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Review

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Prevalence and Antibiotic Resistance of Biofilm-Forming Clinical *Escherichia coli* Isolates in Asia: A Systematic Review and Meta-Analysis

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Abstract: Biofilm formation in *Escherichia coli* promotes persistence, pathogenicity, and antibiotic resistance in the infections of urinary tract, bloodstream, and wounds, highlighting a global public health concern. This systematic review and meta-analysis evaluated the prevalence and antibiotic resistance patterns of biofilm-forming clinical *E. coli* isolates from 17 Asian countries. Following PRISMA guidelines, PubMed and Google Scholar were searched for studies spanning January 2010 and May 2025, and 67 studies were included. In a random-effects meta-analysis of 31 microtiter plate studies (logit-transformed proportions, DerSimonian–Laird τ^2, Hartung–Knapp–Sidik–Jonkman confidence interval), the pooled biofilm prevalence was 63% (95% CI: 51–73%; 95% prediction interval [PI]: 17–93%; $I^2 = 95.6\%$, $p < 0.0001$). Pooled estimates were similar across the detection methods (63–67%). Urinary isolates, which dominated the dataset, had a pooled prevalence of 65% (95% CI: 51–77). Among the biofilm-producing isolates (microtiter plate assay), separately pooled proportions of strong, moderate and weak producers were 26%, 35% and 35%, respectively; non-producers accounted for 35% of all isolates tested (k = 40, any detection method). Among the biofilm producers, pooled resistance proportions were highest for ampicillin (84%) and amoxicillin–clavulanate (78%), and lowest for ertapenem (8%) and meropenem (18%); descriptive class-level means were highest for tetracyclines (66.4%) and fluoroquinolones (60.4%). These in vitro susceptibility data may not establish clinical effectiveness against biofilm-associated *E. coli* infections. Funnel-plot asymmetry was detected for four subgroups (Egger's/Begg's test, $p < 0.10$), and trim-and-fill sensitivity analyses shifted the estimates to inconsistent directions (e.g., total microtiter 50% [37–63%]; urine isolates 53% [37–68%]), consistent with small-study effects and heterogeneity rather than publication bias alone. Overall, the published evidence indicates that biofilm formation and resistance against common antibiotics are frequently reported among clinical *E. coli* isolates in Asia, particularly urinary isolates. However, the high heterogeneity and wide prediction interval indicate that prevalence in individual settings may differ substantially from the pooled estimate; thus, standardized multicenter surveillance is needed to inform catheter care and antibiotic stewardship for *E. coli* infections across Asia.



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Keywords: antibiotic resistance; biofilm; antibiotic stewardship; *Escherichia coli*; urinary tract infections; systematic review; meta-analysis

1. Introduction

Biofilms are organized bacterial populations that are adherent to biotic or abiotic surfaces by an extracellular polymeric matrix self-produced by the bacteria, and this provides protection against antibiotics, disinfectants, and the host immune system [1]. *Escherichia coli* is a diverse, Gram-negative, rod-shaped bacterium commonly found in the mammalian gut, with certain strains possessing an increasingly recognized virulence factor: the ability to form biofilms [2]. *E. coli* is a common pathogen in a variety of diseases and the leading cause of UTIs [3]. *E. coli* biofilms are mostly detected by phenotypic methods like the microtiter plate assay, although their increased production is driven by bacterial genetic elements like adhesins and quorum sensing systems, as well as surface properties and nutritional availability [4]. Geographically and in clinical settings, the prevalence and distribution of biofilm-forming *E. coli* differ, and there is growing concern in regions, particularly Asia, where antibiotic resistance [5] and challenging healthcare environments [6,7] may amplify the clinical impact of these bacteria.

Asia is densely populated, diversified region with a wide range of healthcare systems, antibiotic consumption patterns, and infection control guidelines [8–11]. Asia also bears a high urinary tract infection (UTI) burden, with the highest number of cases reported globally and projections of rising incidence [12]. These variables can affect the clinical settings where biofilms are most harmful, as well as the frequency of strains that produce biofilms [13]. Individual studies from Asian countries have reported widely varying rates of biofilm-positive *E. coli*; f*or* example, reports from Syria, India, Nepal, and Iran have found >80% of isolates are biofilm producers [14], while hospital surveys in Iraq, Saudi Arabia, and China have reported only about 15–20% of isolates form detectable biofilms [11,15–20].

Biofilm formation is strongly associated with elevated antibiotic resistance in *E. coli* isolates. It has been demonstrated that, compared to the planktonic form, biofilm-producing *E. coli* isolates are substantially more resistant to a variety of current antibiotics, such as fluoroquinolones, trimethoprim-sulfamethoxazole, and extended-spectrum cephalosporins [21]. This increased resistance in biofilm is favored by a variety of mechanisms, including the horizontal transfer of resistance genes within the biofilm community, modified bacterial physiology (such as slow growth rates), restricted diffusion of antibiotics through the extra-cellular matrix, and adaptive stress responses [22,23]. Particularly in areas with high antibiotic consumption and a variety of healthcare practices, the pervasiveness of biofilm-forming *E. coli* with alarming antibiotic resistance patterns highlights an immediate public health emergency.

This systematic review and meta-analysis investigated published research on biofilm development in clinical *E. coli* isolates from Asian nations. Particularly, this study focused on estimating the combined prevalence of biofilm-forming *E. coli* in clinical samples, determining the distribution of biofilm production strength, and classifying isolates as strong, moderate, or poor producers when information was available, and assessing antibiotic resistance against different antibiotic classes. The knowledge gained from this research is intended to guide the concerned stakeholders and healthcare systems to manage the pathogen and clinical burden of illnesses linked to *E. coli* biofilms in a more regionally informed manner.

2. Materials and Methods

2.1. Literature Search and Selection

This meta-analysis was conducted according to the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020) [24]. We searched PubMed and Google Scholar for English-language studies on biofilm formation and antibiotic resistance in clinical *E. coli* isolates from Asia published between January 1, 2010 and May 31, 2025. The search combined three concept blocks, namely terms for *E. coli* (e.g., "*Escherichia coli*", "*E. coli*"), biofilm (e.g., biofilm*, "biofilm formation") and antimicrobial resistance (e.g., "antibiotic resistance", "antimicrobial susceptibility"), together with a geographic block comprising "Asia" and the names of individual Asian countries; synonyms were combined with OR within each block and the blocks were combined with AND. Because Google Scholar does not support controlled vocabulary or full Boolean syntax, simplified keyword strings were used, and all retrieved records were screened. Additional records ("other sources") were identified by searching the websites of regional journals that are not indexed in PubMed, such as Nepal Journals Online (NepJOL) and the International Journal of Current Microbiology and Applied Sciences (IJCMAS). The complete search strings, run dates, filters and number of records retrieved from each source are provided in Supplementary Table S2. PubMed was chosen as the principal source because it indexes the major clinical microbiology and infectious-disease journals, and Google Scholar was added to capture regional journals and

reports from Asian countries that are not indexed in PubMed. Only English-language reports were eligible because the review team could not reliably extract data from articles in other languages; this restriction may have introduced language and selection bias, which is considered in the Limitations. Retrieved records were imported into Paperpile (Paperpile LLC, Cambridge, MA, USA), and duplicates were identified with its duplicate-detection function and removed after manual verification. Before screening, records that fell outside the language (English) or date (January 2010–May 2025) limits on inspection of their bibliographic details were also removed; these arose mainly from Google Scholar, which does not allow language filtering. Titles and abstracts and then full texts were assessed against the eligibility criteria by three reviewers (PD, SN and BP) independently, and disagreements were resolved by discussion among the authors. The study selection process is shown in the PRISMA 2020 flow diagram (Figure 1).

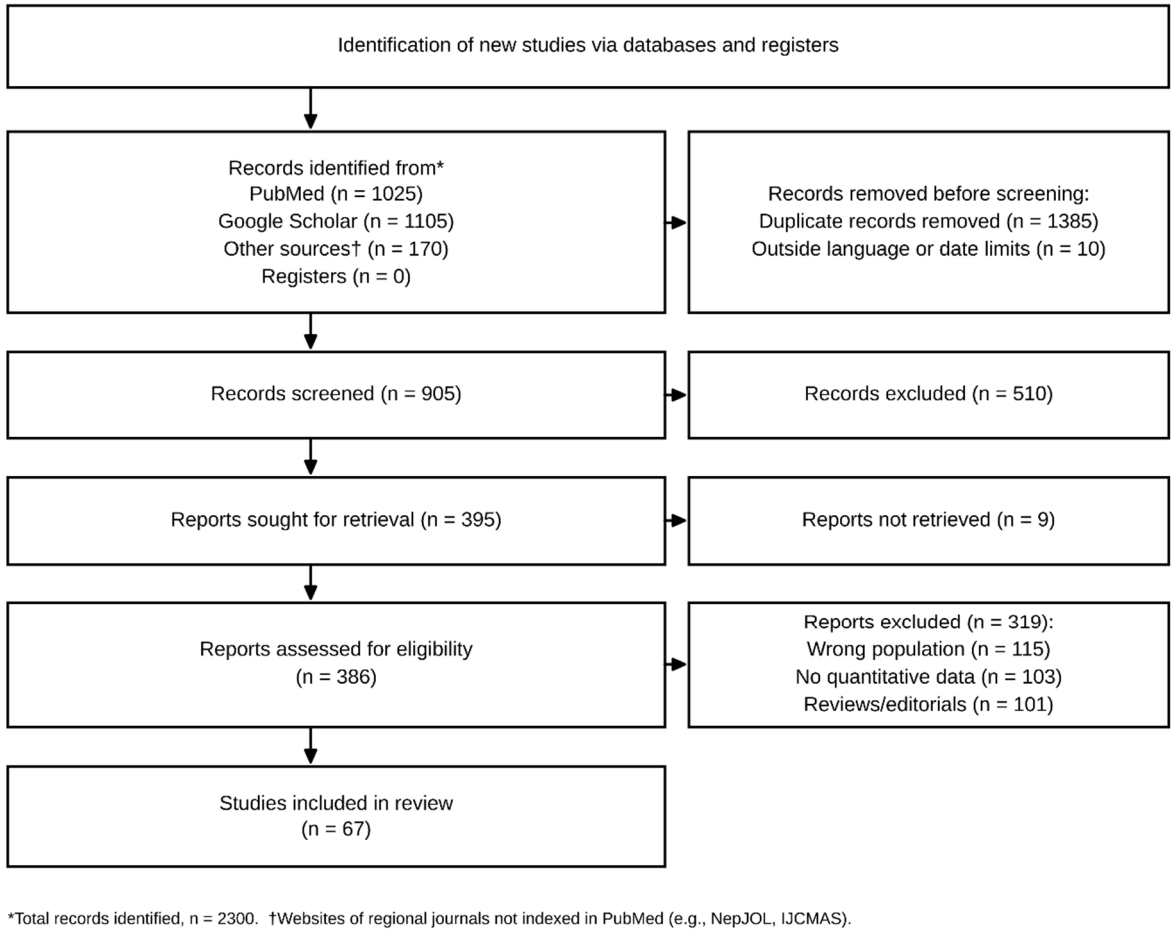


Figure 1. PRISMA 2020 flow diagram of study identification, screening, eligibility assessment and inclusion.

2.2. Inclusion and Exclusion Criteria

Studies were included if they met the following criteria:

Clinical sample: E. coli clinical isolates derived from patient samples from any clinical source. Excluded from consideration were studies that only examined environmental or commensal isolates without a clinical setting.

Exposure/feature of interest: Evaluation of biofilm-forming capacity by any established phenotypic method, such as Congo Red Agar (CRA) assay, tube-adherence method, or microtiter plate/crystal violet assays. Studies had to provide a quantitative classification (strong, moderate, or weak producers, for example) or the percentage of isolates that were biofilm positive.

Outcomes: Studies are required to at least offer one of the following: (1) the percentage or quantity of E. coli isolates found to develop biofilms, (2) the classification of the strength of biofilm formation, or (3) specific information about the detection technique employed.

Antibiotic resistance: Studies contributed to the antibiotic resistance analyses only if they reported susceptibility results specifically for biofilm-forming isolates; studies that reported resistance only for the overall (unstratified) isolate population were not included in these analyses.

Reviews, case studies, and research lacking primary data were not included. We used the more thorough or current report when there were many publications that presented overlapping data from the same cohort. Each

included report was checked by title, DOI, country, study site, sampling period and sample size to ensure that no study or patient cohort was counted more than once. Additionally, studies that used only ambient samples or animal isolates were excluded.

2.3. Data Extraction

Three authors (PD, SN, and BP) separately retrieved important data using a standardized extraction form, cross-checking their work for accuracy. Biofilm detection methods and criteria, number or proportion of biofilm-positive isolates, study design, sample collection period, sample size (total *E. coli* isolates examined), first author, publication year, country, specific study sites, classification of biofilm production strength (non-producer, weak, moderate, and strong), and antibiotic-resistant biofilm-producing colonies were among the variables that were extracted. We made sure that the data was specifically related to *E. coli*. If sample sizes were available, absolute numbers were back calculated when only percentages were reported. Microsoft Excel was used to collect and organize data for statistical analysis. For the resistance analyses, the numerator was the number of biofilm-forming isolates resistant to a given antibiotic and the denominator was the number of biofilm-forming isolates tested against that antibiotic; isolates reported as intermediate were classified as non-resistant, following the interpretive criteria (CLSI or EUCAST) used in each primary study. Antibiotic abbreviations were harmonized before pooling (gentamicin, GEN/CN; levofloxacin, LEV/LE; meropenem, MEM/MRP; cefepime, FEP/CPM); when a study reported the same agent under two codes, the value recorded under the standard code was used. The characteristics of each included study are summarized in Supplementary Table S4.

2.4. Statistical Analysis

Study-level proportions were logit-transformed and pooled with inverse-variance random-effects models, using the DerSimonian–Laird estimator of between-study variance (τ^2) and the Hartung–Knapp–Sidik–Jonkman (HKSJ) 95% confidence interval [25]. Heterogeneity was quantified with Cochran's Q, τ^2 and I^2, and 95% prediction intervals (PIs) were calculated [26]; robustness was examined with the restricted maximum-likelihood and Paule–Mandel estimators [27] and with leave-one-out analysis. Subgroups (detection method, production category and specimen type) were compared descriptively, because individual studies could contribute to more than one subgroup. Strong, moderate and weak producers were expressed as proportions of biofilm-producing isolates, and non-producers as a proportion of all isolates tested. Resistance was analyzed separately for each antibiotic among biofilm-forming isolates, and class-level values are unweighted means of the antibiotic-specific estimates. Small-study effects were assessed with funnel plots and Egger's [28] and Begg's [29] tests ($p < 0.10$; at least 10 studies) [30], with the trim-and-fill method [31] as a sensitivity analysis. Analyses were performed in R version 4.3.3 (meta package, version 8.2-1). Full details, including the handling of zero events, minimum numbers of studies and the trim-and-fill procedure, are given in the Supplementary Methods.

2.5. Quality Assessment

An adapted version of the Newcastle–Ottawa Scale (NOS) for cross-sectional studies [32,33] was used to assess the methodological quality of each included study. The scale comprises three domains: selection (maximum 4 stars), comparability (maximum 2 stars) and outcome assessment (maximum 3 stars); the item-level criteria, as adapted for cross-sectional studies of isolate prevalence, are given in Supplementary Table S3, and study-level scores in Supplementary Table S1. Each study was scored independently by three reviewers (PD, SN and BP), and disagreements were resolved by discussion. Total scores ranged from 4 to 9 out of a possible 9. A minimum total score of 6 was pre-specified as the threshold for inclusion. Two studies scored below this threshold and were retained in error. Bhardwaj et al. [34] (score 4) did not report the denominators needed for any pooled analysis and therefore contributes to none. Dumaru et al. [35](score 5) did not use the microtiter plate assay, and its Congo red and tube-method counts exceeded the number of isolates tested, so it does not contribute to the microtiter, tube or Congo red prevalence analyses; it contributes only to the non-producer analysis (which included studies irrespective of detection method) and to the antibiotic-specific analyses, and excluding it changed the pooled proportion of non-producers from 35% to 33% and antibiotic-specific estimates by at most 3 percentage points.

3. Results

3.1. Study Selection and Characteristics

The searches identified 2300 records (PubMed, n = 1025; Google Scholar, n = 1105; regional journal websites such as NepJOL and IJCMAS, n = 170). After removal of 1385 duplicate records (Google Scholar indexes most

PubMed content, so overlap between the two sources was extensive) and 10 records outside the language or date limits, 905 records were screened by title and abstract, 395 full-text reports were sought, 386 were assessed for eligibility (nine could not be retrieved), and 67 studies from 17 Asian countries met the inclusion criteria [11,13–17,20,34–93] (Figure 1). The reasons for exclusion at full-text stage are outlined in Figure 1. Only a small percentage (n = 3) were from high-income settings, and two were from low-income countries. The bulk of studies were from lower-middle-income countries (n = 39), followed by upper-middle-income countries (n = 23). Individual study sizes ranged from as few as four *E. coli* isolates to as many as 560 isolates, with the median sample size of isolates per study being 103 among the 47 studies that reported it. Urinary tract infections (UTIs) were the focus of most studies (Figure 2A), while some included diverse clinical sources such as blood, wound, or respiratory specimens. Studies that reported isolates from more than one specimen type were counted in each relevant category of Figure 2A; therefore, the category counts (urine, 56; blood, 7; other, 13) sum to more than the number of included studies.

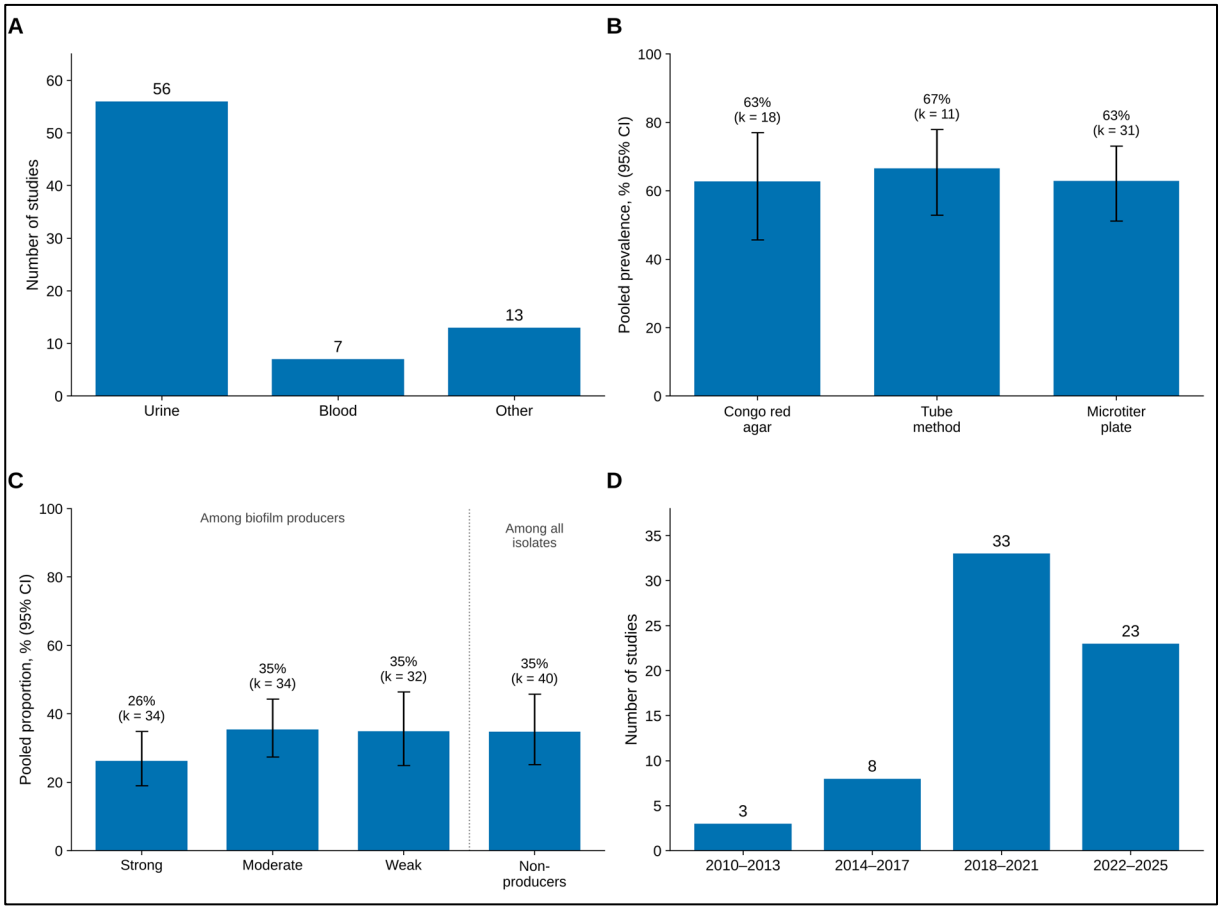


Figure 2. Characteristics of the included studies and pooled estimates. (**A**) Number of included studies by clinical specimen type; studies with isolates from more than one specimen type are counted in each relevant category. (**B**) Pooled prevalence of biofilm-positive isolates by phenotypic assay. (**C**) Pooled proportions of strong, moderate and weak producers among biofilm-producing isolates (microtiter plate assay), and of non-producers among all isolates tested (all phenotypic methods); each category was pooled separately. (**D**) Number of included studies by publication period. In (**B**) and (**C**), bars show random-effects pooled estimates (inverse-variance weighting of logit-transformed proportions, DerSimonian–Laird τ^2, Hartung–Knapp–Sidik–Jonkman 95% CI shown as error bars), and k is the number of contributing studies.

3.2. Pooled Biofilm Prevalence and Subgroup Analyses

The microtiter plate assay (crystal violet staining in 96-well plates) was the most frequently used method (46 studies, compared with 22 for Congo red agar and 14 for the tube method), and there was considerable overlap between the use of CRA assay and tube adhesion testing. With far less research from East and Southeast Asia, the countries most represented geographically were India, Nepal, Iran, Iraq, Indonesia, and numerous Middle Eastern countries.

The pooled prevalence of biofilm formation among clinical *E. coli* isolates tested with the microtiter plate assay (k = 31 studies; 3694 isolates) was 63% (95% CI: 51–73%), with very high heterogeneity (I^2 = 95.6%, τ^2 =

1.00; Q-test $p < 0.0001$) (Figure 3; Table 1). The 95% prediction interval was 17–93%, indicating that the true prevalence in a comparable new setting could lie almost anywhere within this range. The pooled estimate was robust to the choice of between-study variance estimator (REML: 63%, 95% CI: 52–74%, PI: 11–96%; Paule–Mandel: 63%, 95% CI: 52–74%, PI: 11–96%), and omitting any single study in leave-one-out analysis gave pooled estimates between 61% and 65%. One study [85] analyzed isolates from dogs and chickens alongside human clinical isolates; excluding it gave a pooled prevalence of 62% (95% CI: 50–73%). Pooled estimates for the tube method (67%, 95% CI: 53–78%; k = 11; $I^2 = 83.9\%$; PI: 37–87%) and Congo red agar (63%, 95% CI: 46–77%; k = 18; $I^2 = 94.3\%$; PI: 17–93%) were similar to the microtiter estimate, with widely overlapping confidence intervals (Figure 2B); because several studies tested the same isolates with more than one method, these subgroups are not independent and no formal test for subgroup differences was performed. Among biofilm-producing isolates detected by the microtiter plate assay, the pooled proportions of strong, moderate and weak producers were 26% (95% CI: 19–35%; k = 34), 35% (95% CI: 27–44%; k = 34) and 35% (95% CI: 25–46%; k = 32), respectively. Because each category was pooled separately from a different set of studies, these estimates are not additive and should not be interpreted as a distribution within a single common denominator. Non-producers accounted for 35% (95% CI: 25–46%; k = 40; PI: 7–78%) of all isolates tested; this analysis included all studies that reported non-producer counts, irrespective of the detection method (Table 1; Figure 2C).

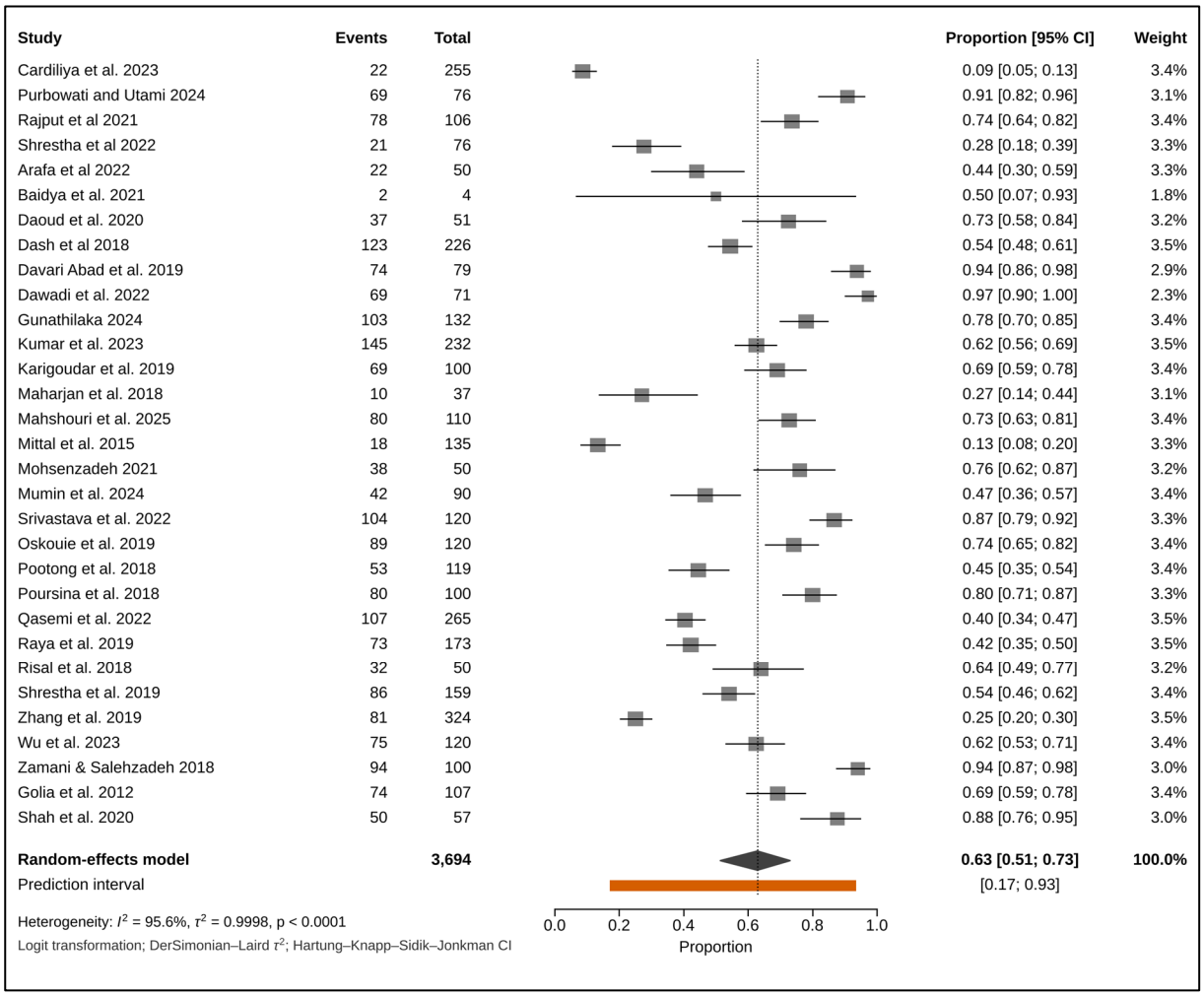


Figure 3. Forest plot of the prevalence of biofilm-positive *E. coli* isolates detected by the microtiter plate method in 31 studies (2012–2025). Study-level proportions are shown with exact 95% CIs. The pooled estimate (63%, 95% CI: 51–73%) was obtained from a random-effects model (inverse-variance weighting of logit-transformed proportions, DerSimonian–Laird τ^2, Hartung–Knapp–Sidik–Jonkman CI); 95% prediction interval, 17–93%. References include [11,14–16,39–41,46,55,56,60,64,65,67–69,71,73,74,77,78,81,83,85,88,89,92–96].

3.3. Specimen Type

Urinary isolates were the most frequently studied specimen type (56 studies; Figure 2A). Among studies of urine isolates tested with the microtiter plate assay, the pooled biofilm prevalence was 65% (95% CI: 51–77%;

k = 24; I^2 = 95.5%; PI: 19–94%) (Table 1). Only seven studies reported bloodstream isolates, and the "other" specimen category was small and heterogeneous; therefore, no formal statistical comparison between urinary and bloodstream isolates was performed, and no conclusion about differences in prevalence by specimen type can be drawn from these data.

Table 1. Pooled prevalence estimates of biofilm-forming *E. coli* in Asian clinical isolates by detection method, biofilm production capacity, and sample type, with random-effects model results and multiple tests for publication bias. Values are pooled proportions [95% CI] from random-effects models (inverse-variance weighting of logit-transformed proportions, DerSimonian–Laird τ^2, Hartung–Knapp–Sidik–Jonkman CI); k, number of studies; PI, 95% prediction interval. * Proportion of biofilm-producing isolates detected by microtiter plate assay (denominator: microtiter-positive isolates). † Proportion of all isolates tested (denominator: all isolates tested), pooled from all studies that reported non-producer counts irrespective of the detection method (k = 40). Each row is a separate meta-analysis with its own set of studies and denominator, so the estimates are not additive and need not sum to 100%. Funnel-plot tests used $p < 0.10$. Trim-and-fill (L_0 estimator) values are sensitivity analyses; k_0, number of imputed studies.

Parameter	Type of Analysis	Random Effect Model	Egger's Test (*p*-Value)	Begg's Test (*p*-Value)	Trim-and-Fill Adjusted Estimate
Type of method for biofilm detection	Total microtiter positive	0.63 [0.51; 0.73] (I^2 = 95.6%, τ^2 = 1.00, $p < 0.0001$); k = 31; PI [0.17; 0.93]	0.0188	0.0151	0.50 [0.37; 0.63] (k_0 = 8)
	Tube Method	0.67 [0.53; 0.78] (I^2 = 83.9%, τ^2 = 0.26, $p < 0.0001$); k = 11; PI [0.37; 0.87]	0.6487	0.2743	—
	Congo Red	0.63 [0.46; 0.77] (I^2 = 94.3%, τ^2 = 0.94, $p < 0.0001$); k = 18; PI [0.17; 0.93]	0.3477	0.3837	—
Production category (strong, moderate and weak: microtiter plate; non-producers: any method)	Strong producer *	0.26 [0.19; 0.35] (I^2 = 88.8%, τ^2 = 0.79, $p < 0.0001$); k = 34; PI [0.05; 0.69]	0.0127	0.0122	0.36 [0.26; 0.48] (k_0 = 8)
	Moderate producer *	0.35 [0.27; 0.44] (I^2 = 88.7%, τ^2 = 0.67, $p < 0.0001$); k = 34; PI [0.09; 0.75]	0.8623	0.8240	—
	Weak producer *	0.35 [0.25; 0.46] (I^2 = 92.1%, τ^2 = 1.02, $p < 0.0001$); k = 32; PI [0.06; 0.81]	0.1067	0.3469	—
	Non-producer (any method) †	0.35 [0.25; 0.46] (I^2 = 95.2%, τ^2 = 0.87, $p < 0.0001$); k = 40; PI [0.07; 0.78]	0.0947	0.1131	0.42 [0.31; 0.55] (k_0 = 6)
Biofilm detection in sample	Sample Type—Urine	0.65 [0.51; 0.77] (I^2 = 95.5%, τ^2 = 0.96, $p < 0.0001$); k = 24; PI [0.19; 0.94]	0.0444	0.0064	0.53 [0.37; 0.68] (k_0 = 6)

3.4. Country-Level Patterns

Country-level estimates were obtained by pooling the microtiter-plate studies from each country separately and are descriptive, because they rest on few studies and are confounded by differences in assay methods, specimen types and study periods. The pooled prevalence was 58% (95% CI: 30–81%; k = 9) for India, 52% (95% CI: 24–79%; k = 7) for Nepal and 79% (95% CI: 58–91%; k = 7) for Iran; China contributed two microtiter studies (25% and 62%), and the remaining countries one or none. Individual studies varied widely within countries, from 9% in a study from the Nilgiris district of South India [94] to 87% in another Indian study, and from 40% to 94% in Iran (e.g., 80% in Isfahan [95]).

3.5. Small-Study Effects and Sensitivity Analyses

To evaluate small-study effects and possible publication bias, we ran Egger's [28] and Begg's tests [29] and created a funnel plot [97] for the meta-analysis of microtiter plate investigations. With a tendency for smaller studies to report higher-than-average prevalences and a relative lack of smaller studies (those with large standard errors) reporting very low prevalences, the funnel plot demonstrated asymmetry (Supplementary Figure S5). Funnel-plot asymmetry tests were below the pre-specified threshold of $p < 0.10$ for the total microtiter (Egger's $p = 0.019$; Begg's $p = 0.015$), strong-producer ($p = 0.013$ and 0.012), non-producer (Egger's $p = 0.095$) and urine ($p = 0.044$ and 0.006) analyses (Table 1). As a sensitivity analysis, we applied the Duval and Tweedie [31] trim-and-fill method (L0 estimator) to each analysis with evidence of funnel-plot asymmetry ($p < 0.10$ on either Egger's or Begg's test). Trim-and-fill imputed eight studies for the total microtiter analysis (adjusted estimate 50%, 95% CI: 37–63%) and six for urine isolates (53%, 95% CI: 37–68%), in both cases on the side of lower prevalence. For strong producers

(k_0 = 8; 36%, 95% CI: 26–48%) and non-producers (k_0 = 6; 42%, 95% CI: 31–55%), the imputed studies fell on the opposite side of the funnel, so the adjusted estimates were higher than the unadjusted values. The inconsistent direction of these adjustments further suggests that the asymmetry does not reflect publication bias alone. Because funnel-plot asymmetry may reflect between-study heterogeneity, differences in assay methodology and clinical populations, or chance rather than publication bias per se [30], these adjusted values are sensitivity analyses rather than bias-corrected prevalences; they are reported alongside the primary estimates in Table 1. Trim-and-fill adjusted funnel plots with imputed studies displayed as open diamonds are provided as Supplementary Figures S1–S4. For most antibiotics, fewer than 10 studies were available, so funnel-plot tests had very low power and were not interpreted (Supplementary Table S5). Among antibiotics with at least 10 studies, Egger's test indicated funnel-plot asymmetry for ampicillin (p = 0.022) and amoxicillin–clavulanate (p = 0.023), and these two estimates should be interpreted with additional caution.

3.6. Antibiotic Resistance among Biofilm-Forming Isolates

Antibiotic-specific pooled resistance proportions among biofilm-forming isolates are shown in Supplementary Table S5. Resistance was highest for ampicillin (84%, 95% CI: 72–92%; k = 21), amoxicillin–clavulanate (78%, 95% CI: 48–93%; k = 11) and norfloxacin (76%, 95% CI: 57–88%; k = 9), and lowest for ertapenem (8%, 95% CI: 1–55%; k = 3), meropenem (18%, 95% CI: 5–46%; k = 8) and imipenem (21%, 95% CI: 12–33%; k = 19). Several estimates based on few studies (e.g., doxycycline, ertapenem and chloramphenicol) had very wide confidence intervals and should be interpreted with caution. As a descriptive summary, the unweighted means of the antibiotic-specific pooled proportions within each class were 66.4% for tetracyclines (two antibiotics), 60.4% for fluoroquinolones (four antibiotics), 53.2% for β-lactams (14 antibiotics), 48.0% for other agents (nitrofurantoin, co-trimoxazole and chloramphenicol) and 38.9% for aminoglycosides (three antibiotics) (Figure 4); these class-level values are not formal pooled estimates.

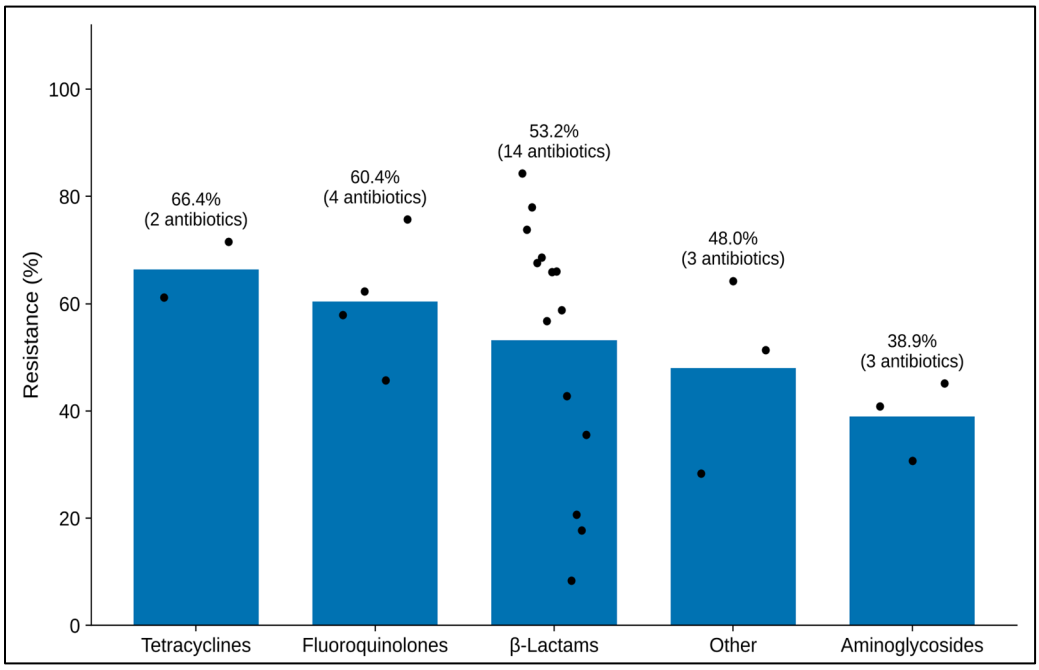


Figure 4. Unweighted mean of antibiotic-specific pooled resistance proportions (%) among biofilm-forming *E. coli* clinical isolates in Asia, by antibiotic class. "Other" category denotes mixed "Other agents" category. Bars show the unweighted mean of the antibiotic-specific pooled proportions in each class and points show individual antibiotics (Supplementary Table S5); this is a descriptive summary, not a formal pooled class-level estimate.

4. Discussion

This systematic review found that biofilm formation is frequently reported among clinical *E. coli* isolates from Asia, with a pooled microtiter-based prevalence of 63%. However, this value should be interpreted as a statistical summary of highly heterogeneous studies rather than as a clinically generalizable prevalence for Asia. The very high heterogeneity (I^2 = 95.6%) and the wide prediction interval (17–93%) indicate that the prevalence expected in an individual hospital, patient population or laboratory may differ substantially from the pooled value, depending on specimen type, patient population (e.g., catheterized vs. non-catheterized patients), assay protocol

and the optical-density cut-off used to define biofilm production. Funnel-plot asymmetry was observed for several outcomes; similar patterns have been reported in other biofilm prevalence meta-analyses, for example by Garousi et al. [98], who reported a global pooled biofilm prevalence of approximately 74% among uropathogenic *E. coli* with wide variation between studies. In the presence of such heterogeneity, however, funnel-plot asymmetry indicates possible small-study effects rather than proof of publication bias [30].

Country-level differences should also be interpreted cautiously, because they are descriptive and are confounded by differences in assay method, specimen type and study period. The lower average reported from China, for example, may reflect differences in sampling (such as a higher proportion of acute-care isolates) or in laboratory methods rather than true differences in biofilm-forming capacity; these explanations could not be tested with the available data. Nevertheless, the frequent detection of biofilm-forming, antibiotic-resistant *E. coli* across diverse settings supports efforts to standardize biofilm detection methods and to strengthen catheter care and antibiotic stewardship in Asian healthcare settings.

4.1. Sample Type and Clinical Source of Isolates

As urinary tract infections (UTIs) offer an opportunity for clinical *E. coli* biofilm research, most included studies focused on urinary isolates of *E. coli* (urine samples). Fifty-six studies included urine isolates, whereas only seven examined blood isolates (Figure 2A). A few more looked at various sample types (labeled as "others", such as wound swabs, sputum, or device-associated samples). Given that *E. coli* is the primary cause of UTIs globally and that biofilm formation is a known contributing factor to recurrent and chronic UTIs, this discrepancy is not surprising. Uropathogenic *E. coli* (UPEC) can create intracellular bacterial populations in the bladder that resemble biofilms, which enables them to endure antibiotic treatment [99]. Additionally, *E. coli* can build dense biofilms on indwelling urinary catheters, which are frequently used in hospitals and long-term care facilities, leading to catheter-associated UTIs. Bacteriuria and biofilms on the catheter surface are nearly always developed by patients with long-term catheters [100]. Our results are in line with these clinical realities: while biofilm-related *E. coli* morbidity is more prevalent in UTIs, research efforts and, consequently, our data are heavily weighted toward urinary isolates.

Even though our analysis only included a small number of papers that examined *E. coli* from bloodstream infections, it is important to discuss how biofilm formation may vary in these isolates. *E. coli* associated bacteremia frequently develops as a side effect of UTI (e.g., pyelonephritis resulting in urosepsis), intra-abdominal infections, and occasionally contaminated intravenous catheters [101]. Before entering the bloodstream in the former case (urosepsis), the *E. coli* strains usually have adhering or biofilm-forming properties in the urinary tract. Bacteremia in the latter case (IV catheter infection) is directly caused by a biofilm on the catheter [99]. Our meta-analysis was unable to provide a reliable comparison of biofilm prevalence vs. urine isolates due to the limited number of blood isolate studies conducted in Asia. The "other" category was also too small and diverse to make definitive inferences. Overall, the distribution of study sample types shows that the majority of *E. coli* biofilm research, as well as the burden of disease, was focused on UTIs.

4.2. Biofilm Detection Methods

The Congo Red Agar (CRA) plate test, the test tube (standing culture) method, and the microtiter plate assay (quantitative crystal violet staining in 96-well plates, also known as the tissue culture plate method) were the three common phenotypic assays that were compared. The random-effects pooled estimates were similar for the three methods (microtiter plate, 63%; tube method, 67%; CRA, 63%; Table 1). These pooled estimates (Figure 2B) should not be interpreted as head-to-head comparisons of assay sensitivity, because different studies, with different specimen types and populations, used different methods. Nevertheless, methodological studies indicate that the microtiter plate assay is the most sensitive and quantitative of the three methods [102]. Even weak biofilm producers are captured by the microtiter technique, which quantitatively evaluates biofilm biomass by optical density [36]. CRA, on the other hand, is a qualitative screen that depends on a characteristic black colony shape (because extracellular matrix polysaccharides bind to Congo red dye) [103]. The CRA approach identified biofilm production in 49% of strains in an Indian UTI isolate investigation, while the microtiter plate found it in 69% of strains, revealing numerous other weak biofilms. Despite demonstrating intermediate sensitivity (55% positive) in that investigation, the test tube approach (visual inspection of pigmented residue on tube walls) failed to detect a subset of strains that the microtiter assay detected [96]. Our results highlight how method selection can affect results; ideally, researchers should employ the microtiter assay or, at the very least, validate CRA results using a quantitative method. Within the microtiter-based studies, more than half of clinical *E. coli* isolates were classified as biofilm producers.

4.3. Biofilm Production Capacity: Strong vs. Weak Producers

Based on quantitative assay results, biofilm-positive *E. coli* isolates are strong, moderate, weak, or non-producers (typically using the conventional OD cutoff definitions). In microtiter-based studies, approximately two-thirds of clinical *E. coli* isolates were classified as biofilm producers (pooled prevalence 63%), and non-producers accounted for 35% of all isolates tested (k = 40, any detection method). Among biofilm producers, 26% were strong producers, whereas moderate and weak producers each accounted for approximately 35% (Table 1; Figure 2C). Our pooled estimate of 63% is broadly consistent with another clinical investigation of UPEC that reported around 60% of isolates produce biofilm [104]. Although *E. coli* can create biofilms, this consistency across investigations suggests that genuinely robust biofilm producers, i.e., those that adhere firmly and build up thick matrices in vitro, are a subset rather than the rule. Under ideal circumstances, most *E. coli* can create some biofilm, although the degree of biofilm formation varies widely.

4.4. Publication Year Trends in Biofilm Research

The number of eligible studies increased over time, from three in 2010–2013 and eight in 2014–2017 to 33 in 2018–2021 and 23 in 2022–2025 (Figure 2D). This increase coincided with growing concern about antimicrobial resistance in *E. coli* in many Asian countries, although our data cannot establish a causal link, and recent studies have increasingly addressed the mechanisms of biofilm formation and anti-biofilm strategies rather than prevalence alone [105].

4.5. Antibiotic Resistance in Biofilm-Forming E. coli

Zhang et al. [37] reported class-level resistance in clinical *E. coli* isolates: penicillins (~84%), cephalosporins (~63%), tetracyclines (~58%) and fluoroquinolones (~62%) showed high resistance, whereas resistance to carbapenems was very low (~3.7%). Our descriptive class means are broadly consistent with this pattern (tetracyclines, fluoroquinolones and β-lactams high; aminoglycosides moderate; carbapenems low), although the β-lactam class mean combines agents with very different activity (e.g., ampicillin 84% vs. ertapenem 8%) and is more informative at the level of individual agents.

Among biofilm-forming isolates, resistance was highest for older agents such as ampicillin, amoxicillin–clavulanate and the tetracyclines, consistent with reports of high tetracycline [38] and quinolone [11] resistance among uropathogenic *E. coli*, whereas nitrofurantoin, amikacin and the carbapenems showed comparatively low resistance proportions [13]. These are antimicrobial susceptibility findings, obtained in most primary studies by standard testing of planktonic cells, and do not by themselves establish clinical effectiveness against biofilm-associated infections, in which antibiotic tolerance can greatly exceed that predicted by conventional susceptibility testing [22,23]. The frequent multi-class resistance observed nevertheless underlines the need for antibiotic stewardship and for alternative strategies against biofilm-associated infections.

4.6. Limitations and Future Directions

This meta-analysis offered a comprehensive overview of biofilm prevalence in clinical *E. coli* isolates across Asia; however, some limitations exist. First, very high heterogeneity (I^2 > 80% for most outcomes) and wide prediction intervals indicate that the pooled estimates should not be interpreted as precise or generalizable prevalence values for Asia. Despite subgroup analyses, heterogeneity persisted, suggesting substantial variations in demographics, laboratory techniques, and regional epidemiology. Many included studies had moderate sample sizes, were conducted at single center, and exhibited mixed quality, as assessed by our adapted Newcastle-Ottawa Scale (SI Table S1). Data from higher-income Asian regions were limited, reducing the robustness of comparisons across economic strata or subregions. Additionally, our focus on phenotypic biofilm assessments excluded molecular data on biofilm-associated genes, which were inconsistently reported and beyond our primary objectives. Regarding antibiotic resistance, the analysis revealed high resistance to tetracyclines, fluoroquinolones, and beta-lactams, but variability in resistance patterns across studies and regions, coupled with limited data on newer antibiotics, restricts generalizability. Second, the search was limited to PubMed and Google Scholar; Embase, Scopus and Web of Science were not searched, and Google Scholar searches are not fully reproducible, so some eligible studies, particularly those in regional journals, may have been missed. Third, only English-language reports were included, which may have introduced language and selection bias, because relevant studies from several of the 17 countries may have been published in local or regional languages. Fourth, most primary studies determined antibiotic susceptibility with standard planktonic methods applied to biofilm-forming isolates rather than biofilm-specific measures such as the minimum biofilm eradication concentration; the resistance

proportions therefore describe biofilm-forming isolates rather than the susceptibility of biofilms themselves. Fifth, class-level resistance values are descriptive averages of antibiotic-specific estimates, not formal pooled estimates. Finally, funnel-plot asymmetry was detected for several outcomes, but in the presence of extreme heterogeneity it cannot be attributed specifically to publication bias. Despite these limitations, the consistently high prevalence of biofilm formation and associated multidrug resistance observed across diverse studies underscore the urgent need for regionally tailored investigations and targeted interventions to mitigate biofilm-associated risks and address antibiotic resistance challenges in clinical *E. coli* isolates in Asia.

5. Conclusions

This systematic review and meta-analysis indicated that biofilm formation is frequently reported among clinical *E. coli* isolates from Asia, with a pooled microtiter-based prevalence of 63% (95% CI: 51–73%). However, the extreme between-study heterogeneity and wide prediction interval (17–93%) suggest that this value is a statistical summary of highly diverse studies rather than a stable or generalizable estimate for the region, and country-level differences are descriptive. Among biofilm-forming isolates, in vitro resistance proportions were high for ampicillin, amoxicillin–clavulanate and several other commonly used agents and lowest for carbapenems; these susceptibility data should not be interpreted as therapeutic recommendations for biofilm-associated infections. Trim-and-fill sensitivity analyses shifted the estimates to inconsistent directions for outcomes with funnel-plot asymmetry, further supporting cautious interpretation. Standardized biofilm detection methods, biofilm-specific susceptibility testing and prospective multicenter surveillance across Asian healthcare settings are needed to generate more robust estimates and to inform catheter care and antibiotic stewardship.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2611111610227153/EESUS-26060194-Supplementary-Materials.pdf. Table S1: Quality assessment of the included studies using the Newcastle– Ottawa Scale adapted for cross-sectional studies. Table S2: Electronic search strategies, dates and numbers of records retrieved from each source. Table S3: Newcastle–Ottawa Scale criteria adapted for cross-sectional studies of biofilm formation and antimicrobial resistance in clinical *E. coli* isolates. Table S4: Characteristics of the 67 included studies. Table S5: Pooled resistance proportions among biofilm-forming *E. coli* clinical isolates, by antibiotic. Figure S1: Trim-and-fill funnel plot (total microtiter positive). Figure S2: Trim-and-fill funnel plot (strong producers among biofilm producers). Figure S3: Trim-and-fill funnel plot (non-producers among all isolates). Figure S4: Trim-and-fill funnel plot (urine isolates, microtiter plate). Figure S5: Contour-enhanced funnel plot (proportion on the logit scale against standard error) for the 31 studies reporting *E. coli* biofilm prevalence by the microtiter plate method.

Author Contributions

M.R.B., L.R.P. and P.D. conceptualized and designed the study. S.N., P.D. and B.P. conducted literature search, identified relevant articles, and performed data extraction. P.D. and S.N. conducted data analysis. B.P., S.N., P.D., S.R., L.R.P. and M.R.B. contributed to data interpretation. P.D., S.N. and M.R.B. contributed to data visualization. B.P. and S.N. wrote the first draft; P.D., S.R., L.R.P. and M.R.B. contributed with edits and revisions to the manuscript. P.D. and L.R.P. reviewed, edited, and revised the final draft. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

All data analyzed in this study, including the extracted study-level data, quality scores and antibiotic-specific estimates, are included in this article and its Supplementary Information (Tables S1–S5).

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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