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Prevalence of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* in wastewater: a systematic review and meta-analysis

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ABSTRACT

Wastewater is considered a hotspot niche of multi-drug and pathogenic bacteria such as *Enterobacteriaceae*-producing extended-spectrum beta-lactamases (ESBL-E). Thus, the aim of this meta-analysis was to evaluate the prevalence of ESBL-E in different wastewater sources. Different databases (Medline, EMBASE, and Cochrane Library) were searched from inception to March 2021. Data were analyzed using random-effects modeling, and subgroup and meta-regression analyses were used to ascertain heterogeneity among the subgroups. Fifty-seven observational studies were selected, and the pooled prevalence of ESBL-E in wastewater was 24.81% (95% CI, 19.28–30.77). *Escherichia coli* had the highest ESBL prevalence. The *bla*CTX-M genes were the most prevalent in the selected studies (66.56%). The pooled prevalence of ESBL was significantly higher in reports from America (39.91%, 95% CI, 21.82–59.51) and reports studying hospital and untreated wastewaters (33.98%, 95% CI, 23.82–44.91 and 27.36%, 95% CI, 19.12–36.42). Overall, this meta-analysis showed that the prevalence of ESBL-E in wastewater is increasing over time and that hospital wastewater is the most important repository of ESBL-E. Therefore, there is a need for developing new sewage treatment systems that decrease the introduction of resistant bacteria and antibiotic residues.

Key words: β-lactamases, bla_{CTX-M}, *Enterobacteriaceae*, ESBL, wastewater

HIGHLIGHTS

- The global prevalence of *Enterobacteriaceae*-producing extended-spectrum beta-lactamases (ESBL-E) in wastewater was found to be 24.81%.
- The pooled prevalence of ESBL-E was significantly higher in reports studying hospital wastewater.
- The highest prevalence of ESBL-E was in America, and the lowest prevalence was in Europe.
- Among ESBL genes, bla_{CTX-M} genes had the highest prevalence, followed by bla_{TEM} and bla_{SHV}.

INTRODUCTION

Enterobacteriaceae are responsible for causing infections in humans and animals. They rely on their ability to resist antibiotics and on their virulence arsenal that facilitates their dissemination. The emergence of antimicrobial resistance in *Enterobacteriaceae* has become a significant concern to public health (Azuma & Hayashi 2020); it is believed that wastewater can contribute to propagating their antibiotic resistance (Meletis 2016). Wastewater is considered a hotspot niche of pathogenic bacteria and genetic exchange of genes encoding antibiotic resistance (Lepuschitz *et al.* 2019). Antibiotics released into wastewater increase the selective pressure on *Enterobacteriaceae*, thus causing antibiotic resistance to proliferate (Fouz *et al.* 2020). Moreover, wastewater treatments do not entirely eliminate the microbial contaminants; therefore, wastewater is mostly discharged to the receiving rivers enabling resistant *Enterobacteriaceae* and genes encoding antibiotic resistance to reach agricultural soils and water bodies used for domestic purposes (Korzeniewska & Harnisz 2013a). From there, these bacteria can disseminate into human and animal populations (Gatica & Cytryn 2013).

β-Lactams are one of the most commonly used antibiotics that are usually released in wastewater; these antibiotics can induce the production of β-lactamases in *Enterobacteriaceae* (Bonomo 2017). Among these β-lactamases, extended-spectrum

beta-lactamases (ESBLs) are among the most widely spread resistance mechanisms (Teklu *et al.* 2019). They are a group of enzymes that can break down penicillins, β-lactamase inhibitors, and third- and fourth-generation cephalosporins and monobactams (Rahman *et al.* 2018). Most of them have been developed through spontaneous mutations of reduced spectrum β-lactamases (Reinthaler *et al.* 2010). Moreover, ESBLs are encoded by genes belonging mostly to three groups called $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} (Pishtiwan & Khadija 2019). These genes are mostly located on conjugative plasmids, facilitating the horizontal gene transfer among bacterial groups (Jesumirhewe *et al.* 2020). Other less-studied types of ESBLs have also been found, including OXA (oxacillinase), VEB (Vietnamese extended-spectrum β-lactamase), PER (*Pseudomonas* extended-resistant), and GES (Guyana extended-spectrum β-lactamase) (Amirkamali *et al.* 2017).

The prevalence of ESBL-producing *Enterobacteriaceae* in wastewater varies with respect to geographical differences, wastewater source, and antimicrobial prescription patterns, most of the studies on ESBL-E in wastewater are limited to geographical areas such as Asia and Europe, and there has been no meta-analysis to the best of our knowledge that evaluated the prevalence of ESBL-E in different wastewater sources. Therefore, this meta-analysis aimed to determine the prevalence of ESBL-producing *Enterobacteriaceae* in different wastewater sources and analyze their influencing factors.

MATERIALS AND METHODS

Study and data collection

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher *et al.* 2009). A comprehensive literature search of PubMed/MEDLINE, EMBASE, and the Cochrane Library was performed until March 2021 to find potentially relevant articles. We also reviewed manually the references cited by the studies included to identify additional studies. The terms applied in electronic searches are listed in Supplementary Material, Table S1.

Inclusion and exclusion criteria

Studies were included if they met the following criteria: observational cross-sectional investigating the prevalence of ESBL-producing *Enterobacteriaceae* in wastewater. Exclusion criteria were included: (1) the absence of the total number of isolates, (2) the ESBL identification method is unclear, and (3) studies with the format of a congress abstract, a review article, or a book chapter.

Data extraction and quality assessment

Two authors independently screened the literature and extracted data, and the following variables were extracted: (1) authors' names, (2) location, (3) year of publication, (4) sample size, (5) the use of treatment or not, (6) prevalence of ESBL isolates, (7) species detected, (8) source of samples, (9) ESBL genes, and (10) ESBL identification methods. Since all the included studies were cross-sectional, each article's quality was assessed using Joanna Briggs Institute's quality assessment checklist (Munn *et al.* 2015) (Supplementary Material, Table S2).

Statistical analysis and data synthesis

STATA 16.0 (StataCorp LP, College Station, TX, USA) was used for the meta-analysis. The prevalence of ESBL-producing *Enterobacteriaceae* was estimated. The heterogeneity among studies was examined by the forest plot and the I^2 heterogeneity test, in which 0–40, 50–60, 50–90, and 75–100% represented low, moderate, substantial, and considerable heterogeneity, respectively. Due to the high level of heterogeneity, the random-effects model was selected for analysis. Freeman–Tukey double arcsine transformation was used to avoid excluding studies where the prevalence of ESBL-E was close to 0% (Freeman & Tukey 1950; Nyaga *et al.* 2014). A subgroup analysis was performed, with a breakdown by the continents, use of treatment, year of publication, and the wastewater origin. A value of $p \leq 0.05$ indicated statistical significance in the pooled effect. Meta-regression was used to ascertain heterogeneity among the subgroups. The selected independent factors are the wastewater origin (hospital wastewater, municipal wastewater, wastewater from rivers, hospital and municipal wastewaters and their receiving rivers, wastewater from other sources (farms and slaughterhouses)), continents (Africa, Europe, Asia, and America), the use of treatment (treated wastewater, untreated wastewater, and treated and untreated wastewater), and year of publication (2007–2015, 2016, 2017, 2018, 2019, and 2020). The number of studies from 2007 to 2015 is small. Therefore, they were put in the same category, so they will not be omitted by the software. The risk of publication bias across the studies was assessed by the funnel plot and the Egger test; the asymmetry of the funnel plot and statistical significance of

Egger's regression test (p-value <0.05) were suggestive of publication bias (Egger *et al.* 1997). Sensitivity analysis was assessed to evaluate the robustness of the meta-analysis.

RESULTS

Eligible articles

The literature search process is shown in Figure 1. The database search yielded a total of 394 publications. After eliminating duplicates, 300 were selected to review their titles and abstracts, resulting in the exclusion of 214 publications; 86 studies were

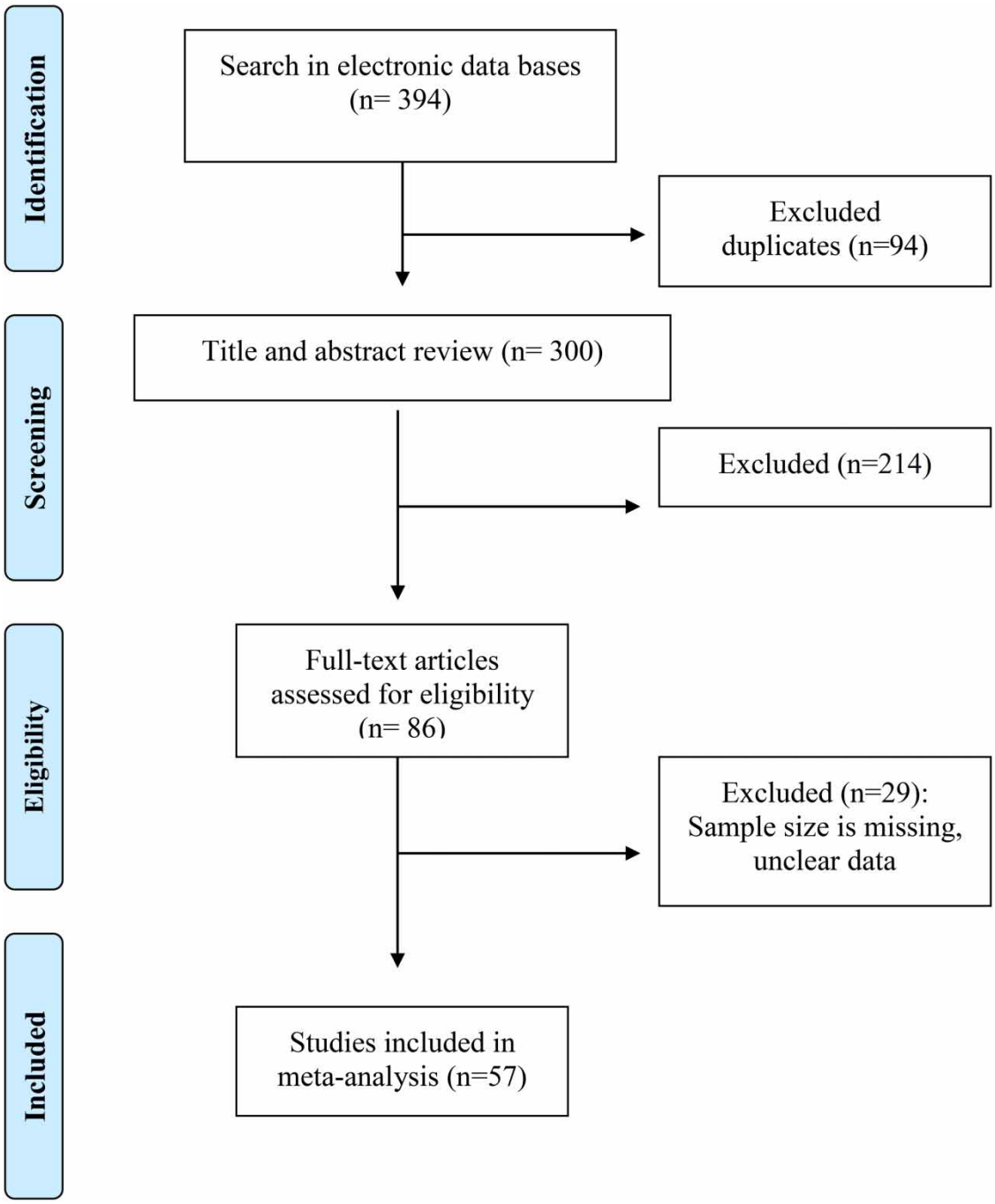

Figure 1 | PRISMA flow diagram of the selection process of the included studies.

assessed then for eligibility by full-text reviewing based on the review's inclusion and exclusion criteria, and 29 were removed. Finally, 57 articles were ultimately included in the analysis.

Characteristics of the eligible studies

The main characteristics of the included studies are summarized in Table 1. The most used method for ESBL-E detection was the double-disk synergy method. In total, 28 countries from four continents studied ESBL-E prevalence in wastewater (Europe: 18, Asia: 18, Africa: 13, and America: 8). Among the 57 included studies, 17 studies examined hospital wastewater, 15 studies examined municipal wastewater, 13 studies examined hospital and municipal wastewaters and their receiving rivers, seven studies examined wastewater effluents in rivers, and five studies examined wastewater from other sources such as farms and slaughterhouses. Moreover, 23 studies examined the occurrence of ESBL-E in untreated wastewater, nine studies in treated wastewater, and 25 studies in untreated and treated wastewater. The selected studies were published between 2007 and 2020, 23.59% were carried out between the years 2007–2015 ($n = 13$), 21% were carried out in 2019 ($n = 12$), and 18.54% were carried in 2020 ($n = 11$).

The most common producer of ESBL in wastewater was found to be *Escherichia coli* (51 studies), followed by *Klebsiella* (29 studies) and *Enterobacter* (18 studies). Moreover, the $bla_{\text{CTX-M}}$ gene was the most studied (30 studies), followed by the bla_{TEM} and bla_{SHV} genes (25 studies).

Pooled prevalence of ESBL-E

A total of 2,618 isolates of ESBL-E from 20,230 *Enterobacteriaceae* were found in the selected publications (Table 2), and the estimated prevalence varied widely across the studies ranging from 0.00% (95% CI, 0.00–7.13) to 85.71% (95% CI, 48.69–97.43) with substantial heterogeneity ($\chi^2 = 4461.79$, $I^2 = 98.74\%$, $p < 0.001$). The random-effect estimated pooled prevalence of ESBL-E was 24.81% (95% CI, 19.28, 30.77). Figure 2 shows the pooled prevalence of ESBL-E.

Variables associated with ESBL-E prevalence

Subgroup meta-analysis results are presented in Table 2. The analysis by continent showed that the highest prevalence of ESBL-E was in America at 39.91%, and the lowest prevalence was in Europe at 10.19% (95% CI, 5.73–15.72). The pooled prevalence of ESBL was significantly higher ($p < 0.001$) in reports studying hospital wastewater (33.98%, 95% CI, 23.82–44.91) and, more specifically, in untreated wastewater (27.36%, 95% CI, 19.12–36.42). The number of reports on ESBL-E prevalence in wastewater increased over time, and the highest number of studies per year was reported in 2019 ($n = 12$). Forest plots of the ESBL-E prevalence among studies stratified by continents, wastewater sources, the year, and the use of treatment are outlined in Figures 3–6.

Among *Enterobacteriaceae* species, *E. coli* had the highest ESBL prevalence (15.02%, 95% CI, 11.12–19.36) (Table 3) followed by *Klebsiella* spp. (9.60%, 95% CI, 19.54 38.29) and *Proteus* spp. (3.80%, 95% CI, 1.61, 6.76). Moreover, among ESBL genes, the $bla_{\text{CTX-M}}$ gene had the highest prevalence (66.56%, 95% CI, 53.98–78.15), followed by bla_{TEM} (49.88%, 95% CI, 35.01–64.76) and bla_{SHV} (21.58%, 95% CI, 13.71–30.49).

Meta-regression evaluating the effect of confounding factors on the prevalence of ESBL-E was performed, and the results are summarized in Table 4. In the univariable meta-regression, an association was found between ESBL prevalence and several factors at $p \leq 0.25$. In the final multivariable meta-regression model, a positive association was found between ESBL-E carriage and studies reported in America (Coef. = 0.22, $p = 0.01$).

Analysis of publication bias and sensitivity

The publication bias among the included papers was evaluated with Egger's test, which showed some evidence of publication bias, with a bias co-efficient of 1.56 ($p = 0.002$). The funnel plot's shape was not symmetrical (Supplementary Material, Figure S1). Thus, there is a potential for publication bias. Moreover, the sensitivity analysis showed no individual study that influenced the overall meta-analysis estimate, which indicates the robustness of the results (Supplementary Material, Table S3).

DISCUSSION

Wastewater is an important environmental supplier of antibacterial-resistant bacteria and antimicrobial-resistance genes (Marti *et al.* 2013). In this study, the occurrence of ESBL-producing *Enterobacteriaceae* in different wastewater sources has been evaluated. There are some big differences between hospital, municipal, and animal wastewaters. Thus, we have

Table 1 | Characteristics of the included studies

References	No. of ESBL	*Enterobacteriaceae*	Wastewater origin	Year	Treatment	Methods/guideline	Country
Prado *et al.* (2007)	20	43	Hospital wastewater	2007	Treated and untreated	CDT, CLSI	Brazil
Sabaté *et al.* (2008)	10	85	Wastewater from other sources (farms and slaughterhouses)	2008	Treated and untreated	E-test, CLSI	Spain
Łuczkiewicz *et al.* (2010)	1	259	Urban wastewater	2010	Treated and untreated	Phoenix Automated Microbiology System, CLSI	Poland
Chagas *et al.* (2011)	96	213	Hospital wastewater	2011	Treated	DDST, CLSI	Brazil
Mokracka *et al.* (2012)	12	1,832	Urban wastewater	2012	Treated and untreated	DDST, CLSI	Poland
Diallo *et al.* (2013)	9	1,248	Wastewater from other sources (farms and slaughterhouses)	2013	Treated	E-test, CLSI	France
Korzeniewska & Harnisz (2013a)	150	310	Hospital wastewater	2013	Untreated	CDT, CLSI	Poland
Korzeniewska & Harnisz (2013b)	34	246	Urban wastewater	2013	Treated and untreated	CDT, CLSI	Poland
Bessa *et al.* (2014)	29	144	Hospital and municipal wastewaters and their receiving rivers	2014	Treated	Disk approximation test, CLSI	Portugal
Chandran *et al.* (2014)	87	190	Hospital wastewater	2014	Untreated	CDT	India
Čornejová *et al.* (2015)	27	104	Urban wastewater	2015	Treated and untreated	Microdilution, CLSI	Slovakia
Kwak *et al.* (2015)	73	1,894	Hospital and municipal wastewaters and their receiving rivers	2015	Treated and untreated	AREB microplates, CLSI	Sweden
Kotlarska *et al.* (2015)	5	774	Hospital and municipal wastewaters and their receiving rivers	2015	Treated and untreated	DDST, EUCAST	Poland
De Oliveira & Van Der Sand (2016)	32	60	Hospital wastewater	2016	Treated and untreated	DDST, CLSI	Brazil
Drieux *et al.* (2016)	25	389	Hospital wastewater	2016	Untreated	DDST, CA-SFM	France
Dropa *et al.* (2016)	7	200	Urban wastewater	2016	Untreated	DDST, CLSI	Brazil
Egbule (2016)	4	96	Hospital wastewater	2016	Untreated	DDST, CLSI	Nigeria
Lenart-Boroń (2016)	23	196	Wastewater-receiving rivers	2016	Untreated	DDST, EUCAST	Poland
Maheshwari *et al.* (2016)	34	103	Hospital wastewater	2016	Untreated	CDT, CLSI	India
Sultana *et al.* (2016)	30	166	Hospital wastewater	2016	Untreated	DDST	Bangladesh
Caltagirone *et al.* (2017)	30	132	Hospital and municipal wastewaters and their receiving rivers	2017	Treated and untreated	DDST, EUCAST	Italy
Conte *et al.* (2017)	55	152	Hospital and municipal wastewaters and their receiving rivers	2017	Treated and untreated	VITEK-2 system and MALDI-TOF, CLSI	Brazil
Lien *et al.* (2017)	115	265	Hospital wastewater	2017	Treated and untreated	CDT, CLSI	Vietnam

(*Continued.*)

Table 1 | Continued

References	No. of ESBL	*Enterobacteriaceae*	Wastewater origin	Year	Treatment	Methods/guideline	Country
Obasi *et al.* (2017)	6	7	Urban wastewater	2017	Untreated	Vitek 2, E-test strips, CLSI	Nigeria
Tafoukt *et al.* (2017)	3	20	Wastewater-receiving rivers	2017	Untreated	DDST, CLSI	Algeria
Adelowo *et al.* (2018)	48	98	Hospital and municipal wastewaters and their receiving rivers	2018	Untreated	DDST, CLSI	Nigeria
Daoud *et al.* (2018)	51	70	Hospital wastewater	2018	Untreated	DDST, E-test ESBL strips, CLSI	Lebanon
Debabza *et al.* (2018)	143	254	Hospital and municipal wastewaters and their receiving rivers	2018	Treated and untreated	DDST, CA-SFM	Algeria
Falodun *et al.* (2018)	30	189	Wastewater-receiving rivers	2018	Untreated	DDST, CLSI	Nigeria
Flach *et al.* (2018)	89	4,028	Urban wastewater	2018	Treated and untreated	DDST, EUCAST	Sweden
Park *et al.* (2018)	14	75	Wastewater from other sources (farms and slaughterhouses)	2018	Treated and untreated	DDST, EUCAST	South Korea
Siddiqui *et al.* (2018)	175	506	Wastewater-receiving rivers	2018	Untreated	PDCT, CLSI	India
Tokajian *et al.* (2018)	21	34	Hospital and municipal wastewaters and their receiving rivers	2018	Untreated	DDST, CLSI	Lebanon
Vital *et al.* (2018)	27	147	Wastewater-receiving rivers	2018	Untreated	DDST, CLSI	Philippines
Adekanmbi *et al.* (2019)	17	58	Hospital wastewater	2019	Untreated	DDST, CLSI	Nigeria
Bartley *et al.* (2019)	20	40	Hospital and municipal wastewaters and their receiving rivers	2019	Treated and untreated	VITEK-2, CLSI	Brazil
Chaudhry *et al.* (2019)	17	112	Hospital wastewater	2019	Untreated	DDST, CLSI	Pakistan
Falodun & Oladimeji (2019)	35	200	Hospital wastewater	2019	Treated and untreated	DDST, CLSI	Nigeria
Haberecht *et al.* (2019)	47	70	Urban wastewater	2019	Treated and untreated	CHROMagar ESBL	USA
Li *et al.* (2019)	50	70	Wastewater from other sources (farms and slaughterhouses)	2019	Treated and untreated	DDST, CLSI	China
Mahato *et al.* (2019)	6	13	Hospital wastewater	2019	Treated	DDST, CLSI	Nepal
Miyagi & Hirai (2019)	141	249	Hospital and municipal wastewaters and their receiving rivers	2019	Untreated	DDST, CLSI	Japan
Paulshus *et al.* (2019)	314	3,123	Urban wastewater	2019	Untreated	AREB microplates, CLSI	Norway
Raven *et al.* (2019)	192	388	Urban wastewater	2019	Treated and untreated	Brilliance ESBL agar, Vitek	UK
Sghaier *et al.* (2019)	58	123	Urban wastewater	2019	Treated	DDST, CLSI	Tunisia
Tesfaye *et al.* (2019)	6	54	Wastewater from other sources (farms and slaughterhouses)	2019	Untreated	DDST, CLSI	Ethiopia

(*Continued.*)

Table 1 | Continued

References	No. of ESBL	*Enterobacteriaceae*	Wastewater origin	Year	Treatment	Methods/guideline	Country
Adekanmbi *et al.* (2020)	12	33	Hospital wastewater	2020	Treated and untreated	DDST, CLSI	Nigeria
Banjo *et al.* (2020)	12	23	Hospital wastewater	2020	Untreated	DDST, CLSI	Nigeria
Khan *et al.* (2020)	35	61	Urban wastewater	2020	Treated	CHROMagar ESBL, DDST, CLSI	UAE
King *et al.* (2020)	14	130	Hospital and municipal wastewaters and their receiving rivers	2020	Treated and untreated	MASTDISCS D68C AmpC/ESBL kits, EUCAST	South Africa
Lenart Boroń *et al.* (2020)	0	50	Wastewater-receiving rivers	2020	Treated	DDST, EUCAST	Poland
Saima *et al.* (2020)	1	10	Wastewater-receiving rivers	2020	Untreated	DDST, CDT, CLSI	Pakistan
Smyth *et al.* (2020)	89	498	Urban wastewater	2020	Treated	DDST, CLSI	Ireland
Surleac *et al.* (2020)	8	34	Hospital and municipal wastewaters and their receiving rivers	2020	Treated and untreated	ChromID ESBL agar, CLSI	Romania
Urano *et al.* (2020)	5	64	Urban wastewater	2020	Treated and untreated	DDST, CLSI	Japan
Urase *et al.* (2020)	13	264	Urban wastewater	2020	Treated	DDST, CLSI	Japan
Zagui *et al.* (2020)	11	34	Hospital and municipal wastewaters and their receiving rivers	2020	Treated and untreated	DDST, CLSI	Brazil

CDT, combined disc diffusion method; CLSI, Clinical & Laboratory Standards Institute; DDST, The Double Disc Synergy Test; PDCT, Phenotypic disc confirmatory test; CA-SFM, Comité de l'Antibiogramme de la société Française de Microbiologie.

chosen to do the subgroup analysis and study the prevalence of ESBL-E in each source separately. In total, the pooled prevalence of ESBL-E in wastewater was substantially important (24.81%), with the highest prevalence in untreated wastewater (27.36%). Untreated wastewater is loaded with different pollutants that can facilitate the proliferation of resistant bacteria (Preisner 2020). However, Du *et al.* (2015) suggested that biological treatments in wastewater treatment plants can also enhance the bacterial proliferation and genetic exchange.

Moreover, hospital wastewater had the highest prevalence of ESBL-E (33.98%). This could be due to the high usage of antibiotics in hospitals compared with public usage. Kümmerer (2009) found that the concentration of antibiotics in hospital effluents is 100 times higher than in municipal effluents. Moreover, the intensive use of antibiotics in hospitals could also contribute in the ESBL emergence (Debabza *et al.* 2018), which turns the hospital into a highly selective environment for ESBL-producing bacteria (Duong *et al.* 2008). Wastewater effluents in rivers had the lowest prevalence of ESBL-E. Wastewaters are usually treated before being released to waterways, which could explain the low prevalence of ESBL-E in rivers (Numberger *et al.* 2019).

Europe had the highest number of studies on ESBL-E and the lowest prevalence of ESBL-E. On the other hand, America (mainly South America because 7/8 of studies are in Brazil) and Asia had the highest prevalence of ESBL-E (39.91 and 33.95%, respectively), and according to the meta-regression results, studies reported in America are significantly associated with the presence of ESBL-E in wastewater. In developing countries, wastewater generated from farming, communities, and hospital effluents does not obtain appropriate treatment, and few functioning treatment facilities are available. This inadequate management can lead to environmental contamination with resistant bacteria such as ESBL-E into the water environment (Behnam *et al.* 2020; WHO 2018).

The highest prevalence of ESBL-E in wastewater was reported in studies undertaken in 2019. Gelband *et al.* (2015) analyzed data from 73 countries over the past 10 years and found that antibiotic use is growing steadily worldwide, which can explain, in part, the increase of ESBL-E in wastewater in the last 2 years. On the other hand, the biggest number of studies on ESBL-E in wastewater was also observed between the years 2019–2020; this could possibly mean that public awareness of the challenges related to the presence of resistant bacteria in wastewater is starting to rise lately.

Table 2 | Stratified pooled prevalence estimates of ESBL-producing *Enterobacteriaceae* in wastewater

Subgroups	No. of studies	No. of ESBL	No. of *Enterobacteriaceae*	Pooled estimate (%) of ESBL	95% Confidence interval	Heterogeneity chi-squared (χ^2)	Heterogeneity test, I^2 (%)	Heterogeneity test, *p*-value
Continents								
Europe	18	1,112	15,700	10.19	5.73, 15.72	1,645.83	98.97	<0.001
Asia	18	830	2,433	33.95	24.05, 44.58	449.28	96.22	<0.001
Africa	13	388	1,285	29.31	17.86, 42.17	250.07	95.20	<0.001
America	8	288	812	39.91	21.82, 59.51	206.09	96.60	<0.001
Overall	57	2,618	20,230	24.81	19.28, 30.77	4,461.79	98.74	<0.001
Wastewater origin								
Hospital wastewater	17	743	2,344	33.98	23.82, 44.91	442.77	96.39	<0.001
Municipal wastewater	15	929	11,267	18.83	11.15, 27.88	1,410.51	99.01	<0.001
Wastewater from rivers	7	259	1,118	13.67	5.36, 24.64	96.29	93.77	<0.001
Hospital and municipal wastewaters and their receiving rivers	13	598	3,969	29.41	15.03, 46.19	1,123.05	98.93	<0.001
Wastewater from other sources (farms and slaughterhouses)	5	89	1,532	18.43	1.04, 48.62	273.15	98.54	<0.001
Overall	57	2,618	20,230	24.81	19.28, 30.77	4,461.79	98.74	<0.001
The use of treatment								
Treated wastewater	9	335	2,614	21.11	7.40, 39.17	649.12	98.77	<0.001
Untreated wastewater	23	1,229	6,350	27.36	19.12, 36.42	956.04	97.70	<0.001
Treated and untreated wastewater	25	1,054	11,266	23.93	15.87, 33.03	2,286.92	98.95	<0.001
Overall	57	2,618	20,230	24.81	19.28, 30.77	4,461.79	98.74	<0.001
Year of publication								
2020	11	200	1,201	19.32	10.31, 30.17	144.56	93.08	<0.001
2019	12	903	4,500	37.62	22.18, 54.43	766.42	98.56	<0.001
2018	9	598	5,401	33.93	14.01, 57.34	1,191.61	99.33	<0.001
2017	5	209	576	34.73	22.53, 47.96	28.30	85.86	<0.001
2016	7	155	1,210	15.51	7.02, 26.45	126.54	95.26	<0.001
2007–2015	13	553	7,342	15.08	7.17, 25.19	1,276.01	99.06	<0.001
Overall	57	2,618	20,230	24.81	19.28, 30.77	4,461.79	98.74	<0.001

The studies included in this meta-analysis reported that *E. coli* was the most isolated ESBL-producing *Enterobacteriaceae* (15.02%). These bacteria are indicators of fecal contamination, agents of several kinds of nosocomial and community-acquired infections, and are often associated with therapy failure when cephalosporins are used (Kola *et al.* 2007; Shakya *et al.* 2017). The high prevalence of these enzymes in *E. coli* is maybe due to the ability of *E. coli* to survive for long periods and multiply in wastewater without being affected with wastewater treatments, which leads to the selection and emergence of ESBL-producing *E. coli* (Jang *et al.* 2017). Moreover, the $bla_{\text{CTX-M}}$ genes were the most prevalent in the selected studies, accounting for a significant percentage of the ESBL genes detected (66.56%). This high prevalence of CTX-M-type ESBL producers could be due to the global spreading of clones with epidemic and pandemic potential, such as the extra-intestinal pathogenic *E. coli* (ExPEC) ST131 known for its ability to produce extended-spectrum β-lactamases, such as CTX-M-15 (Cantón *et al.* 2012; Tanaka *et al.* 2019).

These findings enhance our understanding of ESBL-producing *Enterobacteriaceae* in wastewater. However, some limitations need to be addressed; in some recent studies, ESBL-E isolates were directly isolated using selective media without

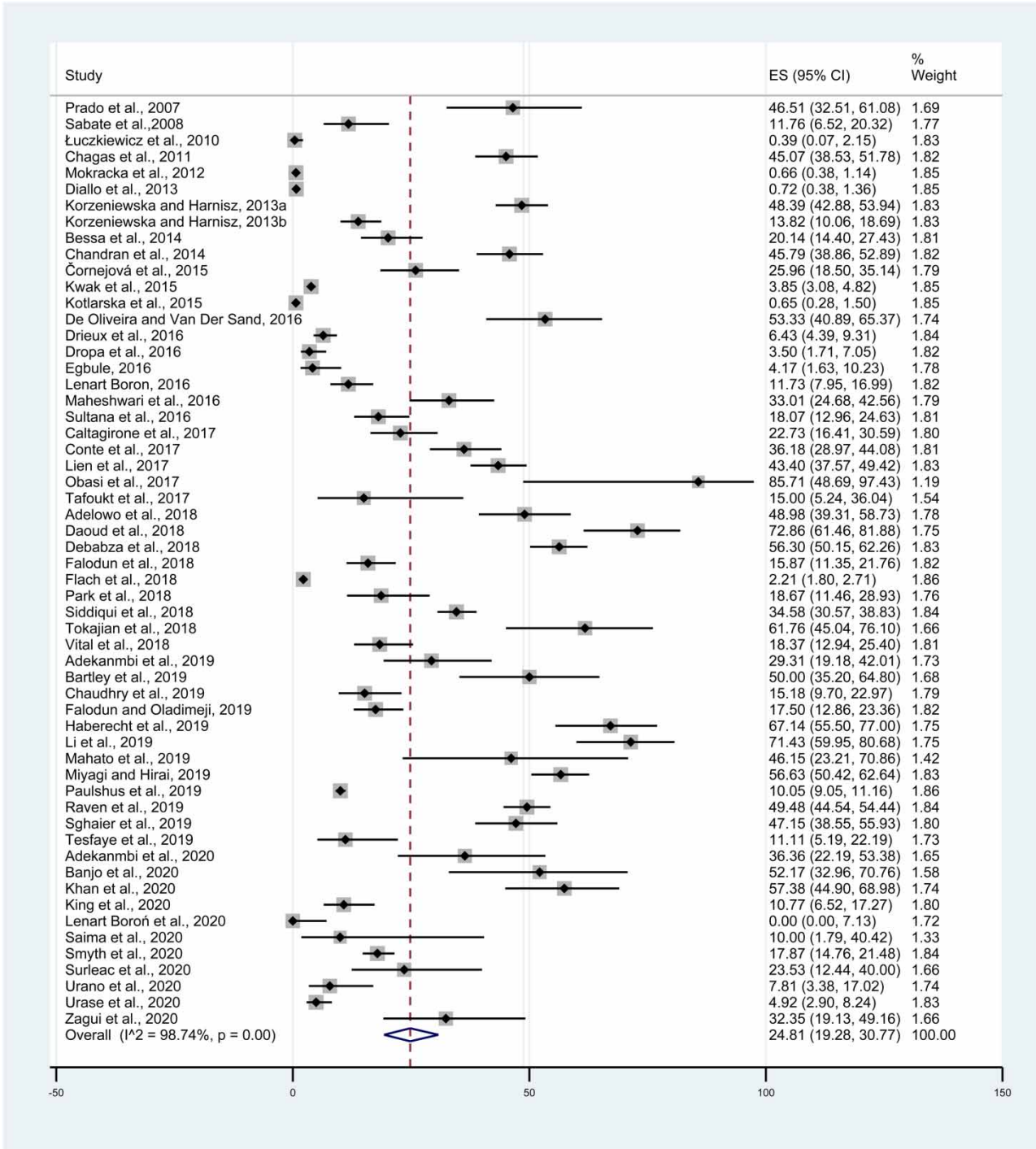

Figure 2 | Pooled prevalence of ESBL-producing *Enterobacteriaceae* in wastewater. Studies are sorted alphabetically, squares represent effect sizes of individual studies and the diamond indicates the estimated pooled effect size. CI, confidence interval.

isolating *Enterobacteriaceae* first, and these studies were not included in our study because the total number of isolates is absent. Moreover, most of the included studies on ESBL-E in treated wastewater did not report the type of treatment used, so we were obligated to put three categories (treated and untreated, treated, and untreated), and another potential limitation of the present study is that statistical results may have been influenced by publication bias based on the visual evaluation

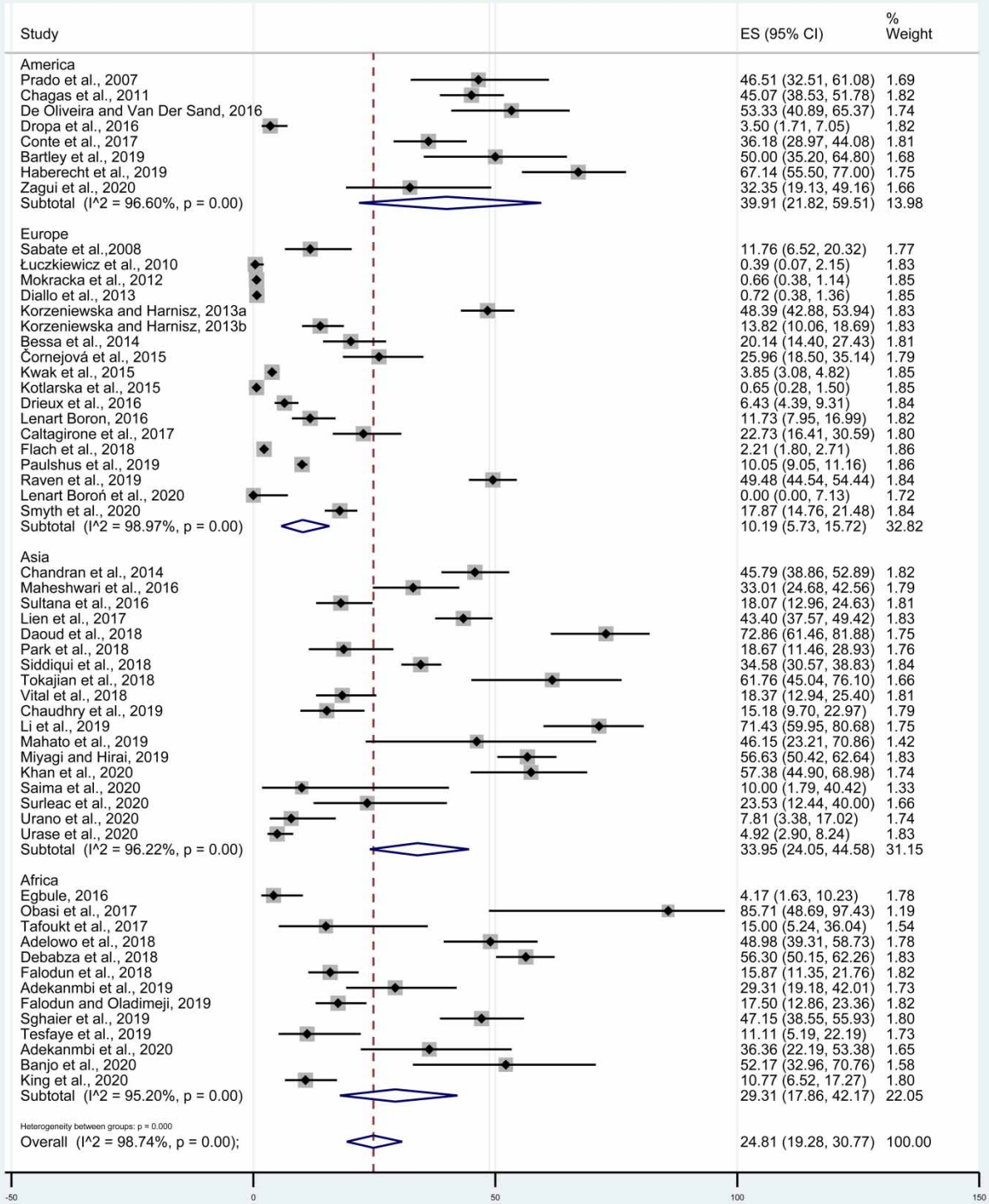

Figure 3 | Pooled prevalence of ESBL-producing *Enterobacteriaceae* stratified by continents.

of the funnel plot and the Egger test results; this is maybe due to the inherent bias toward reporting positive results; despite our effort to use search strategies that enhance the reporting of negative results, we have only found one study (the study of Lenart-Boroń *et al.* (2020)) that reported negative data.

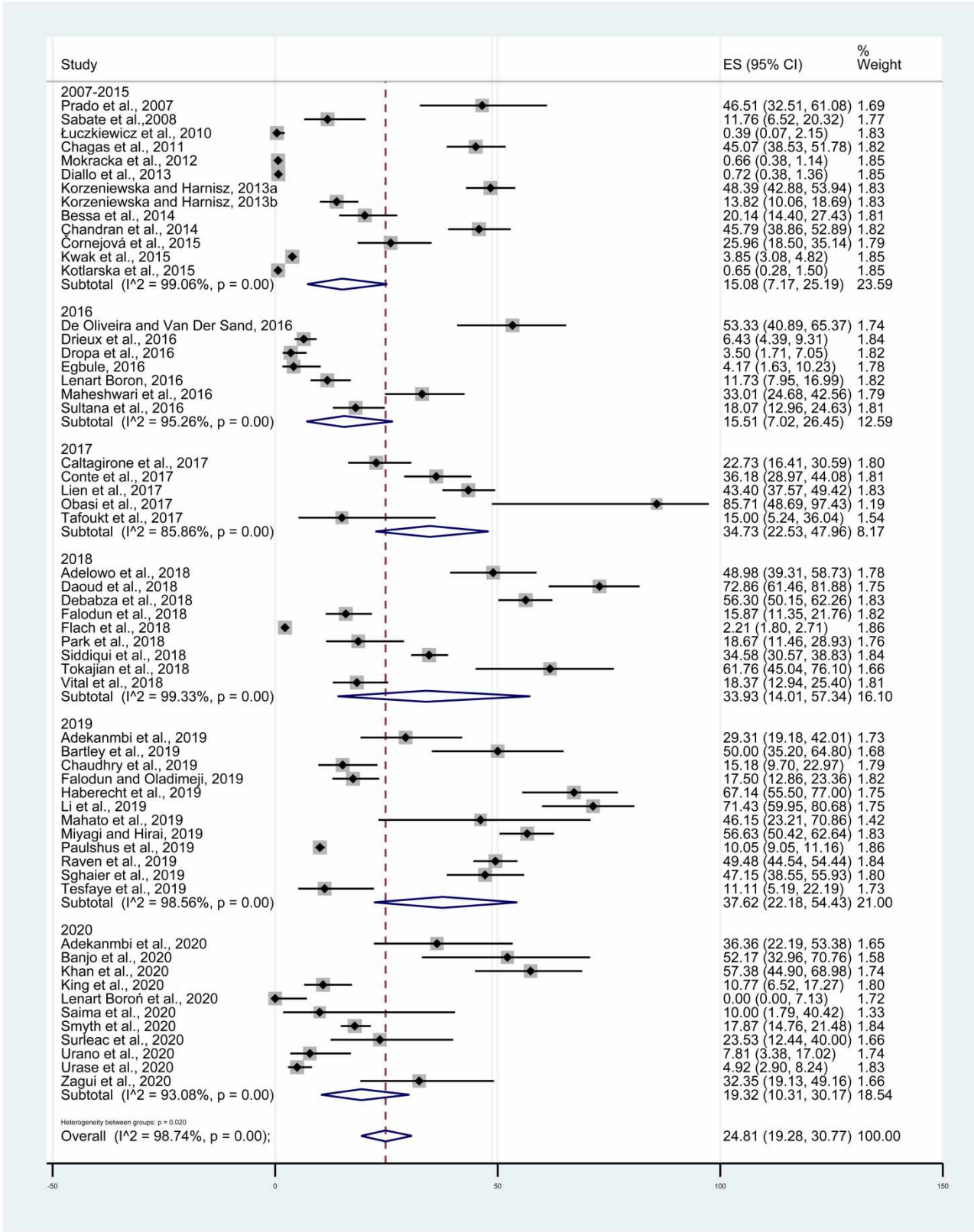

Figure 4 | Pooled prevalence of ESBL-producing *Enterobacteriaceae* stratified by the year of publication.

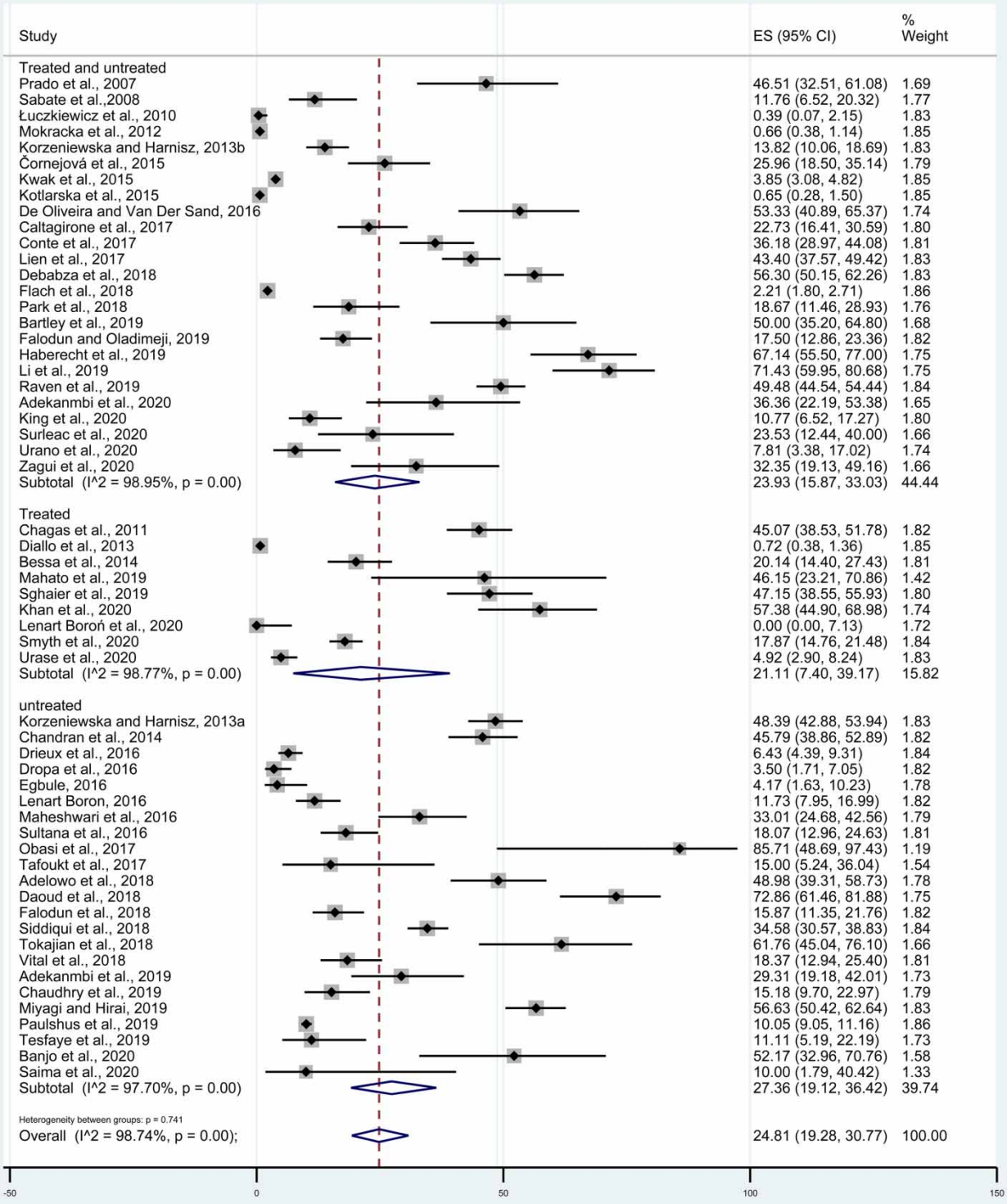

Figure 5 | Pooled prevalence of ESBL-producing *Enterobacteriaceae* stratified by treatments.

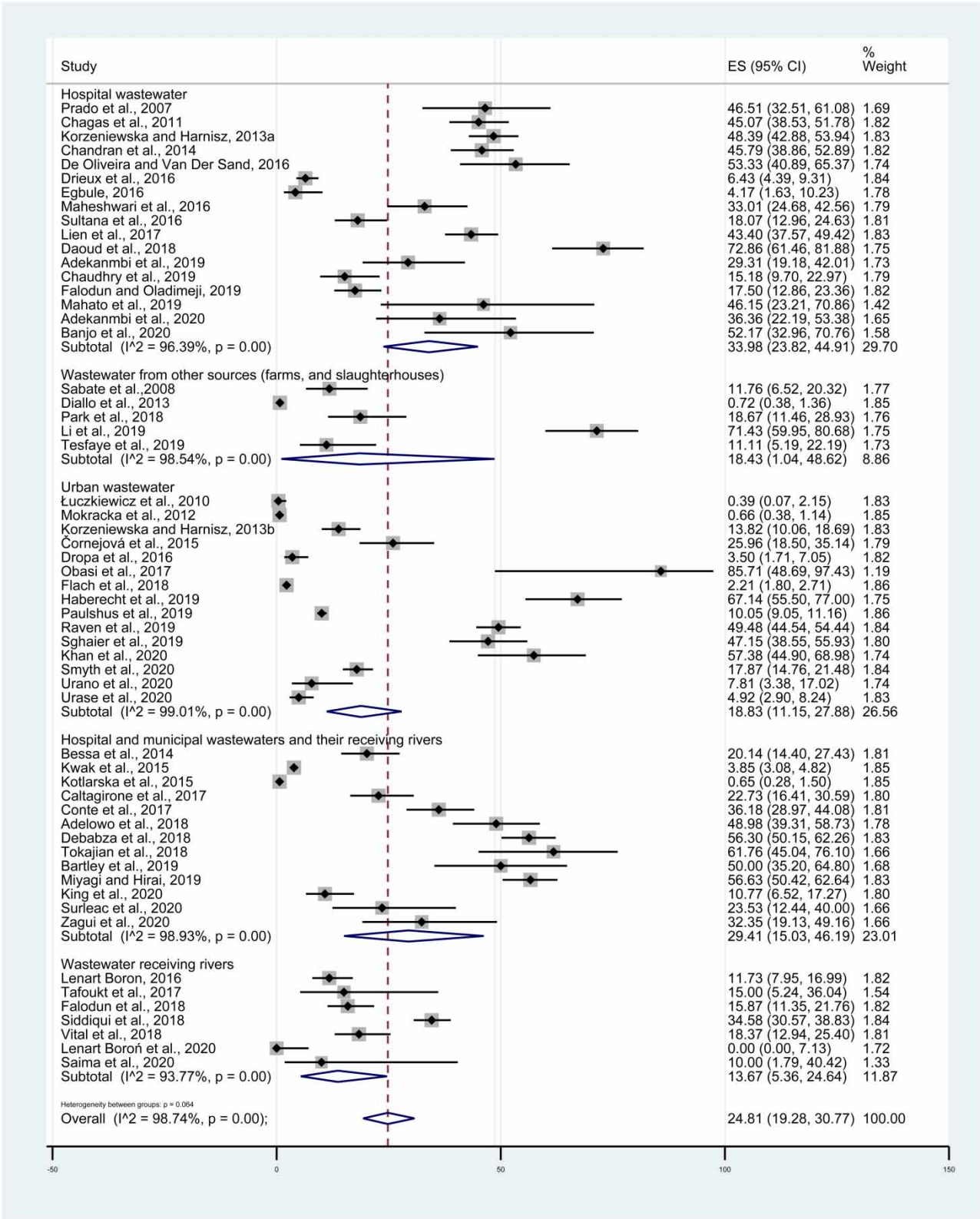

Figure 6 | Pooled prevalence of ESBL-producing *Enterobacteriaceae* stratified by the wastewater origin.

Table 3 | Meta-analysis of ESBL-E at the species level and ESBL-E gene prevalence in wastewaters

Subgroups	No. of studies	No. of ESBL	No. of *Enterobacteriaceae*	Pooled estimate (%) of ESBL	95% Confidence interval	Heterogeneity χ^2	Heterogeneity test, I^2 (%)	Heterogeneity test, *p*-value
***Enterobacteriaceae* species**								
E. coli	51	2,541	19,881	15.05	11.14, 19.41	2,902.78	98.28	<0.001
Klebsiella	29	1,222	5,732	9.66	5.83, 14.25	665.17	95.79	<0.001
Enterobacter	18	1,139	5,125	2.39	1.08, 4.09	149.25	88.61	<0.001
Citrobacter	8	714	1,846	2.69	0.50, 6.23	87.10	91.96	<0.001
Shigella	7	619	1,578	2.10	0.66, 4.19	28.58	79.01	<0.001
Proteus	6	323	900	3.80	1.61, 6.76	18.02	72.26	<0.001
Serratia	5	481	1,036	3.37	0.27, 8.89	48.36	91.73	<0.001
Other species	12	785	2,064	3.07	1.04, 5.96	169.26	93.50	<0.001
ESBL genes	**No. of studies**	**No. of ESBL**	**No. of *Enterobacteriaceae***	**Pooled estimate (%) of ESBL genes**	**95% Confidence interval**	**Heterogeneity χ^2**	**Heterogeneity test, I^2 (%)**	**Heterogeneity test, *p*-value**
$bla_{CTX\text{-}M}$	30	1,621	7,532	66.82%	52.57, 79.79	691.61	96.10	<0.001
bla_{TEM}	25	1,292	4,888	51.01%	35.52, 66.40	661.40	96.37	<0.001
bla_{SHV}	25	1,044	4,875	24.59%	15.42, 34.91	239.93	90.41	<0.001

Table 4 | Summary results of univariable and multivariable meta-regression of the effects of confounding factors on ESBL-E occurrence in wastewaters

	Coef.[b] (95% CI[c])	SE	*p*-value	Coef. (95% CI)	SE	*p*-value
Moderators	**Univariable regression**			**Multivariable regression**		
Continents						
Europe[a]	–	–	–	–	–	–
Asia	0.22 (0.08, 0.35)	0.06	0.002	0.14 (– 0.01, 0.31)	0.08	0.078
Africa	0.16 (0.007, 0.31)	0.07	0.040	0.06 (– 0.11, 0.24)	0.09	0.507
America	0.26 (0.08, 0.44)	0.09	0.003	0.22 (0.04, 0.39)	0.08	0.012
Wastewater origin						
Hospital wastewater[a]	–	–	–	–	–	–
Municipal wastewater	– 0.13 (– 0.27, 0.016)	0.07	0.081	–0.11 (– 0.27, 0.05)	0.08	0.183
Wastewater effluents in rivers	– 0.17 (– 0.37, 0.017)	0.10	0.074	– 0.28 (– 0.41, 0.008)	0.10	0.060
Hospital and municipal wastewaters and their receiving rivers	– 0.03 (–0.19, 0.11)	0.07	0.650	– 0.05 (–0.21, 0.11)	0.08	0.557
Wastewater from other sources (farms and slaughterhouses)	–0.13 (–0.34, 0.08)	0.10	0.222	–0.14 (–0.36, 0.06)	0.10	0.163
Treatments						
Treated and untreated wastewater[a]	–	–	–	–	–	–
Untreated wastewater	0.02 (– 0.10, 0.14)	0.06	0.733	0.016 (– 0.13, 0.16)	0.07	0.829
Treated wastewater	– 0.01 (– 0.18, 0.14)	0.08	0.816	0.04 (– 0.11, 0.20)	0.08	0.604
Year of publication						
2007–2015	–	–	–	–	–	–
2016	– 0.01 (– 0.19, 0.16)	0.09	0.851	– 0.11 (– 0.31, 0.09)	0.10	0.276
2017	0.16 (– 0.06, 0.38)	0.11	0.158	0.08 (– 0.14, 0.31)	0.11	0.483
2018	0.15 (– 0.01, 0.32)	0.08	0.073	0.14 (– 0.06, 0.35)	0.10	0.175
2019	0.19 (0.03, 0.34)	0.08	0.018	0.13 (– 0.03, 0.31)	0.08	0.122
2020	0.02 (– 0.14, 0.19)	0.08	0.784	– 0.01 (– 0.20, 0.18)	0.09	0.911

[a]Ref., reference category; Coef., regression coefficient; CI, confidence interval.

CONCLUDING REMARKS

Based on the 57 studies analyzed in this meta-analysis, we concluded that the prevalence of *Enterobacteriaceae*-producing ESBL in wastewater is increasing over time and that hospital wastewater is the most important repository of ESBL. This study also revealed a high prevalence of the $bla_{\text{CTX-M}}$ genes in *Enterobacteriaceae* isolated from wastewater, which is an alarming indicator of the global spreading of epidemic resistance plasmids.

Hence, these results highlight the need to develop effective strategies and measures adapted for removing ESBL-producing bacteria in wastewater and preventing the dissemination and transmission of antibiotic resistance from wastewater to different aquatic systems. Moreover, further research must focus on developing new sewage treatment systems that decrease the introduction of resistant bacteria and antibiotic residues.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

NZ: conceptualization, writing and editing; SB and NS: investigation and writing.

DATA AVAILABILITY STATEMENT

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

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