–50 mg/l COD 100–180 mg/l Ref: (GoV, 1991) Use WHO standard: pH 6–9 Temperature: ≤30C TSS ≤35 mg/l BOD ≤25 mg/l COD ≤125 mg/l DO: >5 mg/l Ammonium: <0.2 mg/l Phosphorous: ≤10 mg/l Ref: (République du Bénin, 2001; Todedji et al., 2021; WHO, 2014) General Appearance: Clear Color: 5.15 Odour: Unobjectionable Turbidity: 10 NTU Temperature: Ambient PH: 6.5–8.5 TDS: 500 mg/l TSS: 0.75 mg/l Total solid: 1000 mg/l Dissolved oxygen: 3.3 mg/l COD 30 mg/l BOD 6.0 mg/l	(Cevik et al., 2020)
2020	Benin	Fixed bed: activated carbon/potassium permanganate	1	Conductivity 1000 N/CM Sulphate: 500 mg/l Nitrate: 40 mg/l Phosphorous: 3.5 mg/l Chlorides 250 mg/l Alkalinity 461 mg/l Iron: 0.3 mg/l Lead 0.01 mg/l Copper: 1.0 mg/l Zinc 0.2 mg/l FC: 100 CFU/ml E. coli: Negative Total plate count: 3 CFU/ml Ref: (Emeka, 2015) Turbidity: 10 NTU Temperature: <30C PH: 6–9 TDS: 1000 mg/l TSS: 50 mg/L Total solid: 1000 mg/l Dissolved oxygen: 1.0 mg/l COD 250 mg/l BOD 50 mg/l	(Nonfodji et al., 2020)
2019	Nigeria	Cassava peel	1	Conductivity 1000 N/CM Sulphate: 500 mg/l Nitrate: 40 mg/l Phosphorous: 3.5 mg/l Chlorides 250 mg/l Alkalinity 461 mg/l Iron: 0.3 mg/l Lead 0.01 mg/l Copper: 1.0 mg/l Zinc 0.2 mg/l FC: 100 CFU/ml E. coli: Negative Total plate count: 3 CFU/ml Ref: (Emeka, 2015) Turbidity: 10 NTU Temperature: <30C PH: 6–9 TDS: 1000 mg/l TSS: 50 mg/L Total solid: 1000 mg/l Dissolved oxygen: 1.0 mg/l COD 250 mg/l BOD 50 mg/l	(Adeolu and Olayinka, 2019)
2018	Ghana	Packed granular carbo and smooth activated carbon	1	Conductivity 1000 N/CM Sulphate: 500 mg/l Nitrate: 40 mg/l Total Nitrogen: 50 mg/l Total Phosphate: 2.0 mg/l Chlorides: 250 mg/l Iron: 0.1 mg/l Lead 0.1 mg/l Copper: 1.0 mg/l FC: 400 CFU/100 ml	(Tulashie SKK et al., 2018)

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Table 4 (continued)

Year	Country	Type of Treatment steps	#of studies	Effluent discharge standard	Reference
2015	Haiti	Hydrated lime, hydrochloric acid	1	<i>E. coli</i> : 10 CFU/100 ml Ref: (Owusu-Ansah et al., 2015) Follow US EPA standard Ref: (USAID, 2014)	(Sozzi et al., 2015)

microbial removal efficacy of the treatment process is challenging for most microorganisms in this review due to heterogeneity in treatment options, sample collection strategies, laboratory analyses, and selection of target organisms. However, since *E. coli* was the most used indicator organism to assess the removal efficacy, these studies can be compared to evaluate the removal efficacy between the treatments processes included in this review.

The highest microbial reduction was achieved using a combination of various treatment processes (i.e., aqueous solutions of glutaraldehyde and advanced oxidative processes, O<sub>3</sub>, and UV, and the combination of O<sub>3</sub>/UV) in Brazil, with an overall reduction of pathogens of 99.90 % (Kist LTR et al., 2013). In India, the use of 3D electrochemical coagulation (ECC) process along with metal particle electrodes of aluminium and stainless steel removed microorganisms by 97–98 % (Singh SM and Sahana, 2019). Membrane bioreactor (MBR) combined with nanofiltration (NF) also significantly reduced the concentration of microorganisms (log<sub>10</sub> reduction of *E. coli* >6.0 CFU/100 ml) after treatment in Vietnam (Thanh TTB et al., 2019). We found wide ranges of microbial reduction in the effluent after treatment using full-scale conventional wastewater treatment plants (*E. coli* removal range: 240 CFU/100 ml to >98,000 CFU/100 ml) (Table 6).

Very few studies mentioned the level of microorganism removal compared with their national standard in developing countries. Activated sludge treatment process was used in Indonesia (Kristanto and William, 2019) and Vietnam (Duong HAP et al., 2008) to assess the treatment efficacy removing microorganisms. Activated sludge treatment process did not achieve the national standard for effluent discharge after treatment. Similarly, packed granular (GAC) and smooth activated carbon (SAC), hydrated lime, hydrochloric acid, and photo inactivation of bacteria by thiolated iron-doped nanoceria treatment used in three countries (Ghana, Vietnam, and Haiti respectively) and their effluent discharge did not meet relevant national standards. Conversely, another study in Vietnam used Membrane Bio-reactor (MBR) and Nanofiltration (NF), and these technologies achieved 100 % removal of indicator bacteria and achieved their national standard. Although most studies utilized AMR and/or ARG to evaluate the effectiveness of the treatment process, there was a lack of country-specific national standards for AMR/ARG in the effluent. Furthermore, the majority of the studies found that the treatment process did not completely eliminate AMR/ARG from the effluent, leading to the potential spread of AMR bacteria and genes to the aquatic environment through HWW (Table 6).

### 3.5. Risk of bias assessment

The findings from the assessment of bias are detailed in Table 7. Within the sampling realm, a notable proportion of the studies exhibited bias risks. Specifically, seven studies (19.4 %) omitted reporting the sampling dates, while twelve studies (33.3 %) failed to document the sampling frequency. Moreover, twenty studies (55.6 %) neglected to elucidate the pre-post sampling approach utilized during the study.

The evaluation revealed a substantial risk of bias within the treatment domains. Specifically, a high risk of bias was evident in the depiction of available treatment options within LMIC hospitals, observed in 69.4 % (25 studies) of cases, and in the portrayal of treatment scales, found in 47.2 % (seventeen studies) of cases. However, the treatment process itself demonstrated a low risk of bias. Some studies

had an indeterminate risk of bias due to missing data related to sample timing, frequency, sampling points, or treatment efficacy.

>72 % high risk of bias was discerned within the efficacy domains. Of the 22 studies addressing treatment efficacy, twelve (33.3 %) omitted mentioning the national effluent discharge standards adopted in various LMICs. In all encompassed studies, microbiological methods for bacterial identification and resistance detection were disclosed.

## 4. Discussion

The findings of this systematic review indicate a concerning lack of research on HWWT in LMICs. Although there was a significant lack of publications concerning HWWTP in LMICs in the past (year 2000–2015), there has been a promising rise in pertinent research in recent times (year 2016–2022). Furthermore, most research was conducted in high HDI countries, and very few studies were conducted in GNI-classified low-income countries. This may suggest that most of the research grants related to the HWWT were allocated to GNI defined lower/upper middle-income countries (such as India, Brazil, Vietnam, and China), and not focusing on the World Bank defined low-income countries (World Bank, 2023) which may limit the generalizability of the findings to other contexts. These findings underscore the urgent need for more research on HWWT technologies in LMICs, especially in the least developed countries, where the need for effective wastewater treatment is likely to be the greatest (Majumder et al., 2021).

The choice of treatment process used to treat HWW in LMICs varied widely. The majority of the studies examined conventional treatment process (such as activated sludge process and constructed wetlands) (Akiba et al., 2015; Chagas TPS et al., 2011; Dires SB et al., 2018; Khan Naem et al., 2020; Parashar et al., 2022; Teshome AA et al., 2020; Vo, 2016; Zagui GSM et al., 2021). Only Vietnam (Nguyen TTB et al., 2019; Thanh TTB et al., 2019). Brazil (Miranda CCdF et al., 2015; Santoro DOC et al., 2015) piloted advanced wastewater treatment plants to treat HWW. This finding is consistent with the recently published review on global hospital WWTP (Majumder et al., 2021). According to Kumari et al. (2020), a recent evaluation of hospital WWTP revealed that developed countries primarily used advanced technologies for the treatment of HWW, whereas developing countries did not (Kumari et al., 2020). These advanced technologies included membrane bioreactors (MBR), advanced oxidation techniques, and nanotechnologies, and nanostructured catalytic membranes (NCMs) (Kumari et al., 2020). Furthermore, Kaur et al. (2017) reported that developed countries also used nanocomponents which is highly efficient in treating wastewater, and the recovered water met human consumption criteria (Kaur et al., 2017). This indicates the need for more research on the most efficient treatment plants and treatment processes for HWW in LMICs.

The removal of microorganisms, including antimicrobial resistance (AMR) bacteria and antimicrobial resistance genes (ARG), in effluent is not specific to any countries but rather depends on the type of treatment process employed for HWW treatment. For instance, studies conducted in Vietnam and China, utilizing Membrane Bio-reactor (MBR) and Nanofiltration (NF) technologies, demonstrated the potential for achieving 100 % removal of indicator bacteria and meeting the national standard for effluent discharge (Liu et al., 2010; Thanh TTB et al., 2019). Similar technology used in developed countries such as Denmark (Bio-booster, 2016), Australia, Switzerland (Lee et al., 2014) to treat HWW found similar results microbial removals. Similarly, the highest level of

**Table 5**  
Study objectives, treatment approaches and method used in the selected studies between 2000 and 2022.

Short objective	Treatment approaches and process	Samples were collected from	Frequency and date of sample collection	# of samples tested
Prevalence of antibiotic resistant <i>E. coli</i> in three antibiotics from different treatment stages of AS	AS, polystyrene filtration and chlorination	Equalization tank, surface of the sedimentation tank; filtration effluent pipe; and treated water storage	Not specified: Sep-Nov 2015	32
Influence of HWW on the prevalence of antimicrobial-resistant <i>E. coli</i> in Indian STPs	AS	Equalization, aeration, and clarifier tanks, and outlet of STPs	3 seasons: post-monsoon, pre-monsoon, and monsoon	28
Occurrence and behavior of fluoroquinolone antibacterial agents were investigated	AS, Aerobic reactor, biological treatment	Raw sewage, effluent from the AS reactor, secondary effluent, and treated WW	2 times between Sep 2004 and April 2005	15
Antibiotic resistance surveillance in selected WW systems	3 different system: AS, WSP, ST	Influent and effluent from each unit operation/process treatment	3 times between Oct 2018–Apr 2019	66
AMR profile and the occurrence of <i>Klebsiella pneumoniae carbapenemase</i> (KPC) producing Gram-negative rods in sewage samples	Affluent, aeration tank, AS, secondary effluent	Affluent, aeration tank, activated sludge, and effluent	Not mentioned	1
AMR profile and types of beta-lactamases	Aeration, AS, chlorination	Influent, Clarifier tank, chlorine tank	8 times between July-Dec 2008	24
Genotoxicity tests for effluent in different hospitals with or without treatment plants	Filtration, aeration, and chlorination	Influent, Aeration tank, Effluent	4 times between July2007-Jan2008	72
Effectiveness of 8 horizontal subsurface flow pilot scale artificial wetlands in the removal of antibiotic-resistant bacteria from HWW	CW	Inlet and Eight CW outlets	4 times between Sep-Dec2017	40
Assessed the utility of CW for treatment	CW: six different combinations	Inlet and outlet	3 seasons: Summer, rainy, winter seasons	21
HWW was characterized and treated with activated carbon/potassium permanganate in a fixed bed column system	Fixed bed system with activated carbon/potassium permanganate composite	3 points of storage tank manholes and fecal sludge	3 times between Jan-June 2017	162
Prevalence of antibiotic resistance and ARGs in <i>E. coli</i> isolates from HWW	Filtration, Biological, and biochemical process	Before and after Treatment: 2 hospitals	Monthly samples for 12 months	265
Incidence of ARB in hospital-generated recycled water	STP: Equalizer tank, aeration tank, sedimentation tank, sand filter, carbon filter	7 different stages at the treatment plant including influent and effluent	Not mentioned	140
Associations among multiple environmental variables such as STP type, treatment step, climatic effect, gene regulation potency, and presence of antimicrobial resistant <i>E. coli</i> in wastewater samples	STP: equalization, aeration, settling, and outlet	Equalization tanks, aeration tanks, settling tanks, and outlets	2 seasons: Pre-monsoon and monsoon periods of 2013	Not mentioned
Investigation of <i>Pseudomonas aeruginosa</i> isolates from a HWTP, focusing on enzyme-based mechanisms of $\beta$ -lactams resistance and the genetic relatedness among isolates.	STP: pre-treatment, aeration tank, clarifier tank, disinfection	Affluent, aeration tank, sludge, chlorination tank and treated effluent	Not mentioned	Not mentioned
Evaluation of the diversity of Pseudomonads and antibiotic resistance profiles of <i>Pseudomonas aeruginosa</i> in a HWTP	STP: Extended aeration, activated sludge system, chlorination total 5 step treatment	Effluents	Not mentioned: February 2010, from each of five stages of the treatment station	15
Effectiveness of a HWW treatment system that combined a membrane bioreactor (MBR) system with nanofiltration (NF)	MBR and Nanofiltration (NF)	Inlet, after aerobic treatment, outlet	Once daily for 120 days in 3 locations	360
Effectiveness of different disinfection models using UV and chlorine and explored the impact on horizontal gene transfer at low doses while developing a synergistic disinfection approach.	Ultraviolet (UV), chlorine	Before and after treatment	Not mentioned	Not mentioned
Disinfection of hospital materials using aqueous solutions of glutaraldehyde and advanced oxidative processes, O <sub>3</sub> , and UV, and the combination of the latter two.	UV, O <sub>3</sub> and O <sub>3</sub> /UV methods	Septic tank outlet	Not mentioned	3
Performance of ozonation for the removal of ARB and ARGs using <i>E. coli</i> and <i>Pseudomonas aeruginosa</i> carrying ARGs from HWW	4 dose Ozonation: TOD1 = 11 mg/l, TOD2 = 25 mg/l, TOD3 = 37 mg/l, TOD4 = 45 mg/l	Untreated sewerage	Not mentioned	Not mentioned
Decontamination and disinfection of WW by photocatalysis under UV/visible light using nanocatalysts based on Ca-doped ZnO	Nano catalyst based on ZnO-Ca	Untreated WW samples supplied by industrial laundry	Not mentioned	Not mentioned
Treatment of raw HWW without any modification using 3D ECC using metal particle electrodes of aluminium and stainless steel for removing pollutants	3D Electrochemical coagulation (ECC)	Inlet of effluent treatment plant	Not mentioned	Not mentioned
Enhance the HWWT performance and to reduce antibiotic resistance genes production simultaneously by electro-peroxone ( <i>E</i> -peroxone) pretreatment	Electro-peroxone and sequencing batch reactor	Simulated hospital wastewater	Not mentioned	Not mentioned
Assessing, and treating hospital effluent using packed granular (GAC) and smooth activated carbon (SAC)	GAC, SAC	Effluents	3 weeks interval: 2 days per cycle of sampling	Not mentioned
Examined the use of a novel in-situ treatment system to disinfect CTC HWW	Hydrated lime, hydrochloric acid	Not mentioned	Not mentioned	Not mentioned

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Table 5 (continued)

Short objective	Treatment approaches and process	Samples were collected from	Frequency and date of sample collection	# of samples tested
Contamination of environment in isolation wards and sewage in hospital	Pre-processing disinfection (chlorination)	Inlet of the treatment processing and final disinfection pool	Not mentioned February 6 to April 4	41
Thiolated iron-doped nanoceria was synthesised and tested for killing of microbes from hospital effluent.	Photo inactivation of bacteria by thiolated iron-doped nanoceria	Untreated WW	Not mentioned	Not mentioned
The persistence and transmission of <i>Aeromonas</i> in a duckweed aquaculture-based HWW treatment plant	STP: Duckweed aquaculture	Raw sewage water, duckweed lagoon, and fishpond	Not specified: Dec2000-Feb2002	670
Prevalence of AMR in <i>Aeromonas</i> PhP types recovered from different locations in the treatment plant	STP: Duckweed aquaculture	Raw sewage water, duckweed lagoon, and fish pond	Not specified: Dec2000-Feb2002	Not mentioned

microbial reduction, reaching an overall reduction of microorganisms of 99.90 %, was achieved in Brazil through the combination of different treatment processes, including aqueous solutions of glutaraldehyde and advanced oxidative processes such as O<sub>3</sub> and UV, as well as the combination of O<sub>3</sub>/UV (Kist LTR et al., 2013). This combination of different treatment process showed potential for achieving significant pathogen removal in many developed countries to treat HWW (Ferre-Aracil et al., 2016; Lutterbeck et al., 2015). The use of MBR combined with other polishing units has shown promising results in various countries, indicating their potential for achieving high levels of microbial removal in effluent. Furthermore, the importance of advanced treatment technologies is not limited to the removal of indicator bacteria alone but also effective to remove virus, AMR, ARG and pharmaceuticals from the wastewater (Majumder et al., 2021). These findings support the need for MBR based treatment plants to treat highly polluted HWW before discharging.

The efficacy of wastewater treatment is not solely determined by the treatment plant itself but also relies on the presence of well-established sanitation infrastructure, adequate resources, and skilled manpower at both healthcare facilities and community levels (Brault et al., 2022). Developed countries typically possess a comprehensive system of separate drainage and sewer networks, along with centralized treatment plants specifically designed for HWW treatment (Kumari et al., 2020). Conversely, developing countries often face challenges due to limitations and deficiencies in their infrastructure, making it difficult to transport HWW to centralized treatment facilities (Khan et al., 2019). Furthermore, developed countries tend to allocate greater financial resources to healthcare infrastructure, allowing for investments in advanced technologies and the recruitment of skilled personnel for wastewater treatment. Given the infrastructural and resource constraints in developing countries, decentralized (on-site) approaches to HWW treatment are essential (Azuma and Hayashi, 2021).

The use of effluent discharge standards varies widely across LMICs and between LMICs and high-income countries' (HIC) healthcare settings. This review highlights that in developing countries, only a few studies incorporated microorganism removal in line with their national standards, and many treatment technologies failed to meet these standards. For example, studies conducted in Indonesia and Vietnam did not achieve the national standard for effluent discharge after treatment (Duong HAP et al., 2008; Kristanto and William, 2019). In comparison, developed countries have established effluent discharge standards for healthcare facility wastewater, aligning them with their national municipal effluent discharge standards. These standards mandate effective microbial removal and control of antimicrobial resistance (AMR) and antimicrobial resistance genes (ARG). In the United States, the Clean Water Act (1972) and its updated version in 2002 govern the discharge of wastewater into surface water bodies, with the United States Environmental Protection Agency (USEPA) listing contaminants of toxic and harmful nature, including the antibiotic "erythromycin" and five synthetic hormones (USEPA, 2002). Similarly, the United Kingdom has regulations such as the Controlled Waste Regulations (1992) for healthcare discarded pharmaceuticals and the Special Waste Regulations (1996) for monitoring clinical waste from hospitals. The UK also

has dedicated biologic degradation landfills for HWW sludge (UK Government, 1992; UK Government, 1996). European countries utilize the Urban Wastewater Treatment Act of 1997 and have specific strategies to treat HWW, both onsite and within the sewer network (EUEPA, 1991). These examples demonstrate that developed countries have comprehensive regulatory frameworks and specific guidelines in place to address the treatment and discharge of HWW, including the control of pharmaceuticals, clinical waste, and biological degradation. Such measures ensure that effluent discharge meets standards that safeguard public health and the environment. The findings also emphasize the importance of developing and adopting advanced treatment technologies, implementing standardized guidelines for effluent discharge in LMICs, and investing in research and infrastructure to bridge the gap between healthcare facility wastewater treatment practices in developing and developed countries.

Hospital wastewater can undergo various treatment approaches, including direct discharge, co-treatment in a municipal wastewater treatment plant, or on-site treatment before discharge (Pauwels and Verstraete, 2006). The World Health Organization (WHO) recommends the onsite treatment approach (WHO, 2014). However, the WHO guidelines lack specific limits for indicator parameters in hospital effluents, and requirements vary among countries, with limited focus on microbiological indicators. A recent white paper by the Wellcome Trust and CDC emphasizes pre-treatment of hospital sewage to target major antibiotic resistance sources (Diana Aga et al., 2018). In our review, although most studies incorporated AMR and/or ARG evaluation to assess treatment effectiveness, there was a lack of country-specific national standards for AMR/ARG in the effluent (Alam et al., 2021a, 2021b). Additionally, only a few studies mentioned the quality of the effluent in relation to AMR/ARG after treatment, and the majority of the effluent did not completely eliminate AMR/ARG following treatment. This indicates the need for standardized guidelines and regulatory frameworks to address AMR/ARG in healthcare facility wastewater and ensure proper treatment efficacy (Kaloni, 2021). This review highlights the need for developing countries to improve their wastewater treatment processes in healthcare settings to meet national effluent discharge standards.

Limitations exist within the available evidence. Out of the 321 studies screened, fewer than 11 % studies were ultimately included into this review. Many studies were excluded because that were only conducted in laboratory settings, as well as systematic reviews published in peer-reviewed journals. Due to constraints in time and resources, we were unable to incorporate non-English research, unpublished data, and grey literature. This may have resulted in potential gaps in valuable data related to HWW. Reporting of the findings was highly heterogeneous, frequently qualitative or only partially quantitative and susceptible to different forms of bias. Evidence suggests that environmental systematic reviews often lack thorough bias evaluation. For instance, among environmental articles from 2018 to 2020 labeled as systematic reviews, 64 % did not assess bias (Frampton et al., 2022; Whaley and Roth, 2022). Formal ROB assessment scores are not usual in environmental research. The ROB used in this study is aligned with the recently published environmental systematic review (Hassoun-Kheir et al., 2020) that can

**Table 6**  
 Characterization parameters in influent and effluent hospital wastewaters focused on detecting microorganisms, ARBs and ARGs and comparing with national standard.

Name of treatment plant	Lab method used	Target pathogens	Influent	Effluent	Reduction	National standard of HWW	National Standard achieved
AS, polystyrene filtration and chlorination	Culture	Antimicrobial Registrant (AMR) <i>E. coli</i>	Mean AMR <i>E. coli</i> (CFU/ml): $4.6 \pm 2.9 \times 10^4$	Mean <i>E. coli</i> (CFU/ml): $6.4 \pm 8.1 \times 10^2$	$\sim 4.536 \times 10^4 \pm 2.819 \times 10^4$ (CFU/ml)	National standard TC: $\leq 10^3$ CFU/ml	Did not meet national standard: Indonesia (ADB, 2017)
AS	A Kirby–Bauer disk diffusion test	Total viable counts of bacteria (TVC) and total coliforms (TC) AMR <i>E. coli</i>	TVC: $6.3 \log_{10}$ CFU/ml TC: $6.2 \log_{10}$ CFU/ml AMR <i>E. coli</i> : Ampicillin-36 %	TVC: $4.0 \log_{10}$ CFU/ml TC: $3.8 \log_{10}$ CFU/ml AMR <i>E. coli</i> : Ampicillin-Not mentioned	TVC: 1.7 CFU/ml TC: 2.4 CFU/ml AMR <i>E. coli</i> : Not mentioned	National standard TC: $\leq 1000$ CFU/ml	Met national standard: India (MoEF, 2016)
AS, Aerobic reactor, biological treatment	Fluoroquino-lone tosfloxacin	<i>E. coli</i> , AMR: ciprofloxacin (CIP) and norfloxacin (NOR) registrant	>100,000 CFU/100 ml CIP: $25.8 \pm 8.1$ NOR: $8.4 \pm 2.5$ Concentration $\pm$ STD (lg 11)	2000 CFU/100 ml CIP: $3.7 \pm 1.3$ NOR: $1.5 \pm 0.3$ Concentration $\pm$ STD (lg 11)	>98,000 CFU/100 ml CIP: $22.1 \pm 8.4$ NOR: $6.9 \pm 2.5$	National standard TC: $\leq 1000$ CFU/ml	Did not meet National standard: Vietnam (World Bank, 2020)
3 different system: AS, WSP, ST	Kirby-Bauer disk diffusion method	AMR TC, FC, <i>E. coli</i> , <i>Enterococcus</i>	For AS: TC: $5.14 \times 10^8$ FC: $2.45 \times 10^7$ <i>Enterococci</i> : $1.31 \times 10^8$ <i>E. coli</i> : $1.17 \times 10^7$ CFU/100 ml	For AS: TC: $5.14 \times 10^8$ FC: $5.12 \times 10^4$ <i>Enterococci</i> : $3.93 \times 10^5$ <i>E. coli</i> : $2.75 \times 10^4$ CFU/100 ml	For AS: TC: 3.21 FC: 2.68 <i>Enterococci</i> : 2.52 <i>E. coli</i> : 2.62 log CFU/100 ml	-Microorganism not mentioned in the standard	Not applicable: Ethiopia (Haddis et al., 2014)
Affluent, aeration tank, AS, secondary effluent	PCR	Bacteria + AMR: MDR bacteria, KPC-2–producing <i>Aeromonas</i> spp. and several <i>Enterobacteriaceae</i> spp, including <i>Kluyvera</i> spp. TC (MPN 100/ml) FC (MPN 100/ml) Gram-negative bacteria: <i>Aeromonas</i> spp. <i>Citrobacter freundii</i> <i>Chromobacterium violaceum</i> <i>Enterobacter asburiae</i> <i>Enterobacter cloacae</i> <i>Enterobacter</i> spp. <i>Escherichia coli</i> <i>Escherichia hermannii</i> <i>Klebsiella ornithinoly.</i> <i>Klebsiella oxytoca</i> <i>Klebsiella pneumoniae</i> <i>Klebsiella terrigena</i> <i>Pantoea agglomerans</i> <i>Proteus mirabilis</i> <i>Serratia marcescens</i> <i>Serratia rubidaceae</i>	41.6 % Gram-negative bacilli were recovered from the hospital sewage, <i>Enterobacteriaceae</i> (61.2 %), <i>Aeromonadaceae</i> (18.6 %) TC: $7.4 \times 10^5$ FC: $0.8 \times 10^5$ 3 4 0 0 0 3 15 0 24 1 1 4 41 0 10 1 1 1 1 TC: $1.3 \times 10^7 \pm 1.2 \times 10^3$ FC: $1.4 \times 10^5 \pm 5 \times 10^2$	58.4 % Gram-negative bacilli were recovered from the WWTP <i>Aeromonadaceae</i> (58.8 %), <i>Enterobacteriaceae</i> (35.5 %) TC: $0.3 \times 10^5$ FC: $0.07 \times 10^5$ 0 0 0 3 1 2 3 0 2 18 1 6 0 0 0 0 0 TC: $5.1 \times 10^3 \pm 7.1 \times 10^2$ FC: $4.5 \times 10^2 \pm 70$ 50 $\pm$ 12 2 $\pm$ 1 1 $\pm$ 1 6.8 $\pm$ 3 (25.2 %)	16.8 % increased after treatment TC: $7.1 \times 10^5$ FC: $0.73 \times 10^5$	-Did not mention concentration -Microorganism not mentioned in the standard	Not applicable: Brazil (Da Silva et al., 2011; SEMACE, 2002)
Aeration, AS, chlorination	IDEXX, PCR	<i>Escherichia coli</i> <i>Escherichia hermannii</i> <i>Klebsiella ornithinoly.</i> <i>Klebsiella oxytoca</i> <i>Klebsiella pneumoniae</i> <i>Klebsiella terrigena</i> <i>Pantoea agglomerans</i> <i>Proteus mirabilis</i> <i>Serratia marcescens</i> <i>Serratia rubidaceae</i>	0 4 0 24 1 1 4 41 0 10 1 1 1 1 1 1 1 1 TC: $1.3 \times 10^7 \pm 1.2 \times 10^3$ FC: $1.4 \times 10^5 \pm 5 \times 10^2$	0 0 3 3 2 3 0 0 2 18 1 6 0 0 0 0 0 0 0 TC: $5.1 \times 10^3 \pm 7.1 \times 10^2$ FC: $4.5 \times 10^2 \pm 70$ 50 $\pm$ 12 2 $\pm$ 1 1 $\pm$ 1 6.8 $\pm$ 3 (25.2 %)	TC: 7.1 log <sub>10</sub> FC: 5.1 log <sub>10</sub> 3.1 $\times 10^3 \pm 43$	-Microorganism not mentioned in the standard	Not applicable: Ethiopia (Dires, 2018; Teshome AA et al., 2020)
CW	Culture media	TC (MPN/100 ml) <i>Staphylococcus</i> (CFU/ml) <i>Salmonella</i> (CFU/ml) <i>Shigella</i> (CFU/ml) <i>Escherichia coli</i> (AMR)	3.2 $\times 10^3 \pm 55$ 4 $\pm$ 1 5 $\pm$ 2 10.8 $\pm$ 3 (24 %)	50 $\pm$ 12 2 $\pm$ 1 1 $\pm$ 1 6.8 $\pm$ 3 (25.2 %)	TC: 7.1 log <sub>10</sub> FC: 5.1 log <sub>10</sub> 3.1 $\times 10^3 \pm 43$	-Microorganism not mentioned in the standard	Not applicable: Ethiopia (Dires, 2018; Teshome AA et al., 2020)
CW: Six different combinations	Membrane filtration: APHA 2005 method	AMR FC, TC,	Not mentioned	Not mentioned	TC:95 %, FC:98 %	-Did not mention concentration -Microorganism not mentioned in the standard	Not applicable: India (MoEF&CC, 1998) (MoEF, 2016)
Fixed bed system with activated carbon/potassium	Protozoa: optical microscope	AMR-4 Parasites, 7 bacteria, antibiotic susceptibility	FC: $9.50 \times 10^5$ $\pm 0.31 \times 10^5$	Not applicable: Only treated effluent	<i>E. coli</i> : $0.70 \pm 0.04 \log_{10}$ <i>S. typhi</i> $3.82 \pm 0.01 \log_{10}$	-Microorganism not mentioned in the standard	Not applicable: Benin (République du Bénin,

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Table 6 (continued)

Name of treatment plant	Lab method used	Target pathogens	Influent	Effluent	Reduction	National standard of HWW	National Standard achieved
permanganate composite	Bacteria: Membrane filtration	AMR, ARGs: <i>E. coli</i> isolates: (%)	28	19	9		2001; Todedji et al., 2021; WHO, 2014)
Filtration, Biological, and biochemical process	Standard disk diffusion and <i>E</i> -test	Amoxicillin/clavulanic acid	32	23	9	-Did not mention concentration	Not applicable: Vietnam
		Ceftazidime	45	32	13		
		Ceftriaxone	23	17	6		
		Ciprofloxacin	80	60	20		
		Co-trimoxazole	15	0	15		
		Fosfomycin	33	23	10		
		Gentamycin	0	0	0		
STP: Equalizer tank, aeration tank, sedimentation tank, sand filter, carbon filter	A pure culture by spread plate technique	7 organisms: <i>E. coli</i> , <i>Klebsiella</i> , and <i>P. mirabilis</i> , <i>Bacillus... Pseudomonas</i>	<i>E. coli</i> in Collection tank: 205 × 10 <sup>5</sup> CFU/ml	<i>E. coli</i> in end Lagoon: 42 × 10 <sup>5</sup> CFU/ml	163 × 10 <sup>5</sup> CFU/ml	-Microorganism not mentioned in the standard	Not applicable: India (MoEF&CC, 1998) (MoEF, 2016)
		AMR <i>E. coli</i> isolate	<i>E. coli</i> isolates registrant: STP1: 46 % STP2: 25 % STP3: 0 % STP4: 52 %	<i>E. coli</i> isolates registrant: STP1: 9 % STP2: 35 % STP3: 36 % STP4: 8 % Overall Concentration: >3.7 log <sub>10</sub> CFU/ml	<i>E. coli</i> isolates registrant: STP1: 37 % STP2: -10 % STP3: -36 % STP4: 44 %	-Did not mention concentration -Microorganism not mentioned in the standard	Not applicable: India (MoEF&CC, 1998) (MoEF, 2016)
STP: equalization, aeration, settling, and outlet	QuickGene RNA cultured cell kit and Quick- Gene-810 Nuclear Acid Isolation System	6 cytochrome P450 (CYP)	Isolation steps: (1) Grid/ affluent; (2) aeration tank; (3) Sludge; (4) Chlorination tank and b-lactamase genes; 1 (bla <sub>SHV</sub> , bla <sub>KPC</sub> , bla <sub>SPM</sub> , bla <sub>VIM</sub> ) 2(bla <sub>SHV</sub> , bla <sub>VIM</sub> , bla <sub>TEM</sub> , bla <sub>KPC</sub> , bla <sub>CTX-M-1</sub> ) 3(bla <sub>TEM</sub> , bla <sub>KPC</sub> ) 4(bla <sub>TEM</sub> , bla <sub>VIM</sub> , bla <sub>SHV</sub> , bla <sub>KPC</sub> , bla <sub>SPM</sub> )	Isolation step (5) Treated effluent and b-lactamase genes; 5(bla <sub>TEM</sub> , bla <sub>KPC</sub> , bla <sub>VIM</sub> , bla <sub>SPM</sub> )	Isolates carrying bla <sub>TEM</sub> , bla <sub>KPC</sub> , bla <sub>VIM</sub> and bla <sub>SPM</sub> were found in the fifth and final stage of the hospital wastewater.	Not mentioned	Not applicable (Da Silva et al., 2011; SEMACE, 2002)
STP: pre-treatment, aeration tank, clarifier tank, disinfection	PCR	<i>Pseudomonas aeruginosa</i> : AMR + ARGs	Of 29 isolates, 27 were biochemically identified as <i>Ps. aeruginosa</i> (93.1 %): 5 in HWTP 1, 6 in HWTP 2 and 8 in HWTP 3	8 <i>Ps. aeruginosa</i> in HWTP 5	A total of 29 strains were isolated from the wastewater treatment system from sites HWTP 1 (n = 6), HWTP 2 (n = 6), HWTP 3 (n = 8) and HWTP 5 (n = 9)		Not applicable (CLSI, 2011)
STP: Extended aeration, activated sludge system, chlorination Total 5 step treatment	PCR	AMR: antimicrobial Susceptibility e.g., <i>Pseudomonas species</i> AMR <i>E. coli</i>	Coliform: (1,50E+6–4,30E+6) <i>E. coli</i> (1,40E+4–4,50E+4).	0	Log removal value for both bacteria: 1.50 × 10 <sup>4</sup> to 4.30 × 10 <sup>6</sup>	National standard: Coliforms: ≤1000 CFU/ml	Met national standard: Vietnam (World Bank, 2020)
MBR and Nanofiltration (NF)	Not mentioned	<i>E. coli</i> and coliform				National standard: Fecal coliform [individual/L] 500 – 1000 and 100–500 Total Chlorine after disinfection <0.4 - > 2.5 (contact time	
Ultraviolet (UV), chlorine	PCR	ARB and ARGs	Not mentioned	ARGs relative abundance to 16S-rRNA gene, in which aminoglycoside resistance genes, lb-01, strB, lphonamide (sul2), and tetracycline resistance genes had high	log removal: 0.58–1.60 to 1–1.5		Not applicable: China (MoEE, 2002; MoEE, 2006)

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Table 6 (continued)

Name of treatment plant	Lab method used	Target pathogens	Influent	Effluent	Reduction	National standard of HWW	National Standard achieved
UV, O <sub>3</sub> and O <sub>3</sub> /UV methods	Spectrophotometric measurement	TTC	16 × 10 <sup>6</sup> MPN/100 ml	abundance in both pre and post treated samples ml: <18 MPN/100	15 × 10 <sup>5</sup> MPN/100 ml Overall: 99.90 %	≥ 1.2 h) and > 5.5 – 7.5 (contact time ≥ 1.6 h) -Microorganism not mentioned in the standard	Not applicable: Brazil (Da Silva et al., 2011; SEMACE, 2002)
4 dose Ozonation: TOD1 = 11 mg/l, TOD2 = 25 mg/l, TOD3 = 37 mg/l, TOD4 = 45 mg/l	Bacterial culture and PCR	Multidrug-resistant <i>E. coli</i> , <i>Pseudomonas aeruginosa</i> and ARGs	Identified <i>Pseudomonas aeruginosa</i> 99.43 % (resistant gene bla <sub>ctx</sub> , bla <sub>tem</sub> , bla <sub>vim</sub> , Sul, qnrs) and <i>Escherichia coli</i> 97.02 % (resistant gene bla <sub>tem</sub> , Sul, qnrs)	Not found	<i>E. coli</i> : 10 <sup>8</sup> cfu/ml <i>Pseudomonas</i> : 10 <sup>4</sup> cfu/ml and 10 <sup>6</sup> cfu/ml	<i>E. coli</i> : 10 CFU/100 ml	Not applicable
Nano catalyst based on ZnO-Ca	Sol-gel Culture method	Heterotrophic bacteria, TTC and TC	H. Bacteria: 1.30 × 10 <sup>6</sup> TTC: <1.8 TC: 4.60 × 10 <sup>2</sup>	H. Bacteria: 4.45 × 10 <sup>5</sup> TTC: <1.8 TC: <1.8	H. Bacteria: 8.55 × 10 <sup>6</sup> TTC: 0 TC: 4.56 × 10 <sup>6</sup>	-Microorganism not mentioned in the standard	Not applicable: Brazil (Da Silva et al., 2011; SEMACE, 2002)
3D Electrochemical coagulation (ECC)	3D ECC experimental reactor	<i>E. coli</i>	3.5 × 10 <sup>5</sup> CFU/ml	>1600/100 ml	97 % and 98 %	-Microorganism not mentioned in the standard	Not applicable: India (MoEF&CC, 1998) (MoEF, 2016)
Electro-peroxone and sequencing batch reactor	PCR	ARGs	ARG-CIP concentration of 200 mg/L	Not mentioned	E-peroxone pre-treatment can effectively reduce the ARG (reduction rate of 73.0 %)	Did not study indicator organisms	Not applicable
GAC, SAC	WHO Standard of drinking water	TC	S1: 42 × 10 <sup>6</sup> S2: 230 × 10 <sup>6</sup> S3: 138 × 10 <sup>6</sup>	S1: 12 × 10 <sup>6</sup> S2: 17 × 10 <sup>6</sup> S3: 15 × 10 <sup>6</sup>	S1: 3.0 × 10 <sup>7</sup> , S2: 2.13 × 10 <sup>8</sup> , S3: 123 × 10 <sup>6</sup> Overall: 84.39 %,	National Standard: FC: 400 CFU/100 ml <i>E. coli</i> : 10 CFU/100 ml	Did not meet the national standard: Ghana (Owusu-Ansah et al., 2015)
Hydrated lime, hydrochloric acid	Jar-test process	TTC	4.1 × 10 <sup>4</sup> Mean CFU/100 ml	81 Mean CFU/100 ml	4.09 × 10 <sup>4</sup> Mean CFU/100 ml Overall: >99.9 %	FC: 400 CFU/100 ml <i>E. coli</i> : 10 CFU/100 ml	Did not meet the national standard: Haiti (USAID, 2014)
Photo inactivation of bacteria by thiolated iron-doped nanoceria	Culture: Agar media	TC	TC: 7.5 × 10 <sup>3</sup> to 64 × 10 <sup>6</sup> MPN/100 ml.	Not specified	TC concentration were 300 times higher than national standard	TC: ≤1000 CFU/ml	Did not meet national standard (World Bank, 2020)

**Table 7**  
Risk of Bias (ROB).

Study	Sampling						Treatment				Efficacy					
	Location	Dates	Frequency	Sampling point	Tested sample	Lab method	Treatment Type	Treatment plant	Treatment process	Treatment scale	AMR	ARB and ARGs	Microorganisms	Reduction	Standard	Reported treatment efficacy
Parashar 2022	L	L	U	L	L	L	MH	L	L	LM	L	H	L	L	H	LM
Baghal Asghari 2021	L	LM	L	MH	H	L	LM	H	L	MH	H	L	L	L	L	L
Ge 2021	L	L	U	L	L	L	H	H	L	MH	H	H	L	H		LM
Rashid 2021	L	L	L	H	H	L	MH	H	L	H	H	H	U	H		L
Zagui 2021	L	LM	U	H	H	L	LM	H	L	LM	L	L		H		U
Cevik 2020	L	L	L	L	H	L	LM	H	L	L	H	H	U	H		U
Khan 2020	L	L	L	L	H	L	MH	H	L	LM	H	H		H		LM
Nonfodji 2020	L	L	L	H	L	L	LM	H	L	H	H	H	L	LM	H	LM
Teshome 2020	L	L	U	L	L	L	MH	L	L	L	H	H	L	H		U
Wang 2020	L	MH	H	L	H	L	MH	H	L	LM	H	L	H	LM	L	LM
Adeolu 2019	L	H	H	H	H	L	MH	H	L	H	H	H	H	H		L
Fitriany 2019	U	L	L	U	H	H	H	H	L	L	H	H	H	H		L
Khan 2019	U	H	H	MH	H	L	LM	H	L	H	H	L	H	L	H	H
Kristanto 2019	L	LM	L	L	L	L	L	H	L	L	L	H	L	H	L	U
Nguyen 2019	U	H	H	H	H	L	LM	H	L	H	H	H	H	H		LM
Oliveira 2019	H	H	H	H	H	L	LM	H	L	H	H	H	L	LM	H	LM
Singh 2019	L	U	H	MH	H	L	LM	H	L	H	H	H	L	L	H	LM
Thanh 2019	L	U	L	L	L	L	LM	H	L	MH	H	H	L	L	L	LM
Dires 2018	L	L	L	MH	L	L	MH	L	L	MH	H	H	L	L	H	LM
Tulashie 2018	L	U	L	U	H	L	LM	H	L	H	H	H	L	L	L	LM
Zheng 2018	L	U	H	H	H	L	LM	H	L	H	H	L	H	LM	U	LM
Lien 2017	U	L	L	L	L	L		H	L	L	L	L	L	H	MH	U
Kalaiselvi 2016	L	L	U	L	L	L	MH	L	L	L	H	H	L	H	H	U
Vo 2016	L	H	H	L	H	L	MH	H	L	L	H	H	L	H		H
Akiba 2015	L	L	U	L	H	L	MH	H	L	L	H	H	L	U	L	U
Guruge 2015	L	LM	H	L	L	L	L	L	L	L	H	H	H	H	H	H
Miranda 2015	L	L	U	L	H	L	L	L	L	L	L	L	H	U		U
Santoro 2015	L	L	H	H	L	L	L	L	L	MH	L	H	L	U	H	U
Sozzi 2015	H	H	U	H	H	L	LM	L	L	MH	H	H	L	L	L	LM
Kist 2013	L	U	H	H	L	L	MH	H	L	MH	H	H	L	L	H	L
Picão 2013	L	U	H	H	L	L	MH	L	L	L	L	H	L	H	H	U
Chagas 2011	U	L	U	H	L	L	MH	L	L	L	L	H	L	U	H	H
Gupta 2009	U	L	U	H	L	L	H	H	L	MH	H	H	L	U		U
Rahman 2009	L	L	U	H	L	L	MH	H	L	L	L	L	L	H		U
Duong 2008	U	L	U	H	L	L	MH	L	L	L	L	H	L	U	L	U
Rahman 2007	L	L	U	H	L	L		H	L	MH	H	H	L	H		

The evaluation employs color coding: a red color denotes a 'H' classification for "high" risk of bias, 'MH' represents "medium-high" risk of bias, a green shade corresponds to 'LM' indicating "low-medium" risk of bias, 'L' signifies "low risk" of bias, and yellow color represented 'U' for "unclear" risk of bias.

be utilized in the future environmental review studies. There are also limitations to the review process. Due to the absence of standardized guidelines and regulatory frameworks in low and middle-income countries, microbial removal and treatment efficacy were assessed by comparing influent and effluent parameters with national standards, while the lack of reporting standards necessitated additional searches in various databases, which prolonged the review timeframe.

Most studies lack robust methodologies, with few exploring seasonality and using systematic sampling approaches. These limitations may lead to incomplete or misleading conclusions. Most studies did not estimate the sample size needed for treatment efficacy assessment, affecting statistical power. Few studies collected enough wastewater samples, with <100 collected, potentially resulting in biased or unreliable estimates. Developed countries invest in research and infrastructure

for HWW treatment, utilizing advanced technologies and methodologies. They have a dedicated sewer network and a full-scale treatment plant, allowing researchers and evaluators to select appropriate sampling points and compare efficacy between treatment plants. Studies in developed countries have implemented on-site wastewater treatments to improve treatment effectiveness and reduce contamination (Majumder et al., 2021). This proactive approach enhances HWW treatment efficiency, but LMICs need more rigorous methodologies and adequate sample sizes for effective assessment. Future studies should pay attention to these aspects to improve the accuracy and reliability of the results and provide more robust evidence to support decision-making in wastewater treatment in LMICs.

## 5. Conclusion and recommendations

The current review stands out as the first systematic review to explore the status of HWWT plants and their microbial efficacy in LMICs. This systematic review reveals a concerning lack of research on HWW in LMICs, with most studies conducted in high HDI countries and very few in GNI-classified low-income countries. Among the studies, the selected treatment processes varied widely, with most studies using conventional basic treatment plants (i.e., CW and AS). Only a limited number of studies in LMICs have utilized advanced technologies such as MBRs, O<sub>3</sub>, and UV treatment for HWW treatment plants. Our review revealed that when MBR is combined with nanofiltration and/or ozonation, it has demonstrated the highest removal efficiency among the treatment processes. The combination of various treatment processes with conventional WWTPs has shown promise in achieving significant microbial removal in both developing and developed countries when treating HWW. Therefore, further research is crucial to explore and understand suitable additional technologies that can complement existing treatment processes in LMICs.

Overall, there was a lack of consensus on the standards used to evaluate microbial treatment efficacy, with different studies using different guidelines and criteria. The choice of microorganism and effluent standards used to assess the HWW treatment plant efficacy also varied widely across the reviewed studies, and limits the comparability and generalizability of the findings across studies. While most studies explored the use of AMR/ARG and indicator microorganisms, such as *E. coli* and fecal coliforms, only a few studies assessed the removal efficiency of specific pathogens.

The review highlights the need for more research on the most efficient treatment plants and processes for HWW in LMICs, especially in the least developed countries, where the need for effective wastewater treatment is likely to be the greatest. This systematic review highlights the urgent need for more research on effective and efficient and on-site full scale HWW treatment technologies in LMICs, particularly in the least developed countries, including with regards to use of innovative technologies for the treatment of HWW in LMICs to improve the quality of effluent and protect public health and the environment. It is recommended that future studies should include larger sample sizes with appropriate sample size calculations for laboratory analysis, and standardized parameters for assessing microbial treatment efficacy. Governments health officials, policymakers, sanitation practitioners, and international organizations should prioritize funding for HWW treatment plant in LMICs to address this significant public health and environmental issue.

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### CRedit authorship contribution statement

**Nuhu Amin:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Tim Foster:** Conceptualization, Methodology, Writing – review & editing. **Nafeya Tabassum Shimki:** Data curation, Methodology, Writing – review & editing. **Juliet Willetts:** Conceptualization, Methodology, Supervision, Writing – review & editing.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author (NA) utilized Grammarly, QuillBot, and OpenAI's ChatGPT software to check grammar and correct sentences. Additionally, the author employed the iThenticate plagiarism detection service to identify and prevent any instances of plagiarism within the manuscript. Following the use of these tools and services, the author thoroughly reviewed and edited the content as necessary, assuming full responsibility for the publication's content.

## Appendices. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.170994>.

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