



Research article

Prevalence of extended-spectrum beta-lactamase-producing *Escherichia Coli* in broiler farms: A systematic review and meta-analysis

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Abstract

Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL *E. coli*) is a serious public health issue. ESBL *E. coli* in broiler production is known as a potential risk for human health. The aim of this study was to estimate the global pooled prevalence of ESBL *E. coli* in broiler farms. Literature searches were performed via PubMed, Web of Science, and Scopus. A random effect model was used to estimate the pooled prevalence. Heterogeneity was assessed by Cochran's Q test and the I squared statistic (I^2). Subgroup analysis and meta-regression were conducted to investigate the source of heterogeneity. Sensitivity and publication bias analyses were performed. Overall, 872 and 18 additional studies were retrieved from databases and bibliography searches. Thirty-seven studies met the inclusion criteria. Pooled prevalence of ESBL *E. coli* in broiler farms was 26.6% (95% CI = 18.2 to 37.3). The analyzed data showed high heterogeneity ($Q = 1958.87$ and $I^2 = 98.2\%$). The pooled prevalence of ESBL *E. coli* in broiler farms was almost identical between Asia; 33.0% (95% CI = 18.7 to 51.3) and Europe; 32.8% (95% CI = 15.0 to 57.4), while Africa showed the lowest prevalence at 12.4% (95% CI = 5.1 to 27.1). Prevalence of ESBL *E. coli* was found to be highest in fecal samples; 33.0% (95% CI = 21.5 to 46.8), followed by environmental samples; 28.3% (95% CI = 13.5 to 50.1), and other samples; 11.0% (95% CI = 5.0 to 22.8). Results from our study indicated a moderate prevalence of ESBL *E. coli* in broiler farms globally, and it has been fluctuating inconsistently in the last decade.

Keywords: Antimicrobial resistance, Broiler farm, *Escherichia coli*, Extended-spectrum beta-lactamase, Prevalence

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INTRODUCTION

Antimicrobial resistance (AMR) is a current and future threat to humanity. At present, AMR accounts for at least 700,000 deaths annually. The death toll could rise to as high as 10 million in 2050 if the rising rates of AMR could not be slowed down (De Kraker et al., 2016). Beta-lactam is a widely used class of antibiotic, both in the animal sector and the human sector. In the United States, beta-lactam accounts for 65% of all injectable antibiotic use, and nearly half of it consists of cephalosporin (Bush and Bradford, 2016). Cephalosporin has been categorized as “Veterinary Critically Important Antimicrobial Agents and Veterinary Highly Important Antimicrobial Agents” by the World Organization for Animal Health (WOAH) (OIE, 2020). The emergence of resistant enzymes such as extended-spectrum beta-lactamase (ESBL) has raised concern within the human medical and veterinary communities to revise its application of this antibiotic class. ESBL is known as plasmid-encoded enzymes that were found in Enterobacteriaceae, mostly in *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae*. This enzyme has the ability to hydrolyze various drugs of the beta-lactam class including penicillin, the second, third, and fourth generations of cephalosporin, and monobactams. However, carbapenems and cephamycins (e.g. aztreonam) are still effective against ESBL organisms (EFSA, 2011).

The emergence of ESBL-producing bacteria was recognized in the 1980s, with the emergence of ESBL-producing *Klebsiella pneumoniae* in the 1990s as a cause of nosocomial infections and the emergence of ESBL-producing *E. coli* in the 2000s as a cause of community-acquired infections (Chong et al., 2011; Chong et al., 2018). A recent meta-analysis on the prevalence of ESBL *E. coli* in healthy individuals reported a considerably high prevalence in South, Southeast, and East Asia, with an estimated rate of 50%. In contrast, Europe and North America showed considerably lower rates, both estimated at 10% (Karanika et al., 2016). Prevalence of ESBL *E. coli* among healthy individuals poses an ongoing worrisome on public health as the current second, third, and fourth generation cephalosporins are no longer an effective option for treatment of ESBL infected patients (Karanika et al., 2016). Food-producing animals are found to be a reservoir and risk factor for ESBL *E. coli*, especially in poultry production.

During 2000 to 2001, the initial findings of ESBL *E. coli* in poultry was reported in Spain. Since then, there have been frequent reports of ESBL *E. coli* in broiler farms and the broiler production chain (Dierikx et al., 2013). ESBL *E. coli* is also included in the monitoring program of antimicrobial resistance in food-producing animals as recommended by the Food and Agriculture Organization of the United Nations (FAO) and the European Food Safety Authority (EFSA) (EFSA, 2011; FAO, 2019). Several studies have suggested potential transmission of ESBL *E. coli* from poultry productions to humans that could pose a high risk to public health (Bui et al., 2018; Van Hoek et al., 2020; Aworh et al., 2020). Prevalence of ESBL *E. coli* in broiler farms has been reported from various places, however, a global level study on the prevalence of ESBL *E. coli* in broiler farms has not existed yet. Moreover, the indiscriminate and extensive use of antibiotics has contributed immensely to the rise of ESBL *E. coli* in broiler production. Therefore, our systematic review and meta-analysis aim to study the estimated pooled prevalence of ESBL *E. coli* in broiler farms and to assess the heterogeneity of the prevalence.

MATERIALS AND METHODS

Search strategy

This study was conducted based on Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Page et al., 2021). The research question was defined to include all primary research investigating the occurrence of ESBL-producing *E. coli* in broiler farms. The population, intervention, comparison of intervention, and outcome (PICO) framework was applied when designing research questions to ensure all relevant studies were covered.

The systematic literature search was conducted in three databases (PubMed, Web of Science, and Scopus). The advanced search functions were used across all databases to locate literature. A broad list of search terms including related terms, alternative spelling, and synonyms were used and joined by Boolean operator ('OR' and 'AND') to minimize missing records. Within the same domain "OR" was used to join related key terms, whereas "AND" was used to join across domains. Therefore, the search term string: [broiler OR poultry OR chicken] AND [ESBL-producing *E. coli* OR Extended-spectrum β -lactamase-producing *Escherichia coli* OR Extended-spectrum beta-lactamase-producing *Escherichia coli* OR ESBL-producing *Escherichia coli* OR ESBL *E. coli* OR ESBL *Escherichia coli* OR ESBL-producing Enterobacteriaceae OR Extended-spectrum β -lactamase-producing Enterobacteriaceae] was used for systematic literature search. All record yields were limited to the English language only, with a publication year between January 2010 and October 2021.

Inclusion and exclusion criteria

Records yielded from all databases were exported to Endnote, then duplicates were tracked and removed before the records were subjected to inclusion and exclusion assessment. The assessment of eligible studies was performed in two steps. The first step was the screening of relevant titles and abstracts. After title and abstract screening, the full texts of all relevant studies were screened for their eligibility. Studies were included if they were observational studies and reported the prevalence of ESBL-producing *E. coli* in broiler farms. On the other hand, studies were excluded if they were; 1) review articles, 2) experimental studies, 3) lack of targeted population, 4) lack of outcome of interest, 5) case reports or case series, and 6) articles with no clear information on the prevalence of ESBL-producing *E. coli* in broiler farms. At this step, two authors; PO and NA worked independently to screen for eligible studies. Disagreement on selecting some studies was discussed among both authors for consensus.

Data extraction

Data were extracted and recorded into a Microsoft Excel spreadsheet by both authors, who screened the studies for eligibility. Discrepancies between the data obtained by these two authors were discussed with a third author for consensus to avoid bias. The extracted data consisted of; 1) author's surname, 2) year of publication, 3) year of isolation, 4) continents and countries where the study was conducted, 5) study design, 6) sample type, 7) number of samples, 8) number of *E. coli* isolates, 9) number of ESBL *E. coli* isolates, 10) detection method for ESBL *E. coli*, and 11) interpretation guideline for ESBL *E. coli*.

Study quality assessment

Included studies were assessed for their quality using a checklist adapted from Ding et al. (2017). The checklist consisted of five questions which appraise five different aspects of the articles. The checklist contained questions as follows; 1) Was the research objective clearly described and stated?, 2) Was the sampling method described in detail?, 3) Were the period and location of the study clearly stated?, 4) Were the detection method and procedure for ESBL-producing *E. coli* clearly pointed out?, and 5) Were the samples clearly classified into different subgroups?. Each included study was appraised for their compliance with the

checklist, and then a score was given to each aspect of the study (2 = yes, 1 = unsure, 0 = no). The individual score from each aspect was then summed up with a maximum score of 10.

Statistical analyses

Meta-analysis for the prevalence of ESBL *E. coli* in broiler farms was computed using 'R Studio' statistical software (version 4.0.3). The in-built package "meta" was used to conduct meta-analysis. The prevalence of ESBL *E. coli* was calculated by dividing the total number of ESBL *E. coli* positives by the total number of samples.

Pooled prevalence of ESBL *E. coli* in broiler farms

Pooled prevalence and its 95% confidence interval (95% CI) were computed using a random effect model. Cochran's Q test was used to estimate the heterogeneity of pooled prevalence. In addition, I squared statistic (I^2) was used to quantify the degree of heterogeneity between studies, with I^2 value of 25%, 50%, and 75% indicating low, moderate, and high degrees of heterogeneity, respectively (Higgins et al., 2003).

Subgroup meta-analysis

Subgroup meta-analysis was performed to estimate the pooled prevalence among different categories. The pooled prevalence within each subgroup was calculated using a random effect model. The subgroup was divided into three characteristics included; 1) sample type, 2) continent where studies were conducted, and 3) year of publication (2010-2021). There were three groups of sample type; a) fecal sample (feces and cloacal swab), b) environmental swab (boot swab, air, dust, litter, flies, and equipment used in the broiler farms), and c) others (other clinical samples and other sample sources rather than fecal sample and environmental swab). There were five groups of continent including Asia, Europe, Africa, North America, and South America. The year of publication was categorized into nine groups including 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, and 2021.

Meta-regression

Univariable and multivariable meta-regression was conducted to determine the association between ESBL *E. coli* in broiler farms and selected variables in the subgroup analysis. Association between variables and pooled prevalence in the multivariate meta-regression model was assumed when a p -value was less than 0.05 ($p < 0.05$).

Sensitivity analysis

Sensitivity analysis was conducted to assess the robustness of the result. To achieve this, two methods were used in our meta-analyses. First, the result from the random effect model was compared with the result from the fixed effect model. Second, the leave-one-out analysis was used by removing a study with the highest and lowest prevalence one after another and recalculating the pooled prevalence.

Publication bias

Funnel plot and Eggers's test were conducted to measure publication bias, where a $p < 0.05$ suggested publication bias of small study-effects (Egger et al., 1997).

RESULTS

Search results and selection of studies

A total of 872 studies were yielded from primary databases. In addition, 18 more studies were retrieved from various sources through bibliography search. After duplicates were removed, 547 studies were screened through titles and abstracts. In this step, 344 studies failed to meet inclusion criteria and were excluded. Furthermore, 203 studies were screened through full text, and 166 studies were excluded. Finally, 37 studies met the inclusion criteria and were included in the meta-analyses. The selection process and included studies are given in Figure 1.

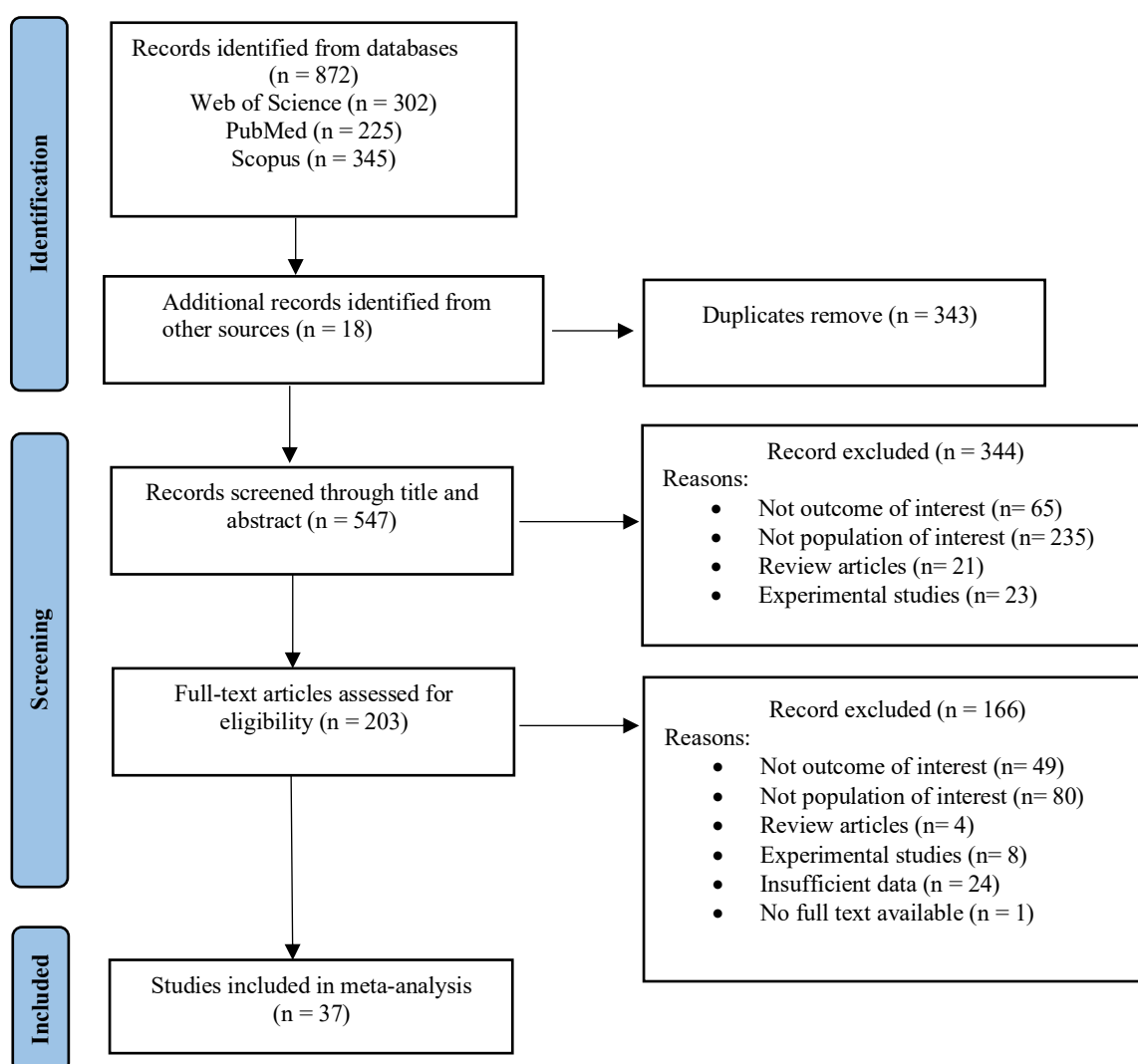


Figure 1 PRISMA flow diagram that indicated the selection process of the included studies.

Characteristics of included studies

Mean score of study quality assessment was 9.48/10 (SD = 0.9). Most studies (15/37, 40.5%) included in the meta-analysis were conducted in Asia followed by Europe (11/37, 29.7%). More than half of the included studies (22/37, 59.4%) were published during 2019-2021. We found that the most used ESBL E.

coli detection method in the included studies was the double disk-synergy test (16/37, 43.2%), followed by the combination disk method (14/37, 37.8%). In Asia, the prevalence of ESBL *E. coli* in broiler farms was reported in nine countries including India, Thailand, the Philippines, Vietnam, Pakistan, China, Lebanon, Bangladesh, and Nepal. In Europe, there were published reports on the prevalence of ESBL *E. coli* in broiler farms in eight countries including Spain, Turkey, Italy, the Netherland, Slovenia, Slovakia, Belgium, and Germany. In Africa, four countries, including Ghana, Tunisia, Nigeria, and Egypt, were found to have the reported prevalence of ESBL *E. coli* in broiler farms. Studies that reported prevalence in South America consisted of reports from two countries including Brazil and Columbia. In North America, we found only one study reported the prevalence of ESBL *E. coli* in broiler farms in Cuba. More details on study characteristics are given in Table 1.

Table 1 Characteristics of the included studies.

Authors and year of publication	Country	Sample type	No. of ESBL positive samples/ total no. of samples (%prevalence)	Methodology	Interpretation guideline
Abreu et al., 2014	Spain	Cloacal swab	237/260 (91.1%)	Double-disk synergy test	CLSI
Apostolakos et al., 2019	Italy	Cloacal swab	227/580 (39.1%)	Double-disk synergy test	CLSI
Baez et al., 2021	Cuba	Cloacal swab	40/100 (40.0%)	Combination disk method	EUCAST
Bhave et al., 2019	India	Feces	10/176 (5.7%)	Combination disk method	CLSI
Blaak et al., 2015	Netherland	Feces, rinse water, air, dust, flies, surface water, soil, run-off gullies, feces of other animals	195/276 (70.6%)	Combination disk method	CLSI
Boonyasiri et al., 2014	Thailand	Cloacal swab	30/80 (37.5%)	Double-disk synergy test	EUCAST
Cardozo et al., 2021	Brazil	Cloacal swab	17/100 (17.0%)	Combination disk method	EUCAST
Carissa et al., 2013	Nigeria	Feces	9/96 (9.4%)	Double-disk synergy test	CLSI
Castellanos et al., 2017	Colombia	Feces, drag swab, cloacal swab	13/45 (28.9%)	PCR	CLSI
Dierikx et al., 2013	Netherland	Cloacal swab	209/600 (34.8%)	Combination disk method	EUCAST
Doregirae et al., 2017	Iran	Cloacal swab	27/471 (5.7%)	Combination disk method	CLSI
Drugdova et al., 2013	Slovakia	Feces	14/83 (16.9%)	PCR	CLSI
Falgenhauer et al., 2019	Ghana	Feces	45/140 (32.1%)	Combination disk method	EUCAST
Ferreira et al., 2014	Brazil	Cloacal swab	19/200 (9.5%)	Double-disk synergy test	CLSI
Gazal et al., 2021	Brazil	Feces, boot swab, water, feed	67/576 (11.6%)	Double-disk synergy test	CLSI
Gundran et al., 2019	Philippines	Cloacal swab, boot swab	69/156 (44.2%)	Combination disk method	CLSI
Hassen et al., 2020	Tunisia	Feces	60/286 (21.0%)	Double-disk synergy test	CLSI
Hinenoya et al., 2018	Vietnam	Cloacal swab, bedding	15/17 (88.2%)	Combination disk method	CLSI
Ilyas et al., 2021	Pakistan	Cecal sample	156/250 (62.4%)	Double-disk synergy test	CLSI
Jouini et al., 2021	Tunisia	Feces	11/80 (13.7%)	Double-disk synergy test	CLSI
De Koster et al., 2021	Belgium, Netherland	Feces	666/779 (85.5%)	Combination disk method	EUCAST

Authors and year of publication	Country	Sample type	No. of ESBL positive samples/ total no. of samples (%prevalence)	Methodology	Interpretation guideline
Kwoji et al., 2019	Nigeria	Cloacal swab	12/48 (25.0%)	Combination disk method	CLSI
Laube et al., 2013	Germany	Feces, litter, boot swab, dust and environmental sample	152/420 (36.2%)	PCR	CLST
Li et al., 2016	China	Feces	142/160 (88.7%)	Combination disk method	CLSI
Mikhayel et al., 2021	Lebanon	Cloacal swab	60/280 (21.4%)	Double-disk synergy test	EUCAST
Moawad et al., 2018	Egypt	Cloacal swab	7/576 (1.2%)	Vitek 2 system	EUCAST
Niero et al., 2018	Italy	Swab at lesion site	7/98 (7.1%)	PCR	CLSI
Ur Rahman et al., 2019	Pakistan	Drinking water, farm floor, feeding and drinking equipment	14/100 (14.0%)	Double-disk synergy test	CLSI
Rodroo et al., 2021	Thailand	Boot swab, feed, water	28/144 (19.4%)	Combination disk method	CLSI
Rojs et al., 2019	Slovenia	Feces, air, internal organ	6/33 (18.2%)	Combination disk method	EUCAST
Rousham et al., 2021	Bangladesh	Feces	26/40 (65.0%)	ESBL selective media	CLSI
Sayin et al., 2020	Turkey	Synovial fluid	8/152 (5.3%)	Vitek 2 system	CLSI
Shafiq et al., 2021	China	Feces	103/210 (49.0%)	Double-disk synergy test	CLSI
Shoaib et al., 2016	Pakistan	Broiler liver	8/150 (5.3%)	Double-disk synergy test	CLSI
Sola-Gines et al., 2015	Spain	Flies from broiler farm	42/682 (6.1%)	Double-disk synergy test	EUCAST
Subramanya et al., 2020	Nepal	Cloacal swab	15/47 (31.9%)	Double-disk synergy test	CLSI
Wibisono et al., 2021	Indonesia	Cloacal swab	46/160 (28.7%)	Double-disk synergy test	CLSI

Pooled prevalence of ESBL *E. coli* in broiler farms

Pooled prevalence of ESBL *E. coli* in broiler farms calculated by a random effect model was estimated at 26.6% (95% CI = 18.2 to 37.3). The overall estimation indicated high heterogeneity (Q value = 1958.87 and $I^2 = 98.2\%$). The total number of samples being observed was 8,651 and the total number of ESBL *E. coli* positive was 2,812. The forest plot indicated the overall pooled prevalence of ESBL *E. coli* is given in [Figure 2](#).

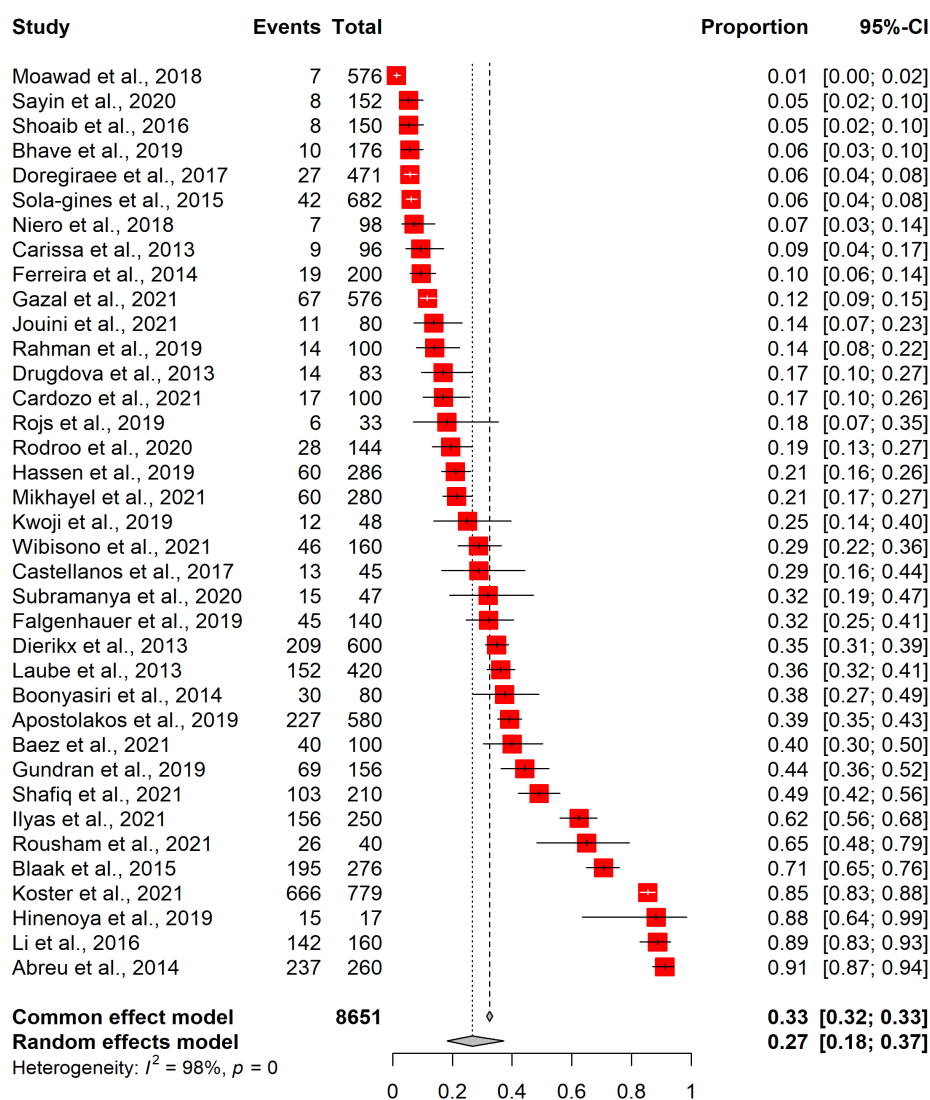


Figure 2 Forest plot indicated the global pooled prevalence of ESBL *E. coli* in broiler farms.

Subgroup analysis

In the continent subgroup, Asia and Europe had almost the same pooled prevalence estimated, 33.0% (95% CI = 18.7 to 51.3) and 32.8% (95% CI = 15.0 to 57.4), respectively. Africa had the lowest pooled prevalence, estimated at 12.4% (95% CI = 5.1 to 27.1). Global distribution of ESBL *E. coli* in broiler farms is shown in Figure 3. In the subgroup of sample type, the highest estimated pooled prevalence of 33.0% (95% CI = 21.5 to 46.8) was found in fecal samples, while the lowest estimated pooled prevalence of 11.0% (95% CI = 5.0 to 22.8) was found in other samples. Pooled prevalence of ESBL *E. coli* within subgroups is given in Table 2.

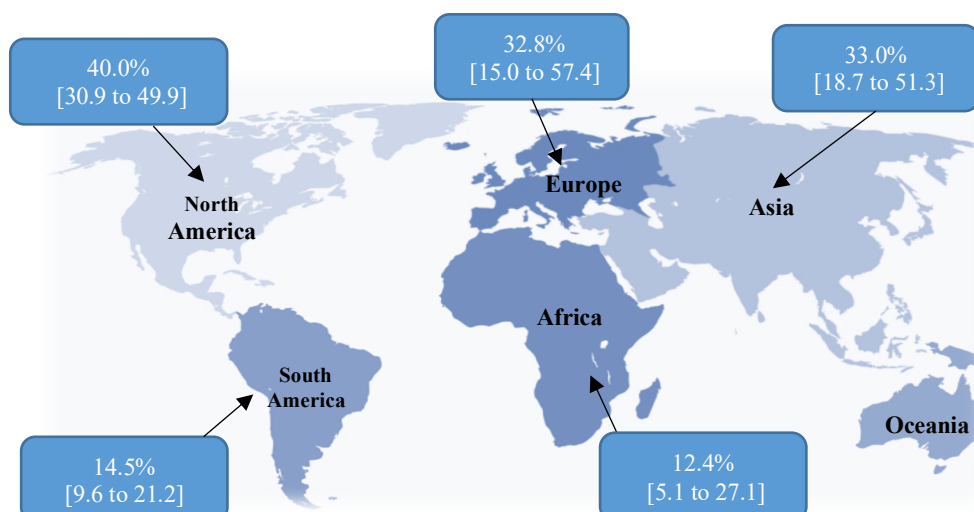


Figure 3 Distribution of the pooled prevalence of ESBL *E. coli* in broiler farms across different continents.

Table 2 The overall pooled prevalence of ESBL *E. coli* in broiler farms and subgroup meta-analysis using a random effects model.

No.	Categories	No. of studies	Pooled prevalence (95% CI)	I^2 (%)	Q	p -value	p -value for subgroup difference
1	ESBL <i>E. coli</i> in broiler farm	37	26.6 (18.2 to 37.3)	98.2	1958.87	$P = 0$	
Sample types							$P < 0.01$
1	Fecal sample	29	33.0 (21.5 to 46.8)	98.2	1543.20	$P < 0.01$	
2	Environmental sample	7	28.3 (13.5 to 50.1)	97.8	267.96	$P < 0.01$	
3	Others	5	11.0 (5.0 to 22.8)	95.5	89.08	$P < 0.01$	
Continent							$P < 0.01$
1	Asia	15	33.0 (18.7 to 51.3)	97.2	500.69	$P < 0.01$	
2	Africa	6	12.4 (5.1 to 27.1)	94.0	83.90	$P < 0.01$	
3	Europe	11	32.8 (15.0 to 57.4)	99.0	995.91	$P < 0.01$	
4	North America	1	40.0 (30.8 to 49.9)	-	-	-	
5	South America	4	14.5 (9.6 to 21.2)	78.2	13.75	$P < 0.01$	
Year-Wise							$P = 0.01$
1	2013	4	22.9 (12.9 to 37.3)	90.6	31.85	$P < 0.01$	
2	2014	3	46.4 (9.1 to 88.2)	99.0	206.32	$P < 0.01$	
3	2015	2	28.4 (3.1 to 82.9)	99.7	302.87	$P < 0.01$	
4	2016	2	39.9 (2.0 to 95.6)	99.2	125.47	$P < 0.01$	
5	2017	2	12.9 (3.8 to 35.6)	95.9	24.46	$P < 0.01$	
6	2018	2	2.8 (0.8 to 9.3)	91.1	11.26	$P < 0.01$	
7	2019	9	27.9 (15.5 to 44.9)	92.7	110.26	$P < 0.01$	
8	2020	3	15.5 (6.4 to 33.0)	90.2	20.38	$P < 0.01$	
9	2021	10	37.3 (22.2 to 55.4)	98.8	736.32	$P < 0.01$	

Meta-regression

In the univariable meta-regression, the association between ESBL *E. coli* in broiler farms to studies reported in Asia (Coef. 95% CI = 1.36 (0.0002 to 2.71), $p = 0.050$), and studies that were published in 2018 (Coef. 95% CI = -2.29 (-4.57 to -0.01), $p = 0.049$) were found (Table 3). In the multivariable meta-regression, the association with ESBL *E. coli* was found in studies reported from Europe and in studies isolated from other types of sample subgroups, as indicated by a $p < 0.05$ (Table 4).

Table 3 Summary results of univariable meta-regression for the prevalence of ESBL *E. coli* in broiler farms.

Variables	Coef. (95% CI)	Standard error	p -value
Sample type			
Environmental sample (ref)	-	-	-
Fecal sample	0.16 (-1.09 to 1.41)	0.64	0.802
Others	-1.24 (-2.98 to 0.50)	0.89	0.162
Continent			
Africa (ref)	-	-	-
Asia	1.36 (0.0002 to 2.71)	0.69	0.050
Europe	1.41 (-0.0071 to 2.83)	0.72	0.051
North America	1.55 (-1.49 to 4.59)	1.55	0.318
South America	0.35 (-1.37 to 2.06)	0.87	0.692
Year-wise			
2013 (ref)	-	-	-
2014	-1.12 (-0.85 to 3.08)	1.00	0.265
2015	0.34 (-1.88 to 2.56)	1.13	0.764
2016	0.89 (-1.36 to 3.14)	1.15	0.439
2017	-0.63 (-2.87 to 1.62)	1.14	0.584
2018	-2.29 (-4.57 to -0.01)	1.16	0.049
2019	0.32 (-1.23 to 1.88)	0.79	0.684
2020	-0.44 (-2.43 to 1.54)	1.01	0.659
2021	0.74 (-0.78 to 2.27)	0.78	0.340

Sensitivity analysis and publication bias

Compared to the results obtained from the random effects model, the pooled prevalence of ESBL *E. coli* in broiler farms was found to be higher, at 32.5% (95% CI = 31.5 to 33.5) when computed by the fixed-effects model. However, the pooled prevalences determined by fixed and random effect models are comparable. The leave-one-out analysis showed a slight change in the pooled prevalence being estimated. The pooled prevalence was estimated at 28.5% (95% CI = 19.9 to 38.9) when the study with the lowest prevalence was excluded from the meta-analysis. On the other hand, the pooled prevalence was estimated at 24.8% (95% CI = 17.1 to 34.6) when the study with the highest prevalence was removed. Thus, removing the studies with the highest and lowest prevalence did not influence the overall pooled prevalence of ESBL *E. coli* in broiler farms. Sensitivity analysis to assess the robustness of the pooled prevalence is given in Table 5.

The existence of publication bias due to the small study effect was suggested by a funnel plot as shown in Figure 4 and Egger's test which indicated a statistically significant coefficient bias (-5.29 ± 2.54 , $p = 0.0449$).

Table 4 Summary results of multivariable meta-regression for the prevalence of ESBL *E. coli* in broiler farms.

Variables	Coef. (95% CI)	Standard error	p-value
Sample type			
Environmental sample (ref)	-	-	-
Fecal sample	0.34 (-0.83 to 1.51)	0.60	0.570
Others	-1.78 (-3.55 to -0.01)	0.90	0.048
Continent			
Africa (ref)	-	-	-
Asia	0.95 (-0.28 to 2.19)	0.63	0.130
Europe	2.35 (0.97 to 3.73)	0.70	0.0008
North America	0.85 (-1.65 to 3.34)	1.27	0.506
South America	-0.09 (-1.74 to 1.57)	0.84	0.917
Year-wise			
2013 (ref)	-	-	-
2014	1.28 (-0.53 to 3.09)	0.92	0.167
2015	0.15 (-1.75 to 2.05)	0.97	0.876
2016	2.22 (-0.05 to 4.49)	1.16	0.055
2017	0.15 (-2.03 to 2.33)	1.11	0.894
2018	-1.17 (-3.19 to 0.85)	1.03	0.256
2019	1.04 (-0.38 to 2.46)	0.72	0.150
2020	0.19 (-1.64 to 2.02)	0.93	0.838
2021	1.24 (-0.28 to 2.76)	0.78	0.11

Table 5 Sensitivity analysis to assess robustness of the pooled prevalence.

Categories	No. of studies or subgroups	Prevalence (95% CI)
Model		
Fixed effects	37	32.5 (31.5 to 33.5)
Random effects	37	26.6 (18.2 to 37.3)
Leave-one-out analysis		
The lowest prevalence ^a	36	28.5 (19.9 to 38.9)
The highest prevalence ^b	36	24.8 (17.1 to 34.6)

^aExcluded study of [Moawad et al. \(2018\)](#) from the meta-analysis.

^bExcluded study of [Abreu et al. \(2014\)](#) from the meta-analysis.

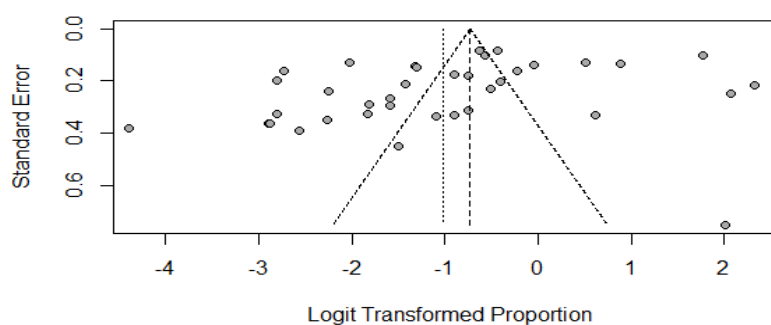


Figure 4 Funnel plot to measure publication bias of the included studies.

DISCUSSION

Antibiotic agents have been extensively used in broiler production for the purposes of growth promotion, prophylaxis (antibiotic is given to exposed herd of healthy animals), metaphylaxis (antibiotic is given to herd of healthy animals for disease prevention), and individual treatment (Page and Gautier, 2012; Roth et al., 2019). Broiler farming is depicted as a pyramidal hierarchy with the narrow top of the pyramid representing the breeding company. Studies revealed that there are only a few breeding companies worldwide, and two companies have a market share of more than 85% of the European market (EFSA, 2011). Our results revealed a moderate pooled prevalence of ESBL *E. coli* in broiler farms worldwide, with an estimated rate of 26.6% (95% CI = 18.2 to 37.3) using a random effects model. The pooled prevalence demonstrated a high level of heterogeneity, with $Q = 1958.9$ and $I^2 = 98.2\%$. There are many risk factors that could potentially lead to the presence of ESBL *E. coli* in broiler farms. ESBL *E. coli* may introduce to the broiler farms via stocking of new chicks, exposure to contaminated air, water or feed, insect or rodent vectors, human-to-animals, and animal-to-animal transmission.

Subgroup analyses showed a significant difference ($p < 0.01$) of ESBL *E. coli* in the continents and sample type subgroup. In a subgroup of continent, the pooled prevalence of ESBL *E. coli* in broiler farms was highest in Asia (33.0%) and lowest in Africa (12.4%). Studies from Asia revealed an extensive use of beta-lactam in broiler production which is a factor contributing to the increased prevalence of ESBL *E. coli* in broiler production (Brower et al., 2017; Coyne et al., 2019). In addition, a study conducted in Chiang Mai and Lamphun provinces in Thailand reported that 96.2% of ESBL *E. coli* isolates were confirmed as multidrug resistant (MDR). Additionally, 7.5% of these MDR isolates were found to be resistant to as many as nine different classes of antimicrobials (Rodroo et al., 2021). This emphasizes the significant impact of ESBL *E. coli* on public health. Moreover, a shift from conventional to commercial farming practices is also attributed to the increase of ESBL *E. coli* in broiler farms (Li et al., 2016; Hinenoya et al., 2018). Our results showed that the prevalence of ESBL *E. coli* in European broiler farms was similar to those in Asia. ESBL *E. coli* has been reported in broiler farms and chicken meat in European countries due to several attributable factors such as heavy use of cephalosporins, lack of monitoring of biosecurity practices, and animals' trade (Liebana et al., 2013; Paivarinta et al., 2016). The European Food Safety Authority (EFSA) has a recommendation that applies to all EU members, which aims to minimize the public health risk of ESBL *E. coli* posed by poultry farming. The guideline calls for the prudent use of the third and fourth generation cephalosporins in poultry farms. The off-label use of these antibiotics is discouraged. The use of third and fourth generation cephalosporins are reserved for animals that have responded poorly to narrow-spectrum antimicrobials. Most importantly, the guideline emphasized biosecurity practices within poultry farms to prevent vertical transmission from the top of the poultry production pyramid (EFSA, 2011). Even though the prevalence of ESBL *E. coli* in Africa was the lowest in our studies, the risk of ESBL *E. coli* remains high. Absence of strict regulations, extensive use of antibiotics in broiler farms, and over-the-counter availability of antibiotics have been reported as associated factors that contributed to the presence of ESBL *E. coli* in African broiler farms (Carissa et al., 2013). In addition, the pooled prevalence of ESBL *E. coli* in broiler farms has fluctuated inconsistently throughout the decade. However, the prevalence has remained below 50%. To the best of our knowledge, we could not find any evidence to support this fluctuation.

We conducted a two-step meta-regression, where univariable meta-regression acts as a screening test and multiple meta-regression takes into account other variables when assessing the association, to investigate the association between ESBL *E. coli* in broiler farms and other variables that might influence the prevalence. The results from the univariable meta-regression reveal

an association between ESBL *E. coli* in broiler farms and studies conducted in Asia ($p = 0.05$), as well as studies published in 2018 ($p = 0.049$). However, the multiple meta-regression reveals an association between ESBL *E. coli* in broiler farms and studies conducted in Europe ($p = 0.0008$), as well as others sample type ($p = 0.048$).

Publication bias analysis by Eggers's test suggested a publication bias of small study-effect. This indicated that studies with small sample sizes included in this study are a major source of publication bias. However, all studies included in the meta-analysis were of good quality, with an average score of 9.48/10 (SD = 0.9). The result from the sensitivity analysis, which involved comparing the pooled prevalence between the fixed and random effects models and the use of leave-one-out analysis, demonstrated that our meta-analyses were both robust and stable.

Our study contains some limitations. Firstly, the systematic literature searches were limited to only three databases. Secondly, all records were limited to studies that were published in English, as a result, we may rule out relevant studies that were published in other languages. Thirdly, subgroups were not well distributed across some characteristics due to the fact that few studies being eligible for analysis. Fourthly, the prevalence of ESBL *E. coli* was limited to samples taken from broiler farms, thus, studies conducted at broiler slaughterhouses were not included. Lastly, publication years may not always accurately reflect the time of sample collection. These could affect the result of the subgroup analysis.

CONCLUSIONS

Our study revealed a moderate prevalence of ESBL *E. coli* from broiler farms over a decade. The estimated pooled prevalence was 26.6% (95% CI = 18.2 to 37.3). This suggests a potential burden of ESBL *E. coli* in public health and broiler production. The use of antibiotics in broiler production requires monitoring to ensure their prudent use within the industry. Regulations regarding the use of antibiotics in broiler production need to be established, especially in areas where such regulations are currently absent. Collective actions among policymakers, broiler producers, veterinarians, and consumers are necessary to achieve sustainable and less antibiotic use in broiler production. Further research on alternatives to antibiotic use in broiler production is encouraged to make it more available and accessible to a broader scale of broiler production.

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AUTHOR CONTRIBUTIONS

NA and SB designed and conceptualized the study. NA, PO and SB performed systematic review and meta-analysis. NA and PO prepared and revised the manuscript. VP revised statistical analysis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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