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SYSTEMATIC REVIEW

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The global prevalence of biofilm-forming *Enterococcus faecalis* in clinical isolates: a systematic review and meta-analysis

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Abstract

Background *Enterococcus faecalis* (*E. faecalis*) is a major cause of healthcare-associated infections (HAIs). It exhibits a strong biofilm-forming ability, which contributes to treatment resistance and persistence. Despite its clinical relevance, the global prevalence of biofilm-forming *E. faecalis* remains poorly defined. This study aimed to estimate the pooled prevalence of biofilm-forming *E. faecalis* in clinical isolates worldwide.

Methods Following PRISMA 2020 guidelines, we systematically searched PubMed, Scopus, ScienceDirect, Google Scholar, and institutional repositories for studies published between 2015 and 2024. A total of 56 studies involving 3,739 clinical isolates met the inclusion criteria. We used a random-effects model to estimate pooled prevalence and conducted subgroup analyses based on WHO region, continent, publication year, specimen type, and biofilm detection method. Meta-regression and sensitivity analyses assessed heterogeneity and robustness. Publication bias was evaluated using Egger's test and corrected with trim-and-fill analysis.

Results The global pooled prevalence of biofilm-forming *E. faecalis* was 68.68% (95% CI: 61.33–76.02%), with significant heterogeneity ($I^2 = 99.30\%$). By WHO region, prevalence ranged from 57.93% (95% CI: 41.01–71.85%) in South-East Asia to 73.66% (95% CI: 63.40–83.92%) in the Eastern Mediterranean. By continent, South America (all from Brazil) showed the highest prevalence at 89.79% (95% CI: 73.02–106.56%). Studies from 2021 to 2024 reported higher prevalence (76.18%, 95% CI: 66.25–86.11%) than those from 2015 to 2020. Among specimens, urine showed the highest prevalence (80.47%, 95% CI: 61.17–99.77%). Among biofilm-positive isolates, 47.92% (95% CI: 39.34–56.51%) were strong producers. Meta-regression identified WHO region ($p = 0.005$) and specimen type ($p = 0.043$) as significant sources of heterogeneity. Egger's test indicated publication bias ($p = 0.0066$), but trim-and-fill analysis yielded a consistent adjusted prevalence of 68.08%.

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Conclusion Biofilm formation is highly prevalent in *E. faecalis* clinical isolates globally, with substantial regional and specimen-based variation. These findings highlight the urgent need for standardized biofilm detection protocols, improved infection prevention and control, tailored antibiotic stewardship, and the development of anti-biofilm therapies to mitigate biofilm-associated resistance and enhance patient outcomes.

Keywords Biofilm, Clinical isolates, *Enterococcus faecalis*, Meta-analysis, Prevalence, Systematic review

Introduction

Enterococcus species, commensal microorganisms of the human and animal gastrointestinal tract, are known for their ability to survive under harsh environmental conditions, including those found in healthcare settings [1–3]. Among them, *Enterococcus faecalis* (*E. faecalis)* and *Enterococcus faecium* (*E. faecium)* are opportunistic pathogens responsible for various infections, including urinary tract infections, wound and intra-abdominal infections, medical device-associated infections, endocarditis, and bacteremia [4, 5]. These two species rank as the third most common cause of hospital-acquired infections, after *Staphylococcus aureus* and *Pseudomonas aeruginosa* [6], with *E. faecalis* accounting for 85–90% of enterococcal infections [7]. The global emergence of multidrug-resistant (MDR) and vancomycin-resistant *E. faecalis* (VRE) strains presents a significant challenge to healthcare systems [8], as these strains can transfer resistance genes to other pathogens, further complicating treatment and endangering both patients and healthcare workers [9, 10].

Given its resistance profile and persistence in clinical environments, *E. faecalis* has been the focus of increasing research interest, particularly regarding its virulence mechanisms. A major contributor to its clinical persistence is the production of virulence factors, most notably proteases and the ability to form biofilms. Proteases facilitate bacterial lysis and the release of extracellular nucleic acid, particularly deoxyribonucleic acid (DNA), which supports biofilm development [11]. Biofilms, structured bacterial communities embedded in a self-produced extracellular matrix, frequently develop on medical devices and within infected tissues such as the urinary tract, skin, and oral cavity, where they lead to chronic, difficult-to-treat infections [12–16]. Compared to planktonic cells, biofilm-associated *E. faecalis* exhibits markedly increased resistance to antimicrobials due to limited drug penetration, protective matrix components, and reduced metabolic activity [17]. These characteristics highlight the pivotal role of biofilm formation and virulence in the pathogenicity and treatment resistance of *E. faecalis* infections [18].

Although numerous primary studies have investigated biofilm formation among *E. faecalis* clinical isolates [12–14, 19–33], findings vary considerably due to differences in detection methods, sample sizes, clinical sources, and geographic focus. Most of the existing data are fragmented, locally focused, and methodologically inconsistent, preventing a comprehensive understanding of the global burden. Furthermore, there is currently no systematic synthesis that quantifies the global prevalence of biofilm-forming *E. faecalis* across varied clinical settings. This gap limits the ability of researchers and clinicians to assess the true public health impact of these infections and to develop standardized infection control and treatment strategies.

Therefore, a global estimate of the prevalence of biofilm-forming *E. faecalis* is urgently needed. Such data are essential to inform the development of standardized biofilm detection protocols, guide antibiotic stewardship programs, support infection control strategies, and prioritize research into anti-biofilm therapies. This systematic review and meta-analysis address this gap by synthesizing data across regions and methodologies to generate a comprehensive understanding of the global distribution and clinical significance of biofilm-producing *E. faecalis*.

Methods

Design and protocol registration

This systematic review and meta-analysis aimed to estimate the global pooled prevalence of biofilm-forming *E. faecalis*. The findings are reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [34]. The protocol for this review has been registered in the PROSPERO database under the registration number CRD42025643477.

Search strategy

Comprehensive and systematic searches were conducted across various databases, including PubMed, Scopus, and ScienceDirect electronic databases, to retrieve relevant published articles. To capture a broader spectrum of relevant studies and minimize publication bias, we also included grey literature sources such as Google Scholar and online institutional repositories, including the World Health Organization Institutional Repository for Information Sharing (IRIS), Africa Journals Online (AJOL), and university repositories including Addis Ababa University, University of Gondar, Bahir Dar University, and Jimma University were also reviewed. These sources were included to minimize publication bias and capture relevant but unpublished or regionally disseminated data. The search targeted studies published in English between January 1, 2015, and December 31, 2024.

A comprehensive strategy was employed using Medical Subject Headings (MeSH) terms and relevant keywords. The search string was structured to ensure correct Boolean nesting for optimal sensitivity, using combinations such as: ((“prevalence” [MeSH Terms] OR “epidemiology” [MeSH Terms] OR “magnitude” [All Fields]) AND (“biofilms” [MeSH Terms] OR “biofilm formation” [All Fields]) AND (“*Enterococcus faecalis*” [MeSH Terms] OR “Enterococcus” [MeSH Terms])). The full search strategy and database-specific strings are provided in (S1 Table).

To ensure rigor, reference lists of all included studies and relevant reviews were also screened for additional eligible studies. Duplicate records and overlapping datasets were identified and excluded. Quality assessment was conducted using the Joanna Briggs Institute (JBI) checklist, as described in the Quality Appraisal section, to eliminate low-quality studies and preprints.

Eligibility criteria

Studies retrieved from the specified databases were imported into EndNote X9 reference management software (Thomson Reuters, Toronto). Following the updated PRISMA guidelines [34] (Fig. 1), duplicates were removed, and the remaining records underwent a multistage screening process, initially by title, followed by abstract and full-text review, conducted independently by two reviewers (ET and ZA). Eligible studies were original research articles reporting on clinical isolates of *E. faecalis*, defined as strains isolated directly from human clinical specimens (e.g., urine, blood, wound swabs, catheter tips, and other body fluids or tissues collected for diagnostic purposes). Only studies involving isolates derived from infected or colonized patients were considered. Environmental, animal-derived, or food-related *E. faecalis* strains were explicitly excluded.

Included studies employed observational designs (cross-sectional, cohort, or case-control), surveillance studies, or laboratory-based experimental studies that used standard or widely accepted biofilm detection methods, such as the microtiter plate assay, Congo red agar, tube method, or polymerase chain reaction (PCR) targeting biofilm-related genes. Only English-language publications were considered. We excluded studies that: focused on environmental, foodborne, or animal isolates, were reviews, case reports, editorials, or commentaries, lacked data on biofilm formation in *E. faecalis* or did not report results separately for this species, used non-standard detection methods or had methodological inconsistencies, or did not report relevant prevalence or outcome measures.

Study selection and quality assessment for risk of bias

Three authors (ET, ZA, MAR) independently identified articles, removed duplicates, and screened titles and abstracts. Full texts of studies reporting the prevalence or epidemiology of biofilm-forming *E. faecalis* were reviewed. Discrepancies between ET and ZA were resolved by MAR. Study quality was assessed by ET, ZA, and MAR using the Joanna Briggs Institute (JBI) critical appraisal tools [35]. Studies scoring 50–75% were rated as good quality, and those above 75% as high quality; only these were included in the review (S2 Table).

Data extraction

An Excel sheet was developed by the authors (ET, ZA, MRA, and AS) to create a data extraction form, which included fields such as the first author’s name, publication year, WHO region, continent, number of *E. faecalis* isolates, biofilm detection methods, and specimen types. For biofilm detection methods, studies were categorized into Crystal Violet (CV) staining, Microtiter Plate (MTP) assays, and the Test Tube method. Since CV staining is frequently integrated within MTP assays, studies employing CV as part of the MTP protocol were classified under MTP assays. CV staining performed in non-MTP formats (e.g., test tubes) was categorized separately.

Given the variability in reproducibility among detection methods, studies using quantitative methods (MTP, CV) were prioritized during synthesis when multiple methods were reported. Although all eligible studies were included regardless of method, no formal statistical weighting was applied across methods. However, as the majority of included studies (over 75%) used quantitative assays, the pooled estimates are expected to reflect these more robust techniques.

A pilot test was conducted using four randomly selected studies to ensure the effectiveness of the extraction form. Following the pilot phase, adjustments were made to the template. Two authors (ET and ZA) independently extracted the data using the revised form. The accuracy of the extracted data was verified by the other two authors (MRA and AS). Any discrepancies between the reviewers were resolved through discussions with a third and fourth reviewer to reach a consensus. Cross-checking with the included studies was performed to minimize errors and correct any inaccuracies.

Primary outcome

The global pooled prevalence of biofilm-forming *E. faecalis* in clinical isolates.

Statistical analysis

Data were initially entered into a prepared Microsoft Excel sheet and then exported to STATA version 17.0 software (StataCorp LLC, College Station, TX, USA) for final analysis. A random-effects model using the DerSimonian and Laird method was applied to estimate the pooled prevalence of biofilm-forming *E. faecalis* clinical

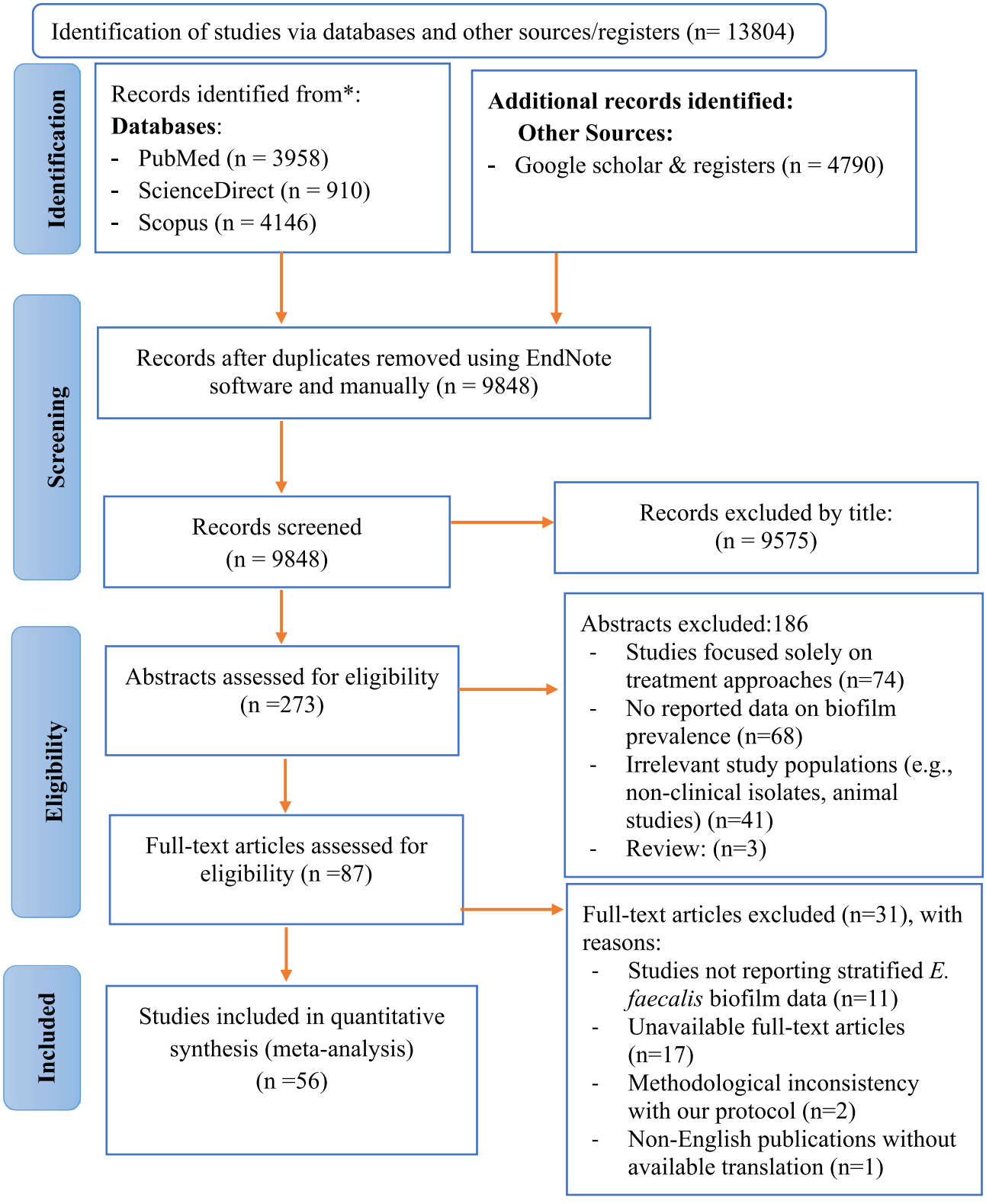

Fig. 1 PRISMA flow diagram showed the results of the search and reasons for exclusion [34]

isolates, accounting for variability between studies. Heterogeneity was assessed with the inverse variance (I^2) statistic and interpreted as follows: 0% (no heterogeneity), 0–25% (low heterogeneity), 25–50% (moderate heterogeneity), and >75% (high heterogeneity) [36]. Subgroup analyses were conducted for studies with high heterogeneity based on publication year, WHO region, continent, and biofilm formation intensity, and specimen types.

To ensure transparency in regional classification, WHO region groupings followed official World Health Organization designations, while continent-level analyses used conventional geographic categorizations. Each study was classified according to the WHO region of the country where it was conducted and independently by continent using standard geographic definitions. For countries spanning multiple WHO regions or continents, classification was based on the primary location reported. This dual classification explains slight differences between WHO region- and continent-based pooled prevalence estimates and provides complementary geographic perspectives.

For studies reporting biofilm formation, isolates were classified as strong, moderate, or weak biofilm producers based on pre-specified optical density (OD) cut-off thresholds, typically using the Crystal Violet microtiter plate assay. The cut-offs followed the formula: $OD \leq ODc$ = non-adherent, $ODc < OD \leq 2 \times ODc$ = weak, $2 \times ODc < OD \leq 4 \times ODc$ = moderate, and $OD > 4 \times ODc$ = strong biofilm producers, where ODc represents the optical density of the negative control plus three standard deviations [37]. These thresholds were largely consistent across the included studies, though minor variations were noted.

To identify sources of heterogeneity, we performed meta-regression using theoretically relevant moderators with sufficient between-study variation. Variables lacking adequate data or minimal variability were excluded to ensure model stability [38]. Egger's regression test was used to assess publication bias, with a significance level of $p < 0.05$. If bias was identified, a trim-and-fill method was applied to adjust the pooled prevalence estimates accordingly. Sensitivity analyses involved the sequential removal of individual studies to evaluate the consistency and reliability of the overall results. To handle cases where studies reported extreme proportions of biofilm formation (0% or 100%), which can cause zero variance and distort the analysis, a continuity correction of 0.5 was added to both the numerator and denominator [39].

Results

Descriptive summary of included studies

A systematic search across PubMed, ScienceDirect, Scopus, Google Scholar, and specialized registers identified 13,804 articles. Following duplicate removal, 9,848 records underwent title screening, which excluded 9,575 entries. Subsequent abstract evaluation of 273 articles led to the exclusion of 186 studies, leaving 87 for full-text review. Of these, 56 met the predefined criteria and were included in the systematic review and meta-analysis (Fig. 1).

The 56 studies encompassed 3,739 *E. faecalis* clinical isolates globally, with 2,325 identified as biofilm-forming. Isolate counts per study ranged from 8 to 274 (biofilm-forming: from 2 to 216). Geographically, the research spanned Africa, Asia, Europe, and South America (Brazil); no studies from North America, the Caribbean, or other South American countries were identified. Thirty-four studies were conducted in Asia [12, 13, 20–28, 30–33, 40–58]. Thirty-two studies were conducted between 2015 and 2020 [13, 14, 16, 21, 22, 25, 27–29, 32, 33, 40–44, 47, 50–57, 59–65], while the remaining 24 were conducted between 2021 and 2024 [12, 18–20, 23, 24, 26, 30, 31, 45, 46, 48, 49, 58, 66–75].

By WHO region, 21 studies originated from the Eastern Mediterranean [12, 13, 18, 20, 21, 23, 24, 32, 33, 43, 44, 46, 53–55, 57, 63, 68, 70, 71, 75], 17 from South Asia [22, 25, 26, 31, 40–42, 45, 47–52, 58, 60, 69], 7 from European [14, 29, 59, 64, 65, 67, 73], 5 from Western-pacific [27, 28, 30, 56], 6 from the Americas (all studies from Brazil, South America) [16, 19, 61, 62, 72, 74] (Table 1). By specimen types, 3 studies reported biofilm formation from blood samples [30, 61, 69], 4 from root canal specimens [46, 53–55], 3 from stool samples [23, 64, 71], and 10 from urine samples [13, 18, 20, 25, 28, 41, 44, 60, 70, 75]. The remaining 35 studies involved mixed clinical specimens from more than one source [12, 14, 16, 19, 21, 22, 26, 27, 29, 31–33, 40, 42, 43, 45, 47–52, 56–59, 62, 63, 65–68, 72–74]. One study based on a semen sample [24] was included in the overall analysis but not sub grouped due to its unique nature. A detailed breakdown of specimen sources across all included studies is provided in (S3 Table).

Meta-analysis

Pooled prevalence of biofilm-forming E. faecalis clinical isolates

This systematic review and meta-analysis revealed a global prevalence of biofilm-forming *E. faecalis* of 68.68% (95% CI: 61.33–76.02%), estimated using a random-effects model due to substantial heterogeneity (I^2 = 99.30%). The prevalence varied significantly across regions, ranging from 7.41% (reported in India) to 99.60% (reported in Brazil). The forest plot (Fig. 2) illustrates this distribution across 56 studies conducted between 2015 and 2024.

Table 1 Characteristics of included studies, from January 2015- December 2024

S. No	Author's name	Publication year	Continent	Country	WHO Region	Biofilm detection method	E. faecalis isolates (*N*)	Biofilm cases (*N*)	Degree of Biofilm formation (*N*)			Study's Quality score
									S	M	W	
1	Talebi et al. [21]	2015	Asia	Iran	EM	MP	58	42	36	6	NR	9
2	Shahveh et al. [13]	2020	Asia	Iran	EM	MP	51	50	40	6	4	9
3	Tsankova et al. [14]	2019	Europe	Bulgaria	EU	MP	72	18	NR	NR	NR	8
4	Manta et al. [19]	2023	South America	Brazil	AM	CVS	13	12	11	1	NR	7
5	Bhardwaj et al. [40]	2017	Asia	India	SEA	MP	39	28	NR	NR	NR	8
6	Garg et al. [25]	2020	Asia	India	SEA	CVS	10	7	NR	NR	NR	8
7	Das et al. [41]	2020	Asia	India	SEA	MP	36	29	7	15	7	8
8	Zheng et al. [27]	2017	Asia	China	WP	CVS	265	125	35	24	66	9
9	Habashneh et al. [12]	2024	Asia	Jordan	EM	MP	23	18	9	4	5	8
10	Soro et al. [66]	2024	Africa	Kenya	AF	CVS	26	24	22	1	1	8
11	Zheng et al. [28]	2018	Asia	China	WP	CVS	113	57	30	NR	27	8
12	Jovanovi´c et al. [67]	2023	Europe	Serbia	EU	CVS	43	42	8	17	17	8
13	Sieńko et al. [59]	2017	Europe	Poland	EU	CRA & TTM	30	4	NR	NR	NR	7
14	Mulik et al. [42]	2016	Asia	India	SEA	MP	147	82	NR	NR	NR	8
15	Kart et al. [60]	2016	Europe	Türkiye	SEA	CVS	12	11	NR	5	6	7
16	George et al. [43]	2020	Asia	Saudi Arabia	EM	MP	220	55	NR	NR	NR	9
17	Anderson et al. [29]	2016	Europe	Germany	EU	MP	82	51	8	43	NR	8
18	Komiyama et al. [16]	2016	South America	Brazil	AM	CMM	101	100	NR	NR	NR	8
19	Andrade et al. [61]	2016	South America	Brazil	AM	CVS	27	11	2	6	3	8
20	Haghi et al. [44]	2019	Asia	Iran	EM	MP	69	62	NR	NR	NR	8
21	Dawood et al. [68]	2024	Africa	Egypt	EM	MP	65	51	10	17	24	8
22	Shet et al. [45]	2022	Asia	India	SEA	MP	18	16	7	9	NR	7
23	ÖZKÖK et al. [69]	2021	Europe	Türkiye	SEA	MP	85	27	NR	NR	NR	8
24	Salih et al. [46]	2024	Asia	Iraq	EM	CVS	39	17	5	8	4	8
25	Yang et al. [30]	2024	Asia	China	WP	CVS	72	66	20	31	15	8
26	Ahmed et al. [70]	2023	Africa	Egypt	EM	MP	43	24	4	10	10	8
27	Biswas et al. [47]	2016	Asia	India	SEA	CVS	146	37	NR	NR	NR	9
28	Soares et al. [62]	2018	South America	Brazil	AM	CVS	124	123	61	44	18	7
29	Hashem et al. [18]	2021	Africa	Egypt	EM	CVS	61	60	37	15	8	8
30	Mubarak et al. [71]	2024	Africa	Egypt	EM	CVS	28	16	11	5	0	8
31	Momotaz et al. [48]	2024	Asia	Bangladesh	SEA	TCP	65	34	NR	NR	NR	8
32	Tawfick et al. [63]	2020	Africa	Egypt	EM	MP	15	5	NR	4	1	8
33	Sikdar et al. [49]	2021	Asia	India	SEA	TTM	274	216	NR	NR	NR	9
34	Khattak et al. [50]	2017	Asia	Pakistan	SEA	MP	51	18	NR	NR	NR	8
35	Ravichandran et al. [51]	2016	Asia	India	SEA	TTM	89	42	NR	NR	NR	7

Table 1 (continued)

S. No	Author's name	Publication year	Continent	Country	WHO Region	Biofilm detection method	E. faecalis isolates (*N*)	Biofilm cases (*N*)	Degree of Biofilm formation (*N*)			Study's Quality score
									S	M	W	
36	Banerjee et al. [52]	2015	Asia	India	SEA	CVS	155	42	NR	NR	NR	9
37	Hussein et al. [53]	2020	Asia	Iraq	EM	NI	8	7	5	2	0	8
38	Nair et al. [31]	2024	Asia	India	SEA	MP	63	58	7	41	10	8
39	Shahi et al. [32]	2020	Asia	Iran	EM	CVS	17	10	NR	NR	NR	8
40	Ramos et al. [72]	2023	South America	Brazil	AM	CVS	13	12	9	2	1	8
41	Gorski et al. [73]	2024	Europe	Croatia	EU	MP	31	30	22	6	2	8
42	Ghaziasgar et al. [33]	2019	Asia	Iran	EM	MP	37	36	27	NR	9	7
43	Aghdam et al. [54]	2017	Asia	Iran	EM	MP	101	100	49	42	9	7
44	Saffari et al. [55]	2018	Asia	Iran	EM	MP	22	18	16	NR	NR	7
45	Khalil et al. [20]	2022	Asia	Saudi Arabia	EM	MP	23	22	10	12	NR	9
46	Weng et al. [56]	2019	Asia	Malaysia	WP	CVS	51	25	14	NR	NR	8
47	Woz´niak-Biel et al. [64]	2019	Europe	Poland	EU	MP	46	45	34	11	0	7
48	Ghazvinian et al. [23]	2024	Asia	Iran	EM	MP	63	50	14	16	20	8
49	Yoo et al. [65]	2017	Europe	Australia	EU	CVS	37	19	10	4	NR	7
50	Shahroodian et al. [24]	2022	Asia	Iran	EM	MP	60	53	22	NR	31	8
51	Sengupta et al. [26]	2021	Asia	India	SEA	MP	27	2	NR	NR	NR	8
52	Shridhar et al. [22]	2019	Asia	India	SEA	MP	87	14	4	5	5	8
53	Tibúrcio et al. [74]	2022	South America	Brazil	AM	MP	42	41	1	40	NR	7
54	Saffari et al. [57]	2017	Asia	Iran	EM	MP	103	38	NR	NR	NR	8
55	Kumar et al. [58]	2022	Asia	India	SEA	MP	41	22	NR	NR	NR	8
56	Atrees et al. [75]	2023	Africa	Egypt	EM	CVS	100	64	35	29	NR	7

Keys: NR = Not Recorded, S = strong, M = moderate, W = weak, EM = Eastern Mediterranean, SEA = South-East Asia, AM = Americas, AF = African, EU = European, WP = Western pacific, MP = Microtiter Plate, CVS = Crystal Violet Staining, TTM = Test tube Method, TCP = Tissue culture plate, CRA = Congo Red Agar, CMM = Cellulose Membrane Method, NI = Not indicated, N = Number

Note: All studies from the Americas/South America were conducted in Brazil only

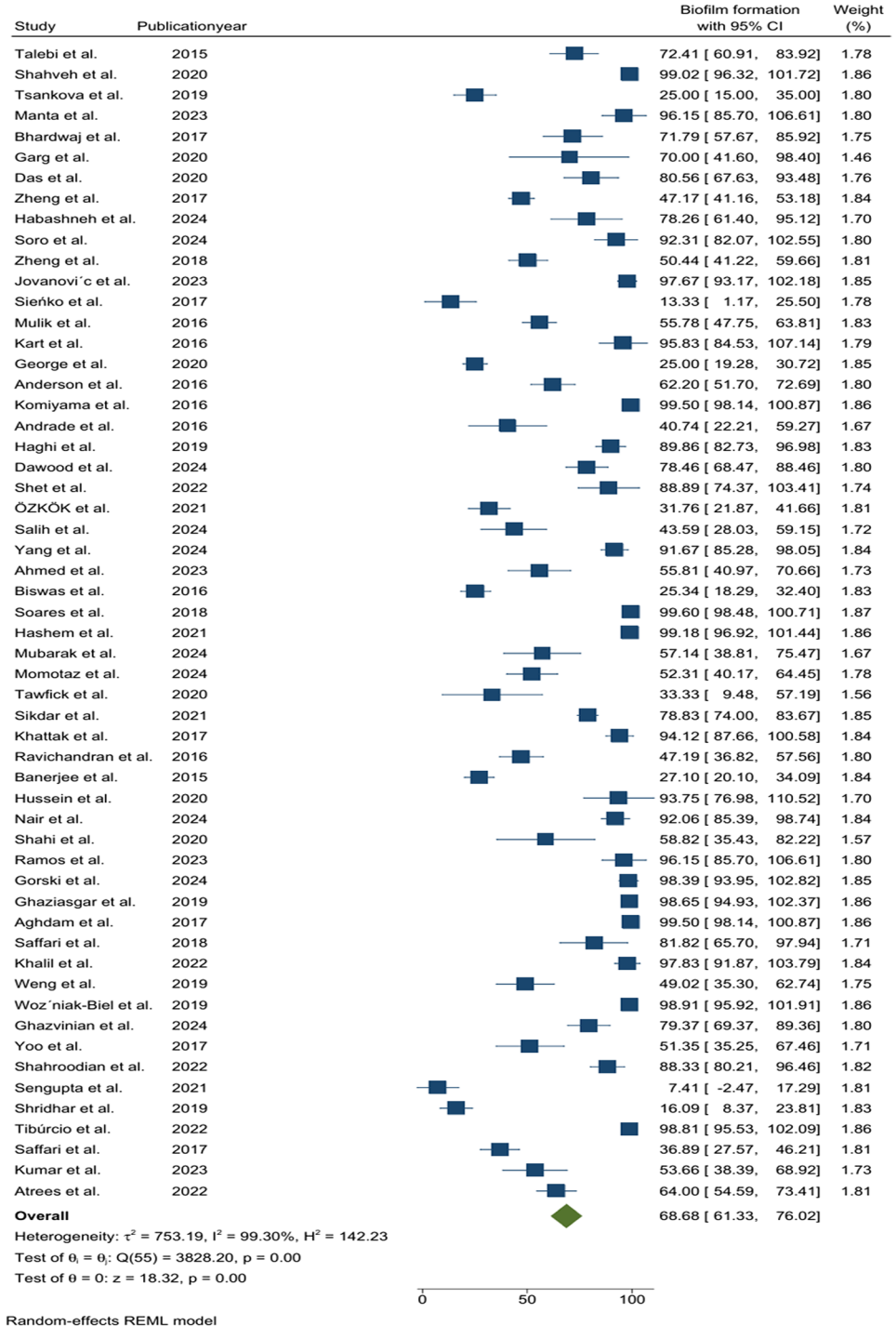

Fig. 2 Forest plot showing the global prevalence of biofilm-forming *E. faecalis* from 2015 to 2024

Publication bias

To evaluate potential publication bias and small-study effects in the pooled prevalence estimates, we employed a funnel plot analysis. Visual inspection of the funnel plot (Fig. 3) revealed asymmetry, suggesting the presence of publication bias. To statistically confirm this observation, we performed Egger's linear regression test under a random-effects meta-regression framework.

The results are summarized in (Table 2). The slope coefficient (β_1) was −3.37, suggesting an inverse relationship between study size and reported effect size, characteristic of small-study effects. The standard error (SE) of β_1 was 1.240, and the corresponding z-value was

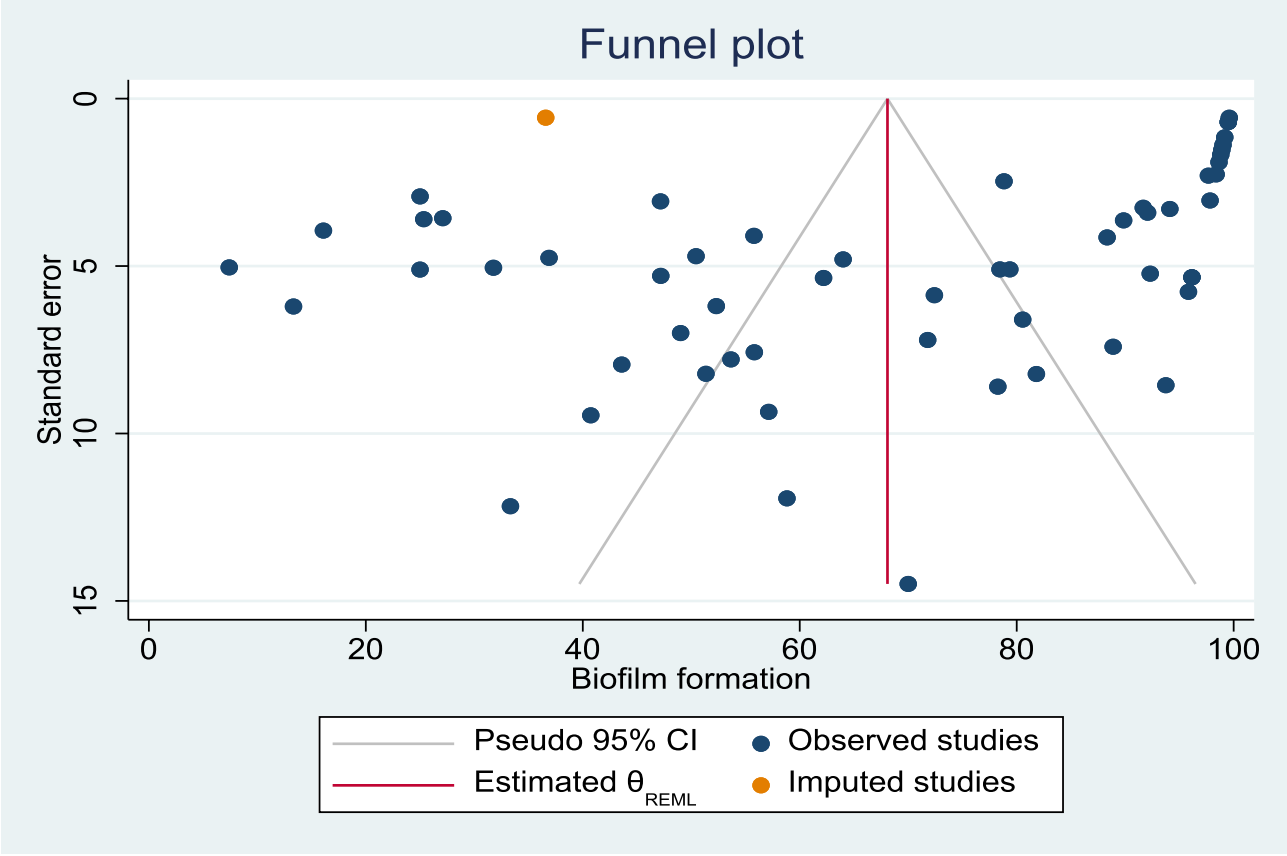

Fig. 3 Funnel plot assessing potential publication bias among included studies reporting the prevalence of biofilm-forming *E. faecalis* clinical isolates

Table 2 Results of the Egger test for small-study effects in the meta-analysis of biofilm formation studies

Statistics	Value	Interpretation
beta1 (Slope Coefficient)	-3.37	A negative slope suggests small-study effects, where smaller studies tend to report larger effect sizes.
SE of beta1 (Standard Error of Beta1)	1.240	The SE indicates the precision of the slope estimate. A smaller SE would suggest a more precise estimate of the effect.
z-value	-2.72	The negative z-value indicates a statistically significant negative relationship between study size and effect size.
p-value	**0.0066**	The p-value is less than 0.05, indicating that the small-study effects are statistically significant and not due to chance.

−2.72. The test yielded a statistically significant *p*-value of 0.0066, confirming the presence of small-study effects and publication bias.

Trim-and-fill analysis of publication bias

Publication bias was assessed using the nonparametric trim-and-fill method (linear estimator). The analysis identified 1 imputed study on the left side of the funnel plot, suggesting mild asymmetry potentially attributable to publication bias favoring smaller effect sizes. Following this adjustment, the pooled prevalence of biofilm formation decreased marginally from 68.68% (95% CI: 61.33–76.02%) to 68.08% (95% CI: 60.78–75.39%), indicating minimal influence of the imputed study (Fig. 4).

Subgroup analysis of the biofilm-forming *E. faecalis*

Subgroup analysis by WHO region

Subgroup analysis by WHO region included studies from the Americas, Eastern Mediterranean, Europe, South-East Asia, and Western Pacific regions. The highest pooled prevalence of biofilm-forming *E. faecalis* isolates was reported in the Americas (89.79%, 95% CI: 73.02–106.56), based on six studies conducted in Brazil. The lowest pooled prevalence (57.93%, 95% CI: 41.01–71.85) was observed in the South-East Asia region.

The African region (AFRO) was excluded from subgroup comparisons, as only one study was available from this region [66], limiting its analytic value. High heterogeneity was detected in the Eastern Mediterranean (I^2 = 98.79%) and European regions (I^2 = 99.23%), indicating substantial variability among studies. The Western Pacific region also showed considerable heterogeneity (I^2 = 96.48%), despite having fewer studies. The test for subgroup differences by WHO region was

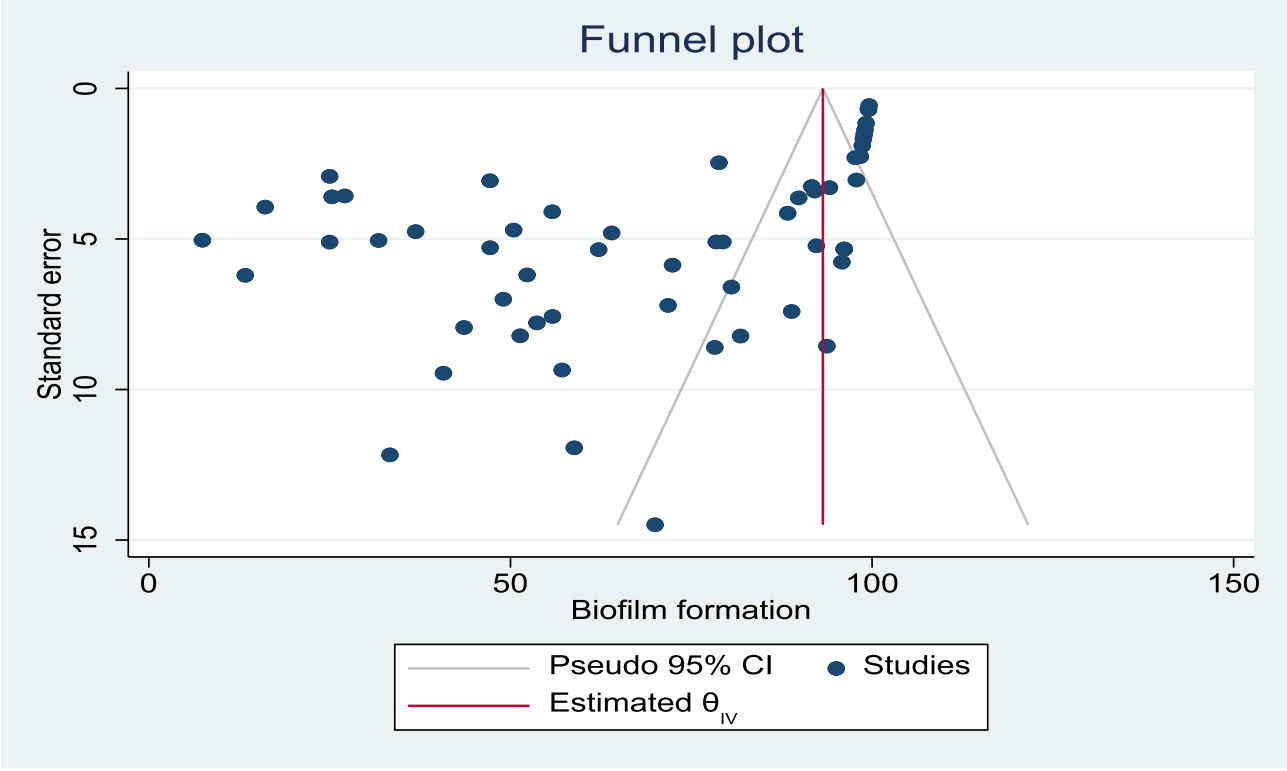

Fig. 4 Funnel plot assessing publication bias with left-side trim-and-fill imputation applied to studies on biofilm-forming *E. faecalis* clinical isolates

statistically significant ($p = 0.04$), suggesting variation between regions (Table 3; Fig. 5, and S1 figure).

Subgroup analysis by continent

A separate subgroup analysis by continent included studies from Africa, Asia, Europe, and South America. The highest pooled prevalence of biofilm-forming *E. faecalis* isolates was observed in South America at 89.79% (95% CI: 73.02–106.56%), based on six studies conducted in Brazil. The lowest pooled prevalence was reported in Europe at 64.11% (95% CI: 41.15–87.08%). This pattern contrasts with the WHO region-based analysis, where the lowest prevalence was observed in the South-East Asia region. High heterogeneity was detected in all continental subgroups: Asia (I^2 = 98.66%), Europe (I^2 = 99.10%), Africa (I^2 = 95.36%), and South America (I^2 = 99.61%). The test for subgroup differences between continental groups was not statistically significant ($p = 0.10$) (Table 3 and S2 figure).

Subgroup analysis by publication year

A subgroup analysis based on publication year categorized studies into two periods: 2015–2020 and 2021–2024. For 2015–2020 (32 studies), the prevalence ranged from 13.33 to 99.60%, with a pooled prevalence of 63.04% (95% CI: 52.86–73.22). For 2021–2024 (24 studies), the prevalence ranged from 7.41 to 99.18%, with a higher pooled prevalence of 76.18% (95% CI: 66.25–86.11). Both subgroups exhibited substantial heterogeneity (I^2 = 99.51% and 98.15%, respectively). Although there was an apparent increase over time, the difference between periods was not statistically significant ($p = 0.07$) (Table 3 and S3 figure).

Subgroup analysis by biofilm detection methodologies

A subgroup analysis was performed based on biofilm detection methodologies, comparing Crystal Violet staining (19 studies) [18, 19, 25, 27, 28, 30, 32, 46, 47, 52, 56, 60–62, 65, 66, 71, 72, 75], Microtiter Plate assays (31 studies) [12–14, 20–24, 26, 29, 31, 33, 40–45, 50, 54, 55, 57, 58, 63, 64, 67–70, 73, 74], and the Test Tube method (2 studies) [49, 51]. Four additional studies were excluded from subgroup comparisons as they represented single-study methodologies (Sieńko et al., Komiyama et al., Momotaz et al. and Hussein et al.). The pooled prevalence of biofilm-forming *E. faecalis* was 66.42% (95% CI: 54.52–78.31) for Crystal Violet, 70.88% (95% CI: 60.72–81.04) for Microtiter Plate assays, and 63.36% (95% CI: 32.36–94.36) for the Test Tube method. High heterogeneity was present across all methods (Crystal Violet: I^2 = 98.80%; Microtiter Plate: I^2 = 99.11%; Test Tube: I^2 = 96.59%). The test for subgroup differences between detection methods was not statistically significant ($p = 0.81$) (Table 3 and S4 figure).

Table 3 Subgroup analysis of biofilm-forming *Enterococcus faecalis* prevalence by study characteristics

Variable	Characteristic	Number of Studies	Prevalence Range (%) *	Pooled Prevalence (95% CI)	I^2 (%)	*p*-value
WHO Region	Eastern Mediterranean	21	33.33–99.50	73.66 (63.40–83.92)	98.79	**< 0.001**
	South-East Asia	17	7.41–95.83	57.93 (41.01–71.85)	97.60	**< 0.001**
	European	7	13.33–98.91	64.21 (37.45–90.97)	99.28	**< 0.001**
	America	6	40.74–99.60	89.79 (73.02–106.56)	99.61	**< 0.001**
	Western Pacific	4	47.17–91.67	59.83 (38.45–81.21)	96.48	**< 0.001**
Continent	Asia	34	7.41–99.50	65.98 (56.79–75.17)	98.66	**< 0.001**
	South America	6	40.74–99.60	89.79 (73.02–106.)	99.61	**< 0.001**
	Africa	7	33.30–99.18	70.25 (53.76–86.74)	95.36	**< 0.001**
	Europe	9	13.33–98.91	64.11 (41.15–87.08)	99.10	**< 0.001**
Publication Year	2015–2020	32	13.33–99.60	63.04 (52.86–73.22)	99.51	**< 0.001**
	2021–2024	24	7.41–99.18	76.18 (66.25–86.11)	98.15	**< 0.001**
Detection Method	Crystal Violet Staining	19	25.34–99.60	66.42 (54.52–78.31)	98.80	**< 0.001**
	Test Tube Method	2	47.19–78.83	63.36 (32.36–94.36)	96.59	**< 0.001**
	Microtiter Plate	31	7.41–99.50	70.88 (60.72–81.04)	99.11	**< 0.001**
Biofilm Strength	Strong	35	2.41–91.67	47.92 (39.34–56.51)	93.92	**< 0.001**
	Moderate	32	4.17–96.39	38.06 (30.03–46.10)	91.94	**< 0.001**
	Weak	24	4.17–58.49	26.88 (19.89–33.87)	88.52	**< 0.001**
Sample types	Blood	3	31.76–91.67	55.17 (17.94–92.40)	97.39	**< 0.001**
	Mixed	35	7.41–99.60	63.64 (53.67–73.60)	99.32	**< 0.001**
	Root Canal	4	43.59–99.50	80.13 (55.48–104.78)	94.44	**< 0.001**
	Stool	3	57.14–98.91	79.85 (56.65–103.04)	94.61	**< 0.001**
	Urine	10	50.44–99.18	81.12 (69.15–93.09)	97.62	**< 0.001**

Note: *Prevalence range reflects the lowest and highest point estimates from individual studies in each subgroup. All studies from the Americas/South America were conducted in Brazil only

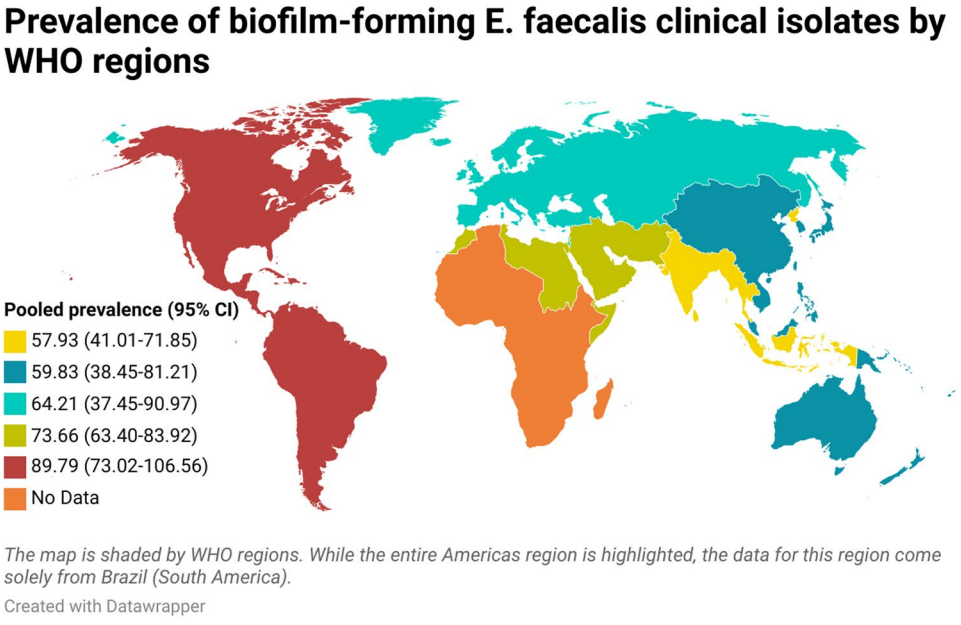

Fig. 5 Subgroup analysis by WHO regions of biofilm-forming *E. faecalis* clinical isolates prevalence. Pooled prevalence estimates (%; 95% CI) are stratified by WHO region, with shading intensity reflecting prevalence levels. Data for the Americas derive exclusively from Brazil (South America). Regions without data are indicated. Ranges: 57.93% (41.01–71.85) to 89.79% (73.02–106.56)

Subgroup analysis by sample type

The pooled prevalence of biofilm-forming *E. faecalis* was highest in urine samples at 81.12% (95% CI: 69.15–93.09), followed by root canal samples at 80.13% (95% CI: 55.48–104.78), stool at 79.85% (95% CI: 56.65–103.04), mixed samples at 63.64% (95% CI: 53.67–73.60), and blood at 55.17% (95% CI: 17.94–92.40). One study based on semen was excluded from subgrouping. Although differences were observed, they were not statistically

significant ($p = 0.16$), and heterogeneity remained high across all subgroups (I^2 = 94.44–99.32%) (Table 3 and S5 Figure).

Biofilm formation strength

Biofilm-forming *E. faecalis* isolates by strength level showed a pooled prevalence of 47.92% (95% CI: 39.34–56.51) for strong producers (35 studies), 38.06% (95% CI: 30.03–46.10) for moderate producers (32 studies), and 26.88% (95% CI: 19.89–33.87) for weak producers (24 studies). These categories were analyzed from distinct subsets of studies that reported stratified biofilm strength data; therefore, the percentages are based on different denominators and are not additive. High heterogeneity was observed across all subgroups (I^2 range: 88.52–93.92%, $p < 0.001$) (S6-S8 Figure). The overlapping confidence intervals (CI), particularly between moderate and weak producers, suggest a lack of statistically significant difference between these subgroups.

Meta-regression analyses

Meta-regression identified WHO region (β = -7.96, $p = 0.005$) and sample type (β = 4.56, $p = 0.043$) as significant contributors to biofilm prevalence heterogeneity, explaining 11.93% and 5.64% of variability, respectively. Continent, publication year, and detection method showed no significant effects (Table 4). However, high residual heterogeneity (I^2 >99%) indicates other factors also influence prevalence variation.

Sensitivity analysis

The leave-one-out sensitivity analysis demonstrated consistent pooled prevalence estimates across all iterations, ranging narrowly from 68.09% (95% CI: 60.69–75.49) to 69.83% (95% CI: 62.69–76.97). This minimal variability (a range of 1.74% points) confirms the stability of our meta-analysis results, as no single study exerted disproportionate influence on the overall estimate (Fig. 6).

Table 4 Meta-regression analysis of factors contributing to heterogeneity in the prevalence of biofilm formation among clinical *E. faecalis* isolates

Moderator	Coefficient	95% CI	R^2 (%)	τ^2	I^2 (%)
WHO region	-7.96	[-13.57, -2.36]	11.93	663.3	99.15
Specimen type	4.56	[0.15, 8.98]	5.64	717.6	99.22
Publication year category	13.03	[-1.53, 27.60]	3.80	724.6	99.23
Continent	5.57	[-3.48, 14.62]	0.96	746.0	99.18
Biofilm detection method	2.39	[-11.43, 16.21]	0.00	732.3	99.00

Note: The table presents the regression coefficient, 95% confidence interval (CI), proportion of variance explained (R^2), between-study variance (τ^2), and residual heterogeneity (I^2) for each moderator variable

Discussion

Biofilm formation, responsible for 65–80% of persistent infections such as dental caries and endocarditis [76–81], is a key survival mechanism for *E. faecalis*. Our review shows a global prevalence of 68.68% (95% CI: 61.33–76.02%), indicating that biofilm formation is not incidental but central to its resilience as a health-care associated pathogen.

Biofilms enhance antimicrobial resistance by forming a protective extracellular polymeric substance (EPS) matrix and harboring dormant persister cells, leading to treatment failure and recurrence [82, 83]. *E. faecalis* commonly colonizes urinary catheters and medical devices in polymicrobial biofilms [83], aided by virulence factors like *esp* and *efaA* that promote adhesion and immune evasion [56, 84]. Vancomycin-resistant strains often form stronger biofilms, compounding clinical challenges [3, 85]. This dual threat underscores the urgent need for biofilm-targeted diagnostics and therapeutics in clinical settings.

Over all, our analysis reveals that biofilm formation in *E. faecalis* is both widespread, with a striking prevalence of 68.68% (95% CI = 61.33%-76.02%), and highly variable. The heterogeneity among studies (I^2 = 99.30%) indicates that this variation stems from genuine biological differences in strain virulence [32, 86] as well as inconsistencies in the methodologies used for biofilm detection and quantification [37, 58]. Without uniform detection protocols, comparisons across studies, and their translation into clinical risk stratification, remain limited.

The extreme heterogeneity warrants further exploration of underlying drivers such as the genetic variability of isolates, differences in sample sources, and inconsistent definitions or thresholds used to classify positive biofilm formation [87–89]. For instance, studies varied in their interpretation of optical density cut-offs, and subjectivity in Crystal Violet staining interpretation may further exacerbate measurement variability [89]. Patient-related factors such as age, comorbidities, and hospitalization status may also contribute to inter-study differences, alongside regional disparities in healthcare infrastructure and laboratory capacity [90]. International consensus on biofilm assay standardization, possibly led by bodies such as CLSI or WHO, would significantly improve data comparability.

Biofilm intensity varied widely: 47.92% of producers were strong (95% CI: 39.34–56.51%), 38.06% moderate (95% CI: 30.03–46.10%), and 26.88% weak (95% CI: 19.89–33.87%), indicating a spectrum rather than a binary trait. Different detection methods—microtiter plate, crystal violet staining, and test tube assays, along with inconsistencies in incubation, washing, and reagent use, limit comparability [58]. Despite subgroup analyses by region, year, and method, high heterogeneity (I^2:

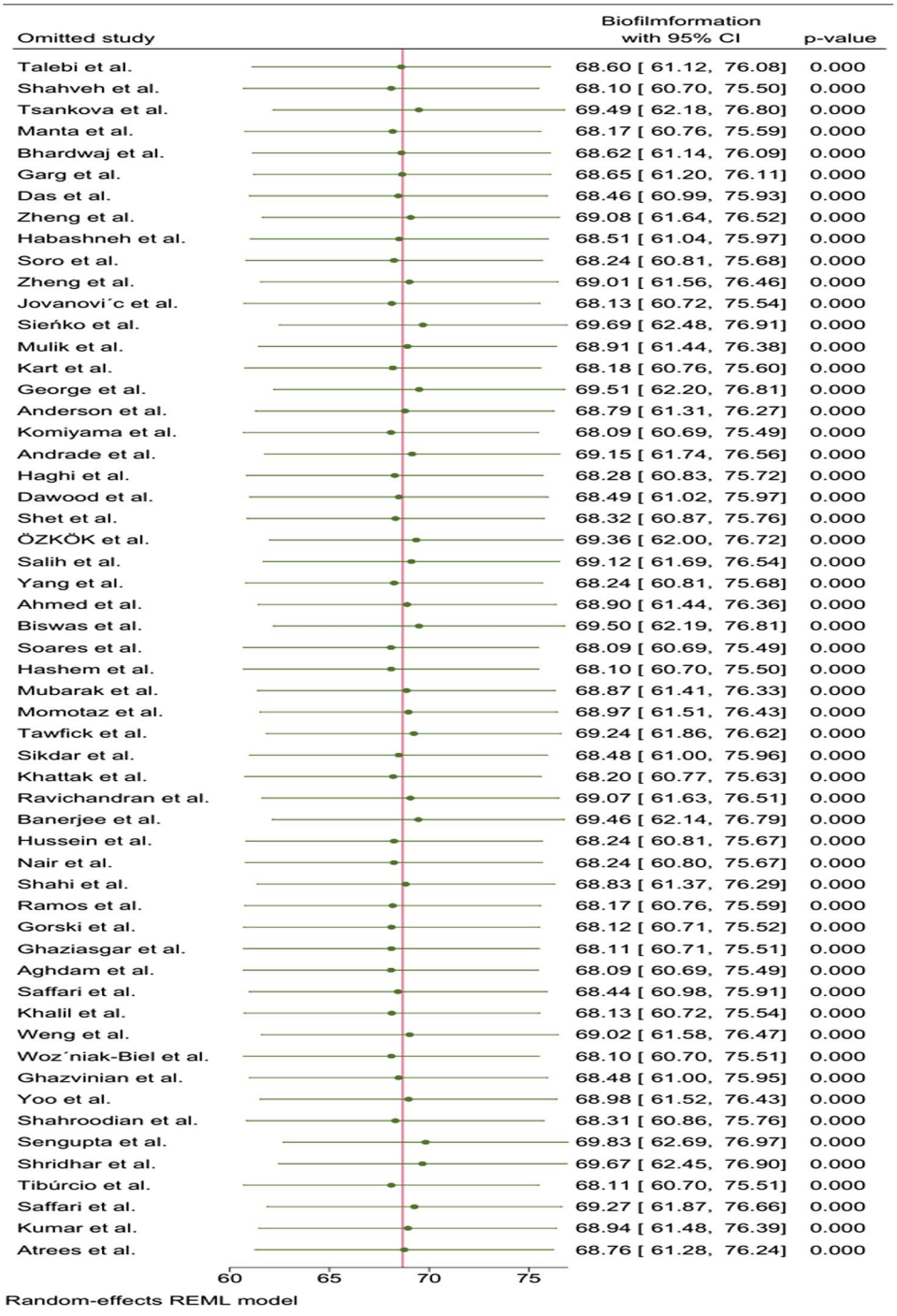

Fig. 6 A forest plot of the leave-one-out analysis, displaying the pooled prevalence estimate (with 95% CIs) obtained after sequentially excluding each study. The estimates cluster tightly around the original pooled prevalence of 68.46%, visually reinforcing the robustness of our findings

88.52–99.61%) persisted, emphasizing the need for standardized protocols to enhance reproducibility and clinical applicability.

Many studies did not report biofilm formation strength [14, 16, 25, 26, 32, 40, 42–44, 47–52, 57–59, 69], limiting understanding of its clinical relevance. Strong biofilm producers are often linked to persistent infections, higher antimicrobial tolerance, and increased treatment failure, particularly in conditions like endocarditis, chronic wounds [27], and CAUTIs [13], while weak producers may respond better to standard therapies. Future studies should routinely report biofilm strength and stratify outcomes. Incorporating molecular typing methods like multilocus sequence typing (MLST) or whole-genome

sequencing (WGS), can further link biofilm capacity to specific *E. faecalis* lineages, enhancing insight into pathogenicity, resistance, and regional patterns.

Evidence of publication bias complicates interpretation. Funnel plot asymmetry and Egger's test suggest small study effects, likely due to overrepresentation of studies with higher prevalence [91, 92]. Bias may also stem from methodological differences, selective regional reporting, and exclusion of gray literature [93]. However, extreme heterogeneity (I^2 = 99.30%) can itself distort funnel plots and bias tests, even without true publication bias [94]. Although the trim and fill method showed minimal correction, its validity is limited under high heterogeneity [95]. Future meta-analyses should include gray literature and data from underrepresented regions [96], particularly Africa, North America, and Australasia, to enhance generalizability. Strengthening research capacity in regions like sub-Saharan Africa should be a global priority.

Subgroup analyses revealed significant variation in the prevalence of *E. faecalis* biofilm formation across WHO regions, ranging from 57.93% (95% CI: 41.01–71.85) in South-East Asia to 89.79% (95% CI: 73.02–106.56) in the Americas ($p = 0.04$). Similarly, continental data showed South America with the highest prevalence and Europe the lowest ($p < 0.001$). These differences likely reflect a combination of biological factors, such as the presence of more virulent or biofilm-prone *E. faecalis* strains, and variations in healthcare infrastructure, including diagnostic capacity, infection control, and laboratory resources. Additionally, methodological inconsistencies in detection techniques and sampling strategies contribute to this variability [97, 98]. These findings highlight the importance of standardized biofilm detection protocols and the integration of genomic surveillance to better distinguish biological diversity from healthcare system influences driving regional differences.

Studies from 2015 to 2020 (32 studies) reported a pooled biofilm prevalence of 63.04% (95% CI: 52.86–73.22), and those from 2021 to 2024 (24 studies) showed 76.18% (95% CI: 66.25–86.11), with no significant temporal trend ($p = 0.07$). By method, microtiter plate assays (31 studies) had the highest pooled prevalence at 70.88% (95% CI: 60.72–81.04), followed by crystal violet staining (19 studies) at 66.42% (95% CI: 54.52–78.31), and the test tube method (2 studies) at 63.36% (95% CI: 32.36–94.36), though the small sample size limits confidence ($p = 0.81$). Variations likely stem from protocol differences, such as optical density thresholds and subjective interpretation, with quantitative methods generally more objective than qualitative ones [37, 99, 100]. This methodological heterogeneity underscores the need for standardized biofilm detection protocols to improve consistency and comparability.

Specimen-level analysis showed higher pooled biofilm prevalence in isolates from urine (81.12%; 95% CI: 69.15–93.09), root canals (80.13%; 95% CI: 55.48–104.78), stool (79.85%; 95% CI: 56.65–103.04), and blood (55.17%; 95% CI: 17.94–92.40), compared to mixed sources (63.64%; 95% CI: 53.67–73.60), which represent a broad categorization. However, the differences were not statistically significant ($p = 0.16$). The overrepresentation of urinary isolates may have inflated the overall estimate. Notably, semen was reported by a single study only, limiting interpretability. Inconsistencies in specimen reporting further underscore the need for disaggregated and standardized classification in future biofilm research.

Despite these subgroup differences, heterogeneity remained high across all strata (I^2 >95%), suggesting that these variables explain only part of the variability. Unaccounted-for sources, such as isolate genetic variability, differences in patient immunity, and methodological inconsistency, remain important contributors [101–103]. This underscores the need for integrated approaches combining epidemiological, clinical, and genomic data in future surveillance.

Meta-regression identified WHO region ($p = 0.005$, R^2 = 11.93%) and specimen type ($p = 0.043$, R^2 = 5.64%) as significant contributors to heterogeneity. In contrast, publication year, continent, and detection method were not statistically significant. Interestingly, specimen type emerged as significant in meta-regression despite being non-significant in subgroup analysis, likely due to the regression model's capacity to detect linear trends and control for between-study variation [38]. This highlights the value of using complementary statistical tools in systematic reviews [104].

Importantly, our sensitivity analyses confirm the robustness of our findings. Leave-one-out iterations indicate that no single study disproportionately influenced the pooled prevalence, as all iterations remained within the overall 95% confidence interval. This consistency reinforces the reliability of the meta-analytic results while simultaneously emphasizing the significant regional and methodological variability present in the literature.

Despite the global scope of this review, a significant geographic imbalance was observed among the included studies. Specifically, 60.70% (34 out of 56) originated from Asia, whereas only 7 studies were from Africa. Notably, no studies were identified from North America or Australasia. This uneven representation may limit the global generalizability of our findings and obscure regional variations in biofilm prevalence that could be influenced by local healthcare infrastructure, infection control practices, or strain-specific factors. In particular, the underrepresentation of regions such as sub-Saharan Africa raises concerns about potential gaps in understanding the true burden and behavior of biofilm-forming

E. faecalis in those settings. Future systematic reviews should prioritize balanced geographic inclusion to ensure more equitable surveillance and to inform region-specific strategies for prevention and control.

In summary, while our meta-analysis reinforces the high global prevalence of biofilm-forming *E. faecalis*, it also highlights the need for standardized detection methodologies and more comprehensive, regionally diverse data. Future research should address these methodological disparities and explore the underlying socioeconomic and healthcare-related factors contributing to geographic variability. Additionally, integrating molecular epidemiology approaches will be essential to elucidate strain-level genetic determinants of biofilm formation and their potential associations with clinical outcomes. Such efforts will be crucial in developing targeted strategies to mitigate the clinical impacts of biofilm-associated infections.

Strength and limitations

This systematic review and meta-analysis provide a detailed global estimate of biofilm formation in *E. faecalis*, revealing a pooled prevalence of 68.68% (95% CI: 61.33–76.02%). Drawing on data from more than fifty studies spanning multiple WHO regions, the large sample size strengthens the generalizability of the findings. The study adhered to strict PRISMA guidelines and employed advanced meta-analytic approaches, including sensitivity analyses, to ensure the robustness of the results. Additionally, identifying specimen type and geographic region as contributors to heterogeneity offers important directions for future research efforts.

However, several limitations must be acknowledged. High heterogeneity (I^2 = 99.30%) reflects differences in biofilm detection methods, assay types, sample sizes, and strain characteristics. Most studies used in vitro models under static, nutrient rich conditions, limiting clinical relevance. The lack of standardized biofilm assessment protocols hampers comparability. Clinical metadata such as age and comorbidities were often missing, precluding detailed subgroup analyses.

Additionally, the review included only English language studies, and geographic representation was uneven. 60.70% were from Asia, while North America, Australasia, and parts of Africa and South America were underrepresented. Inclusion of small sample studies may have inflated variability, and the 2015 to 2024 timeframe, while current, excludes earlier data that could provide historical insight. These limitations highlight the need for standardized methodologies, improved reporting, and more geographically balanced, clinically integrated research.

Conclusions and recommendations

In conclusion, this meta-analysis confirms a high global prevalence of biofilm-forming *E. faecalis* (pooled prevalence 68.68%, 95% CI: 61.33–76.02%) with marked methodological heterogeneity (I^2 = 99.30%) and notable geographic disparities, as prevalence varied significantly across WHO regions. Our findings also highlight variability related to specimen types and detection methods, underscoring the need for standardized biofilm assessment protocols. To improve clinical relevance and comparability, future research should integrate molecular typing to clarify strain-level differences. Targeted infection prevention and antibiotic stewardship in high-burden regions are critical to curbing biofilm-associated antimicrobial resistance. Furthermore, developing biofilm-disrupting therapies is essential to enhance treatment outcomes. These integrated strategies are vital for effectively managing biofilm-related infections in healthcare settings worldwide.

Future perspectives

Given the high global prevalence and substantial regional disparities of biofilm-forming *E. faecalis*, future research should prioritize the development and implementation of standardized and reproducible biofilm detection methods to allow for accurate comparisons across studies and over time. Large-scale multicenter investigations are urgently needed in underrepresented regions including sub-Saharan Africa, North America, and Australasia to close existing geographic data gaps and enhance the global applicability of findings. Additionally, greater inclusion of countries from South America and parts of the Middle East would improve representativeness and help uncover potential region-specific drivers of biofilm prevalence.

Longitudinal surveillance systems should be established to monitor temporal trends in biofilm formation and related antimicrobial resistance phenotypes. Integration of genomic and transcriptomic technologies may provide deeper insights into the molecular pathways underpinning biofilm-associated persistence. Finally, translational research focused on innovative antibiofilm strategies such as bacteriophage therapy, quorum sensing inhibitors, and biofilm disrupting enzymes holds significant promise for improving treatment outcomes in diverse clinical settings.

Abbreviations

MDR	Multidrug Resistance
VRE	Vancomycin-resistant *E. faecalis*
WHO	World Health Organization

Supplementary Information

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Supplementary Material 1.

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Author contributions
E.T. led the systematic review and meta-analysis, overseeing the study's conceptualization, article selection, data extraction, statistical analysis, and manuscript preparation. E.T., A.G., A.S., E.G., Y.G., M.D., B.K., Z.A., and MAR played a pivotal role in searching for relevant articles, conducting data extraction, performing statistical analysis, and contributing to manuscript drafting. E.T., A.S., A.G., Z.A., and MAR were involved in statistical analysis consultation of the overall process of this systematic review and meta-analysis. G.K., M.N., A.A., A.B., E.T., M.G., S.G., B.B.A., Z.T., A.J., Z.D., T.M., W.A., B.M., and W.T., M.K., involved in data mining, data extraction, in statistical analysis, manuscript writing, editing, and ensuring accuracy and completeness. Additionally, all authors actively engaged in critically.

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Data availability
All data analyzed in this study are included in this published article and its supplementary information files.

Declarations

Ethical approval and consent to participate
Not applicable as this is a systematic review and meta-analysis of published studies.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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