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Review Article

Tetracycline Resistance among *Escherichia coli* Isolates from Broilers in Iran: A Systematic Review and Meta-Analysis

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Abstract

Background: Avian colibacillosis are infections caused by Avian Pathogenic *Escherichia coli* which causes high morbidity, high mortality, and significant economic losses. Antibiotics such as tetracycline are an important treatment choice for colibacillosis in Iran. However, the irrational use of antibiotics has caused high antibiotic resistance. The aim of this study was to estimate the tetracycline resistance rate among *E. coli* isolates from broilers in Iran.

Methods: PubMed, Scopus, Web of Science, Google Scholar, Islamic World Science Citation, Scientific Information Database, and MagIran databases were searched from 2013 and 2023. Risk of bias assessment was assessed with Appraisal tool for Cross-Sectional Studies. For meta-analysis, a random effects model with Freeman-Tukey double arcsine transformation was used. Subgroup analysis for the year of publication and sensitivity analysis to assess the influence of individual studies were conducted and a province-level map of point estimates was drawn.

Results: Among 13 included studies, the pooled tetracycline resistance was 85% (95% CI: 76-92%) and I-squared was 94.29%. In subgroup analysis, resistance rate was higher for articles published in 2018-2023 (91%) compared to articles published in 2013-2017 (76%), but heterogeneity between groups was not statistically-significant (P=0.12). sensitivity analysis showed that the omission of individual studies caused no apparent change. The province-level map showed that most of the studies belonged to northern parts of Iran with Ardabil (99.65%) having the highest value.

Conclusion: Resistance against tetracycline in *E. coli* isolates from broilers in Iran is alarming. The sensible use of tetracycline is advised.

Keywords: Antimicrobial resistance; *Escherichia coli*; Broiler; Iran

Introduction

Antimicrobial resistance (AMR) is a significant global threat to human and animal health (1, 2). For decades, antimicrobials have been used in medicine and veterinary medicine to treat diseases, promote growth, and prevent diseases in food animals, particularly poultry broilers (3, 4).

Avian colibacillosis is referred to infections caused by Avian Pathogenic *Escherichia coli (*APEC), which can result in extraintestinal infections such as acute septicemia, air vasculitis, chronic respiratory diseases, cellulitis, pericarditis, peritonitis, salpingitis in poultry. These infections can cause high morbidity and mortality levels and significant economic losses for the global poultry industry due to costs associated with disinfection and antimicrobial treatment (5). Foods of animal origin might be a major source of human-acquired multi-drug resistant pathogenic *E. coli* (E. coli). Poultry and meat products can be extensively tainted with pathogenic or non-pathogenic groups of *E. coli* of animal origins. Livestock including broiler chickens is deemed to be the primary source of food contamination and the prime cause of sundry foodborne outbreaks ascribable to the consumption of food tainted with pathogenic *E. coli* (6, 7). It has been shown that broiler meat can contain tetracycline-resistant *E. coli* (8). Poultry meat including broiler chickens is an inherent source of *E. coli* infections in humans because of the higher possibility of exposure to *E. coli* via direct contact during food preparation or poor cooking (4, 7).

Tetracycline is a widely used broad-spectrum antibiotic that targets gram-positive and gram-negative bacteria by inhibiting protein synthesis (9, 10). Tetracycline is frequently used in the poultry industry due to its low cost, high efficacy, widespread availability, and few adverse side effects. However, its extensive use has led to the emergence of tetracycline-resistant bacteria, limiting its effectiveness (9). In Iran, the use of tetracycline as a standard antibiotic in farming practices is widespread. However, the overuse of this antibiotic in poultry has led to an alarming increase in the development of tetracycline-resistant strains of *E. coli* (11).

Numerous studies have been conducted on the antibiotic resistance of *E. coli* from broilers in different parts of Iran (12). However, no study has estimated the overall resistance rate. Therefore, the aim of this systematic review and meta-analysis was to estimate the resistance rate against tetracycline among *E. coli* isolated from broilers in Iran between 2013 and 2023.

Materials and Methods

This systematic review and meta-analysis was designed and reported according to PRISMA 2020 statement (13).

Search strategy

The databases of PubMed, Scopus, Web of science, Google Scholar, Islamic World Science Citation Database (ISC), Scientific Information Database (SID), and MagIran were searched by the keywords "Escherichia coli" OR "colibacillosis" AND "broiler" AND "resist*" OR "suscept*" OR "Drug Resistance, Microbial" OR "tetracycline" AND "Iran" both in English and Persian. The search process and the selection of relevant articles are presented in the PRISMA flowchart (Fig. 1).

Title and abstract screening

Two review authors (AFD and HH) independently screened the titles and abstracts of the results to identify relevant studies based on the inclusion and exclusion criteria. The inclusion criteria was cross-sectional studies which assessed tetracycline resistance in broilers in Iran between January 2013 and January 2023 and the exclusion criteria was studies that did not contain original data, were written in languages other than Persian or English, or had no available full-text. If conflict arose whether to include a study, the final decision was made by consensus. The two review authors (AFD and HH) retrieved the full-text of the relevant studies and screened their references in order to maximize the comprehensiveness of the search and to identify any relevant articles that were missed by the search.

Risk of bias (quality) assessment

Two review authors involved in title and abstract screening (AFD and HH) independently assessed the risk of bias of all the relevant studies with Appraisal tool for Cross-Sectional Studies (AX-

IS), which consists of 20 questions that are answered with "Yes", "No", or "Unclear" (14). Conflicts risen on the quality status of an article were resolved by consensus.

Data extraction

Two review authors (AFD and PK) independently performed the data extraction. The two review authors resolved the discrepancies in the extracted data with consensus. The data of the relevant studies including, the number of tetracycline-resistant Escherichia coli, the total number of *E. coli* isolates, age range, the location of sampling, the last name of the first author, and the year of publication of the paper were extracted into a modified Cochrane Collection form. If a study reported several age groups of the same population, the results of the age groups were combined.

Statistical analysis

For meta-analysis, a random effects model was used and Freeman-Tukey Double arcsine transformation was applied to stabilize the variance. Funnel plot was drawn to assess the presence of publication bias. Subgroup analysis was carried out between studies published between 2013-2018 and 2019-2023. Sensitivity analysis was conducted to determine the influence of individual studies on the overall pooled estimation. Province-level point estimates of resistance rate were calculated by dividing the sum of numerators by the sum of denominators of studies of each province and they were mapped. All the statistical analysis was done by STATA12.1. The results of the risk of bias assessment table were illustrated with RevMan 5.4.1. software. Province-level point estimate map was drawn with ArcGIS 10.3.

Results

Eligibility Studies and Search Results

Overall, 124 results were found, among which 16 papers were duplicates. From the remaining 108 articles, 19 articles were deemed to be relevant in abstract screening. The full-texts of the papers were retrieved and the relevance was re-checked. It was revealed that four articles did not meet the inclusion criteria. Also, two papers were suspected to be duplicates due to the similarity of their authors, methods and materials, and reported results with two other papers that were already included in the meta-analysis. The corresponding authors of these six papers were contacted via email. However, they did not respond. Therefore, the six papers were not included in the meta-analysis. The screening of titles and abstracts of the reference lists of the relevant articles returned no new relevant articles (Fig. 1). Finally, 13 articles were included in meta-analysis. The summary of the characteristics of the included studies is presented in Table 1.

Risk of bias assessment

The results of risk of bias assessment are presented in Fig. 2.

Table 1: Study characteristics of the included studies of tetracycline resistance in Escherichia coli isolates from broilers in Iran 2013-2023

	Author	Year of publication	Number of resistant *E. coli* isolates	Total number of *E. coli* isolates	Age range	Location	
						Province	City
1	Rahimi (29)	2013	131	154	5-42 days	Kermanshah	-
2	Kazemnia (30)	2014	15	25	-	-	-
3	Seifi (31)	2015	57	80	-	Mazandaran	-
4	Seifi (32)	2016	73	100	-	Mazandaran	-
5	Kheiri (33)	2016	24	50	-	Alborz	Karaj
6	Azizpour (34)	2017	177	178	14-42 days	Ardabil	-
7	Asadi (35)	2018	112	117	-	Kerman	Shahr-e babak
8	Mohammadi (36)	2018	35	44	-	West Azerbaijan	-
9	Esmaeelzade Dizaji (37)	2020	96	100	7-42 days	Sistan and Baluchestan	Zabol
10	Rezatofighi (38)	2021	85	100	-	Semnan	-
11	Kalantari (39)	2021	251	300	1-day-old, 30-day-old, 1-day before slaughter	Fars	Shiraz
12	Azizpour (11)	2022	111	111	14-49 days	Ardabil	Ardabil city
13	Goudarztalejerdi (20)	2022	160	200	-	Hamedan	-

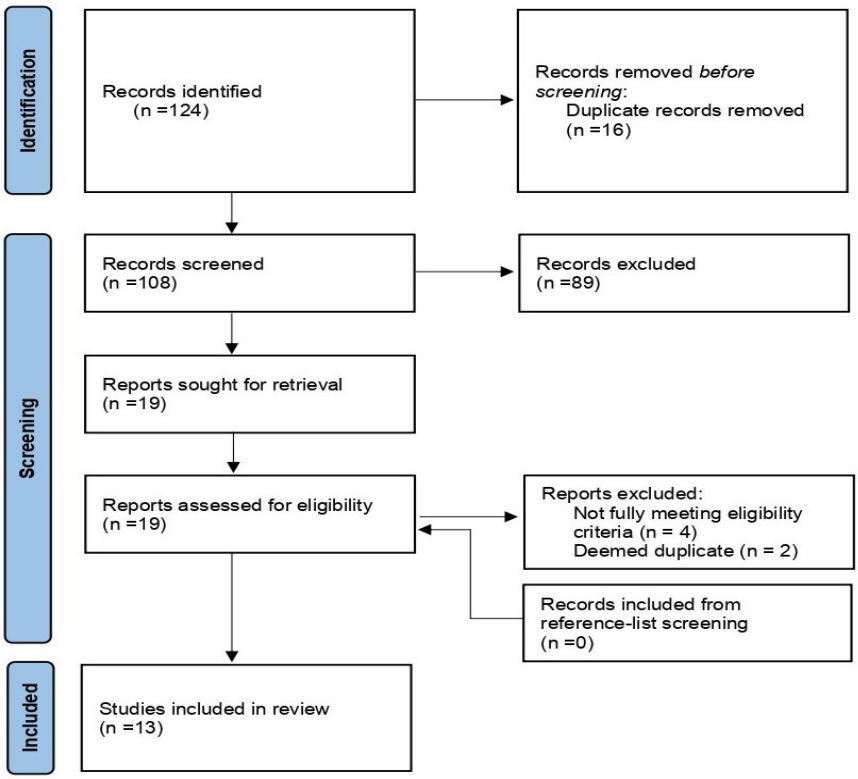

Fig. 1: Flow chart of studies

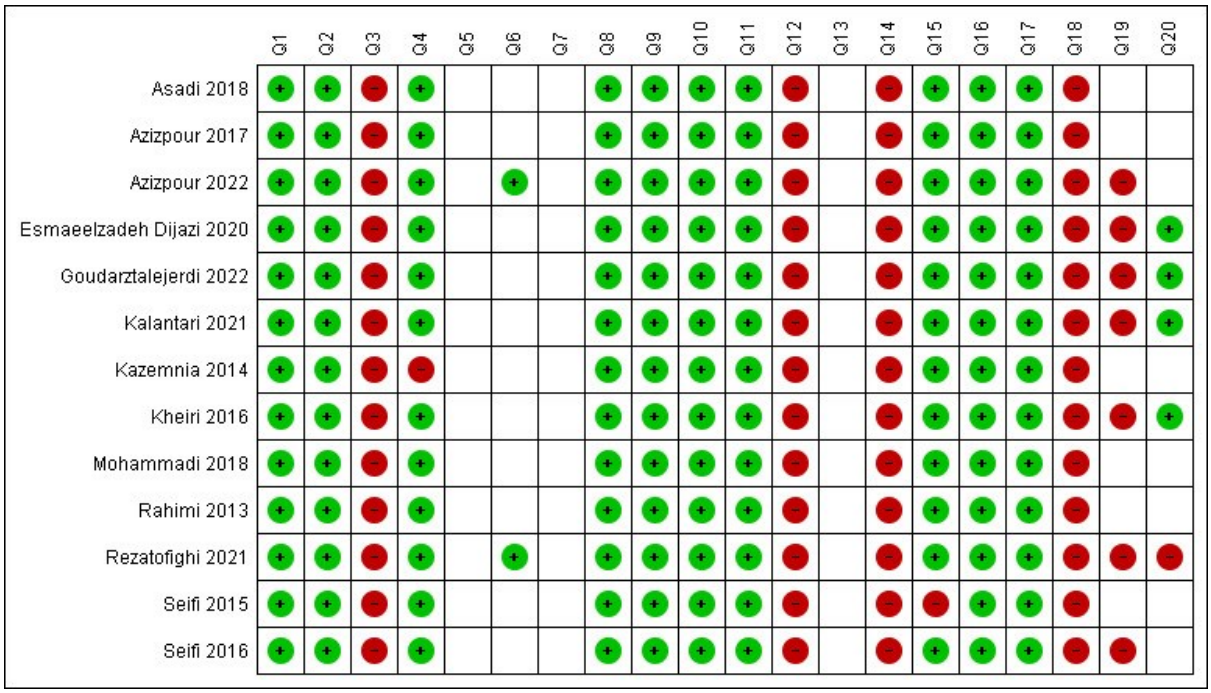

Fig. 2: Results of risk of bias assessment with AXIS tool of included studies of tetracycline resistance in *E. coli* isolates from broilers in Iran 2013-2023. Green=Yes, Red=No, Blank=Unclear

Pooled estimation

The pooled estimation of tetracycline resistance among *E. coli* in broilers in Iran was 85% (95% CI: 76-92%) and I-squared was 94.29%. In subgroup analysis, the resistance rate was higher in studies published in 2018-2023 (91%) in comparison with studies published in 2013-2017 (76%). But, heterogeneity between groups was not statistically significant (*P*=0.12). The forest plot and subgroup analysis is presented in Fig. 3. In the estimation of province-level prevalence, one study was excluded due to not reporting the location of the study. Ardabil (99.65%), Sistan and Baluchestan (96%), and Kerman (95.72%) had the highest province-level prevalence. The results of province-level point estimates of tetracycline resistance rate are presented in Table 2 and Fig. 4.

Table 2: Province-level point estimates of tetracycline resistance rate in Escherichia coli isolates from broilers in Iran 2013-2023

	Province	Prevalence (%)
1	Kermanshah	85.06
2	Mazandaran	72.22
3	Alborz	48
4	Ardabil	99.65
5	Kerman	95.72
6	West Azerbaijan	79.54
7	Sistan and Baluchestan	96
8	Semnan	85
9	Fars	83.66
10	Hamedan	80

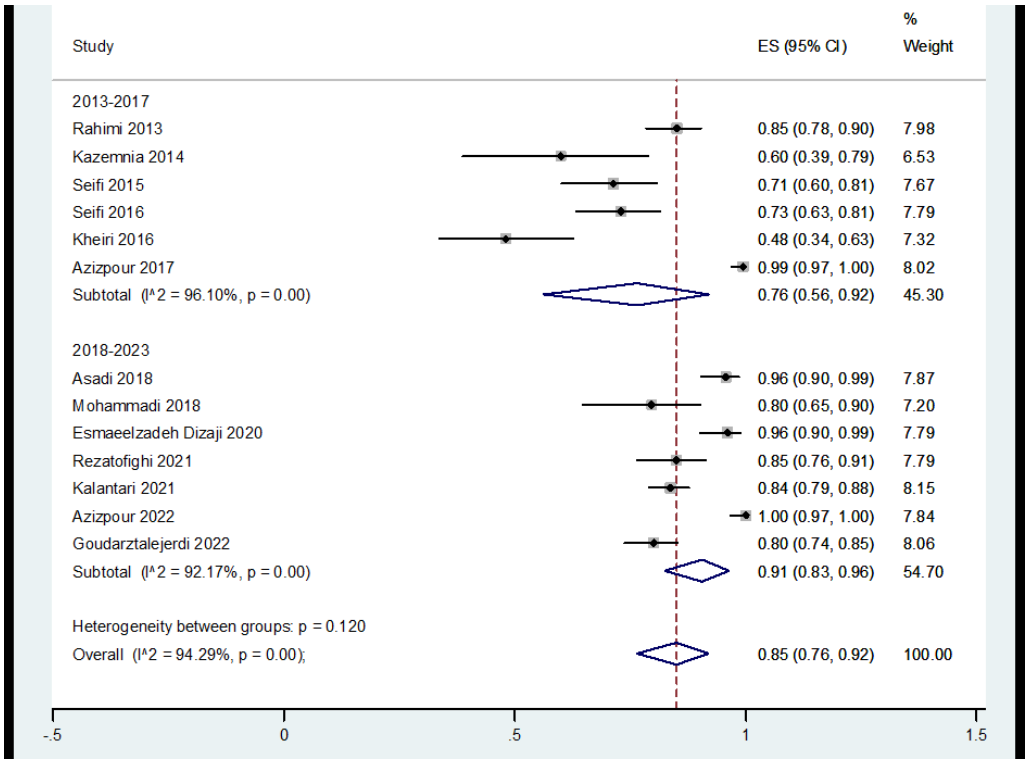

Fig. 3: Forest plot and subgroup analysis of tetracycline resistance in E. coli isolates from broilers in Iran 2013-2023

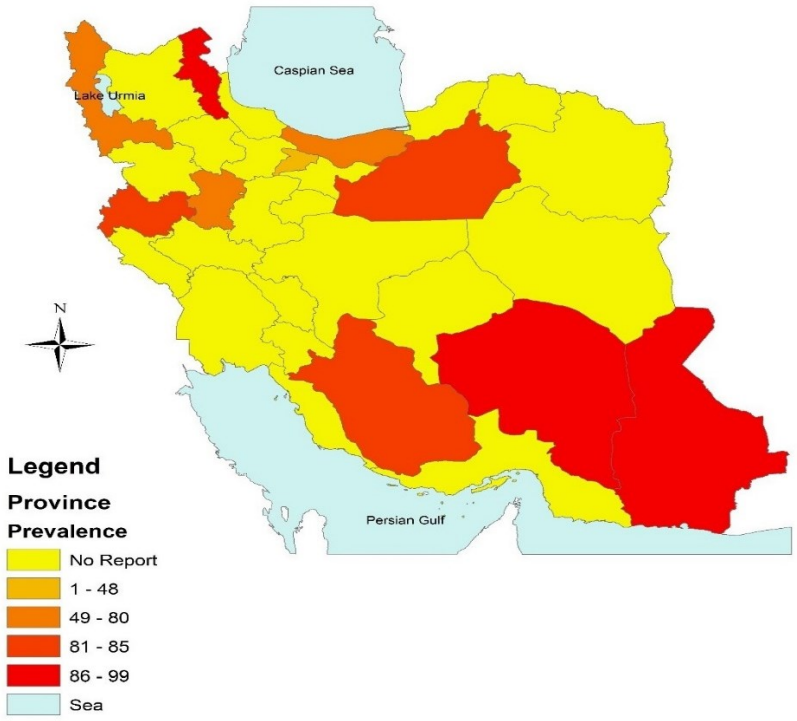

Fig. 4: Province level of resistance rate of tetracycline resistance in included studies of tetracycline resistance in E. coli isolates from broilers in Iran 2013-2023

Funnel plot

The funnel plot is presented in Fig. 5.

Sensitivity analysis

The results of influential analysis are presented in Fig. 6.

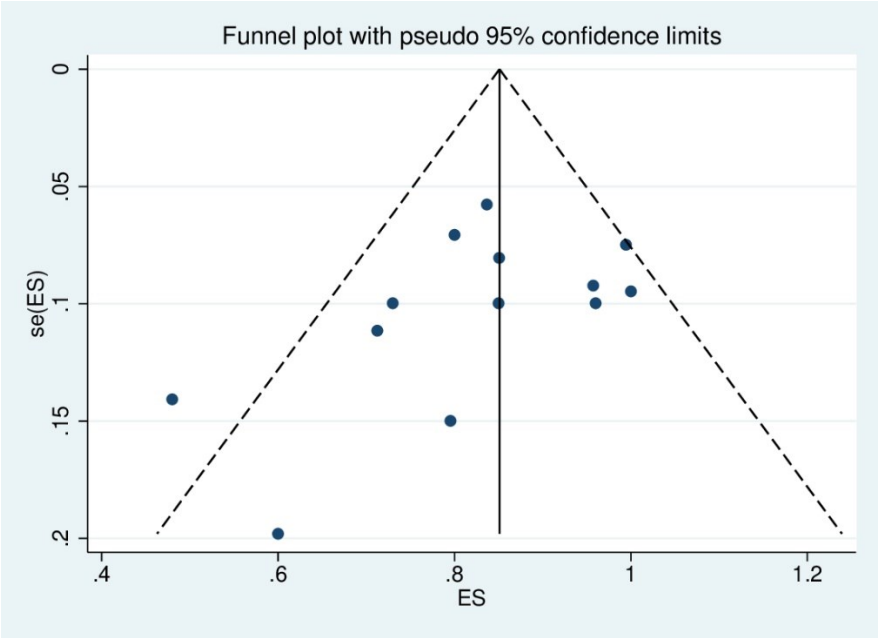

Fig. 5: Funnel plot of tetracycline resistance in E. coli isolates from broilers in Iran 2013-2023

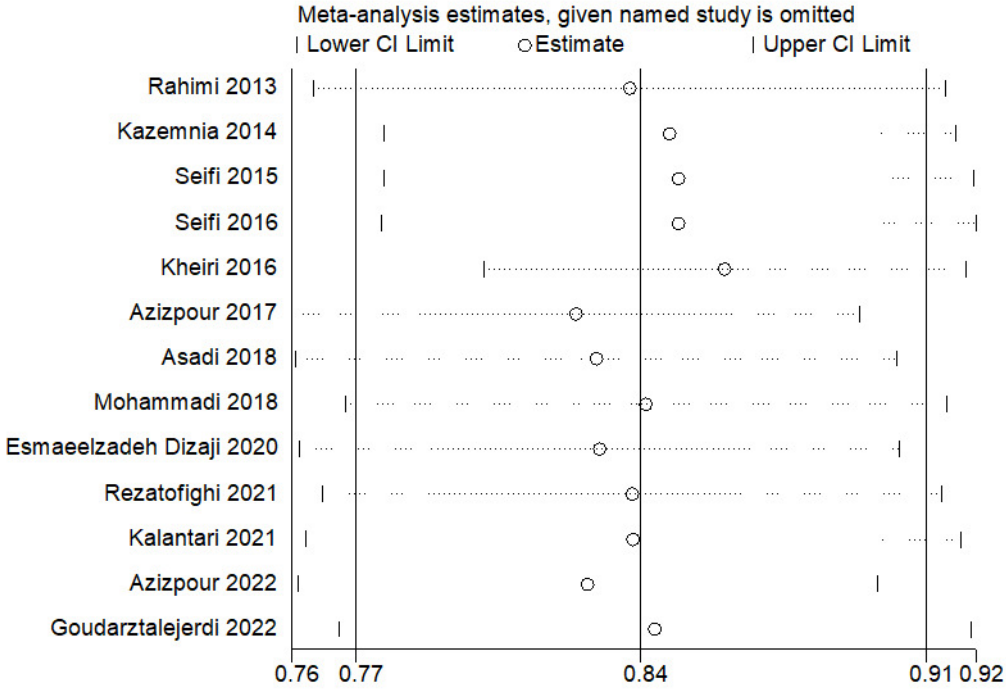

Fig. 6: Sensitivity analysis of the influence of each study on the overall pooled estimation of tetracycline resistance in E. coli isolates from broilers in Iran 2013-2023

Discussion

AMR is one of the most vital health-threatening issues for both humans and animals worldwide (15). Among animals, the largest volume of antimicrobial agents are consumed in food animals (16). The use of antimicrobial agents in animals increases AMR in humans (17). The aim of the current systematic review and meta-analysis was to estimate the resistance rate against tetracycline in *E. coli* isolates collected from broilers in Iran from 2013-2023.

Based on our findings, the pooled estimation of tetracycline resistance among *E. coli* isolated from broilers in Iran between January 2013 and January 2023 was 85% (95% CI: 76-92%). I-squared was 94.29% indicating significant heterogeneity. Due to the high pooled estimate of tetracycline resistance rate in *E. coli* isolates recovered from broilers, it is suggested that tetracycline is not an effective antimicrobial agent for the treatment of avian colibacillosis in broilers in Iran. The funnel plot was asymmetrical, suggesting that publication bias may be present. Overall, the included studies provided few details about the sampling techniques and the sample frame used in the studies. Therefore, it was unclear whether or not sampling frame and sample selection were able to draw representative samples in the individual studies. The map of province-level resistance rate point estimates showed that resistance was different among provinces among which Ardabil (99.65%), Sistan and Baluchestan (96%), and Kerman (95.72%) had the highest values, respectively. This finding could suggest that between provinces, there are differences between the tetracycline resistance rates of *E. coli* isolates recovered from broilers. However, most of the included studies belonged to northern parts of Iran with only two papers conducted in southern parts and no studies in central parts of Iran. Therefore, it is recommended to conduct more investigations especially in provinces without reports to have a better view of the tetracycline resistance rate in *E. coli* isolates from broilers.

In subgroup analysis, although the resistance rate was higher in studies published between 2018 and 2023 (91%) compared to 2013-2017 (76%), heterogeneity between groups was not statistically significant. This finding suggests that subgroups based on studies published between 2013 and 2017 and studies published between 2018 and 2023 might not be a source of heterogeneity. In sensitivity analysis, the omission of Kheiri 2016 had the highest influence on the overall pooled estimate. However, no apparent change was observed. Therefore, the pooled estimate was relatively robust.

Tetracycline is an important and common antibiotic in both medicine and veterinary medicine (18). Among food animals, tetracyclines have been found to be the most frequently used antimicrobial agent overall (33,305 tonnes) worldwide, which has been predicted to increase by 9% by 2030 (19).

In Iran, antimicrobial agents such as tetracycline are frequently used for treatment, control, and prevention of colibacillosis outbreaks and for growth promotion in broilers (20). Moreover, Farmers frequently utilize antibiotics without the prescription of veterinary personnel (21). The high density of flocks, improper sanitisation, and unregulated use of broad spectrum antimicrobial agents are beneficial for the spread of antimicrobial resistance at the farm level (22). The majority of hotspot areas with antimicrobial use intensity in food animals have been found to be in Asia (67%), among which, Northwest of Iran is a hotspot alongside eastern China, southern India, Central Java (Indonesia), central Thailand, eastern coast line of Viet Nam, western South Korea, eastern India and Bangladesh, and Pakistan. Moreover, Iran was among the top ten countries with antimicrobial use in food animals in 2020 (19).

In this systematic review and meta-analysis, there were limitations: First, due to lack of reporting of demographic data of the flocks from which *E. coli* strains were isolated and the absence of further molecular analysis of *E. coli* strains in the individual studies, it was not feasible to conduct sub-

group analysis or meta-regression to explore heterogeneity on the aforementioned variables. Second, although publication bias may be present, it could not be assessed.

To our knowledge, this is the first systematic review and meta-analysis conducted to determine the pooled tetracycline resistance rate in *E. coli* isolates from broilers in Iran. In the systematic review of the antimicrobial resistance of *E. coli* isolates from poultry in Bangladesh, the prevalence of resistance against tetracycline ranged from 17.7% to 100% (95% CI: 12.8%-100%) (23), which had a broader range of reported point estimates of the resistance rates compared to our findings.

In the systematic review of antimicrobial resistance in food producing animals in West Africa French-speaking countries, the median resistance (MR) and the interquartile range (IQR) of resistance against tetracycline among *E. coli* strains from poultry between 2000-2019 were 97% and 80.65- 98.5%, respectively (24).

In the systematic review and of antimicrobial resistance in Enterobacteriaceae isolates from humans, animals, and the environment in Vietnam, the resistance rate of *E. coli* isolates against tetracycline ranged from 60.9–84.6% and the highest level of resistance was observed in animals (25).

In the systematic review of antimicrobial resistance of foodborne pathogens in Middle East between 2011 and 2020, MR and IQR against tetracycline among *E. coli* isolates were 80.6% and 58.97–88.63%, respectively (26).

Mesa-Varona et al. investigated the resistance of clinical and non-clinical *E. coli* isolates from broilers and turkeys in Germany and reported that among isolates from broilers, resistance to tetracycline in clinical isolates for 2014-2017 were 44.4%, 17.3%, 14.0%, and 31.7% respectively. And, in non-clinical samples from broilers in 2014 and 2016, resistance rate against tetracycline were reported to be 33.5% and 27.7%, respectively (27).

Willis et al. surveyed antimicrobial resistance in *E. coli* and Salmonella isolates in chicken products from the UK in 2021 and reported that among the 113 samples which were found positive for E. coli, antimicrobial resistance rate against tetracycline was 23.6% (28).

Conclusion

The high pooled estimate of tetracycline rate is indicative of tetracycline not being an effective antimicrobial agent in the treatment of *E. coli* infections in broilers in Iran. Rational use of tetracycline is advised. More investigations should be conducted in Iran especially in areas without reports to have better view upon tetracycline resistance in *E. coli* isolates.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of Interest

The authors declare no conflict of interest.

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