

The Global Prevalence of Biofilm-forming *Enterococcus faecalis* in clinical isolates: A systematic review and meta-analysis

Ephrem Tamrat ^{1*}, Zelalem Asmare^{1,2}, Alene Geteneh¹, Assefa Sisay¹, Ermias Getachew¹, Brhanu Kassanew¹, Mesfin Desale¹, Yalewayker Gashaw¹, Abdu Jemal¹, Muluken Gashaw¹, Alembante Bazezew¹, Solomon Gedfie¹, Woldeteklehaymanot Kassahun¹, Wagaw Abebe¹, Zelalem Dejzmach¹, Tadesse Misganaw¹, Agenagne Ashagre¹, Marye Nigatie¹, Abebe Adisu Damtie¹, Bewuketu Belete Alemu¹, Zewdu Tefera¹, Bahriew Mezgebu¹, Getinet Kumie¹, Mulugeta Kiros⁴, Melese Abate Reta^{1,3}

¹Department of Medical Laboratory Sciences, College of Health Sciences, Woldia University, Woldia, Ethiopia.

²Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Injibara University, Injibara, Ethiopia.

³Research Centre for Tuberculosis and Department of Medical Microbiology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

⁴Department of Medical Laboratory Sciences, College of Health Sciences, Raya University, Maichew, Ethiopia

*Corresponding Author: E-mail address: ephremtamrat445@gmail.com

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S1 Table: Search strategy and searching strings for the databases

This table outlines the detailed search strategy, including keywords, Boolean operators, and filters applied across the selected electronic databases to identify relevant studies.

#	Database	Searching strings	Number of articles
1	Scopus	prevalence OR epidemiology OR magnitude AND biofilms AND enterococcus AND faecalis OR e.faecalis OR enterococcus AND species AND clinical AND isolates AND PUBYEAR > 2014 AND PUBYEAR < 2025 AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))	4146
2	ScienceDirect	(Prevalence OR epidemiology OR magnitude) AND (biofilms) AND (enterococcus faecalis OR E.faecalis OR enterococcus species) AND (clinical isolates) Filter: Year: 2015-2024 Language: English Article: Research article	910
3	PubMed	((("prevalence"[MeSH Terms] OR "epidemiology"[MeSH Terms] OR ("magnitude"[All Fields] OR "magnitudes"[All Fields])) AND "biofilms"[MeSH Terms] AND "enterococcus faecalis"[MeSH Terms]) OR "enterococcus"[MeSH Terms]) AND ("humans"[MeSH Terms] AND 2015/01/01:2024/12/31[Date - Publication] AND "english"[Language])) AND ((humans[Filter]) AND (2015/1/1:2024/12/31[pdat]) AND (english[Filter]))	3958
4	Google Scholar	("prevalence" OR "epidemiology" OR "magnitude") AND biofilms AND ("Enterococcus faecalis" OR "E. faecalis" OR "Enterococcus species") AND ("clinical isolate" OR "clinical isolates") AND ("antimicrobial resistance" OR "virulence" OR "infection") AND "human" AND ("microbiology" OR "clinical microbiology" OR "laboratory study") AND ("pathogenesis" OR "biofilm formation") AND ("in vitro" OR "in vivo") Filter: Year: 2015-2024 Language: English Article: Any type	4790
Total			13804

S2 Table: Quality appraisal result of included studies; Using Joanna Briggs Institute (JBI) quality appraisal checklist for prevalence studies.

Authors	1 Appropriate sample frame	2 Appropriate sampling	3 Adequacy of sample size	4 Study subjects and setting described in detail	5 Data analysis conducted with sufficient coverage of the identified sample	6 Valid methods for the identification of the condition	7 Condition measured in a standard, reliable way	8 Appropriate statistical analysis	9 Adequacy of response rate	Total score/9
Talebi et al.	1	1	0	1	1	1	1	1	1	9
Shahveh et al.	1	1	1	1	1	1	1	1	1	9
Tsankova et al.	1	1	0	1	1	1	1	1	1	8
Manta et al.	0	0	0	1	1	1	1	1	1	7
Bhardwaj et al.	0	1	1	1	1	1	1	1	1	8
Garg et al.	1	1	0	1	1	1	1	1	0	8
Das et al.	1	1	0	1	1	1	1	1	1	8
Zheng et al.	1	1	1	1	1	1	1	1	1	9
Habashneh et al.	1	1	0	1	1	1	1	1	1	8
Soro et al.	1	0	0	1	1	1	1	1	1	8
Zheng et al.	1	1	0	1	1	1	1	1	1	8
Jovanović et al.	1	1	0	1	1	1	1	1	1	8
Sieńko et al.	1	0	0	1	1	1	1	1	1	7
Mulik et al.	1	1	0	1	1	1	1	1	1	8
Kart et al.	1	0	0	0	1	1	1	1	1	7
George et al.	1	1	1	1	1	1	1	1	1	9
Anderson et al.	1	1	0	1	1	1	1	1	1	8

Komiyama et al.	1	1	1	1	1	1	1	1	1	1	8
Andrade et al.	1	0	0	0	1	1	1	1	1	1	8
Haghi et al.	1	1	0	1	1	1	1	1	1	1	8
Dawood et al.	1	1	0	1	1	1	1	1	1	1	8
Shet et al.	1	0	0	0	1	1	1	1	1	1	7
ÖZKÖK et al.	1	1	0	1	1	1	1	1	1	1	8
Salih et al.	0	1	0	1	1	1	1	1	1	1	8
Yang et al.	1	1	1	1	1	1	1	1	1	0	8
Ahmed et al.	1	1	1	1	1	1	1	1	1	0	8
Biswas et al.	1	1	1	1	1	1	1	1	1	1	9
Soares et al.	1	1	0	1	1	1	1	1	1	0	7
Hashem et al.	1	1	0	1	1	1	1	1	1	1	8
Mubarak et al.	1	1	0	1	1	1	1	1	1	1	8
Momotaz et al.	1	1	0	1	1	1	1	1	1	1	8
Tawfick et al.	0	1	0	1	1	1	1	1	1	1	8
Sikdar et al.	1	1	1	1	1	1	1	1	1	1	9
Khattak et al.	0	1	0	1	1	1	1	1	1	1	8
Ravichandran et al.	1	0	0	0	1	1	1	1	1	1	7
Banerjee et al.	1	1	1	1	1	1	1	1	1	1	9
Hussein et al.	0	1	0	1	1	1	1	1	1	1	8
Nair et al.	1	1	0	1	1	1	1	1	1	1	8
Shahi et al.	1	1	0	1	1	1	1	1	1	1	8
Ramos et al.	1	1	0	1	1	1	1	1	1	0	8
Gorski et al.	1	1	0	1	1	1	1	1	1	1	8

Ghaziasgar et al.	0	1	0	1	1	1	1	1	0	7
Aghdam et al.	0	1	0	1	1	1	1	1	0	7
Saffari et al.	0	1	0	1	1	1	1	1	0	7
Khalil et al.	1	1	1	1	1	1	1	1	1	9
Weng et al.	1	1	0	0	1	1	1	1	1	8
Woz'niak-Biel et al.	0	1	0	1	1	1	1	1	0	7
Ghazvinian et al.	1	1	0	1	1	1	1	1	1	8
Yoo et al.	0	1	0	1	1	1	1	1	0	7
Shahroodian et al.	0	1	0	1	1	1	1	1	1	8
Sengupta et al.	0	1	0	1	1	1	1	1	1	8
Shridhar et al.	1	0	0	1	1	1	1	1	1	8
Tibúrcio et al.	1	0	0	1	1	1	1	1	0	7
Saffari et al.	1	1	1	1	1	1	1	1	0	8
Kumar et al.	0	1	0	1	1	1	1	1	1	8
Atrees et al.	0	1	0	1	1	1	1	1	0	7

1: Yes ; 0: No

S3 Table: Characteristics of included studies, from January 2015- December 2024.

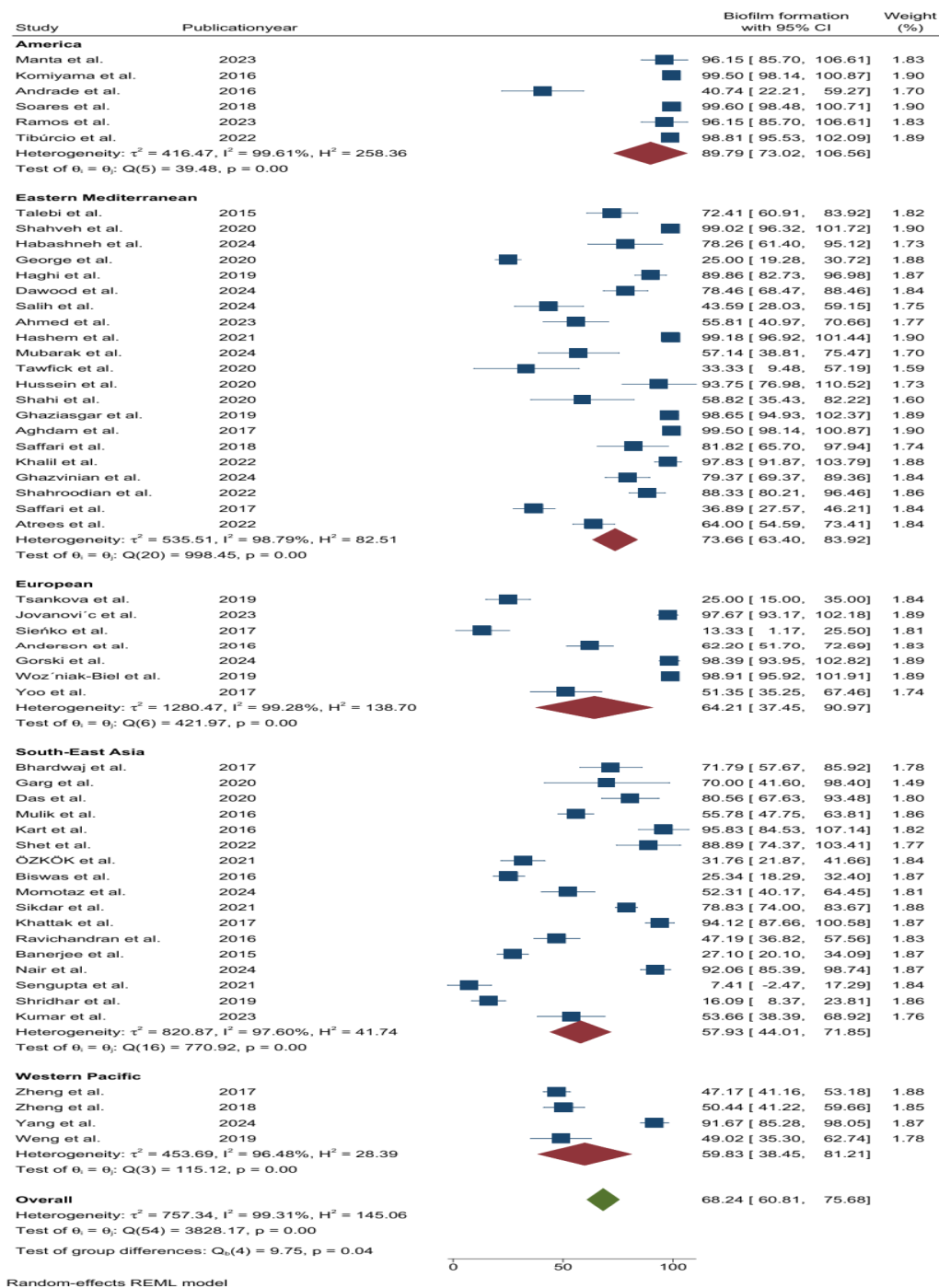
S. No	Author's name	Publication year	Country	Sample type
1	Talebi <i>et al.</i> [21]	2015	Iran	Mixed
2	Shahveh <i>et al.</i> [13]	2020	Iran	Urine
3	Tsankova <i>et al.</i> [14]	2019	Bulgaria	Mixed
4	Manta <i>et al.</i> [19]	2023	Brazil	Mixed
5	Bhardwaj <i>et al.</i> [39]	2017	India	Mixed
6	Garg <i>et al.</i> [25]	2020	India	Urine
7	Das <i>et al.</i> [40]	2020	India	Urine
8	Zheng <i>et al.</i> [27]	2017	China	Mixed
9	Habashneh <i>et al.</i> [12]	2024	Jordan	Mixed
10	Soro <i>et al.</i> [65]	2024	Kenya	Mixed
11	Zheng <i>et al.</i> [28]	2018	China	Urine
12	Jovanovi'c <i>et al.</i> [66]	2023	Serbia	Mixed
13	Sieńko <i>et al.</i> [58]	2017	Poland	Mixed
14	Mulik <i>et al.</i> [41]	2016	India	Mixed
15	Kart <i>et al.</i> [59]	2016	Türkiye	Urine
16	George <i>et al.</i> [42]	2020	Saudi Arabia	Mixed
17	Anderson <i>et al.</i> [29]	2016	Germany	Mixed
18	Komiyama <i>et al.</i> [16]	2016	Brazil	Mixed
19	Andrade <i>et al.</i> [60]	2016	Brazil	Blood
20	Haghi <i>et al.</i> [43]	2019	Iran	Urine

21	Dawood <i>et al.</i> [67]	2024	Egypt	Mixed
22	Shet <i>et al.</i> [44]	2022	India	Mixed
23	ÖZKÖK <i>et al.</i> [68]	2021	Türkiye	Blood
24	Salih <i>et al.</i> [45]	2024	Iraq	Root Canal
25	Yang <i>et al.</i> [30]	2024	China	Blood
26	Ahmed <i>et al.</i> [69]	2023	Egypt	Urine
27	Biswas <i>et al.</i> [46]	2016	India	Mixed
28	Soares <i>et al.</i> [61]	2018	Brazil	Mixed
29	Hashem <i>et al.</i> [18]	2021	Egypt	Urine
30	Mubarak <i>et al.</i> [70]	2024	Egypt	Stool
31	Momotaz <i>et al.</i> [47]	2024	Bangladesh	Mixed
32	Tawfick <i>et al.</i> [62]	2020	Egypt	Mixed
33	Sikdar <i>et al.</i> [48]	2021	India	Mixed
34	Khattak <i>et al.</i> [49]	2017	Pakistan	Mixed
35	Ravichandran <i>et al.</i> [50]	2016	India	Mixed
36	Banerjee <i>et al.</i> [51]	2015	India	Mixed
37	Hussein <i>et al.</i> [52]	2020	Iraq	Root Canal
38	Nair <i>et al.</i> [31]	2024	India	Mixed
39	Shahi <i>et al.</i> [32]	2020	Iran	Mixed
40	Ramos <i>et al.</i> [71]	2023	Brazil	Mixed
41	Gorski <i>et al.</i> [72]	2024	Croatia	Mixed
42	Ghaziasgar <i>et al.</i> [33]	2019	Iran	Mixed
43	Aghdam <i>et al.</i> [53]	2017	Iran	Root Canal

44	Saffari <i>et al.</i> [54]	2018	Iran	Root Canal
45	Khalil <i>et al.</i> [20]	2022	Saudi Arabia	Urine
46	Weng <i>et al.</i> [55]	2019	Malaysia	Mixed
47	Woz'niak-Biel <i>et al.</i> [63]	2019	Poland	Stool
48	Ghazvinian <i>et al.</i> [23]	2024	Iran	Stool
49	Yoo <i>et al.</i> [64]	2017	Australia	Mixed
50	Shahroodian <i>et al.</i> [24]	2022	Iran	Semen
51	Sengupta <i>et al.</i> [26]	2021	India	Mixed
52	Shridhar <i>et al.</i> [22]	2019	India	Mixed
53	Tibúrcio <i>et al.</i> [73]	2022	Brazil	Mixed
54	Saffari <i>et al.</i> [56]	2017	Iran	Mixed
55	Kumar <i>et al.</i> [57]	2022	India	Mixed
56	Atrees <i>et al.</i> [74]	2023	Egypt	Urine

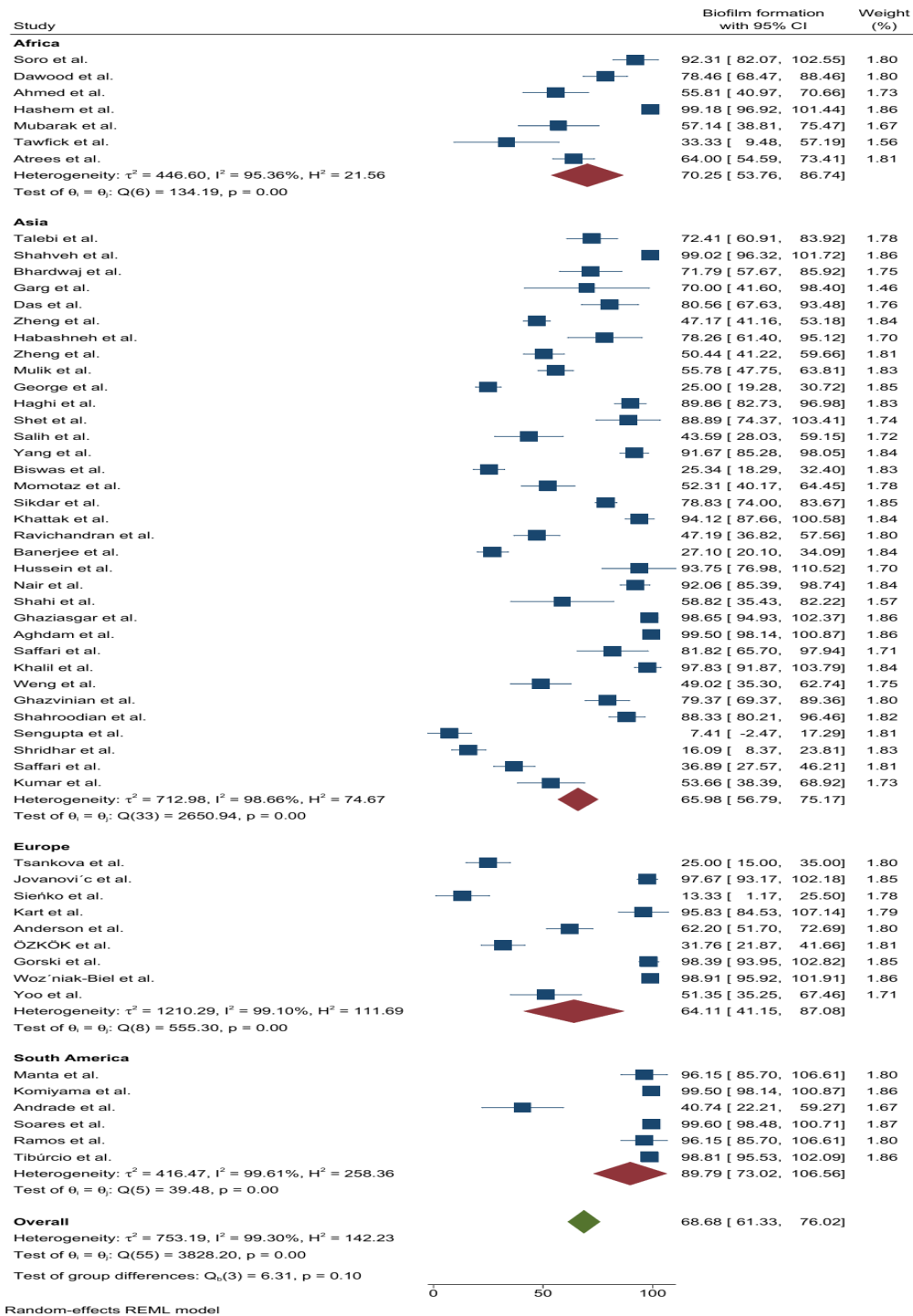
S4 Table: Trim-and-Fill Analysis of Publication Bias for Biofilm-Forming *E. faecalis*.

Imputation Direction	No. of Studies Observed	No. of Studies Imputed	Pooled Prevalence (%)	95% Confidence Interval (%)
Left	56	1	68.08	60.78 – 75.39
Right	56	0	68.68	61.33 – 76.02



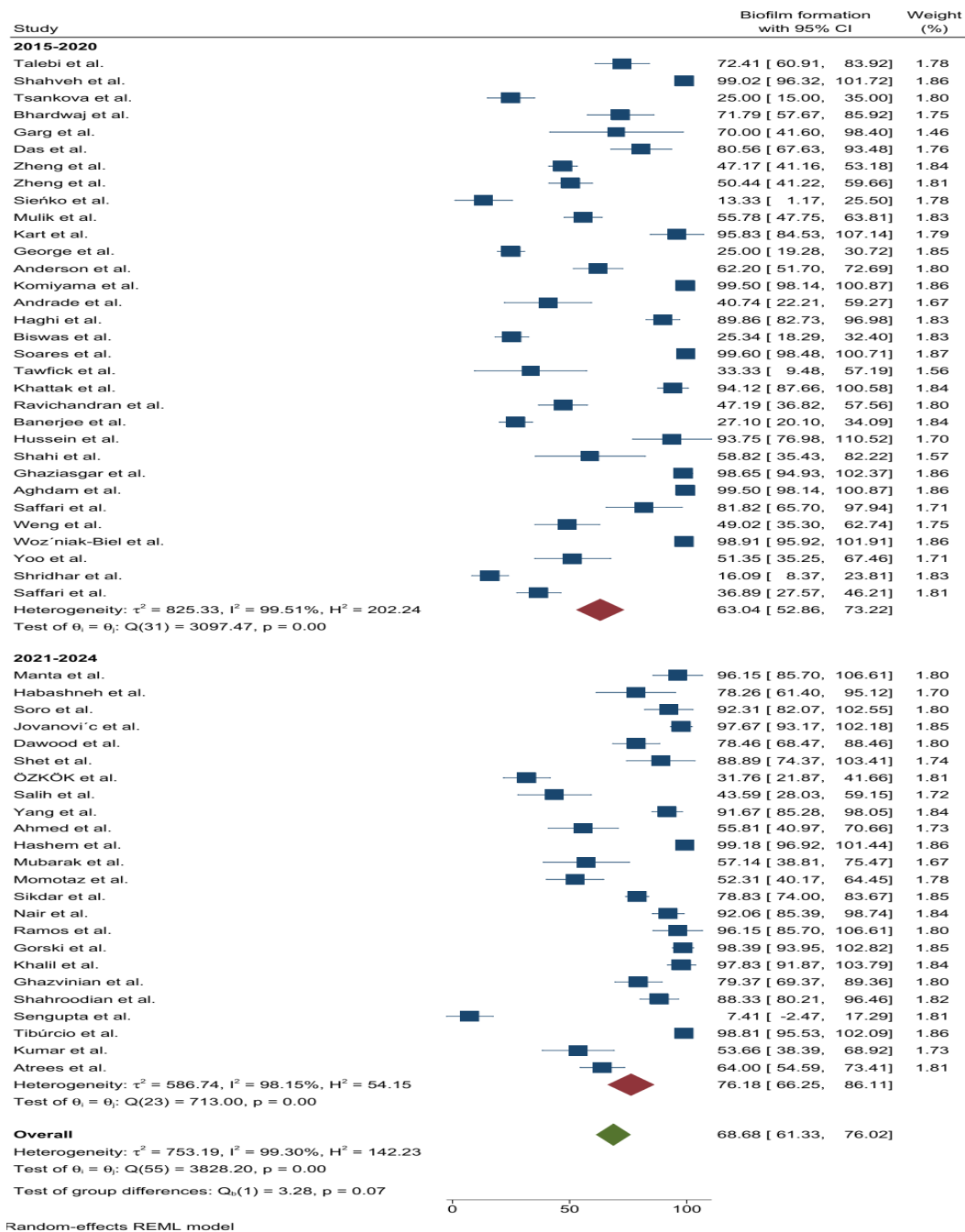
S1 Figure: Subgroup analysis of Biofilm-forming *E. faecalis* clinical isolates by WHO region.

This forest plot presents pooled prevalence estimates of biofilm-forming *E. faecalis* isolates based on WHO region. The highest prevalence was reported in the Americas (89.79%; 95% CI: 73.02–106.56), while the lowest was in the South-East Asia Region (57.93%; 95% CI: 41.01–71.85). The pooled prevalence in the Eastern Mediterranean Region was 73.66% (95% CI: 63.40–83.92). High heterogeneity was observed across all subgroups ($I^2 > 96\%$, $p < 0.001$). **Note:** All studies from the Americas/South America were conducted in Brazil only.



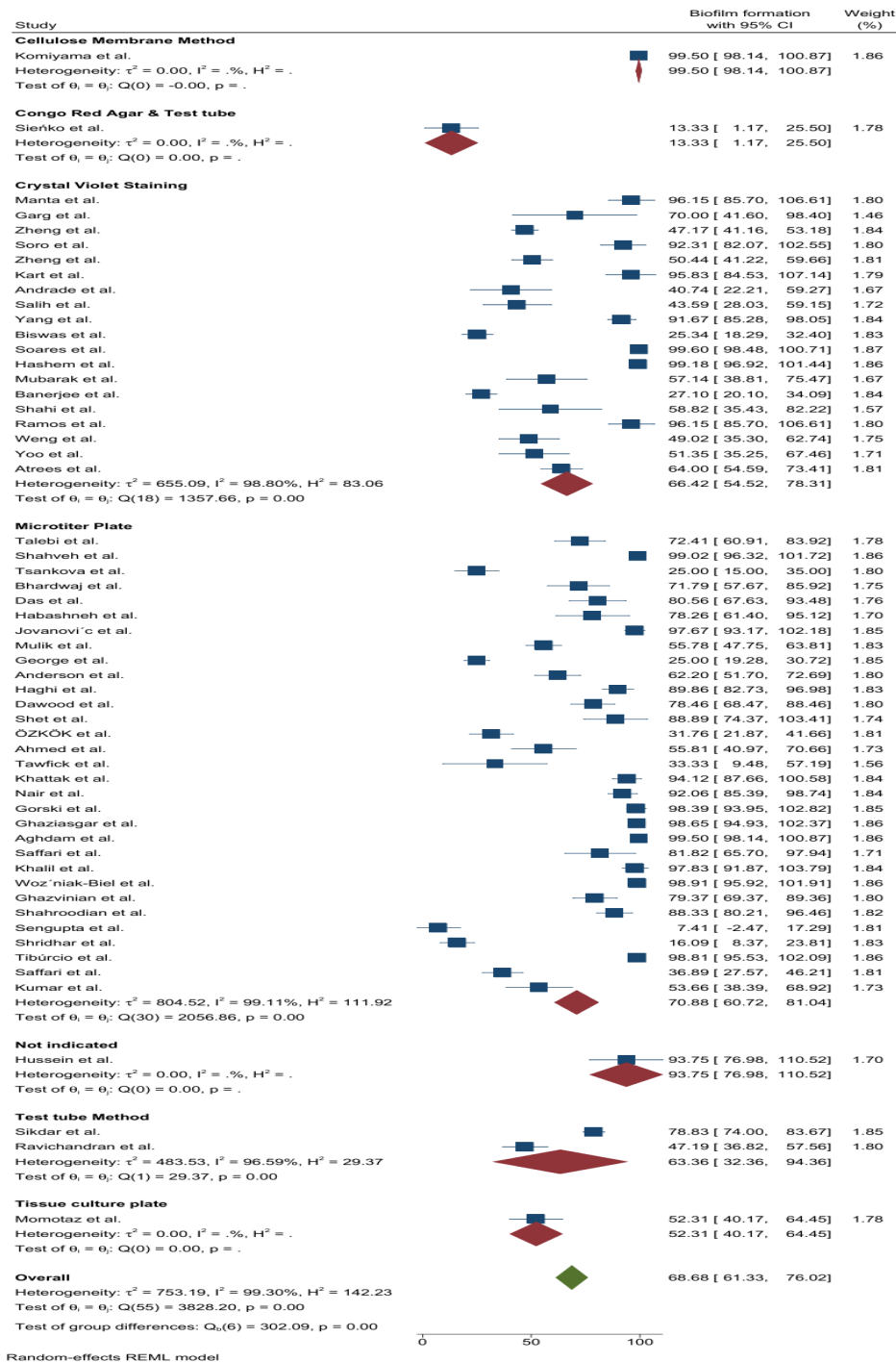
S2 Figure: Subgroup analysis of Biofilm-forming *E. faecalis* clinical isolates by continent.

This forest plot shows pooled prevalence estimates by continent. South America had the highest prevalence (89.79%; 95% CI: 73.02–106.56), followed by Africa (70.25%; 95% CI: 53.76–86.74), Asia (65.98%; 95% CI: 56.79–75.17), and Europe (64.11%; 95% CI: 41.15–87.08). All subgroups exhibited high heterogeneity ($I^2 > 95\%$, $p < 0.001$).



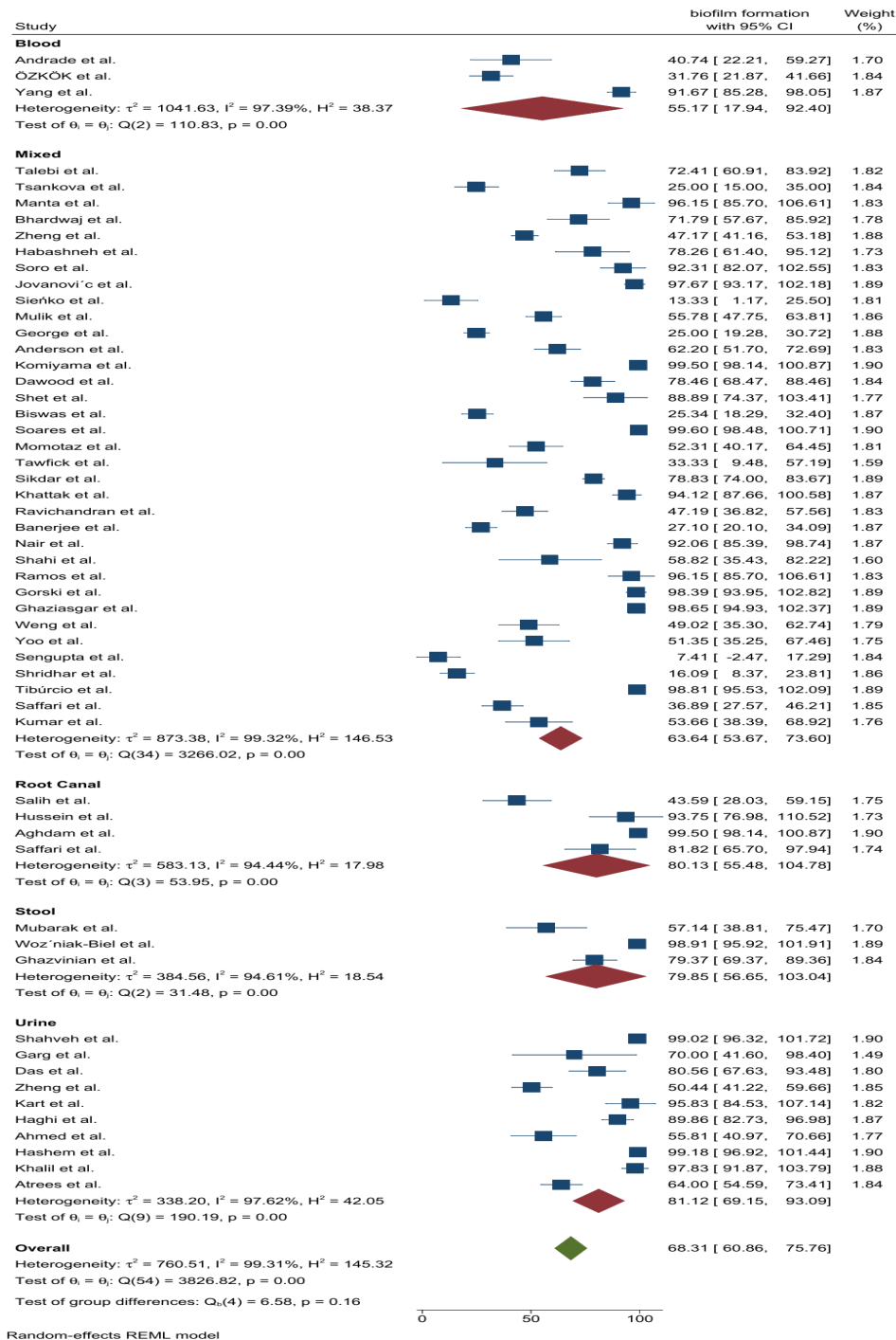
S3 Figure: Subgroup analysis of Biofilm-forming *E. faecalis* clinical isolates by Publication year.

This figure presents pooled prevalence estimates over time. Studies published between 2021–2024 reported a higher prevalence of biofilm formation (76.18%; 95% CI: 66.25–86.11) compared to those published between 2015–2020 (63.04%; 95% CI: 52.86–73.22). Both subgroups exhibited substantial heterogeneity ($I^2 > 98\%$, $p < 0.001$), potentially reflecting methodological improvements or regional differences. Despite the observed increase, the difference between the two time periods was not statistically significant ($p = 0.07$).



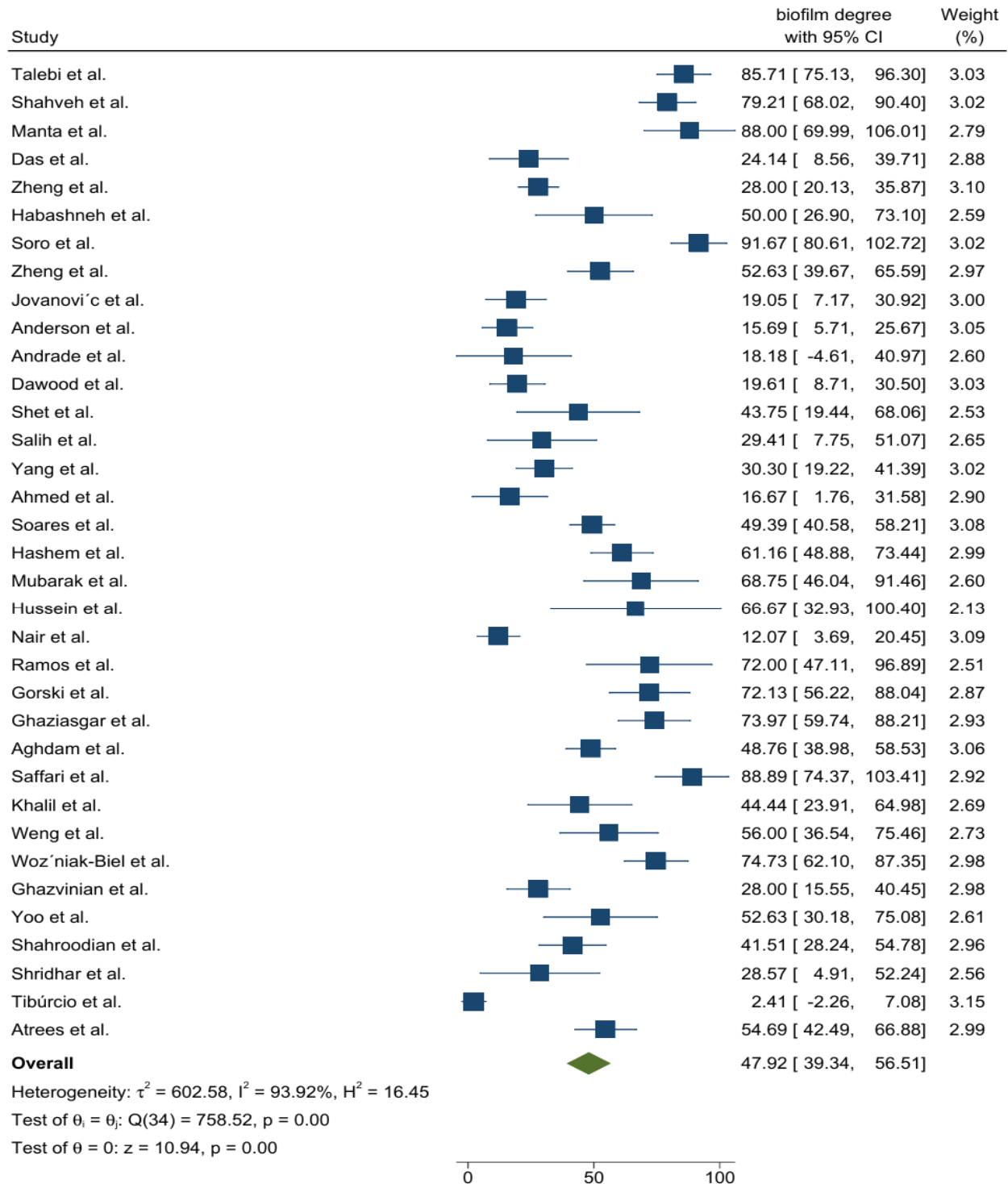
S4 Figure: Subgroup analysis of Biofilm-forming *E. faecalis* clinical isolates by Biofilm detection methods.

This plot compares pooled prevalence by detection method. The Microtiter Plate method had the highest prevalence (70.88%; 95% CI: 60.72–81.04), followed by the Crystal Violet Staining method (66.42%; 95% CI: 54.52–78.31) and the Test Tube method (63.36%; 95% CI: 32.36–94.36). Substantial heterogeneity was noted across all methods ($I^2 > 96\%$, $p < 0.001$). However, the test for subgroup differences was not statistically significant ($p = 0.81$).



S5 Figure: Subgroup analysis of Biofilm-forming *E. faecalis* clinical isolates by Sample type.

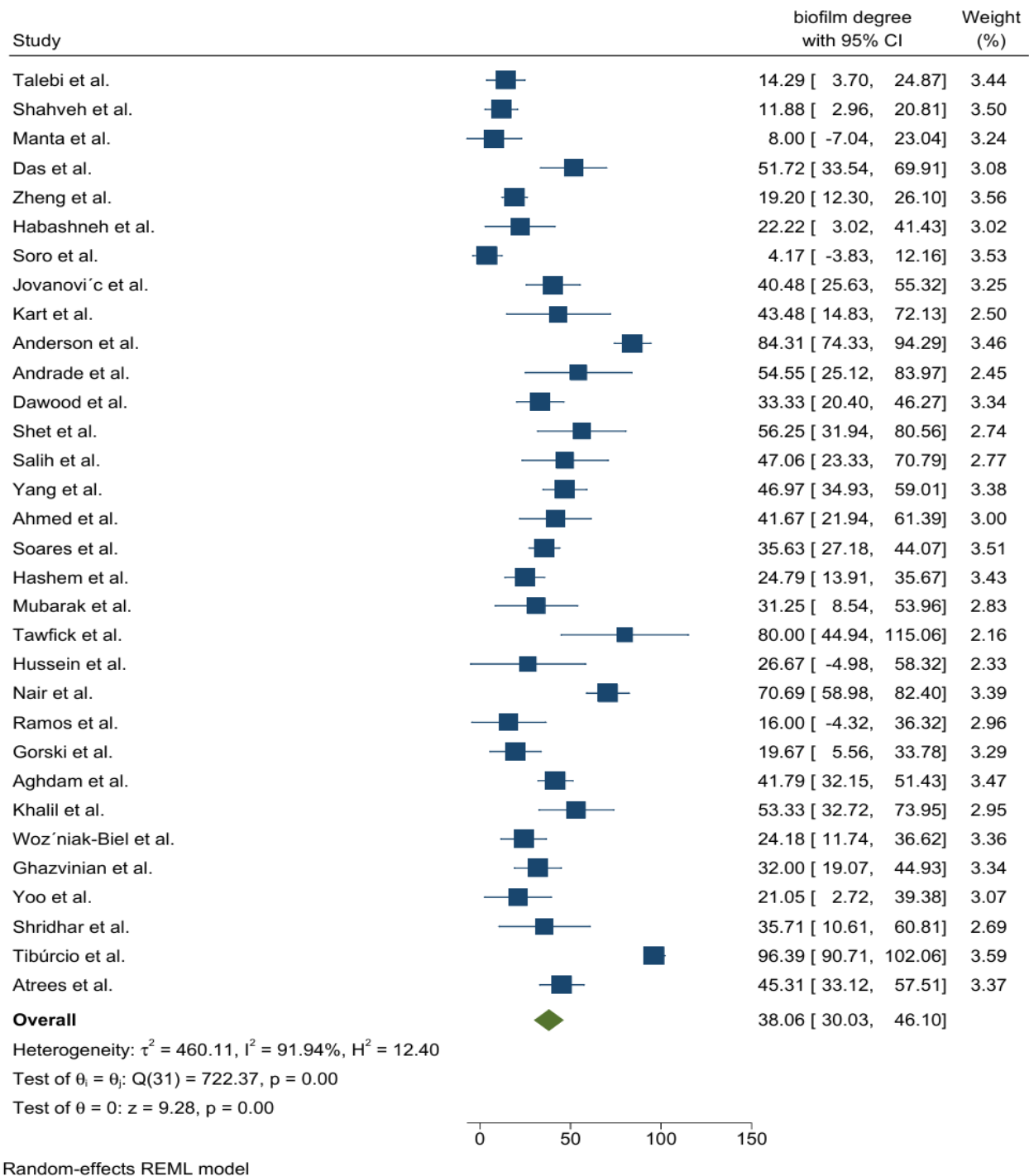
The highest pooled prevalence was observed in urine (81.12%) and root canal samples (80.13%), while blood samples showed the lowest (55.17%). Heterogeneity was high across all subgroups ($I^2 > 94\%$), and subgroup differences were not statistically significant ($p = 0.16$).



Random-effects REML model

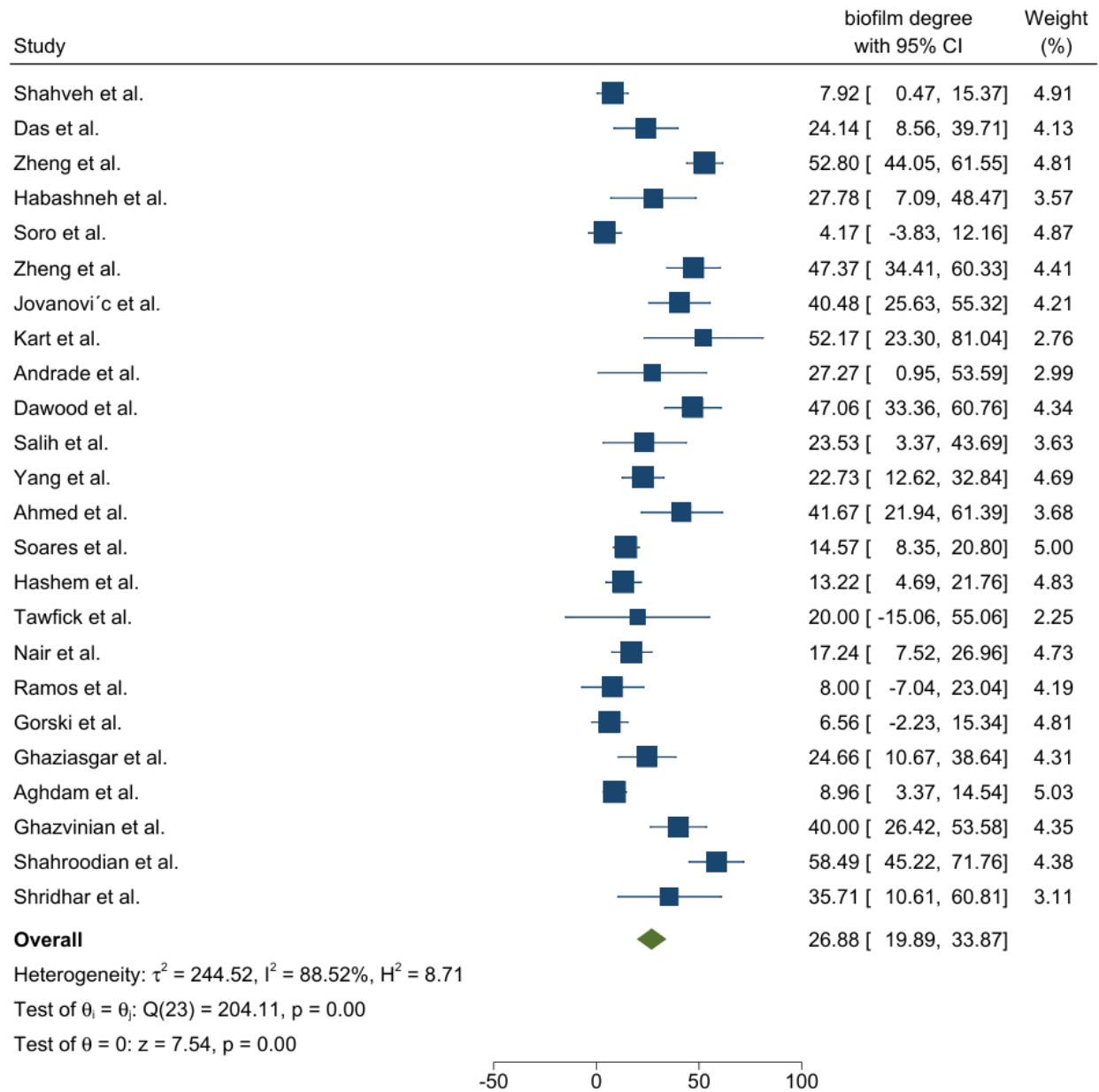
S6 Figure: Prevalence of Strong Biofilm-forming *E. faecalis* clinical isolates.

This forest plot displays the pooled prevalence of *E. faecalis* isolates classified as strong biofilm producers across included studies. The estimated prevalence was 47.92% (95% CI: 39.34–56.51), with substantial heterogeneity observed ($I^2 = 93.92\%$, $p < 0.001$).



S7 Figure: Prevalence of Moderate Biofilm-forming *E. faecalis* clinical isolates.

This forest plot shows the pooled prevalence of *E. faecalis* isolates identified as moderate biofilm producers. The estimated prevalence was 38.06% (95% CI: 30.03–46.10), with substantial heterogeneity ($I^2 = 91.94\%$, $p < 0.001$).



Random-effects REML model

S8 Figure: Prevalence of Weak Biofilm-forming *E. faecalis* clinical isolates.

This figure presents the pooled prevalence of weak biofilm-forming *E. faecalis* clinical isolates, estimated at 26.88% (95% CI: 19.89%–33.87%). The meta-analysis reveals significant heterogeneity among the included studies, with an I^2 value of 88.52% ($p < 0.001$), indicating substantial variability in prevalence estimates across studies.