Prevalence and Antibiotic Resistance of Clostridioides (*Clostridium difficile*) In Meat and Meat Products: A Systematic Review and Meta-Analysis

Zahra Esfandiari [1], Parisa Shoaei [2], Bahareh Vakili [3], Ziba Farajzadegan[4], Mohammad Javad Tarrahi [5], Zahra Emami [6], *Yadolah Fakhri [7], J. Scott Weese [8], Trias Mahmudiono [9]

1. Nutrition and Food Security Research Center, Department of Food Science and Technology, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran
2. Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
3. Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
4. Community and Preventive Medicine Department, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
5. Epidemiology Department of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran
6. Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
7. Food Health Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
8. Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada
9. Department of Nutrition, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia

*Corresponding Author: Email: Ya.fakhri@gmail.com

Abstract

Background: Meat and meat products are introduced as one of the frequent sources of *Clostridioides difficile*. We aimed to determine the prevalence and antibiotic resistance of *C. difficile* isolates in meat and meat products using a systematic review and meta-analysis.

Methods: A literature search was performed in the primary international and bibliographic databases such as MEDLINE (PubMed), Cochrane Library, Embase, Scopus, and Web of Science to achieve all articles related to the prevalence and antibiotic resistance rates from 2007 to 2022.

Results: The 278 retrieved articles were reduced to 54 worldwide eligible studies after screening and matching inclusion/ exclusion criteria. *C. difficile* was examined in different types of samples and its resistance to 10 antibiotics. The pooled prevalence of *C. difficile* was 3.4% in all samples. *C. difficile* pooled prevalence was detected in fish, poultry, and red meat groups with 6.9%, 5.2%, and 3.2%, respectively. Regarding antibiotic resistance, the highest pooled prevalence was for ciprofloxacin (86.6%), followed by clindamycin (42.6%) and erythromycin (34%). The lowest pooled prevalence was observed in metronidazole (7.6%), vancomycin (6.6%), and chloramphenicol (6%).

Conclusion: Low resistance was found to commonly used drugs for *C. difficile* infection (CDI) treatment. Since each antibiotic can be predisposing cause for CDI development, this finding possibly will be warning from a One Health viewpoint about the misuse of antibiotics in the chain of farm to fork including agriculture, animal husbandry and the food industry and also their injudicious use in medicine.

Keywords: Clostridioides difficile; Meat; Antibiotic resistance; Systematic review; Meta-analysis

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Introduction

Clostridium difficile was first identified in a study of gut microbiota of babies about 88 years ago (1). This bacterium is a clinically significant Gram-positive, toxin-producing, spore-forming, anaerobic bacillus with a relatively large size (about 3-5 µm in length) (2). *C. difficile* is slow-growing compared to other bacteria. Its name comes from its characteristics and stability, making its isolation difficult in the laboratory. In a recent reclassification, it was named *Clostridioides difficile* (2).

The most critical virulent factors of this bacterium are two strong toxins, namely toxin A and B, with glucosyltransferase properties. Both toxins cause mucosal damage and neutrophil infiltration into the large bowel lumen. Through a monoglycosylated reaction, these toxins destroy intercellular junctions in the human body and cause diarrhea and *C. difficile* infection (CDI) (3). Another toxin, known as binary toxin, is secreted by *C. difficile*, with a stronger ability to cause CDI. This toxin generally leads to actin depolymerization in the structural part of the cell and allows the bacterium to adhere and colonize. The binary toxin (ADP ribosyl transferase) consists of two proteins encoded by *cdtA* (catalytic subunit) and *cdtB* (binding subunit) genes (4). So far, toxin A, and B have been isolated from newly found strains of *C. difficile*; besides, these genes are associated with a more severe infection. Overall, the binary toxin plays a vital role in the morphological changes of intestinal epithelial cells, leading to increased bacterial adhesion to the intestine (5).

The literature has proposed the infection and transmission of *C. difficile* among animals, humans, and the environment through secondary and cross-secondary contamination (5). In addition, various studies have found this bacterium in food (4, 6). Consequently, concerns have been raised regarding the epidemiology of CDI in the community due to possible transmission to humans from sources other than hospitals (5). Intensified CDI in the community can be due to the appearance of therapeutic strains with the same molecular structure in different sources, such as food products of animal origin (6). Accordingly, some studies have hypothesized that *C. difficile* is zoonotic or food-borne (5).

The resistance of microorganisms to antibiotics is a primary global concern and a health threat to the WHO (7). Epidemiological studies indicate the high prevalence of *C. difficile* resistance to common antibiotics. Almost all antibiotics can cause CDI (5, 8). Common antibiotics, such as lincomycin and clindamycin, can cause diarrhea in approximately 10% of patients and pseudomembranous colitis in 1% (9).

The characteristics of *C. difficile* in meat and its products have been widely studied worldwide, but the results remain inconsistent (6). Such information is crucial for risk assessment and improving the evidence of *C. difficile* as a cause of infection in the community through meat consumption. Accordingly, the current systematic review and meta-analysis study summarizes the epidemiological characteristics of *C. difficile* isolated from meat and meat products, including its prevalence and antibiotic resistance rate.

Methods

Search Strategy

The inclusion criteria of studies were outlined according to PRISMA guidelines (Fig. 1) (10). We performed a literature search in the primary international and bibliographic databases, including MEDLINE (PubMed), Cochrane Library, Embase, Scopus, and Web of Science to achieve all articles reporting the prevalence and antibiotic resistance rates with no time restriction. The keywords were "food" OR "meat" OR "meat products" AND "prevalence" OR "occurrence" OR "incidence" AND "*Clostridium difficile*" OR "*Clostridioides difficile*" OR "toxigenic" OR "antibiotic resistance". These terms were searched in articles' titles, abstracts, or keywords.
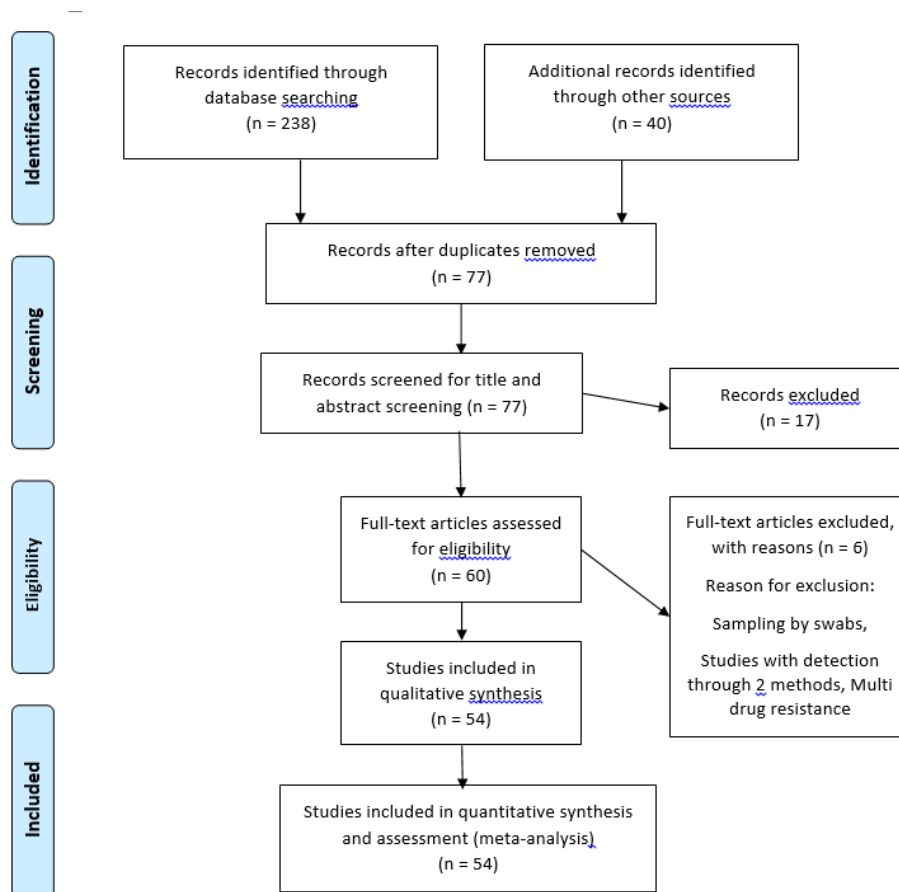
Fig. 1: PRISMA flow chart detailing review process

The articles found during the initial search were downloaded, followed by forward citation tracking in Scopus to retrieve additional papers. The articles were merged, and duplicates were deleted. The articles were primarily screened by abstract and title to remove irrelevant studies. We included full-text studies (in-press or published) and review papers and excluded conference proceedings and chapter books. The potentially eligible data of the selected articles were retrieved and classified based on *C. difficile* prevalence in meat and meat products (fish, poultry, and red meat). The studies included in the current paper were meant to report the antibiotic resistance or prevalence rates of *C. difficile* in meat and meat products at the slaughterhouse or retail stage.

Inclusion/exclusion criteria

The inclusion criteria included 1) full-text English articles, 2) observational studies (cross-sectional or descriptive), 3) reporting laboratory confirmation, 4) providing data on positive and total sample sizes, 5) presenting the toxigenic isolates of *C. difficile*, and 6) antibiotic resistance of positive isolates.

The exclusion criteria were 1) studies on the prevalence of *C. difficile* in meat and meat products without reporting the prevalence rate or antibiotic type and the frequency rate of resistance or susceptibility, 2) studies on development and improvement of methods for detecting *C. difficile* and antibiotic resistance, 3) restrictions applied in the selection of studies in terms of report for the type of antibiotics in less than 3 articles 4) the studies showing multidrug resistance isolates and 5) non-original studies such as different kinds of reviews.

No restriction was applied in the selection of resistance definition breakpoints (EUCAST or CLSI) due to variation in these criteria.

Data extraction

The required data included was shown in Table 1 from 2007 to 2022. The prevalence rate in meat and meat products was based on culture and phenotypic tests (cycloserine cefotaxime fructose/mannitol taurocholate/lysozyme agar, *C. difficile* moxalactam norfloxacin broth, and Columbia agar) and toxin analysis (Polymerase Chain Reaction (PCR) or Remel Xpect CD toxin A/B test or ELFA-VIDAS-CDAB Kit or Vero tissue culture or MLVA). Several methods were used to determine the antimicrobial resistance, which can be performed by the standard disc diffusion test or by determining the minimum inhibitory concentration (MIC) [The e-test, agar dilution, broth microdilution, and evaluator strips]. The frequency of antibiotic resistance was defined as resistance to one drug in antimicrobial classes. The search and screening were performed by three reviewers (Z. E, P. S, and B. V). Any dissimilarities or discrepancies were resolved by consultation with a fourth investigator (Z. F, M.J.T, Z.E, J.S.W, Y.F, and T.M). The researchers made communications if data were unavailable or unclear in an article.

Quality Assessment of articles

The articles' quality was assessed by a checklist prepared by the Joanna Briggs Institute (JBI) (11). It assesses different methodological structures of studies, such as the study population's representativeness, statistical analysis, and study setting (Data not shown).

Statistical analysis

Fixed and random-effects models were done to determine the pooled prevalence of *C. difficile* in various meat and meat products, considering a 95% Confidence Interval (CI). The effect size measure was considered as the prevalence rate. The forest plots showed the distribution of the individual effect sizes and pooled effect sizes. Differences in the trial-level prevalence ratios were assessed using Q-statistics following a Chi-square distribution with a (k-1) degree of freedom. The proportion of the total variation explained by heterogeneity, rather than sampling error, was calculated with I^2 (inverse variance index). The I^2 values smaller than 25% were regarded as low heterogeneity, 25-50% moderate heterogeneity, and more than 50% high heterogeneity (12). The random-effects model was considered valid after obtaining substantial heterogeneity based on Q-statistics and I^2 values; otherwise, we employed the fixed-effects model.

Funnel plots evaluated publication bias. Asymmetrical scattering of effect sizes related to articles and standard errors were considered the signs of publication bias (13). Funnel plots were reported when more than three articles were included in the meta-analysis. The analyses were performed by Comprehensive Meta-Analysis (Biostat, Englewood, NJ, USA). $P < 0.05$ and funnel plot asymmetry suggested significant publication biases in the meta-analysis.

Results

Data on the prevalence rates were gathered from the studies using molecular and cultural methods. When both methodologies were used, the overall prevalence rate was collected and applied for the meta-analysis in each product, including red and white meat, such as fish, poultry, and red meat. Table 1 indicates the full results of the included papers. Different types of meat samples and 10 out of 38 antibiotics were considered for the meta-analysis. Most studies (n=23) were performed in Asia, whereas the others were performed in the USA (n=17), Europe (n=12), and Africa (n=2).

The prevalence of *C. difficile* was assessed in all meat samples from 54 studies (Fig. 2), and the pooled prevalence was 3.4% (0.034; 95% CI: 0.024-0.048). A significant heterogeneity was detected among studies ($I^2 = 91.83\%$; $tau^2 = 1.301$; P value < 0.001).

Table 1: Main characteristics of *C. difficile* in meat and meat products in the included studies

Reference	Food Groups	Country	Sample Size	Positive samples in culture	Positive Toxigenic samples
(23)	Fish	Cambodia	25	4	3
(24)	Red Meat	Saudi Arabia	100	4	4
(25)	Red Meat	Italy	216	2	1
(26)	Poultry	Saudi Arabia	250	11	ND[1]
(6)	Poultry	Germany	364	51	43
(27)	Poultry and Red	Brazil	192	17	0 [2]
(28)	Fish	Iran	184	11	11
(29)	Poultry and Red	Japan	468	10	3
(30)	Red Meat	Turkey	319	22	22
(31)	Poultry	Turkey	185	69	59
(32)	Fish	Italy	702	113	75
(33)	Poultry	Iran	30	2	ND
(34)	Fish	Iran	820	26	26
(35)	Poultry	Egypt	150	ND	ND
(36)	Red Meat	Korea	415	45	2
(37)	Poultry and Red	Turkey	101	2	ND
(38)	Red Meat	Saudi Arabia	600	9	9
(39)	Red Meat	Brazil	80	ND	ND
(40)	Red Meat	Turkey	100	5	3
(41)	Red Meat	Iran	100	30	9
(42)	Poultry	Iran	65	10	8
(43)	Poultry and Red	Pennsylvania	300	2	ND
(21)	Fish	Italy	925	36	19
(44)	Red Meat	Iran	570	6	5
(45)	Poultry	Turkey	310	25	13
(46)	Red Meat	Iran	300	79	17
(4)	Red Meat	Iran	110	7	7
(47)	Red Meat	Iran	200	8	8
(48)	Red Meat	Belgium	240	8	7
(49)	Red Meat	Iran	660	13	12
(50)	Red Meat	Cote d ^ 'Ivoire	395	49	Not Examined
(51)	Poultry and Red	Pennsylvania	303	31	25
(52)	fish	Texas	67	3	0
(53)	Poultry and Red	Costa Rica	200	4	4
(54)	Red Meat	USA	956	0	0
(55)	Poultry	Iran	120	19	Not Examined
(56)	Poultry	Iran	240	25	Not Examined
(57)	Red Meat	USA	102	13	13
(58)	Poultry and Red	USA	1755	0	Not Examined
(59)	Red Meat	Manitoba, Can-	48	3	3
(60)	Red Meat	Pennsylvania	50	4	3
(61)	fish	Italy	53	26	15
(62)	fish	Italy	6	4	2
(63)	fish	Canada	119	5	4
(64)	Poultry and Red	Netherlands	500	8	5
(65)	Red Meat	Canada	393	7	6
(66)	Red Meat	Switzerland	46	0	ND
(67)	Red Meat	France	176	2	2
(68)	Poultry	Canada	203	26	26
(69)	Red Meat	Canada	230	28	28
(70)	Poultry and Red	Austria	84	0	0
(71)	Poultry and Red	USA	88	37	37
(72)	Poultry and Red	Sweden	82	2	2
(73)	Red Meat	Canada	60	12	11

[1] Not Detected

[2] The 80 observed colonies in plate from 17 positive samples were reported for the presence of toxin-encoding genes. Therefore, no exact data existed in this study about the total toxigenic isolate in 17 positive samples.

A funnel plot was created for the outcome to analyze the publication bias, following Duval and Tweedie's trim and fill approach with Begg and Mazumdar rank correlation. Publication bias using

funnel plots was not observed in the current study. In the sensitivity analysis, the pooled prevalence of *C. difficile* in meat showed no substantial change if one study or a few studies were omitted, showing that the obtained results are robust. A subgroup analysis was conducted according to the type of meat (fish, poultry, and meat) and their products (Table 2 and Fig. 2). The highest pooled prevalence of *C. difficile* was found in fish (6.9%), followed by poultry (5.2%) and red meat (3.2%), and while the lowest prevalence was observed in the article assessing both poultry and red meat (1.6%) groups.
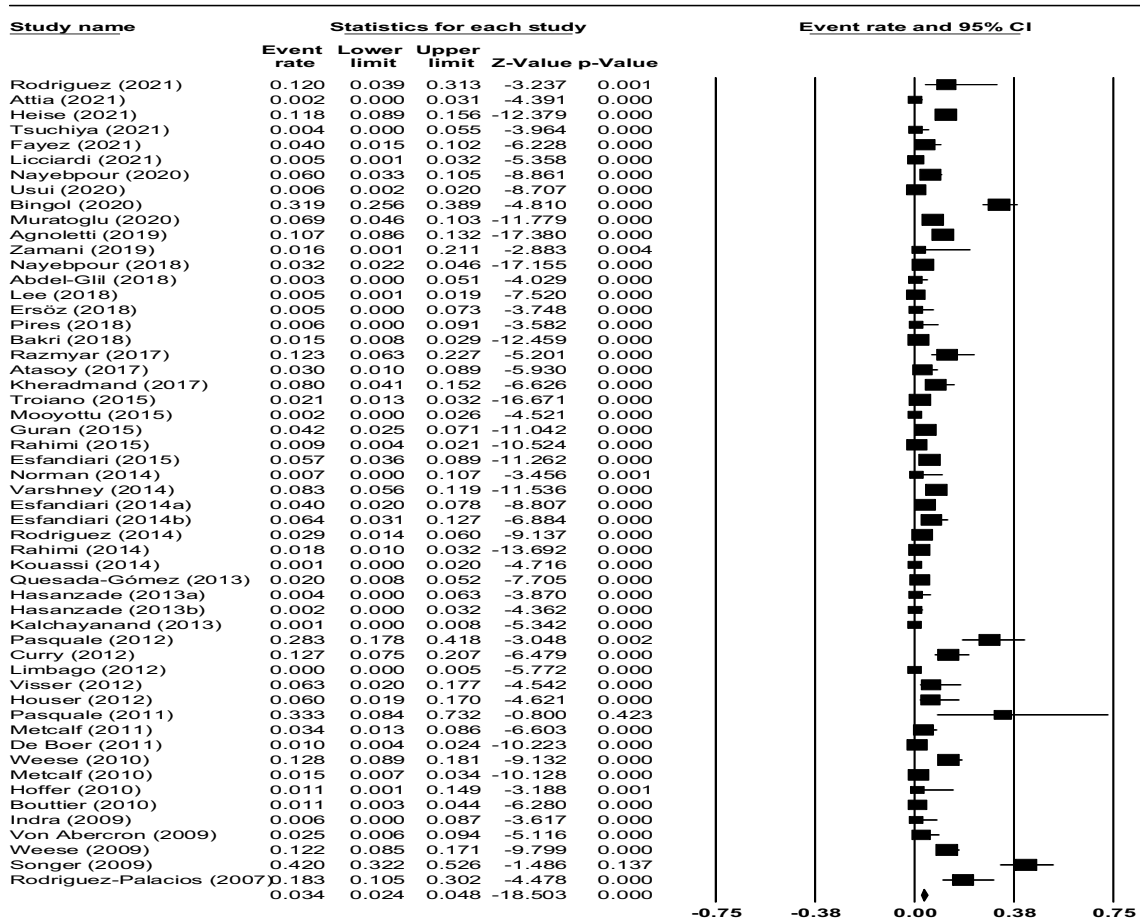
Study name	Event rate	Lower limit	Upper limit	Z-Value	p-Value
Rodriguez (2021)	0.120	0.039	0.313	-3.237	0.001
Attia (2021)	0.002	0.000	0.031	-4.391	0.000
Heise (2021)	0.118	0.089	0.156	-12.379	0.000
Tsuchiya (2021)	0.004	0.000	0.055	-3.964	0.000
Fayez (2021)	0.040	0.015	0.102	-6.228	0.000
Licciardi (2021)	0.005	0.001	0.032	-5.358	0.000
Nayebpour (2020)	0.060	0.033	0.105	-8.861	0.000
Usui (2020)	0.006	0.002	0.020	-8.707	0.000
Bingol (2020)	0.319	0.256	0.389	-4.810	0.000
Muratoglu (2020)	0.069	0.046	0.103	-11.779	0.000
Agnoletti (2019)	0.107	0.086	0.132	-17.380	0.000
Zamani (2019)	0.016	0.001	0.211	-2.883	0.004
Nayebpour (2018)	0.032	0.022	0.046	-17.155	0.000
Abdel-Glil (2018)	0.003	0.000	0.051	-4.029	0.000
Lee (2018)	0.005	0.001	0.019	-7.520	0.000
Ersöz (2018)	0.005	0.000	0.073	-3.748	0.000
Pires (2018)	0.006	0.000	0.091	-3.582	0.000
Bakri (2018)	0.015	0.008	0.029	-12.459	0.000
Razmyar (2017)	0.123	0.063	0.227	-5.201	0.000
Atasoy (2017)	0.030	0.010	0.089	-5.930	0.000
Kheradmand (2017)	0.080	0.041	0.152	-6.626	0.000
Troiano (2015)	0.021	0.013	0.032	-16.671	0.000
Mooyottu (2015)	0.002	0.000	0.026	-4.521	0.000
Guran (2015)	0.042	0.025	0.071	-11.042	0.000
Rahimi (2015)	0.009	0.004	0.021	-10.524	0.000
Esfandiari (2015)	0.057	0.036	0.089	-11.262	0.000
Norman (2014)	0.007	0.000	0.107	-3.456	0.001
Varshney (2014)	0.083	0.056	0.119	-11.536	0.000
Esfandiari (2014a)	0.040	0.020	0.078	-8.807	0.000
Esfandiari (2014b)	0.064	0.031	0.127	-6.884	0.000
Rodriguez (2014)	0.029	0.014	0.060	-9.137	0.000
Rahimi (2014)	0.018	0.010	0.032	-13.692	0.000
Kouassi (2014)	0.001	0.000	0.020	-4.716	0.000
Quesada-Gómez (2013)	0.020	0.008	0.052	-7.705	0.000
Hasanzade (2013a)	0.004	0.000	0.063	-3.870	0.000
Hasanzade (2013b)	0.002	0.000	0.032	-4.362	0.000
Kalchayanand (2013)	0.001	0.000	0.008	-5.342	0.000
Pasquale (2012)	0.283	0.178	0.418	-3.048	0.002
Curry (2012)	0.127	0.075	0.207	-6.479	0.000
Limbago (2012)	0.000	0.000	0.005	-5.772	0.000
Visser (2012)	0.063	0.020	0.177	-4.542	0.000
Houser (2012)	0.060	0.019	0.170	-4.621	0.000
Pasquale (2011)	0.333	0.084	0.732	-0.800	0.423
Metcalf (2011)	0.034	0.013	0.086	-6.603	0.000
De Boer (2011)	0.010	0.004	0.024	-10.223	0.000
Weese (2010)	0.128	0.089	0.181	-9.132	0.000
Metcalf (2010)	0.015	0.007	0.034	-10.128	0.000
Hoffer (2010)	0.011	0.001	0.149	-3.188	0.001
Bouttier (2010)	0.011	0.003	0.044	-6.280	0.000
Indra (2009)	0.006	0.000	0.087	-3.617	0.000
Von Abercron (2009)	0.025	0.006	0.094	-5.116	0.000
Weese (2009)	0.122	0.085	0.171	-9.799	0.000
Songer (2009)	0.420	0.322	0.526	-1.486	0.137
Rodriguez-Palacios (2007)	0.183	0.105	0.302	-4.478	0.000
	0.034	0.024	0.048	-18.503	0.000

Fig. 2: Forest plot of meta-analysis of *C. difficile* prevalence in meat and meat products

Table 2: Meta-analysis of *C. difficile* prevalence based on subgroups

Type of meat	n	%Pooled Prevalence	95% Confidence Interval	Q	I²	Tau²	P-value
Fish	9	0.069	0.035-0.133	102.679	92.209	0.952	<0.001
Poultry	10	0.052	0.025-0.108	103.879	91.336	.301	<0.001
Red meat	22	0.032	0.021-0.05	121.157	82.667	0.771	<0.001
Poultry and Red Meat	13	0.016	0.005-0.046	228.165	94.741	3.420	<0.001

Table 3 shows the meta-analysis results of antimicrobial resistance in *C. difficile*. Based on the pooled prevalence of antimicrobial resistance, the most resistance was to ciprofloxacin (86.8%), whereas the less resistant was to chloramphenicol (6%). Resistance to vancomycin and metronidazole was assessed in 559 positive isolates of *C. difficile*.

Clindamycin, moxifloxacin, erythromycin, tetracycline, and ampicillin resistance were examined in 554, 363, 293, 214, and 210 isolates of *C. difficile*. A limited number of positive isolates of *C. difficile* were assessed for their resistance to rifampicin and ciprofloxacin (n= 171 and 113, respectively).

Table 3: Meta-analysis of antimicrobial resistance in *C. difficile*

Antibiotic	Number of studies	% Pooled Prevalence (95% Confidence Interval)	Q	I²	Tau²	P-value
Ciprofloxacin	6	86.8 (0.547-0.973)	20.448	75.595	3.175	0.029[a]
Clindamycin	26	42.6 (0.278-0.587)	131.521	80.992	1.795	0.369[a]
Erythromycin	10	34 (0.176-0.553)	41.203	78.157	1.190	0.137[a]
Ampicilline	9	33.9 (0.147-0.605)	50.075	84.024	2.142	0.231[a]
Moxifloxacin	18	26.3 (0.138-0.441)	69.49	75.536	1.721	0.011[a]
Tetracycline	13	25.9 (0.119-0.475)	40.351	70.261	1.982	0.03[a]
Rifampicin	4	8.1 (0.046-0.14)	2.61	0	0	0[b]
Metronidazole	26	7.6 (0.039-0.144)	64.029	60.995	1.721	0[a]
Vancomycin	27	6.6 (0.034-0.125)	55.997	53.569	1.680	0[a]
Chloramphenicol	5	6 (0.025-0.137)	0.449	0	0	0.987[b]

[a] *P*-value for random-effects model
[b] *P*-value for fixed-effect model

Discussion

C. difficile is a significant cause of disease in humans, especially in hospitalized people. Human and animal exposure to antibiotics is a significant risk factor for causing CDI (14). On the other hand, three main factors have raised concerns regarding its potential as a cause of the food-borne disease: A rising detection of community-associated CDI, identifying *C. difficile* in animals and food, and similarities in *C. difficile* collected from food, humans, and animals (5). This is the first meta-analysis study with a worldwide perspective that evaluates the prevalence and antibiotic resistance rates of *C. difficile* in meat and meat products.

The most common contamination occurred in fish and seafood (6.9%) (Table 2). This result is consistent with the only meta-analysis study, reporting that seafood poses the highest risk for the presence of this bacterium (14). Undesirable human activities in aquatic environments have caused problems for the health of humans and fish. Because of sewage discharge and water pollution in coastal areas and rivers, the marine environments are exponentially contaminated with various microorganisms, especially of fecal origins, such as *C. difficile* (15).

Poultry and its products also had *C. difficile* prevalence of 5.2% in the current meta-analysis study (Table 2). Besides the high nutritional value of poultry and birds, they are of worldwide interest due to their affordable prices and availability. However, enteric disorders caused by clostridial diseases have had devastating effects on the poultry industry, resulting in high poultry mortality. Therefore, *C. difficile* spores should be controlled in critical points, including poultry breeding on farms, preparation in the slaughterhouse's production lines, distributors, transportation systems, storage places, supermarket suppliers, and consumers (6).

In this study, the overall pooled prevalence of *C. difficile* was 3.2% in meat samples (Table 2).

In the included studies, red meat and derived products were from domestic animal meat, including camel, cow, goat, pork, buffalo, veal, and other products such as ready-to-eat foods, doner, meatballs, salami, and sausages, making the interpretation difficult. In current study, the prevalence was not very high in meat and its products. Nonetheless, most ready-to-eat foods containing meat and meat products are consumed immediately after purchase, without pre-heating or cooking. Such foodstuffs make public health concerns, especially for children, vulnerable elderly, immunocompromised individuals, and pregnant women. Additionally, the higher resistance of spores than vegetative forms of *C. difficile* makes it difficult to destroy the bacterium (16).

Regarding red and poultry meat, the pooled prevalence of *C. difficile* was 1.6% in 13 studies (Table 2). Animal-based foods are a significant source of zoonotic pathogenic bacteria, and foods derived from these animals can be a significant transmission source of bacteria to humans. In this regard, meat is a primary source of human exposure to zoonotic bacteria. About 60% of animal-related diseases in humans and approximately 75% of new human infections are transmitted to humans through vertebrates (17). Although no reports have confirmed that *C. difficile* is food-borne or zoonotic, it is necessary to observe food health and safety protocols and establish food safety management systems such as Good Manufacturing Principles (GMP), Good Hygiene Principles (GHP) and Hazard Analysis and Critical Control Point (HACCP) system (4, 16).

Antibiotic therapy is the most serious factor for CDI as it upsets the balance of the natural flora of the gastrointestinal tract (18). The most important antibiotics that has this function include clindamycin, cephalosporins, fluoroquinolones, ampicillin/amoxicillin, macrolides, cotrimoxazole and tetracyclines. The emergence of antibiotic has made the treatment of CDI more problematic. Antibiotic resistance is a universal menace to the health of animals and humans, and a global effort is needed to combat its spread (18). Because antimicrobials are used to treat CDI, data should be obtained on the resistance profiles of circulating *C. difficile*

strains in sources other than hospitals, such as food (19).

Based on the current analysis, ciprofloxacin (86.6%), clindamycin (42.6%), and erythromycin (34%) showed the highest pooled prevalence for antibiotic resistance of *C. difficile* collected from meat and meat products (Table 3). The aforementioned antibiotics are categorized in fluoroquinolone, lincosamide, and macrolide classes, respectively, which carry the highest risk for *C. difficile* resistance (20). In some reports, the emergence of ribotype 027 is related to its fluoroquinolone-resistant sub-lineages. This situation elucidates the evolutionary potential of *C. difficile* in response to the environment. It seems that there is a potential role of genetic recombination among animal and human-originated isolate of *C. difficile* in the emergence of new drug-resistance lineages (5).

The use of antibiotics is limited in antibiotic stewardship interventions to reduce multidrug resistance (19). Physicians and vets need to be trained about the risk of CDI associated with these antibiotics' prescriptions. On the other hand, antimicrobial agents such as vancomycin and oral fidaxomicin are recommended as the drugs of the first choice in clinical trials of CDI treatment. Metronidazole is no longer recommended as first-line therapy for adults. The meta-analysis of 26 and 27 studies showed that antibiotic resistance for vancomycin and metronidazole was 7.6% and 6.6%, respectively (18). As resistance to antibiotics commonly used to treat CDI in humans can make it challenging to treat, the widespread application of these antibiotics in humans and even the low resistance of *C. difficile* should be noticed. As fidaxomicin was reported in one study, it was impossible to discuss it in the meta-analysis (21). A systematic review and meta-analysis of antibiotic resistance in *C. difficile* isolates from patients, healthy humans, and animals was 2.1% (95% CI: 0–5.1%) for vancomycin and 1.9% (95% CI: 0.5–3.6%) for metronidazole (22). The current study showed higher pooled resistance for vancomycin [6.6% (95% CI: 0.034-0.125)] and metronidazole [7.6% (95% CI: 0.039-0.144)]. The difference may be related to patient-originated and animal originated

isolates of *C. difficile*. As all antibiotics are the influencing risk factor for CDI through gut microbiota alteration (20), we cannot overlook the resistance to ampicillin (33.9%), moxifloxacin (26.3%), tetracycline (25.9%), rifampicin (8.1%), and chloramphenicol (6%) among *C. difficile* isolates in meat and meat products (19). Some of these antibiotics are highly applied as antimicrobials in agriculture, resulting in antimicrobial selective pressure in this sphere. For antibiotic susceptibility testing, it has proposed agar dilution as the standard gold methods. It was indicated that e-test method could underestimate the MIC for antibiotics (19). This situation can misclassify some isolates as susceptible. Additionally, very different breakpoints for antibiotics are recommended by EUCAST and CLSI. Therefore, heterogeneity between the studies for culture method and antibiotic susceptibility testing implies that the summary occurrence should be interpreted with caution. This situation is as a limitation of the current study.

As another limitation of this study, the scarcity or lack of representative studies in several countries may cause a misconception about the actual prevalence and antibiotic resistance of *C. difficile*. Additionally, due to the limited number of meta-analysis studies of *C. difficile* in food, it was not possible to discuss some findings.

Conclusion

Different types of meat and derived products may act as reservoirs of *C. difficile* spores, and its capability to survive, persist, and make cross-contamination can be influential in its spread. Considering the epidemiological complications in the transmission cycle of *C. difficile* spores among various carriers, it is crucial to take preventive actions, like reinforced hygiene measures in food processing plants or even at retail, improved hygiene measures by farmers, and controlling actions to decrease cross-contamination. Inadequate investigations have impeded a comprehensive estimation globally; nonetheless, a pooled prevalence could be obtained for different types of meat and their products. The frequencies of these foodstuffs indicated a low risk for consumer health.

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.

Conflict of Interest

The authors declare that there is no conflict of interests.

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