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The Prevalence of Fluoroquinolone-Resistant *E. coli* in Animals and Animal Products: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Biological contamination of foods is a serious problem for human health. Animal and animal products may be contaminated by these biological and chemical contaminants. One of the most important causes of foodborne illness in humans is *Escherichia coli*. Fluoroquinolones can be used as a suitable treatment for enteric infections in food-producing livestock. We aimed to evaluate the current status of resistance of *E. coli* strains isolated from animals and animal products to fluoroquinolone in Iran.

Methods: A systematic search was conducted using the Web of Science, PubMed, Embase, Google Scholar, and Scopus databases from 2000 to Oct 2020. Nineteen studies were selected based on the inclusion criteria and analysis by Comprehensive Meta-Analysis.

Results: Based on the data analysis, The rates of antibiotic resistance in animal strains were as follows: Flumequine (75.1%), Enrofloxacin (55.2%), Danofloxacin (48.1%), Ciprofloxacin (48.4%), and Norfloxacin (52.9%). Next, the rates of quinolone resistance among *E. coli* strains isolated from animal products were Norfloxacin (45.5%), Ciprofloxacin (44.5%), and Enrofloxacin (60.9%). Based on the funnel plots and Egger's test, there was no significant publication bias.

Conclusion: We finally concluded that antibiotic resistance in commensal *E. coli* is related to the overuse of antibiotics in livestock, especially fluoroquinolones.

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Introduction

Animals and animal products are at risk of physical, biological, or chemical contamination, with equal importance to human health (1). Food contamination with biological hazards is a serious health problem, and their outbreaks often bring about considerable morbidity and mortality. World Health Organization (WHO) reports estimated that in 2010, 31 foodborne diseases caused approximately 600 million illnesses and 420,000 deaths worldwide (2).

The main microorganisms that cause food poisoning or foodborne illness are bacteria, viruses, and parasites. Some bacteria, such as *Staphylococcus aureus*, *Campylobacter*, *Bacillus cereus*, *Salmonella*, *Listeria monocytogenes*, *Clostridium*, and *Escherichia coli* (*E. coli*), are responsible for the majority of foodborne illnesses (2, 3). Most *E. coli* strains are nonpathogenic flora, but some strains contain virulence factors known as pathogenic *E. coli*. Pathogenic strains of *E. coli* are a prominent cause of human foodborne and waterborne illness, with a spectrum of complications (4). *Escherichia coli* is a gram-negative flagellated, facultative, non-sporulating bacterium commonly found in humans and domestic animals' intestines (5).

Prevention strategy and suitable treatment are essential factors in the of *E. coli* infections limitation. In this regard, the livestock and poultry industry widely employ antibiotics for treatment, prevention, or increasing the growth of animals (6, 7). But these applications raise some growing concerns, such as the possible antimicrobial residues in animal products and the antibacterial-resistant strains advent (8-10).

Fluoroquinolones have been effective in treating respiratory diseases and intestinal infections in food-producing and companion animals. Their low toxicity, beneficial pharmacokinetic properties, and antimicrobial activity against a wide range of pathogenic bacteria have made them more widely used in farm animals (11). Fluoroquinolones are broad-spectrum antibiotics from the quinolone family and are used to treat severe or resistant infections. Norfloxacin, Pefloxacin, Enoxacin, Fleroxacin, Ciprofloxacin, and Ofloxacin have been marketed and are still in use. Unfortunately, the incorrect and extensive administration of fluoroquinolones has resulted in the increasing global rate of resistant strains in different bacterial species, especially *E. coli* (12, 13).

Because of the significance of fluoroquinolones as an important choice for human and animal infections, the increasing prevalence of fluoroquinolone-resistant commensal bacteria is a prominent public health consideration as one of the main causes of cross-contamination between animals and humans (14, 15). Our aim was to evaluate the current status of fluoroquinolone resistance of *E. coli* strains isolated from animals and animal products in Iran by meta-analysis and systematic review.

Search strategies

The literature search was conducted consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to identify all published studies related to the research question. We performed a systematic review by searching the Web of Science, PubMed, Embase, Scopus, and Google Scholar from 2000 to Oct 2020. Besides, reference lists of relevant primary studies were manually searched for any additional studies. The search strategy included keywords and a combination of "Fluoroquinolone" OR "Quinolone" AND "*Escherichia coli*" OR "*E. coli*" AND "Animal" OR "Animal product" OR "Animal food" AND "Iran," as well as spelling alternatives and related terms.

Study selection

Two authors independently conducted an initial screen of titles and abstracts based on keywords. Then, two authors retrieved and reviewed the full text of selected articles in parallel to apply for qualification for inclusion. Disagreements were settled through discussion; and, if necessary, a third reviewer was consulted. We did not use any language restrictions. Regarding the cross-sectional studies, we included those meeting the inclusion criteria and indexed them in the Web of Science, PubMed, or Scopus. The inclusion criteria include studies that report the prevalence of quinolone-resistance *E. coli* among animals or animal products, samples originating from Iran, and the prevalence of fluoroquinolone resistance. Articles were excluded if the sample size was smaller than ten isolates or samples derived from the environment (e.g., water, soil, and animal handler) or clinical sources. Other excluded criteria were reviews, case reports, and conference abstracts lacking primary data.

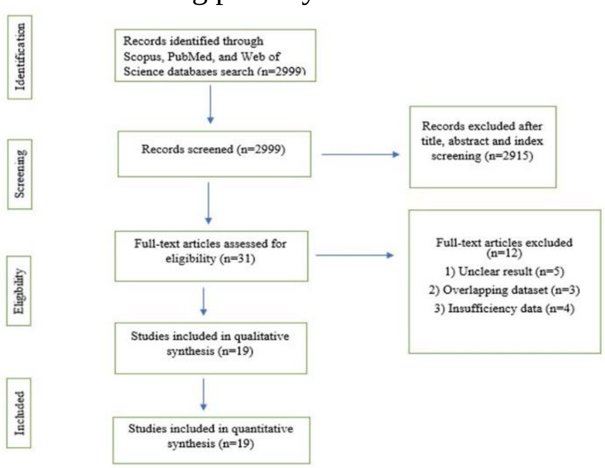

Fig 1. PRISMA flow chart of study selection.

Quality assessment and data extraction

Utilizing the Joanna Briggs Institute (JBI) 9-Point Critical Appraisal Checklist, quality checks were performed for reported outbreak studies. Two authors scored the articles independently, and the reviewers resolved the disagreements by mutual consensus, and if consensus was not reached, we consulted an independent researcher. We included studies that met over half of the quality assessment parameters. Two reviewers used a standardized form to extract the following data independently, including the first author, publication year, study year, study area, sample size, sample type, antibiotic susceptibility testing method, and prevalence of fluoroquinolone resistance.

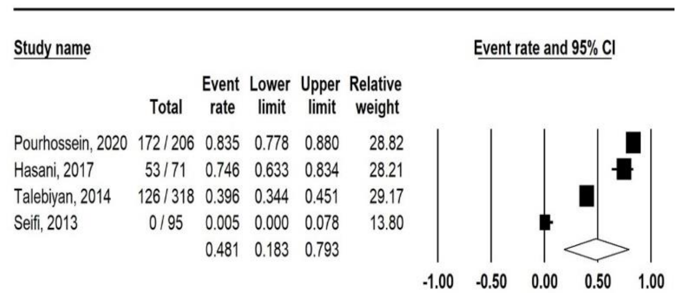

Fig 2. Subgroups analysis results of Danofloxacin resistant *E. coli* in Animal.

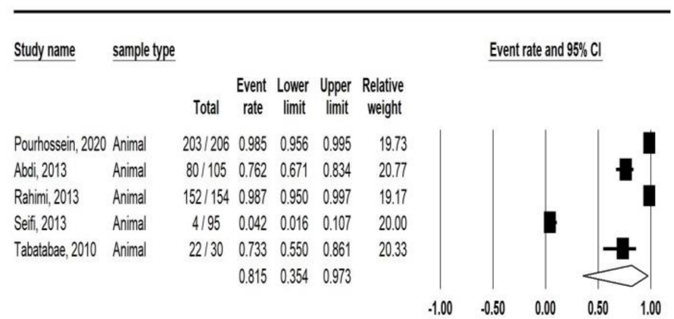

Fig 3. Subgroups analysis results of Flumequine resistant *E. coli* in Animal.

Statistical analysis

Data analysis was performed by Comprehensive Meta-Analysis Software Version 2.2 (BioStat Company). We performed Meta-analysis using a random-effects model to estimate the pooled prevalence and corresponding 95% Confidence interval (CI). We evaluated the statistical heterogeneity between and within groups using the Q statistic and the I2 index. Utilizing the funnel plot, Begg's rank correlation test, and Egger's weighted regression tests, we evaluated possible publication bias (considering P<0.05 a statistically meaningful publication bias). Also, we employed Chi-square tests to detect the importance of the differences via SPSSTM software, version 21.0 (IBM Corp., USA). We regarded a difference statistically meaningful if the p-value was less than 0.05. We designed the current study consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Results

The systematic review determined a total of 19 studies that reported the prevalence of quinolone-resistance *E. coli* isolates derived from animals or animal products. A flow diagram demonstrating the literature search and selection procedures is shown in Figure 1. Table 1 lists extracted data from included articles. In a total of nineteen, fourteen studies reported the quinolone resistance of animal strains, and five studies also documented data on the quinolone resistance of animal product strains. In all, nine reports were from the western regions, three from the northern, three from the central, three from the southern, and one from the east of the country.

Table 1. The main characteristics of the articles.

First Author	Performed Year	Sample size	Sample type	Sample	Region	References
Pourhossein,	2020	206	Animal	Poultry	Rasht/ North	(16)
Dehdashti,	2019	145	Animal	Livestock	Kerman/south	(17)
Mohammadi	2018	44	Animal	Poultry	West Azerbaijan/west	(18)
Ranjbar	2018	64	Animal products	Milk and dairy products	Isfahan / center	(19)
Boroomand	2010	44	Animal	Poultry	Ahvaz/south	(20)
Bakhshi	2017	100	Animal	Poultry	Yazd, center	(21)
Safavi	2017	89	Animal	Livestock	Kermanshah/west	(22)
Sharafati	2016	11	Animal products	Milk Yoghurt Cheese	Kashan/center	(23)
Hasani	2013	71	Animal	Poultry	Tabriz/west	(24)
Kheiri	2015	150	Animal	Poultry and livestock	Karaj/north	(25)
Bonyadian	2014	120	Animal products	Raw milk, Cheeses	Shahrekord/west	(26)
Talebiyan	2012	318	Animal	Poultry	Shahrekord/west	(27)
Abdi	2013	105	Animal	Poultry	Shiraz/south	(28)
Rahimi	2012	154	Animal	Poultry	Kermanshah/west	(29)
Seifi	2012	95	Animal	Poultry	Southern Caspian Sea/north	(30)
Momtaz	2011	146	Animal products	Chicken meat	Shahrekord /West	(31)
Rad	2010	36	Animal	Poultry	Mashhad/east	(32)
Momtaz	2010	360	Animal products	Chicken meat	Chaharmahal and Bakhtiari /west	(33)
Tabatabae	2010	30	Animal	Poultry	West Azerbaijan/west	(34)

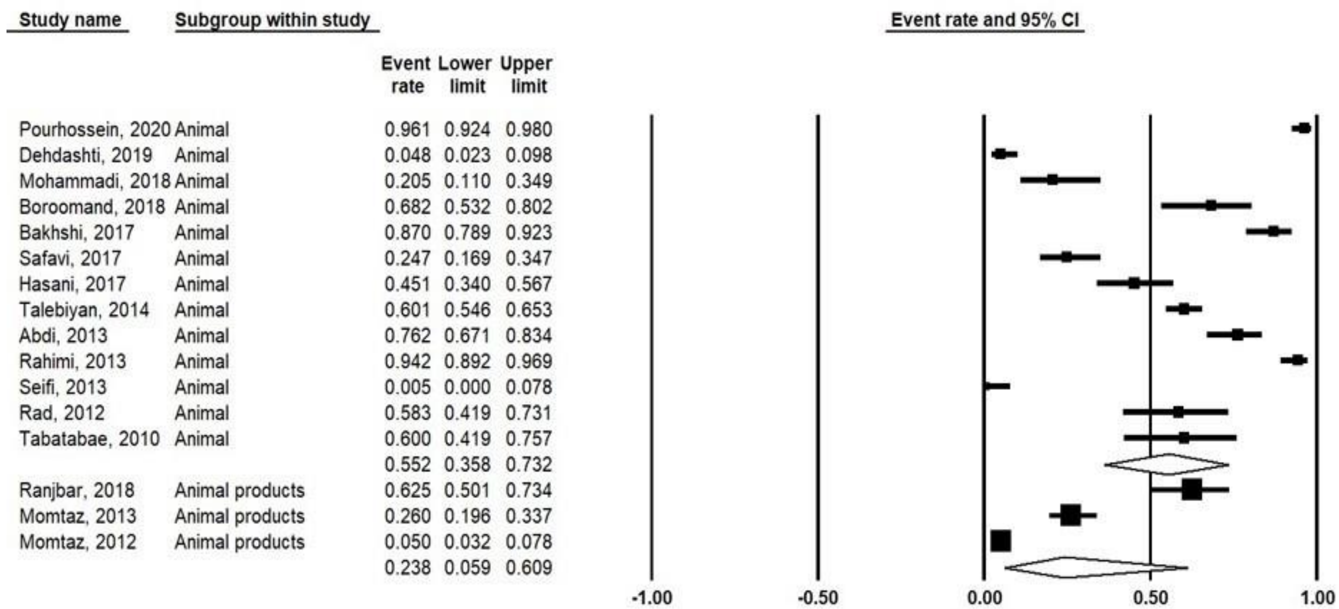

Fig 4. Subgroups analysis results of Enrofloxacin resistant *E. coli* in animal and animal products.

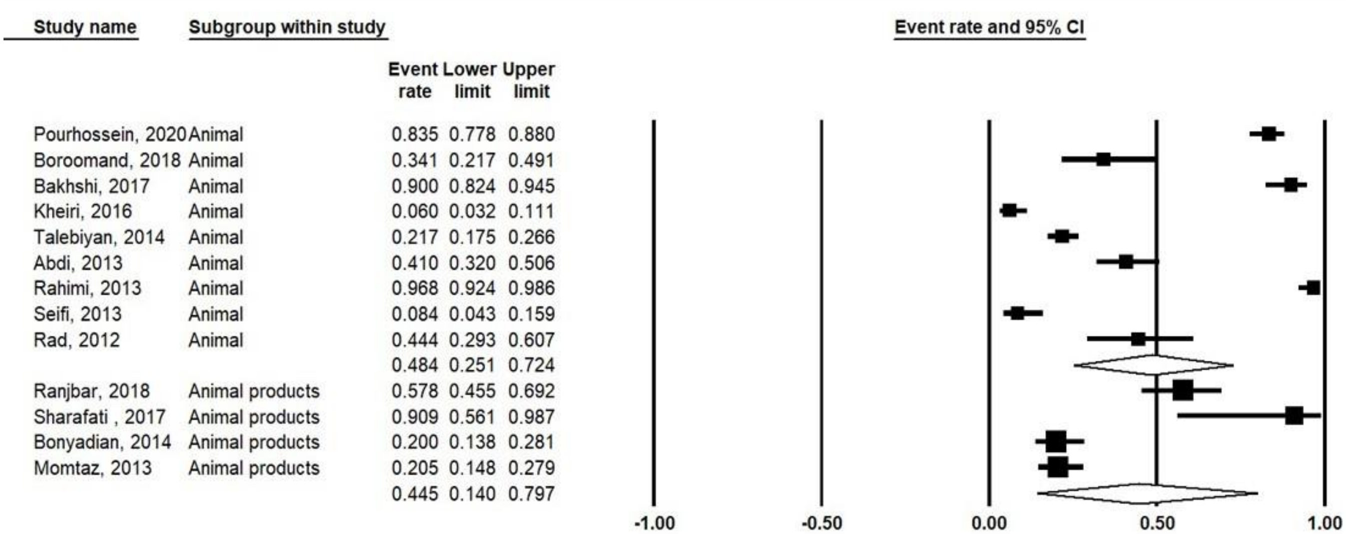

Fig 5. Subgroups analysis results of Ciprofloxacin resistant *E. coli* in animal and animal products.

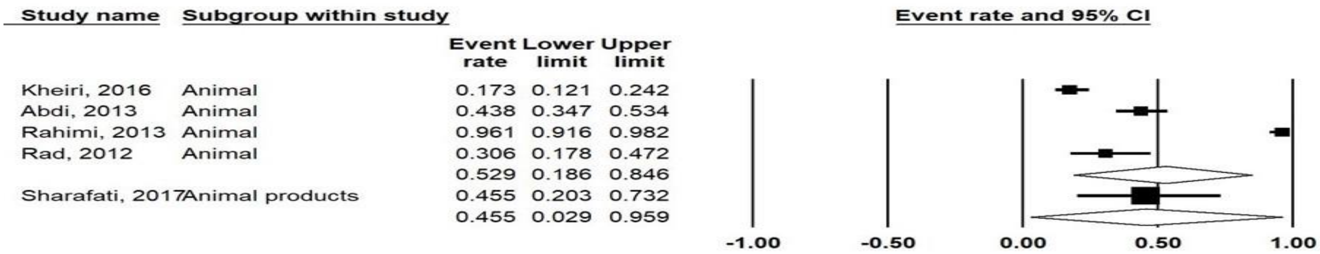

Fig 6. Subgroups analysis results of Norfloxacin resistant *E. coli* in animal and animal products.

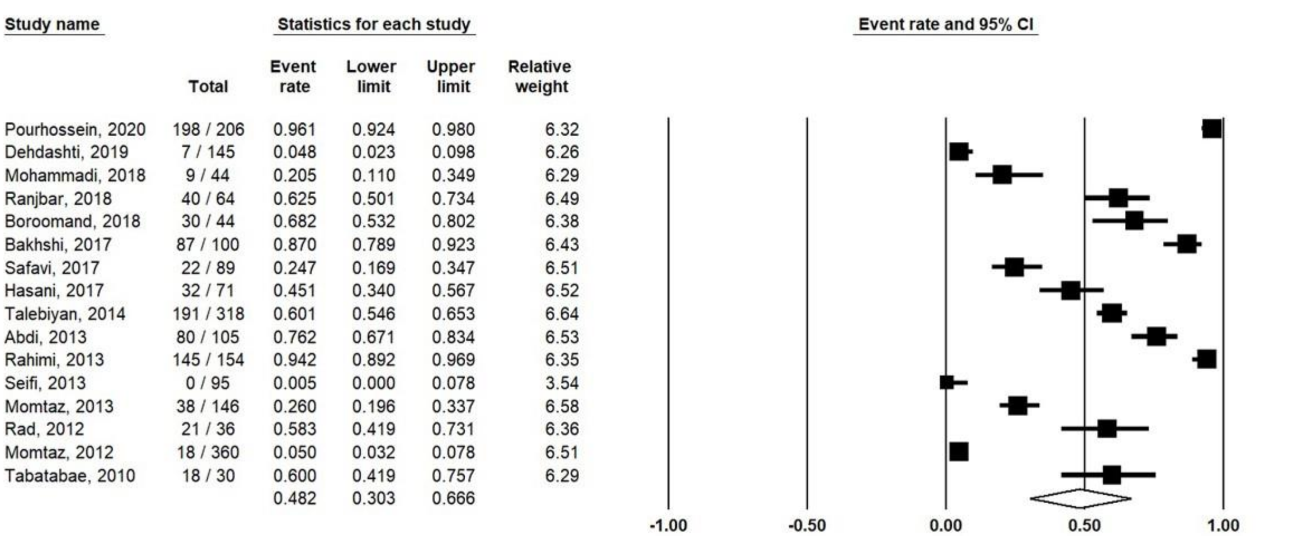

Fig 7. Pooled analysis results of Enrofloxacin resistant *E. coli*.

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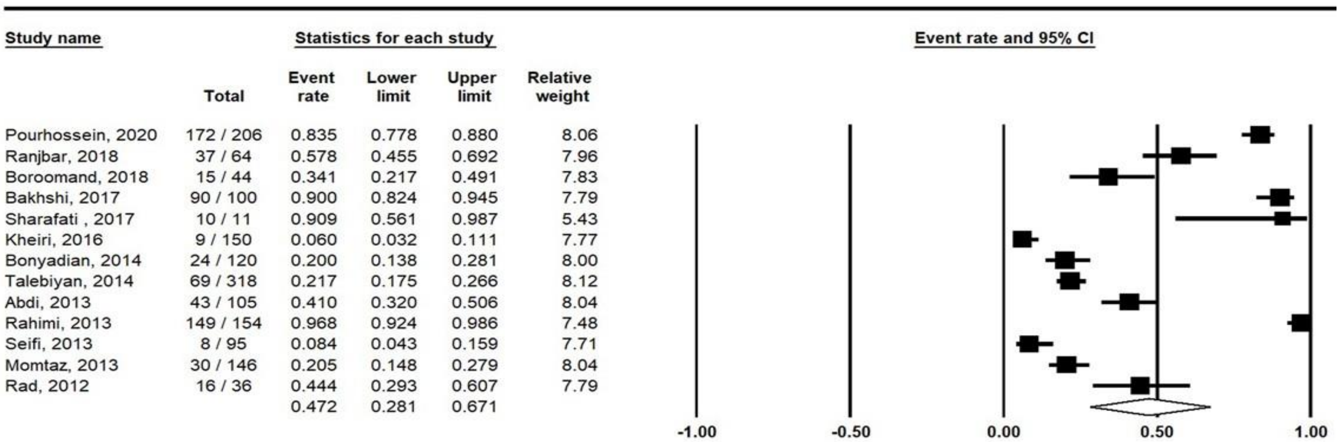

Fig 8. Pooled analysis results of Ciprofloxacin resistant *E. coli* .

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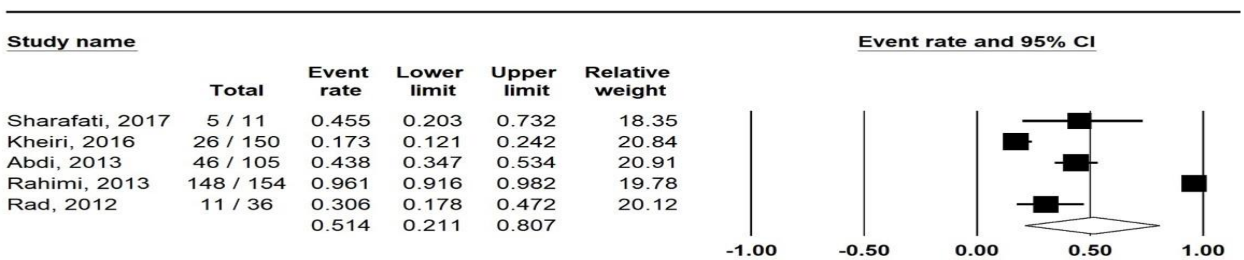

Fig 9. Pooled analysis results of Norfloxacin resistant *E. coli*.

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Table 2. The complete results of heterogeneity and publication bias examination.

Variable	Number of report/s	Pooled prevalence %	95% CI		Heterogeneity			Egger's regression	
			Lower limit %	Upper limit %	X^2	*p*-value	I^2	*P*-value	*t*-value
Enrofloxacin	16	48.2	30.3	66.6	521.521	<0.001	97.124	0.849	0.192
Danofloxacin	4	48.1	18.3	79.3	111.841	<0.001	97.318	0.894	0.150
Ciprofloxacin	13	47.2	28.1	67.1	419.489	<0.001	97.139	0.417	0.843
Flumequine	5	81.5	35.4	97.3	117.786	<0.001	97.604	0.785	0.296
Norfloxacin	5	51.4	21.1	80.7	105.928	<0.001	96.224	0.428	0.912

Antibiotic resistance in animal source

Subgroup analysis showed the rates of antibiotics resistance in animal strains were 75.1% (95% CI:68.2-80.8) for Flumequine, 55.2% (95% CI: 35.8%-73.2%) Enrofloxacin, 48.1% (95% CI: 18.3%-79.3%) Danofloxacin, 48.4% (95% CI: 25.1%-72.4%) Ciprofloxacin, and 52.9% (95% CI: 18.6%-84.6%) Norfloxacin (Figure 2-4).

Antibiotic resistance in animal products source

The rates of quinolone resistance among *E. coli* strains isolated from animal products were 45.5% (95% CI: 2.9%-95.9%) Norfloxacin, 44.5% (95% CI: 14%-79.7%) Ciprofloxacin and 23.8% (95% CI: 5.9%-60.9%) Enrofloxacin (Figure 5, 6).

Antibiotic resistance in both source

These articles evaluated five quinolone antibiotics, including Enrofloxacin, Danofloxacin, Ciprofloxacin, Flumequine, and Norfloxacin. From those studies in total, the highest resistance rates were estimated against Flumequine 81.5% (95% CI: 35.4%-97.3%), followed by Danofloxacin 48.1% (95% CI: 18.3%-79.3%), Enrofloxacin 48.2% (95% CI: 30.3%-66.6%), Norfloxacin 51.4% (95% CI: 21.1%-80.7%), and Ciprofloxacin 47.2% (95% CI: 28.1%-67.1%) (Figure 7-9).

Publication bias

As for sensitivity analysis and publication bias, the study exclusion did not affect the results. Funnel plots were used for visual bias, and all funnel plots were symmetric and didn't show any bias. The Egger test showed no considerable evidence of publication bias in the current meta-analysis (Table 2).

Discussion

Avian pathogenic *E. coli* strains that cause generalized infection may be resistant to antibiotics. They can transfer resistance genes to other bacteria in the human gut (35). Due to the administration of antibiotics in raising livestock and poultry, the bacteria that are naturally present in their bodies gradually become resistant to these antibiotics, and with human consumption of meat and animal products, these resistant strains enter the human body and can provide the basis for the development of resistance in the strains of the human body. (36, 37).

Thus, the fluoroquinolone-resistant *E. coli* bacteria in animals and animal products can transfer resistance to human strains. The current systematic review-based study assessed the pooled prevalence of fluoroquinolone-resistant *E. coli* in Iranian animals and animal products. It seems that the prevalence of resistance to different antibiotics varies greatly in different sources the main characteristics of comparing the outcomes of our review study in Iran with other countries enrolled in the study are summarized in Table 2. The highest prevalence of Ciprofloxacin resistance in *E. coli* was 75% in animal strains of Nigeria and 60.9% in animal product strains of Great Britain, while it has been found at 42.7% in animal strains and 29.5% in animal product strains in our research. The highest prevalence of Enrofloxacin resistance in *E. coli* was 86.27% in animal strains of Algeria, while it has been found at 60.6% in animal strains and 23.2% in animal product strains in our investigation. The highest prevalence of Flumequine resistance in *E. coli* was 91.5% in animal strains, while it has been found at 75.1% in animal strains in our research. The prevalence of Norfloxacin resistance in *E. coli* was 22.8% in animal strains of Japan, while it has been found at 38.8% in animal strains and 45.5% in animal product strains in our investigation. The prevalence of Danofloxacin resistance in *E. coli* was 23.6% in animal strains of Japan, while it has been found at 56% in animal strains in our study.

Impressively high variability of PMQR gene variants found on five distinct plasmid types (IncHI2, ColE, IncI1, IncN, and IncX2) indicates broad circulation of resistance determinants among strains. Therefore, the spread of these factors determining resistance in food-producing animals can be the origin of the observed resistance prevalence (38-40). The results strongly imply that transmission of resistant clones and resistance plasmids of *E. coli* from poultry to humans occurs commonly (41, 42). Also, a range of silent mutations and two major mutations in *gyrA* were identified in resistant isolates (43).

Conclusion

The current study is the first systematic review and meta-analysis of antibiotic-resistant (ABR) in animals and animal products in Iran. ABR has received considerable attention and is a crucial hazard to health and food security in Iran. This study brought interesting findings on ABR that can be beneficial in devising measures to hinder this farm-to-plate threat in Iran. We therefore strongly suggest following the One Health approach and guidance advocated by the WHO, OIE, and FAO to limit the administration of antibiotics and consequently curb the spread of ABR and to help preserve the health of animals and humans.

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Ethics approval and consent to participate

I hereby declare all ethical standards have been respected in the preparation of the submitted article.

Conflict of interest

The authors declare that they have no conflicts of interest.

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