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Wastewater-based epidemiology of *Campylobacter* spp.: A systematic review and meta-analysis of influent, effluent, and removal of wastewater treatment plants

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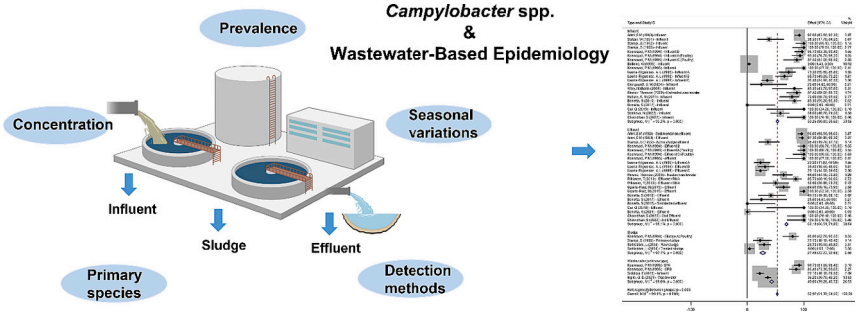
HIGHLIGHTS

- Meta-analysis for the prevalence, concentration, and decay of *Campylobacter* spp. in wastewater.
- The prevalence is 53.26 % and 69.18 % in influent and effluent wastewater.
- *Campylobacter* concentration in influent and effluent is 3.31 and 2.22 log10 GC or MPN/100 mL.
- qPCR-based methods showed the highest sensitivity and thus are recommended for its use in WBE.
- *Campylobacter jejuni* was identified as the most prevalent species (62.34 %) in wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Campylobacter spp. is one of the four leading causes of diarrhoeal diseases worldwide, which are generally mild but can be fatal in children, the elderly, and immunosuppressed persons. The existing disease surveillance for *Campylobacter* infections is usually based on untimely clinical reports. Wastewater surveillance or wastewater-based epidemiology (WBE) has been developed for the early warning of disease outbreaks and the detection of the emerging new variants of human pathogens, especially after the global pandemic of COVID-19. However, the WBE monitoring of *Campylobacter* infections in communities is rare due to a few large data gaps. This study is a meta-analysis and systematic review of the prevalence of *Campylobacter* spp. in various wastewater samples, primarily the influent of wastewater treatment plants. The results showed that the overall prevalence of *Campylobacter* spp. was 53.26 % in influent wastewater and 52.97 % in all types of wastewater samples. The

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mean concentration in the influent was 3.31 ± 0.39 log10 gene copies or most probable number (MPN) per 100 mL. The detection method combining culture and PCR yielded the highest positive rate of 90.86 %, while RT-qPCR and qPCR were the two most frequently used quantification methods. In addition, the *Campylobacter* concentration in influent wastewater showed a seasonal fluctuation, with the highest concentration in the autumn at 3.46 ± 0.41 log10 gene copies or MPN per 100 mL. Based on the isolates of all positive samples, *Campylobacter jejuni* (62.34 %) was identified as the most prevalent species in wastewater, followed by *Campylobacter coli* (30.85 %) and *Campylobacter lari* (4.4 %). These findings provided significant data to further develop and optimize the wastewater surveillance of *Campylobacter* spp. infections. In addition, large data gaps were found in the decay of *Campylobacter* spp. in wastewater, indicating insufficient research on the persistence of *Campylobacter* spp. in wastewater.

1. Introduction

Thermotolerant *Campylobacter*, as one of the leading pathogens causing bacterial gastroenteritis, causes great public concern worldwide (European Food Safety et al., 2019; WHO, 2022). Among the 13 pathogenic *Campylobacter* spp. known to be related to human infections, *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are the top two species that are responsible for >95 % of infections worldwide (Cribb et al., 2022; Igwaran and Okoh, 2019; Zhang et al., 2021). Before the COVID-19 pandemic, the incidence and cases of campylobacteriosis reported in developed countries had been consistently high, while some countries, such as France and Japan, reported an increasing trend (Liu et al., 2022). Clinical surveillance and monitoring of *Campylobacter* spp. are essential tools for minimising the extent of the disease outbreak. However, clinical testing is often limited to individuals seeking treatment, resulting in under-reporting of disease prevalence and untimely indicators of community outbreaks (Van and Hochberg, 2017). Since the first outbreak of COVID-19 in 2019, wastewater-based epidemiology (WBE) has received much attention for its successful applications in monitoring and providing early warning of emerging outbreaks (Abdeldayem et al., 2022; Anand et al., 2022; Zahedi et al., 2021). WBE studies have been often reported towards various viral (e.g., severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and norovirus) and protozoa (e.g., *Cryptosporidium* spp. and *Giardia* spp.) (Chacón et al., 2021; Hemalatha et al., 2021; Zahedi et al., 2021). However, very limited reports exist for the WBE studies of *Campylobacter* spp.

Previous studies have confirmed that obvious concentration increases of target pathogens could be detected one to two weeks before the disease outbreak in communities. Our recent study also detected a concentration increase of *Campylobacter* in wastewater two weeks earlier than a clinically reported disease outbreak caused by *Campylobacter* infection (Zhang et al., 2023a). This study indicated that, although the onset time of *Campylobacter* infection is only 2–5 days, semiweekly sampling surveillance can achieve early detection of *Campylobacter* concentration augment in wastewater before the patient goes to the hospital for treatment. In addition, one global epidemiology of campylobacteriosis study has shown that, in most countries for which epidemiological data were available for 2020, the COVID-19 pandemic has reduced the reported incidence of campylobacteriosis, which further reveals the urgent demand for the improvement of existing fragile and case report-dependent clinical surveillance of *Campylobacter* infection (Liu et al., 2022). WBE-based *Campylobacter* surveillance can be used as a reference and a replacement under the pandemic situation for clinical records to correct its disease prevalence estimation and support future surveillance. Furthermore, WBE-based *Campylobacter* surveillance could be an indirect, cost-effective, and macroscopical reflection of the *Campylobacter* contamination situation in the whole community environment. Since the detected wastewater can contain pathogens from various sources, including contaminated food in addition to the shedding from patients, thus could reflect the comprehensive risk of *Campylobacter* contamination within the communities covered by wastewater treatment plants, which might contribute to the WBE-based early warning for infection outbreaks. Therefore, WBE-based *Campylobacter* surveillance might be an ideal and low-cost approach to help both developed and developing countries achieve more comprehensive *Campylobacter* infection monitoring. Although the infectious risk of *Campylobacter* is not as essential as SARS-CoV-2, WBE-based *Campylobacter* surveillance is still a significant and cost-effective approach to improve the existing surveillance system of *Campylobacter* infection, especially for developing countries lacking the systematic clinical surveillance. By mapping the spatial and seasonal variation of *Campylobacter* concentration, it is reasonable to believe that the abnormal augment of *Campylobacter* (compared to the previous data at the same season and site) in wastewater can represent a potential disease outbreak. In addition, once the WBE-based *Campylobacter* surveillance is established, it can act as a surveillance model of other foodborne bacterial pathogens, which also have low infection does and high health risks. However, further studies and wastewater data are needed to draw the optimal surveillance scheme.

The WBE back-estimation of SARS-CoV-2 prevalence in communities and the artificial neural network-based estimation of COVID-19 case numbers have been established in previous studies and have been successfully deployed in the monitoring of other enteric viruses towards various environmental water samples (Guo et al., 2022b; Jiang et al., 2022; Li et al., 2021; Miao et al., 2022). Parameters, including the viral RNA concentration in wastewater (C_{RNA}) and the air and wastewater temperature, have been identified as essential factors that can induce significant variances in the WBE back-estimation and the prevalence prediction. The recovery efficiency of detection methods and the in-sewer decay of viruses are also important, although their contributions to the overall variances in the WBE back-estimation and the prevalence prediction have not been determined. The extension of these new advancements in WBE to the wastewater surveillance of bacterial pathogens such as *Campylobacter* spp. has not been assessed yet. There is a lack of systematic understanding of *Campylobacter* spp. in terms of the prevalence, concentration, and persistence of *Campylobacter* spp. in wastewater matrices (Murphy, 2017). Most previous studies for *Campylobacter* spp. in wastewater focused on the removal efficiency of wastewater treatment plants and the environmental transmission risk of the treated effluent and its use for irrigation (Farhadkhani et al., 2020; Strakova et al., 2022). To date, no report has systematically reviewed previous studies to summarize wastewater-related parameters of *Campylobacter* spp. for supporting its WBE applications.

This study conducted a systematic review and meta-analysis of the *Campylobacter* spp. prevalence and concentrations in various wastewater samples, such as the influent, effluent, and sludge, obtained in wastewater treatment plants (WWTPs) worldwide. Data analysis of *Campylobacter* spp. prevalence and concentration, considering the detection methods, the sampling seasons, and the dominating species in wastewater, were carried out to provide a comprehensive understanding of the wastewater data of *Campylobacter* spp. The findings of this study can be utilized in further evaluation and application of the established WBE approaches for the wastewater surveillance of *Campylobacter* spp. The estimated *Campylobacter* concentration in influent wastewater of this study further supported the parameter sensitivity evaluation of WBE-based back-estimation of *Campylobacter* prevalence in communities (Zhang et al., 2023b).

Table 1
Information of the 28 studies identified for investigating the prevalence of *Campylobacter* spp. in different types of wastewater samples.

Country	Sample type	Concentration (log_{10} GC or MPN/100 mL)	Positive ratio	WWTP capacity (Population equivalents, PE) or flow (m^3/day)	WWTP removal efficiency (%)	Detection method	Sampling period	Species	Reference
	Inflow of sewage	3.57	–	–	99.5	Culture	July 1985–July 1986	Distinctly more *C. coli* than *C. jejuni* were isolated	(Höller, 1988)
Germany	Raw sewage A	3.02	5/13 (38.5 %)	WWTP A: 30,000 m^3/day	Plant A: 88	Culture	–	Sewage treatment plant A: *C. jejuni* 83.3 % (165/198) *C. coli* 16.7 % (33/198)	(Stelzer and Jacob, 1991)
	Effluent of activated sludge tank A	1.76	–						
	Effluent A	2.11	–						
	Raw sewage B	1.71	–						
	Effluent after first oxidation pond B	0	–	WWTP B: 1100 m^3/day	Plant B: 100				
	Effluent B	0	–						
British	Incoming sewage	3.55 ± 0.32	69/75 (92 %)	–	99.9	Culture	Autumn (Sep)	*C. jejuni* (68.4 %), Unknown (31.6 %)	(Arimi et al., 1988)
	Primary sedimentation effluent	3.26 ± 0.19	72/75 (96 %)						
	Final effluent	0.87 ± 0.24	73/75 (97.3 %)						
Italy	Incoming sewage	3.21 (3.03 ± 0.44)	22/22 (100 %)	–	100	Culture	June 1990–June 1991	*C. jejuni* 66 %; *C. coli* 34 %	(Stampi et al., 1992)
	Active sludge tank effluent	1.4 ± 0.57	8/22 (36.4 %)					*C. jejuni* 30.3 %; *C. coli* 69.7 %	
	Incoming sewage	3.24 ± 0.43	15/15 (100 %)	500,000 PE	100	Culture	February 1991–February 1991	*C. jejuni* 70.6 % (24/34) *C. coli* 29.4 % (10/34)	(Stampi et al., 1993)
	Domestic sewage	–	6/192 (3.1 %)	–	–	Culture	1985–1992	*C. jejuni* 16.7 % (1/6) *C. coli* 83.3 % (5/6)	(Baffone et al., 1995)
	Primary sludge	*C. jejuni* 2.44 log10 MPN/g *C. coli* 3.15 log10 MPN/g	5/22 (22.7 %)	600,000 PE	99.8 % (total coliforms)	Culture	One year	–	(Stampi et al., 1999)
	Influents (24 composite)	–	10/12 (83.3 %)	3 WWTPs: 2500,000 PE; 60,000 PE; 8000 PE	–	PCR	Spring—May 2014, Summer—July 2014, Autumn—October 2014 and Winter—February 2015	*C. jejuni* 60 % (6/10) *C. coli* 30 % (3/10) *C. jejuni* 20 % (1/5) *C. coli* 60 % (3/5)	(Bonetta et al., 2016)
	Effluent (24 composite)		5/12 (41.7 %)						
	Influent (24 composite)	–	0/4 (0 %)	60,000 PE	–	PCR	Summer–July 2015; Autumn–November 2015; Winter–January 2016; Spring–April 2016	*C. coli* 100 % (1/1)	(Bonetta et al., 2017)
	Effluent (24 composite)		1/4 (25 %) (Autumn)						
	Disinfected effluent (24 composite)		0/4 (0 %)						
	Effluent	–	0/18 (0 %)	3 WWTPs: 34,000 PE; 8000 PE; and 8000 PE		PCR	September 2017, November 2017, January 2018, March 2018, May 2018 and July 2018	–	(Bonetta et al., 2021)
	Influent A (poultry)	3 (3.1 ± 0.77)	28/30 (93.3 %)	46,000 PE	1 log10 reduction	Culture	April 1991–April 1993	–	(Koenraad et al., 1994)
	Effluent A	2 (2.16 ± 0.52)	30/30 (100 %)						
	Sedimented sludge	1.5–4.4	24/30 (80 %)						
	Influent B	2.3 (2.38 ± 0.73)	29/30 (96.7 %)	130,000 PE	0.6 log10 reduction				
	Effluent B	<1.5 (1.58 ± 0.09)	30/30 (100 %)						

(*continued on next page*)

Table 1 (*continued*)

Country	Sample type	Concentration (log_{10} GC or MPN/100 mL)	Positive ratio	WWTP capacity (Population equivalents, PE) or flow (m^3/day)	WWTP removal efficiency (%)	Detection method	Sampling period	Species	Reference
	Influent C (poultry)	2.6 (2.49 ± 0.54)	14/ 16 (87.5 %)	280,000 PE	–		February 1992–April 1993		
	Effluent C	2 (1.95 ± 0.43)	16/16 (100 %)						
Netherlands	Influent	–	8/8 (100 %)	30,000 PE	–	Culture and PCR	26 May - 28 July 1993	–	(Koenraad et al., 1995a)
	Effluent	–	8/8 (100 %)		–				
	SPA (poultry abattoir; industrial wastewater)	–	55/60 (91.7 %)	60,000 PE	–	Culture and PCR	One year	*C. jejuni* 80 % (44/55) *C. coli* 20 % (11/55)	(Koenraad et al., 1995b)
	SPB (industrial wastewater)	–	38/44 (86.4 %)	130,000 PE	–			*C. jejuni* 78.9 % (30/38) *C. coli* 21.1 % (8/38)	
Brazil-South America	Influent A	–	22/30 (73.3 %)	700,000 PE	–	Culture	May 1990–May 1991	*C. jejuni* 36.4 % (8/22); *C. coli* 36.4 % (8/22) Un-typable 27.2 %(6/22)	(Lauria-Filgueiras and Hofer, 1998)
	Effluent A		7/30 (23.3 %)					*C. jejuni* 0 % (0/7); *C. coli* 28.6 % (2/7); Un-typable 71.4 % (5/7)	
	Influent B		23/35 (65.7 %)	90,000 PE			September 1990–May 1991	*C. jejuni* 47.8 %(11/23); *C. coli* 26.1 % (6/23); *C. lari* 4.3 % (1/23); Untypable 21.7 %(5/23)	
	Effluent B		11/35 (31.4 %)					*C. jejuni* 27.3 % (3/11); *C. coli* 18.2 % (2/11); Un-typable 54.5 %(6/11)	
	Influent C		23/65 (35.4 %)	–			February 1990–November 1991	*C. jejuni* 56.5 % (12/23); *C. coli* 21.7 % (5/23); Un-typable 21.7 % (5/23)	
	Effluent C		15/65 (23.1 %)					*C. jejuni* 33.3 %(5/15); *C. coli* 13.3 % (2/15); Un-typable 53.3 %(8/15)	
South Africa	Raw sewage	3	1/4 (25 %)	–	–	Culture	–	–	(Diergaardt et al., 2004)
India	Sewage	2.16 ± 0.51	–	–	–	Culture	–	–	(Baserisalehi et al., 2004)
	Influent	3.71 ± 0.39	9/9 (100 %)	3 WWTPs: 9000–13,000 PE	–	qPCR	August 24, 2021, August 31, 2021, and September 7, 2021 (Autumn)	Only targeting *C. coli* 100 % (9/9)	(Chowdhari et al., 2022)
	Secondary effluent	2.53 ± 0.35	9/9 (100 %)						
	Tertiary effluent	2.33 ± 0.35	9/9 (100 %)						
Sweden	Raw sludge	–	19/64 (29.7 %)	8 WWTPs: 3000–200,000 PE	–	Culture	July 2000–June 2001	*C. jejuni* 68.4 % (13/19) *C. coli* 31.6 % (6/19)	(Sahlström et al., 2004)
	Treated sludge		3/69 (4 %)					*C. jejuni* 66.7 % (2/3) *C. coli* 33.3 % (1/3)	
France	Wastewater (24 composite)	4.22 ± 0.92	5/6 (83.3 %)	13, 000 m^3/day	–	qPCR	Dry weather	–	(Wery et al., 2008)

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Table 1 (*continued*)

Country	Sample type	Concentration (log_{10} GC or MPN/100 mL)	Positive ratio	WWTP capacity (Population equivalents, PE) or flow (m^3/day)	WWTP removal efficiency (%)	Detection method	Sampling period	Species	Reference
Switzerland	Influent (24 composite)	4.65 ± 0.59	21/24 (87.5 %)	23 WWTPs	–	qPCR	–	–	(Rinsoz et al., 2009)
	Effluent (24 composite)	3.28 ± 0.18	16/25(64 %)						
Northwest Florida, USA	Raw sewage	3.13 ± 1.12	15/19 (79 %)	19 sites	–	qPCR	July 2008–September 2009	*C. jejuni* 26.7 % (4/15) *C. coli* 13.3 % (2/15) *C.* spp. 60 % (9/15)	(Hellein et al., 2011)
Finland	Effluent	4.54 ± 1.07	12/14 (85.7 %)	10 WWTPs	–	RT-qPCR	April, May, and October 2012	*C. jejuni* 100 %	(Pitkänen et al., 2013)
	Effluent	3.56 ± 0.36	7/14 (50 %)			qPCR			
Pakistan	Wastewater	–	32/145 (22 %)	–	–	PCR	–	–	(Siddiqui et al., 2015)
Spain	Effluent	–	32/50 (64 %)	–	–	Culture	November 2010–November 2013	*C. jejuni* 27.4 % (20/73)	(Ugarte-Ruiz et al., 2015)
			50/50 (100 %)			qPCR		*C. coli* 72.6 % (53/73)	
Australia	Influent-WWTP1	1.85	–	1500 PE	–	Culture	October 2013; March, May, July and September 2014	–	(Sheludchenko et al., 2016)
	Effluent-WWTP1	0.82							
	Influent-WWTP2	–		1500 PE					
	Effluent-WWTP2	–							
	Influent-WWTP3	3.98		1000–2500 PE			October 2013 and September 2014		
	Effluent-WWTP3	3.9							
China	Sewage	4.37 ± 0.14	2/2 (100 %)	2 WWTPs: 15,000 m^3/day; 15,0000 m^3/day	–	qPCR	10:00 a.m. and 16:00 p.m. on 18 October 2016 (Autumn)	*C. jejuni* 100 % (4/4)	(Cui et al., 2019)
	Sewage effluent	0.41 ± 0.11	2/2 (100 %)						
Canada	Wastewater	–	96/265 (36.2 %)	2 WWTPs	–	PCR	One year (2008–2009)	Only targeting *C. jejuni*	(Inglis et al., 2021)
Czech Republic-Europe.	Influent	–	17/29 (58.6 %)	–	–	Multiplex PCR	April 2018–November 2019	*C. jejuni* 58.8 % (10/17); *C. coli* 88.2 % (15/17); *C. jejuni* & *C. coli* 41.2 % (7/17)	(Strakova et al., 2022)

2. Methods

2.1. Search strategy

A comprehensive search on the presence and decay of *Campylobacter* spp. in wastewater was conducted in three electronic international databases: PubMed, Scopus, and Web of Science, following the guidelines for the Preferred Reporting Project for Systematic Evaluation and Meta-Analysis (PRISMA). The following keywords were used to search the databases: "(*Campylobacter* spp. OR *Campylobacter*. jejuni OR *Campylobacter*. coli OR *Campylobacter*. lari) AND (prevalence OR occurrence OR detection OR quantification OR Decay) AND (Wastewater OR sewage OR influent)". Articles with any of these keywords in any field were included.

2.2. Data extraction

The country, the sample type (influent, effluent and sludge), the positive rate and concentration of *Campylobacter* spp., the detection methods and the identified species of each study were extracted from selected papers. The available sampling dates were also extracted for generating the analysis of seasonal distribution. Graphical data were extracted by using the GetData Graph Digitizer software. The 95 % confidence interval (CI) of the positive rate of each study was calculated by using an online calculator with the recommended Wilson method (https://epitools.ausvet.com.au/ciproportion).

2.3. Statistical data analysis

The extracted data were analyzed by using the Stata® 15.0 software (StataCorp LP., College Station, Texas, USA). The I^2 and Chi-square (p-value) tests were used to measure statistical heterogeneity. The heterogeneity identification requires $p < 0.05$ and $I^2 > 50$ %. Following the heterogeneity test, the prevalence and the concentration estimation were calculated using a random-effects model (Mohammadpour et al., 2018).

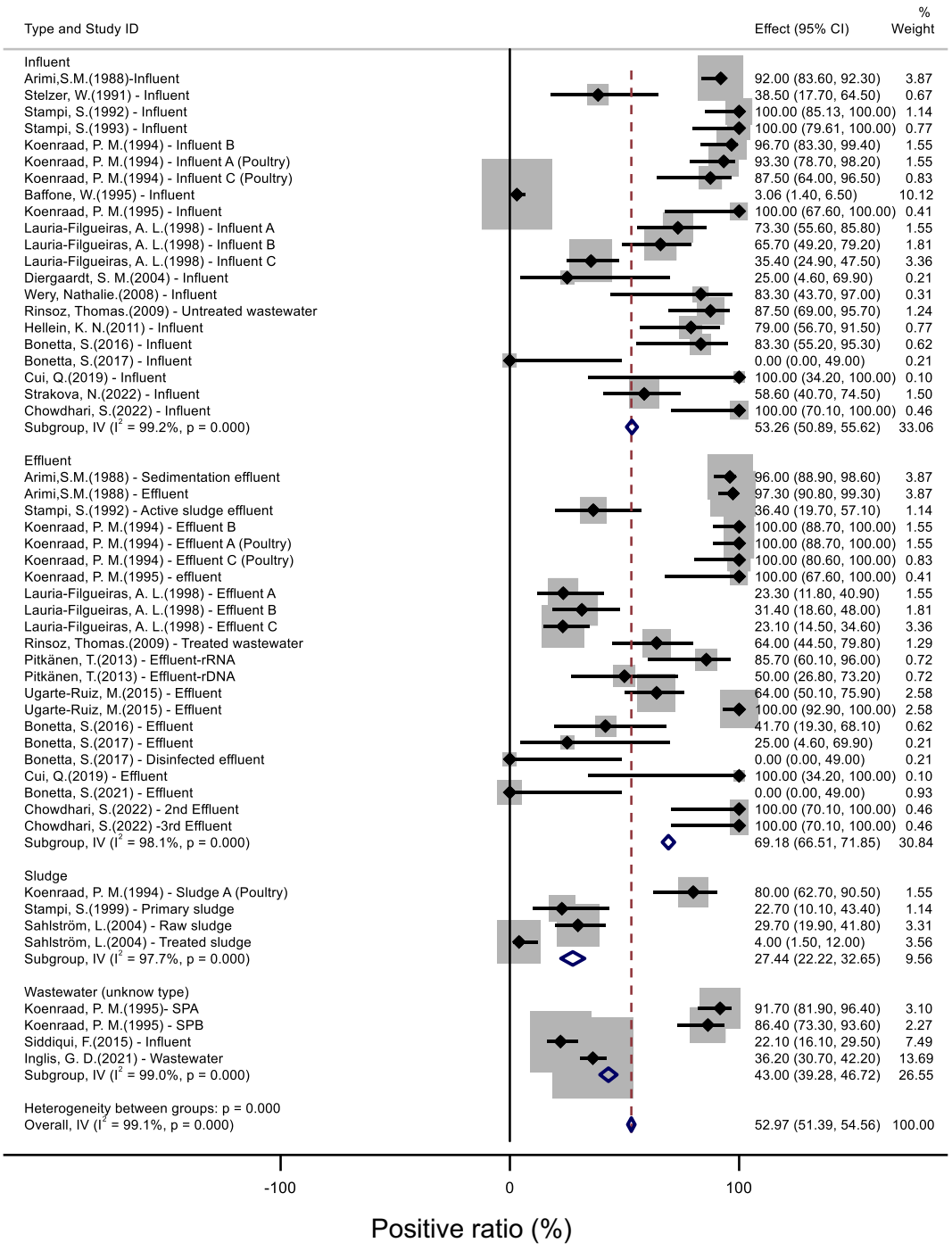

Fig. 1. Forest plot for the *Campylobacter* spp. prevalence (positive ratio, %) in different wastewater and sludge subgroups, including influent, effluent, unknown wastewater type, and sludge. The solid black diamonds and their whiskers represent the average positive rate of each study and their 95 % confidence interval. The size of the grey squares represents the weight of each study. The blue empty diamonds represent the overall estimated positive rate of all studies included and the estimated positive rates of each subgroup. The horizontal lateral tips of the empty diamonds represent the 95 % confidence interval of the estimated positive rate. The solid black vertical line represents the zero positive rate. The dashed red line also represents the overall estimated positive rate of this study. IV: Instrumental-Variable heterogeneity.

3. Results and discussion

3.1. Study selection and a summary of data

In total, 637 articles were selected and involved in the further selection. All involved articles were screened by the titles, abstracts, and full articles in turn. The flowchart of the study selection is shown in Fig. S1. Review articles, duplicated articles, and full-text unavailable articles were excluded. Finally, 29 articles were selected for data extraction. Twenty-eight articles investigated the prevalence of *Campylobacter* spp. in different types of wastewater samples, including influent and effluent wastewater and sludge samples collected in wastewater treatment plants. Only one paper investigated the decay of *Campylobacter* spp. in wastewater-seeded freshwater. Studies on wastewater from poultry and slaughterhouses were excluded since the aim of this study is to investigate the *Campylobacter* spp. prevalence in

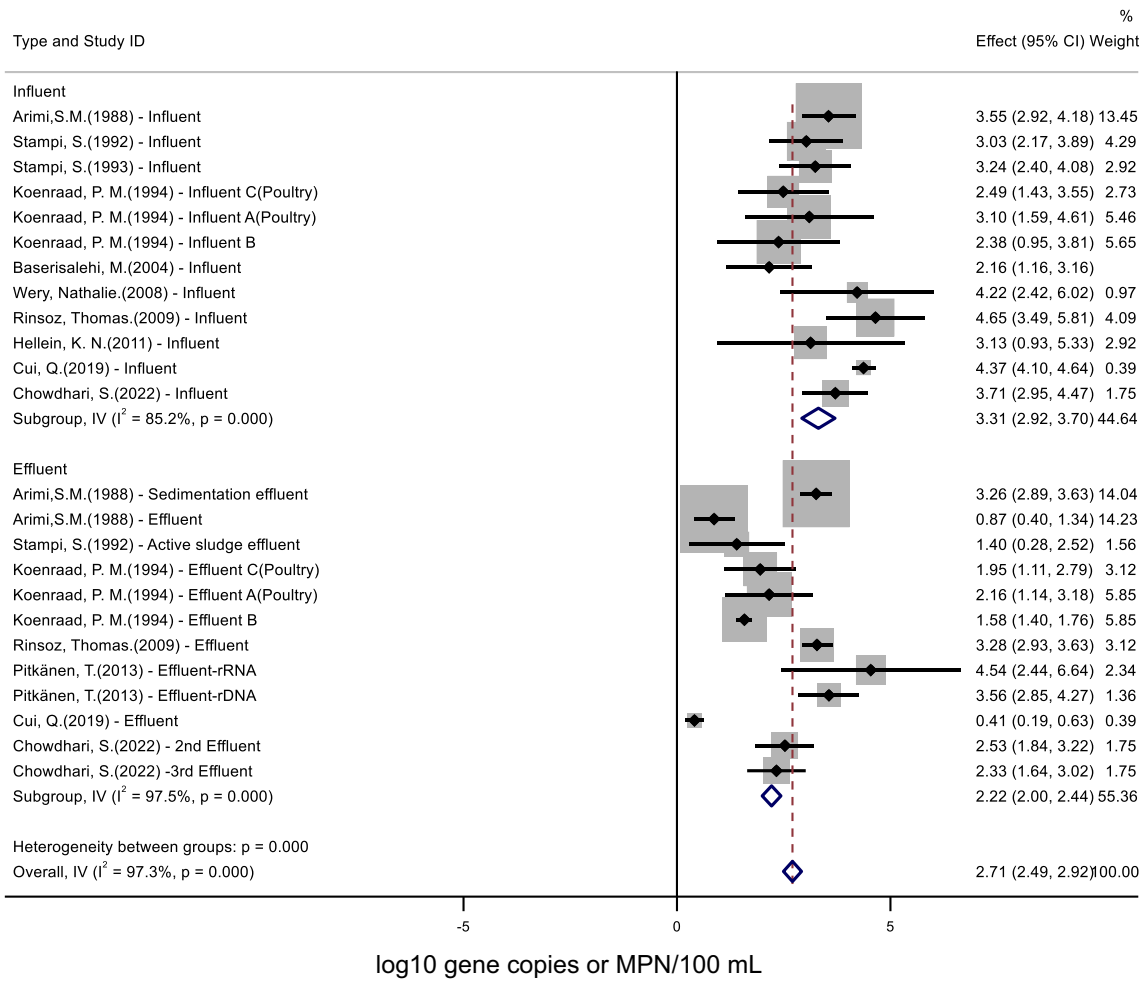

Fig. 2. Forest plot for the *Campylobacter* spp. concentration (log10 gene copies or MPN/100 mL) in different wastewater samples, including influent and effluent. The solid black diamonds and their whiskers represent the average concentration of each study and their 95 % confidence interval. The size of the grey squares represents the weight of each study. The blue empty diamonds represent the overall estimated concentration of all studies included and the estimated concentration of each subgroup. The horizontal lateral tips of the empty diamonds represent the 95 % confidence interval of the estimated concentration. The solid black vertical line represents negative results. The dashed red line also represents the overall estimated concentration of this study. IV: Instrumental-Variable heterogeneity.

wastewater to support the wastewater surveillance of *Campylobacter* infections in the community. Data extraction was carried out on the 28 articles that investigated *Campylobacter* spp. prevalence in wastewater. The information of each study, including the authors, country, positive rate (positive number/total number), mean concentration, sampling seasons, the capacity and removal efficiency of WWTPs, and the species classification, was extracted and listed in Table 1.

3.2. Overall Campylobacter spp. prevalence in different types of wastewater samples

Based on the meta-analysis of the 28 studies, the prevalence of *Campylobacter* spp. in all types of wastewater-relevant samples, as the positive ratio of the total sample number of each study, was calculated as 52.97 % (as 55.67 %, excluded sludge samples) (Fig. 1). For each type of sample, the prevalence of *Campylobacter* spp. in influent, effluent, unknown type wastewater and sludge was estimated at 53.26 %, 69.18 %, 43 %, and 27.44 %, respectively. According to the estimation, more than half of the influent wastewater was detected as positive for *Campylobacter* spp., which indicated a high prevalence of *Campylobacter* in raw wastewater. Interestingly, the results also showed a higher occurrence of *Campylobacter* in the effluent than influent, which might indicate a high risk of *Campylobacter* transmission from effluent wastewater to the environment (Farhadkhani et al., 2020). The low prevalence of *Campylobacter* in sludge may be due to the limited data available from only three studies.

The overall concentration of *Campylobacter* spp. in wastewater samples was estimated by weighting the positive sample numbers of each study and was calculated as 2.71 (95 % CI 2.49–2.92) log10 gene copies or cells per 100 mL (Fig. 2). The *Campylobacter* spp. concentration was 3.31 (95 % CI 2.92–3.7) log10 and 2.22 (95 % CI 2.00–2.44) log10 gene copies or cells per 100 mL in the influent and effluent wastewater, respectively. The results indicated a reduction of around 1.1 log10 gene copies or cells per 100 mL of *Campylobacter* spp. concentration through wastewater treatment, which is consistent with the removal efficiency of WWTPs (Arimi et al., 1988; Höller and Schomakers-Revaka, 1994). The *Campylobacter* concentration of sludge samples was reported between 1.5 and 4.4 log10 cells /100 mL (Koenraad et al., 1994) and 2.44–3.15 log10 MPN/g of *C. jejuni* and *C. coli* (Stampi et al., 1999). It is worth noting that, although a higher prevalence of *Campylobacter* spp. was reported in effluent samples than in influent and sludge samples, the concentration detected in the effluent was lower than in influent and sludge samples. This might be due to the higher likelihood of false-negative detection results caused by the more complex raw wastewater and sludge matrix. The higher positive ratio of effluent samples with an average concentration of 2.22 log10 gene copies or cells per 100 mL in this study indicated that a large volume of *Campylobacter*-positive treated wastewater was released into the environment, which might

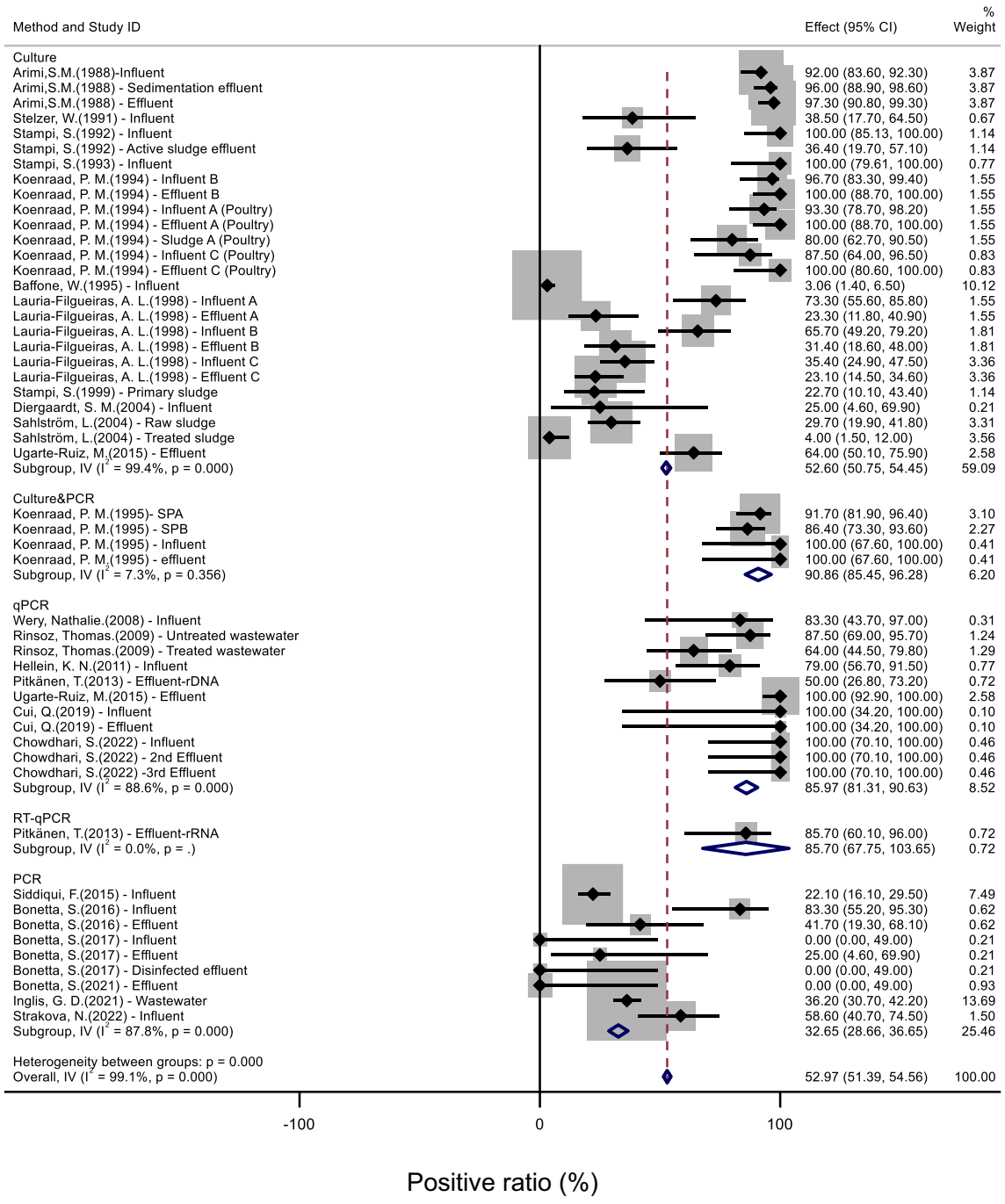

Fig. 3. Forest plot of *Campylobacter* spp. prevalence (positive ratio, %) in wastewater depending on the detection methods. The solid black diamonds and their whiskers represent the average positive rate of each study and their 95 % confidence interval. The size of the grey squares represents the weight of each study. The blue empty diamonds represent the overall estimated positive rate of all studies included and the estimated positive rates of each subgroup. The horizontal lateral tips of the empty diamonds represent the 95 % confidence interval of the estimated positive rate. The solid black vertical line represents the zero positive rate. The dashed red line also represents the overall estimated positive rate of this study. IV: Instrumental-Variable heterogeneity.

pose a high risk to public health.

3.3. *Campylobacter spp. prevalence and concentration depending on the detection methods*

Culture, qPCR, and PCR-based methods were the main detection methods that have been widely used to detect and identify *Campylobacter* spp. prevalence and concentration in wastewater samples. According to the meta-analysis results in Fig. 3, the method that combined the culture and PCR yielded the highest prevalence rate of *Campylobacter* spp. (90.86 %) in wastewater (Koenraad et al., 1995a; Koenraad et al., 1995b). However, considering the low heterogeneity (I^2 = 7.3 %) of this subgroup, more studies should be conducted to confirm this demonstration. For single detection methods, qPCR methods got a relatively higher estimated prevalence of 85.97 %, followed by RT-qPCR (85.7 %), culture (52.6 %) and PCR (32.65 %) methods. These results suggest that to avoid underestimating the prevalence of *Campylobacter* in wastewater, the combination of bacterial culture and species-specific gene detection should be adopted.

In addition, the detected *Campylobacter* spp. concentration by

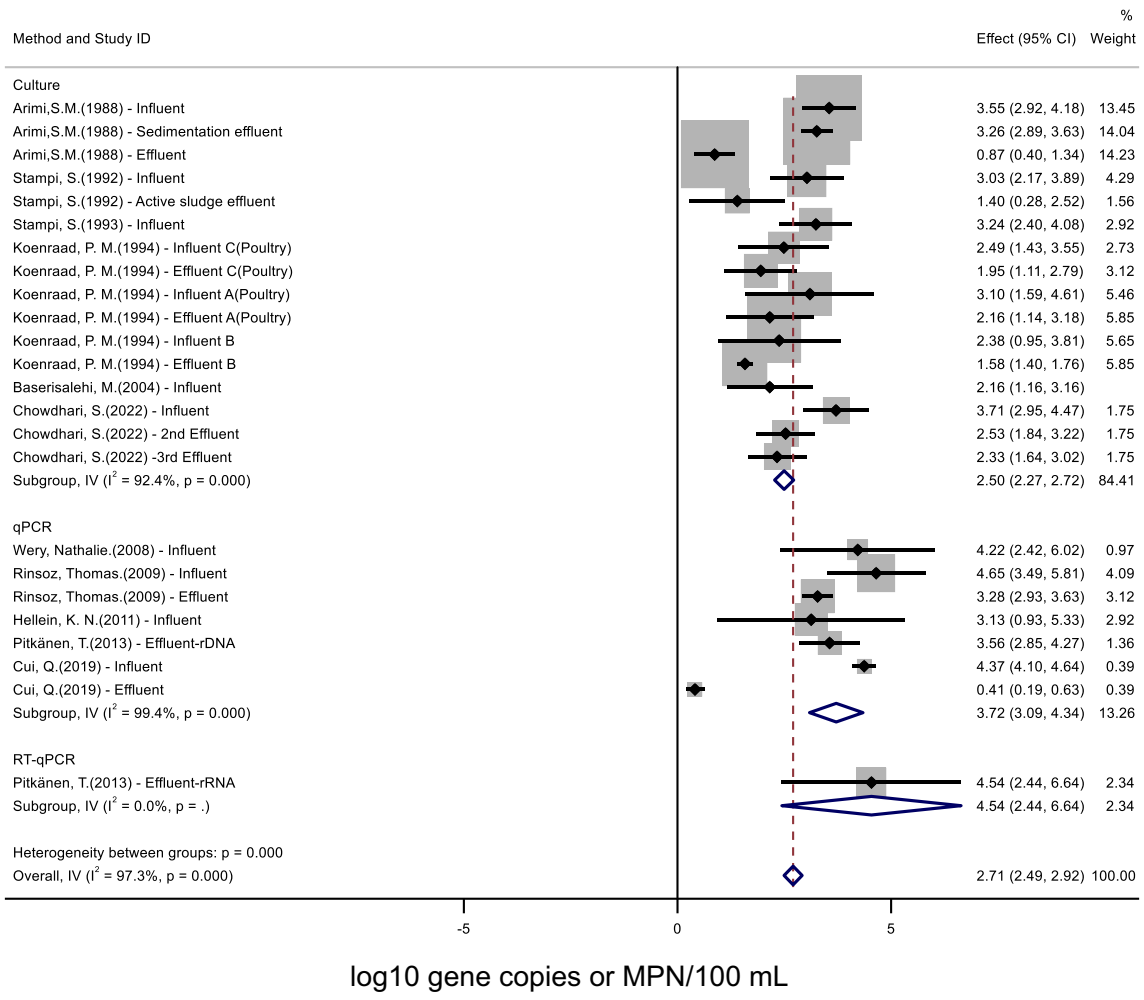

Fig. 4. Forest plot for meta-analysis of the overall and the subgroup *Campylobacter* spp. concentration (log10 gene copies or MPN/100 mL) in influent and effluent samples considering the detection methods. The solid black diamonds and their whiskers represent the mean concentration of each study and their 95 % confidence interval. The size of the grey squares represents the weight of each study. The blue empty diamonds represent the overall estimated concentration of all studies included and the estimated concentration of each subgroup. The horizontal lateral tips of the empty diamonds represent the 95 % confidence interval of the estimated concentration. The solid black vertical line represents negative results. The dashed red line also represents the overall estimated concentration of this study. The IV: Instrumental-Variable heterogeneity.

different methods was also analyzed, and the results were shown in Fig. 4. The *Campylobacter* spp. concentration in influent and effluent wastewater samples was pooled and weighted by the total positive sample numbers of each study. The PCR-based methods can only provide identification results rather than quantification results, thus were excluded from this analysis. qPCR-based methods yielded a higher measured concentration of *Campylobacter* spp. in wastewater samples of 3.72 (95 % CI 3.09–4.34) log10 gene copies /100 mL than culture-based methods at 2.5 (95 % CI 2.27–2.72) log10 MPN/100 mL. This is consistent with a higher estimated prevalence rate from the qPCR-based method than the culture-based method, suggesting that the culture-based method may lead to an underestimation of *Campylobacter* concentrations in wastewater. The qPCR-based method is thus recommended for the detection of *Campylobacter* in wastewater, especially in wastewater surveillance. The qPCR-based methods are more suitable for evaluating the *Campylobacter* spp. prevalence and concentration in wastewater samples. In addition, Pitkänen et al. (2013) reported that, compared to the rDNA-based qPCR method, using the rRNA-based RT-qPCR method significantly increased the detection sensitivity of *Campylobacter* spp. in environmental waters including wastewater effluents, and the detection result of the rRNA-based method was in better agreement with the culture-based method.

In terms of epidemiology and public health, the effluent from WWTPs also raises a high risk of further infections due to wastewater-contaminated environmental water and wastewater-irrigated vegetables (Chen et al., 2020; Moazeni et al., 2017). Therefore, the positive rate and concentration of effluent by different methods were further analyzed. The results are shown in Figs. S2 and S3. The culture-based method showed a prevalence of 68.69 % and an average concentration of 2.01 (95 % CI 1.78–2.24) log10 MPN/100 mL of alive *Campylobacter* in effluent. Although *Campylobacter* can only live for a short time in soil or on crops, considering the low infection dose (500 cells; 50 % infection dose, $\leq 10^2$ CFU) of *Campylobacter*, it is reasonable to claim that the *Campylobacter*-positive wastewater effluent confirmed by the culture-based method is a potential risk for public health through contaminated drinking water and food (Shuval and Fattal, 2003; Tribble David et al., 2010).

3.4. Seasonal variations of *Campylobacter* spp. concentration in influent wastewater

Campylobacter spp. concentration in influent wastewater in different seasons was further analyzed to map the seasonal variations of *Campylobacter* spp. prevalence and concentration in wastewater.

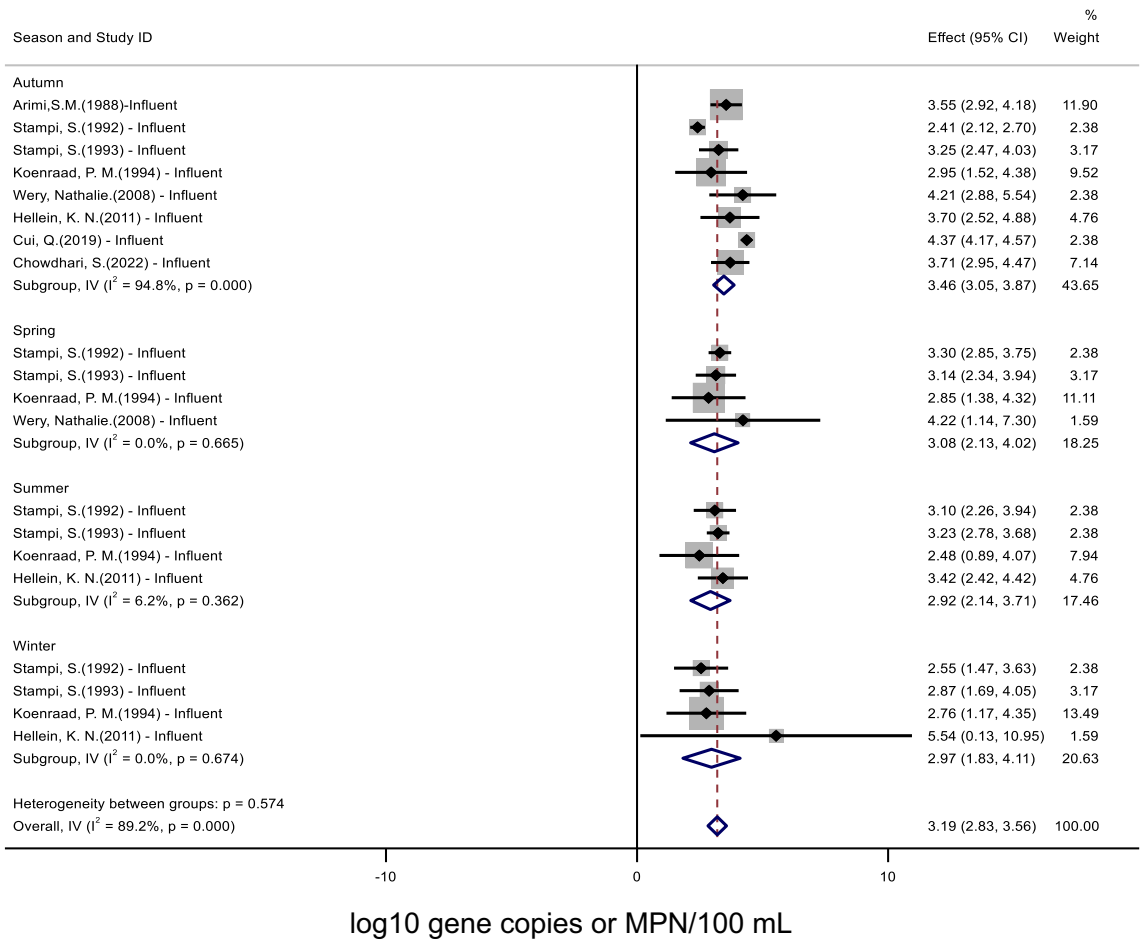

Fig. 5. Forest plot of the *Campylobacter* spp. concentration (log10 gene copies or MPN/100 mL) in influent wastewater depending on the sampling season. The solid black diamonds and their whiskers represent the mean concentration of each study and their 95 % confidence interval. The size of the grey squares represents the weight of each study. The blue empty diamonds represent the overall estimated concentration of all studies included and the estimated concentration of each subgroup. The horizontal lateral tips of the empty diamonds represent the 95 % confidence interval of the estimated concentration. The solid black vertical line represents negative results. The dashed red line also represents the overall estimated concentration of this study. IV: Instrumental-Variable heterogeneity.

Campylobacter spp. concentration in influent wastewater was pooled by considering the sampling seasons and was weighted by the positive sample numbers of each study in the meta-analysis. As shown in Fig. 5, the influent wastewater yielded the highest *Campylobacter* spp. concentration in autumn at 3.46 (95 % CI 3.05–3.87) log10 gene copies or MPN/100 mL, followed by spring, summer, and winter at 3.08 (95 % CI 2.13–4.02), 2.92 (95 % CI 2.14–3.71), and 2.97 (95 % CI 1.83–4.11) log10 gene copies or MPN/100 mL, respectively. This result is consistent with the previous study and indicates that there is a seasonal variation of *Campylobacter* spp. concentration in influent wastewater and the seasonal prevalence peak happened in autumn (Strakova et al., 2022). It is worth noting that, except for the results of the autumn season, the heterogeneity (I^2) of the other three seasons was all below 10 %. Thus, more data is needed to increase the reliability of this finding. In addition, this seasonal variation of wastewater concentration might be because of the seasonal *Campylobacteriosis* infection in communities. Lake et al. (2019) explored the *Campylobacter* seasonality across Europe between 2008 and 2016 by using The European Surveillance System (TESSy). According to their reports, seasonal *Campylobacteriosis* infection peak was found in mid- to late summer in most European countries, which is slightly earlier than the *Campylobacter* spp. concentration peak in influent wastewater identified in this meta-analysis.

3.5. Primary Campylobacter species in wastewater

To analyse the dominating prevalent *Campylobacter* species in wastewater samples, the prevalence rate of each species was pooled and weighted by the total number of *Campylobacter* spp. positive samples in each study. According to the meta-analysis results of the *Campylobacter* species in wastewater (Fig. 6), *C. jejuni* was the most prevalent species in all types of wastewater samples with a prevalence rate of 62.34 % (95 % CI 59.04 % – 65.64 %), followed by *C. coli* and *C. lari* of 30.85 % (95 % CI 27.6 % – 34.09 %) and 4.40 % (95 % CI -5.70 % – 14.50 %), respectively. However, since there was only one study that reported the prevalence of *C. lari*, and the heterogeneity (I^2) of this subgroup was zero, the prevalence of *C. lari* estimate in this study is not meaningful. In addition, since *C. jejuni* and *C. coli* were reported as the top two species that are associated with the most infections worldwide, most of the included studies only investigated these two species, especially the studies adopting the qPCR-based methods. Therefore, the identified dominating *Campylobacter* species in wastewater might be biased in this study.

3.6. Decay of Campylobacter spp. in wastewater

In this review, only one study investigated the decay of *Campylobacter* spp. in wastewater samples which is consistent with the report of the Global Water Pathogen Project in 2017 that there is a large knowledge gap in the persistence of *Campylobacter* spp. in wastewater

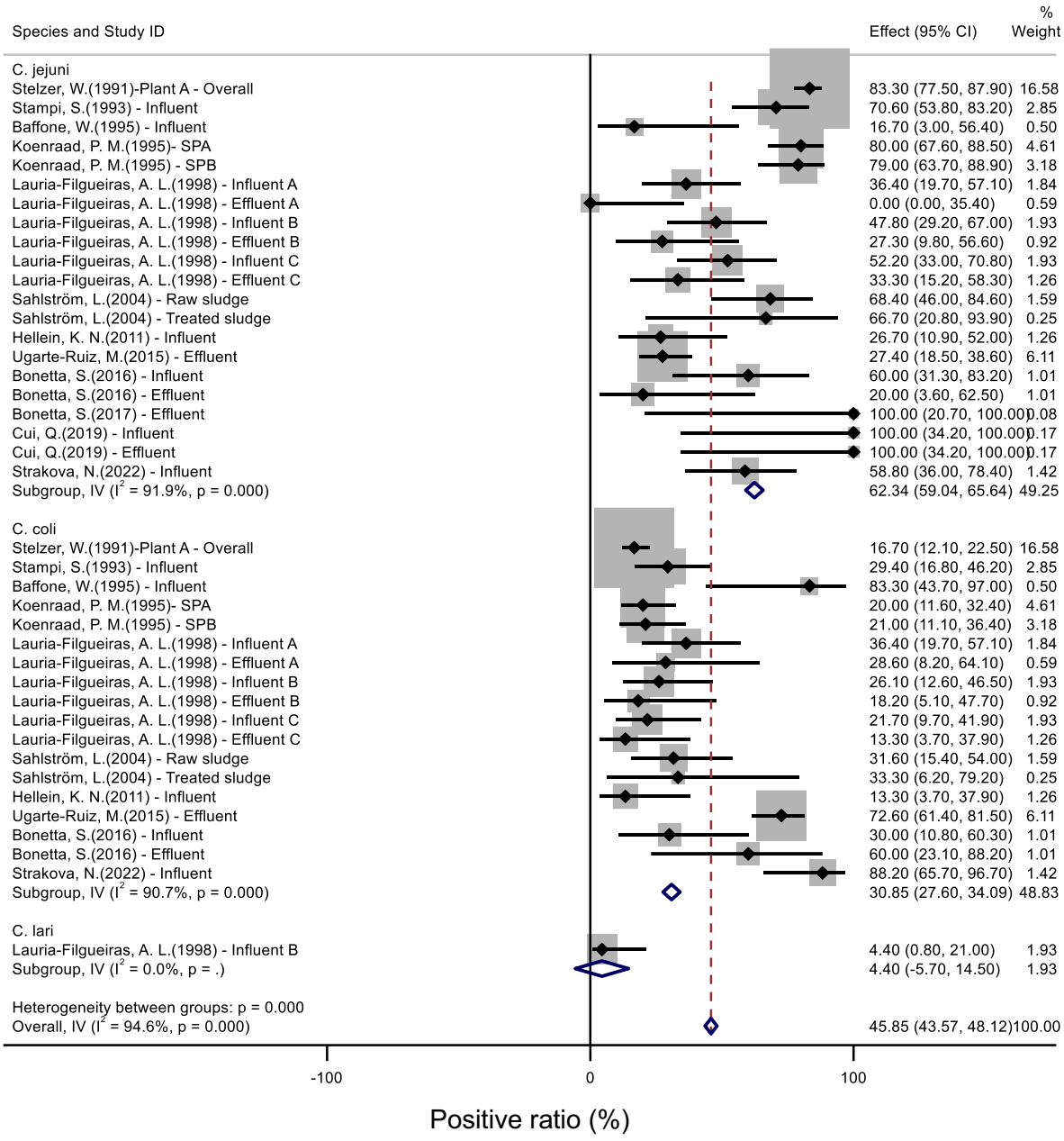

Fig. 6. Forest plot for meta-analysis of the *Campylobacter* spp. prevalence (positive ratio, %) in wastewater samples considering species. The solid black diamonds and their whiskers represent the average positive rate of each study and their 95 % confidence interval. The size of the grey squares represents the weight of each study. The blue empty diamonds represent the overall estimated positive rate of all studies included and the estimated positive rates of each subgroup. The horizontal lateral tips of the empty diamonds represent the 95 % confidence interval of the estimated positive rate. The solid black vertical line represents the zero positive rate. The dashed red line also represents the overall estimated positive rate of this study. IV: Instrumental-Variable heterogeneity.

(Murphy, 2017). Ahmed et al. (2021) investigated the decay rates of several potential pathogens, including *Campylobacter* spp., in artificial microcosms (75 %: 25 % (*v*/v) of freshwater: fresh raw sewage) (Ahmed et al., 2021). The decay rate (decay constant *k*) of *Campylobacter* spp. identified in their study was between 0.069 and 0.102 d^{-1} in the unfiltered raw wastewater seeded freshwater. Another study also found that a significant data gap exists for *Campylobacter* spp. decay in real sewers, which is possible to cause under-estimation of the disease infections for WBE at high temperatures (Guo et al., 2022b). Overall, the knowledge of *Campylobacter* decay in raw wastewater and during the in-sewer transport is still largely unknown.

3.7. Implications for the application in WBE

This meta-analysis is the first study about the prevalence, concentration, and speciation of *Campylobacter* spp. in different wastewater samples. The results of this systematic review fill a significant data gap for the wastewater surveillance of *Campylobacter* diseases. The analysis of wastewater effluent highlighted its potential risk of causing further contamination of the environment and food chain and revealed the insufficient disinfection of *Campylobacter* in some WWTPs. The summarized *Campylobacter* spp. prevalence and concentration in influent wastewater could be further employed to evaluate the feasibility of WBE back-estimation in *Campylobacter* spp. study (Guo et al., 2022a). However, the low heterogeneity of some data reported in this study revealed the limited published data for *Campylobacter* spp. prevalence in

wastewater. Only 13 out of the total 28 involved studies were carried out after 2010, which implies the more recent trend of the *Campylobacter* spp. prevalence in wastewater might not be captured adequately. *Campylobacter* spp. prevalence was not analyzed according to the country of the included studies. This is because, except for the studies carried out in Italy and Netherlands, only one or two studies were reported in other countries involved in this meta-analysis. In addition, the decay and persistence of *Campylobacter* spp. in wastewater under different environmental and sewer conditions are still unknown. In future, to develop the WBE-based surveillance of *Campylobacter* spp., more research should be carried out to delineate i) the recovery efficiency of *Campylobacter* spp. from wastewater by different detection and quantification methods; ii) the decay rate of *Campylobacter* spp. in influent wastewater under different environmental conditions e.g., the air and wastewater temperature; iii) the decay and partition of *Campylobacter* spp. to sewer biofilms during the in-sewer transport.

4. Conclusions

In conclusion, the high prevalence of *Campylobacter* spp. in the influent and effluent wastewater highlighted the significance of developing wastewater surveillance for *Campylobacter* spp. The reported prevalence rate and concentration could further support the WBE back-estimation of *Campylobacter* spp. prevalence in communities and evaluate the sensitivity of the WBE parameters for inducing variances to the back-estimation. qPCR-based methods are recommended for future wastewater-based studies based on the meta-analysis. Consistent with the clinical study, *C. jejuni* and *C. coli* were identified as the top two prevalent species in wastewater samples. In addition, seasonal variation was observed for the *Campylobacter* spp. concentration in wastewater of this study, which is consistent with the previous report of *Campylobacteriosis* infection in communities. This also indicates the feasibility of using wastewater-based epidemiology to monitor *Campylobacter* spp. associated infections in communities.

CRediT authorship contribution statement

Shuxin Zhang – Study design, Data extraction & analysis and Writing – original draft.
Jiahua Shi – Writing – review.
Xuan Li – Writing – review.
Ananda Tiwari – Writing – review.
Shuhong Gao – Writing – review.
Xu Zhou – Writing – review.
Xiaoyan Sun – Writing – review.
Jake W. O'Brien – Writing – review.
Lachlan Coin – Writing – review.
Faisal Hai – Writing – review.
Guangming Jiang - Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Guangming Jiang reports financial support was provided by Australian Research Council. Shuxin Zhang reports financial support was provided by University of Wollongong. Jake W. O'Brien reports financial support was provided by National Health and Medical Research Council.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166410.

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