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Presence of *Clostridioides difficile* in poultry meat and meat products

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ABSTRACT

Clostridioides difficile, a Gram-positive spore-forming bacterium, has emerged as a significant cause of healthcare-associated infections (HAIs) on a global scale. While initial investigations predominantly linked *C. difficile* transmission to hospital settings, recent reports indicate a worrisome increase in community-acquired *C. difficile* infections (CDIs), irrespective of factors such as prior hospitalization or age. The CDC's 2021 Annual Report for *Clostridioides difficile* infection underscores this shift, revealing a slightly higher prevalence of CDIs in the community (55.9 cases/100.000 people) compared to healthcare settings (54.3 cases/100.000 people). These statistics highlight the substantial role of non-hospital sources in CDI transmission. Ongoing studies posits zoonotic pathways, particularly the consumption of contaminated food, as pivotal in community-acquired CDI transmission. Research findings indicate the detection of *C. difficile* in both raw and heat-treated meat, as well as meat products, raising significant concerns. Present investigations emphasize a noteworthy potential for the transmission of *C. difficile* to humans through the consumption of poultry meat. Although no traces of this bacterium have been identified in heat-treated poultry meat and products thus far, the risk of latent transmission through cooked poultry products should not be dismissed. Despite the absence of identified cases in processed poultry meat, the plausible transmission of *C. difficile* through these products underscores the exigency for further investigation in this field. This review provides an in-depth screening of studies on *C. difficile* contamination in poultry meat and its products worldwide. It also summarizes the risk factors associated with *C. difficile* infection through poultry meat consumption and outlines preventive measures to mitigate this risk.

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Introduction

Clostridioides difficile (previously known as *Clostridium difficile*) is a significant spore-forming enteropathogen that is associated with serious gastrointestinal disorders all over the world (Cohen et al., 2010). It is the primary agent responsible for nosocomial diarrhea and pseudomembranous colitis in individuals who have been subjected to antimicrobial treatment in the year 1978, *Clostridium difficile* infection (CDI) has been acknowledged as a hospital-acquired affliction (George et al., 1978; Hampikyan et al., 2018).

Initially, CDI was associated with hospitalized patients treated with antibiotics that are effective against a wide variety of bacteria. It has been held responsible for 20-30% of diarrhea cases caused by antimicrobial drugs (McFarland, 2007) and has been defined as a dangerous disease that can result in

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pseudomembranous colitis, toxic megacolon and even death. A notable shift in the epidemiology of CDI was observed during the initial years of the 21st century. The hyper-virulent fluoroquinolone-resistant strain of *C. difficile*, known as NAP1/BI/027, initially emerged in North America (He et al., 2013). Subsequently, there emerged accounts of CDI outbreaks attributed to the strain *C. difficile* RT 027 in both the United States and Europe, with cases exhibiting a twofold increase (Zilberberg et al., 2008; Jones et al., 2013). The incidence of CDI, which was previously rare and defined as community-acquired CDI (CA-CDI) and is independent of risk factors such as long-term antibiotic treatment, advanced age, a weakened immune system, gastric acid suppression, and previous hospitalization, has begun to increase significantly (Hampikyan et al., 2018; Song and Kim, 2019).

Especially, during the 2010s, there was a notable rise in the incidence of *C. difficile* contamination in both food and the surrounding environment (Rodriguez Diaz et al., 2018; Knight et al., 2015). Based on the findings presented in the CDC's Emerging Infections Program 2021 Annual Report for *Clostridioides difficile* Infection (CDC, 2023), it is observed that the incidence of community-associated cases slightly surpasses that of healthcare-associated cases. Specifically, the rate of community-associated cases stands at 55.9 cases/100.000 individuals, while healthcare-associated cases are reported at a rate of 54.3 cases/100.000 individuals. These statistics suggest that sources beyond the hospital environment play a substantial role in the transmission of CDI.

Ever since its initial discovery in birds and mammals during a scientific investigation carried out in Antarctica in the year 1960 (McBee, 1960), *C. difficile* has emerged as a causative agent for enteric disturbances and diarrheal episodes in numerous animal species, including poultry (Bingol et al., 2020). Numerous studies have substantiated the notion that animals possess the capacity to serve as vectors for the dissemination of the bacterium to human beings, either through direct contact or via indirect transmission facilitated by the consumption of raw sustenance or the ingestion of contaminated water sources (Songer and Anderson, 2006; Rupnik and Songer, 2010). The spread and resistance of *C. difficile* in poultry meat and their products, as well as the molecular linkages between strains isolated from poultry meat and humans, should be considered when evaluating the risk of *C. difficile* presence in poultry meat and investigating the possible threat it poses.

General features

Clostridioides difficile is classified as Gram-positive, toxigenic, and obligate anaerobic bacterium that possesses the ability to undergo spore formation (Akkaya and Hampikyan, 2019; Heise et al., 2021). It flourishes in an oxygen-deprived environment by metabolizing a diverse array of carbon and nitrogen sources, alongside simple nutrients such as trace elements (Rui et al., 2024). The bacterium's optimal growth occurs at temperatures ranging from 35 to 40 ° C; it is capable of fermenting amino acids to produce energy (adenosine triphosphate) while also metabolizing sugars (Gibbs, 2009). Nutrient deficiency, intercellular communication, and harsh environmental conditions activate the survival mechanisms of *C. difficile*. This triggers the activation of the vital Spo0A protein, which initiates endospore formation. These endospores gather around a dehydrated nucleus containing DNA, the bacterium's genetic material, transfer RNA required for protein synthesis, ribosomes, and essential enzymes for metabolic processes that initiate germination. Comparable to lifeboats, these endospores ensure the survival of the bacterium's core components, keeping them secure and intact until the environment becomes hospitable again (Lawler et al., 2020). A sub-lethal thermal shock of approximately 75–80°C for 10 minutes, or alternative stimuli such as high pressure or acidic environments, are requisite for the swift germination of these spores (Gibbs, 2009). Additionally, bile salts and certain amino acids induce spore germination. All these conditions lead to the transformation of dormant *Clostridioides difficile* endospores into exospores. These exospores then undergo germination into active vegetative cells (Lawler et al., 2020; Rui et al., 2024). Their vegetative forms do not survive prolonged exposure to oxygen outside the body. In the context of individuals who are in good health, the presence of stomach acid and commensal intestinal flora serves as a protective mechanism against the invasion of harmful microorganisms. The ingestion of vegetative *C. difficile* cells is effectively neutralized by the low pH environment, often ranging from pH 1 to 2. However, it is important to note that this acidic condition does not exhibit the same lethal effect on *C. difficile* endospores. Furthermore, *C. difficile*, a bacterium that typically resides in the gastrointestinal tracts of both humans and animals, generates toxins as a consequence of the disruption of the typical microbial community caused by prolonged and consistent antibiotic administration (Akkaya and Hampikyan, 2019). Therefore, *C. difficile* is capable of spreading throughout the gastrointestinal tract, causing a variety of gastrointestinal symptoms including diarrhea that can range in severity from moderate to severe. In specific instances, individuals

who are afflicted with severe illness may even die (De Boer et al., 2011). The CDC's Antibiotic Resistance Threats in the United States 2019 report states that in 2017, hospitals in the United States admitted approximately 223,900 adult people, and regrettably, this infection resulted in a minimum of 12,800 deaths (CDC, 2024). In contrast, the infant gut shows a natural resistance to *C. difficile* toxins. The low incidence of clinical infection in this demographic group is proof of this resistance. Colonization rates in healthy infants decline from birth and stabilize at a level corresponding to typical adult levels by the age of three years. There may be possible reasons for this. Initially, the lack of specific receptors in intestinal cells prevents toxins from binding to them. In addition, because the signaling pathways in the gastrointestinal tract of infants are incomplete, harmful agents cannot act sufficiently. Breast milk is a rich source of antibodies and other protective proteins, which neutralize toxic substances. Finally, the unique composition of the intestinal microflora in infants creates a line of defense that prevents the proliferation and activation of pathogens (Kociolek et al., 2019; Li et al., 2023). Therefore, the incidence of clinical infections is low and, in particular, infants younger than one year of age are often asymptomatic carriers of the bacteria, with more than 40% of individuals in this age group being such carriers (Stoesser et al., 2017). Despite all these, *Clostridioides difficile* is a commonly encountered bacterium in pediatric medicine and its negative effects on child health should not be ignored. According to population-based surveillance done by the CDC Emerging Infections Program in 2019, the rate of community-associated *Clostridioides difficile* infection in children was 25.8 per 100,000 and accounting for 75% of all CDI cases in children. This suggests that emerging infections are more likely to be community-associated than healthcare-associated (Shirley et al., 2023; CDC 2024).

The virulent strains of *C. difficile* are known to generate two substantial clostridial toxins, namely toxins A (*tcdA*) and B (*tcdB*) and these toxins are encoded by the genes *tcdA* and tcdB, respectively (Hensgens et al., 2012). The *tcdA* gene encodes the production of toxins A (enterotoxin), which leads to an increase in colonic fluid and cellular damage. Similarly, the *tcdB* gene encodes the production of toxins B (enterotoxin) which also contributes to cellular damage. These toxins are crucial in the pathogenesis of CDI. Certain strains also have cdtA/B genes, which encode the creation of binary toxins (actin-specific ADP-ribosyl transferase) (Barbut et al., 2005). Even though binary toxins by themselves have not been shown to cause disease (Eckert et al., 2015), their presence has been linked to more severe illness (Barbut et al., 2005). The presence of these toxins is largely responsible for the pathogenicity of this bacteria (Usui et al., 2020). Certain strains of *C. difficile* lack the ability to produce toxins, resulting in the absence of CDI symptoms (Jöbstl et al., 2010; Mooyottu et al., 2015). Nevertheless, non-toxigenic strains of *C.* difficile may have the ability to acquire toxins through horizontal gene transfer (Brouwer et al., 2013). Certain strains of *C. difficile* possess a DNA segment known as the pathogenicity locus (PaLoc), which harbors genes responsible for producing toxins A and B. Some strains of *C. difficile* with PaLoc can cause illness. *C. difficile* strains that possess this DNA sequence are toxigenic, indicating that they are capable of causing sickness. In spite of the fact that certain strains are only capable of including a single gene for the toxins (A−B+ or A+B−), it has been shown that they nonetheless cause serious sickness in people (Bolton and Marcos, 2023). However, not every strain of *C. difficile* possesses this specific DNA region. Therefore, PaLoc-free *C. difficile* strains are non-toxigenic and typically do not result in illness. Nevertheless, certain non-toxigenic strains of *C. difficile* have the ability to obtain this genetic material from another *C. difficile* strain that possesses PaLoc. Therefore, *C. difficile* that was previously non-toxigenic can undergo a transformation and begin generating toxins, thereby becoming toxigenic. There is concern that non-toxigenic strains of *C. difficile*, especially those that are resistant to many treatments, may obtain PaLoc and become toxic. This raises concerns that certain strains may be more resistant to treatment and potentially more harmful (Mooyottu et al., 2015).

Some *C. difficile* ribotypes have higher toxin production and effective sporulation, which renders them hypervirulent. In this subgroup, human pathogenic ribotypes such as RT027 and RT078 are prominent and are recognized as the cause of human CDI (Barbut et al., 2005; Rahimi et al., 2015; Hampikyan et al., 2018). The RT027 strain exhibits elevated rates of sporulation, resistance to fluoroquinolone antibiotics, heightened secretion of toxins A and B, and the ability to produce binary toxin, also known as *C. difficile* transferase (Lyon et al., 2016). The community-associated ribotypes RT027, RT078, and RT017 of *C. difficile* have additionally been identified in food products and farm animals (Goorhuis et al., 2008; Janezic et al., 2012; Rodriguez et al., 2014).

Ribotype 078 has been identified as the predominant etiological agent responsible for

Clostridium difficile-associated community-acquired diarrhea and infection (CACDI) in the Northern Hemisphere (Knight et al., 2015). The strain in question has been detected in ground turkeys located in Pennsylvania, USA (Varshney et al., 2014), as well as chicken carcasses situated in Ontario, Canada (Weese et al., 2010).

Presence of *C. difficile* in foods

The detection of genetically similar *C. difficile* strains in food and humans has led to an increased awareness of the potential for *C. difficile* as an unspecific foodborne agent (Songer and Anderson, 2006; Goorhuis et al., 2008; Rupnik et al., 2009; Knight et al., 2015). Numerous hypotheses have been proposed thus far regarding the transmission of *C. difficile*. Research conducted on various continents have exhibited escalating apprehension regarding the potential role of food as a reservoir for CDI. Food products were deemed to possess the capacity to function as a medium for the proliferation of *C. difficile* endospores. However, it appears that the ingestion of metabolically dormant endospores is primarily responsible for the transmission of CDI, likely due to the obligate anaerobic nature of the bacterium (Akkaya and Hampikyan, 2019). In addition, the rising prevalence of community-acquired *C. difficile* infection (CA-CDI) among younger individuals who have not been hospitalized suggests that retail foods may serve as significant reservoirs for this pathogen (Usui et al., 2020).

C. difficile has been detected in the environment (soil and water), a diverse array of sources/reservoirs including food animals (cattle, poultry, pigs, and sheep), meat and meat products (beef, chicken, lamb, pork, turkey, and veal), seafood (clams, mussels, salmon, and shrimp), vegetables, fruits, and packaged foods, according to studies conducted to date (Gould and Limbago, 2010; Knight et al., 2015; Hampikyan et al., 2018; Bingol et al., 2020). Recent meta-analytical studies have shed light on the relationship between various food types and the presence of *C. difficile*. A synthesis of 79 studies conducted from 1981 to 2019 by Rodriguez-Palacios et al. (2020) revealed a 4.1% prevalence in commonly consumed foods, including seafood, green leafy vegetables, and meats such as beef, pork, and poultry. These findings suggest not only an increasing incidence of *C. difficile*, but also a worrying trend of rising infection rates linked to these food products. Similarly, Borji et al. (2023) conducted a meta-analysis of 60 studies over a ten-year period from 2009 to 2019, focusing on the prevalence of *C. difficile* in commonly consumed foods, including seafood, poultry, red meat, dairy products, vegetables, salads, and other foods. Their research reported a *C. difficile* prevalence of 6.3 percent across all food types. According to the results of the analysis, seafood had the highest *C. difficile* prevalence with 10.3%, followed by poultry (6.2%), salads (6.1%), and red meat (5.5%). The study also calculated risk ratios, and seafood emerged as the highest risk carrier with a ratio of 12.88, indicating a risk approximately thirteen times greater than that of side dishes, which pose the least hazard for *C. difficile* contamination. Other significant risk carriers included ready-to-eat red meats and cooked poultry, which had risk ratios of 9.75 and 7.75, respectively, followed by salads and raw poultry meat.

C. difficile is acknowledged to be harbored by food animals. Studies conducted in Türkiye (Hampikyan et al., 2018), Belgium (Rodriguez et al., 2014), Canada (Rodriguez et al., 2019; Weese et al., 2009), Australia (Jöbstl et al., 2010), Costa Rica (Quesada-Gómez et al., 2013), and United States (Mooyottu et al., 2015) have detected *C. difficile* in various meat products, including beef, pork, veal, and sheep carcasses. In their study, Weese et al. (2009) successfully detected the presence of specific toxin genes (tcdA, tcdB, and cdtA/B) in the samples they analyzed. The presence of tcdA, tcdB, and cdtA/B was later confirmed in the findings of Rodriguez et al. (2014), although in a smaller percentage of pork and beef samples. It also identified a group of samples that tested positive for tcdA and tcdB but did not have the binary toxin genes, with one sample completely devoid of the genes in question. According to a study by Hampikyan et al. (2018), tcdB was discovered in more than half of the cattle isolates, and tcdA in nearly half of them. Furthermore, the majority of the isolates contained cdtA/B. Their study emphasized that a significant number of cattle and sheep samples contained all three virulence genes, while a small portion of sheep carcass isolates did not have any. Quesada-Gómez et al. (2013) discovered isolates positive for cdtA and tcdB but did not have the binary toxin genes. On the other hand, studies conducted by Jöbstl et al. (2010) and Mooyottu et al. (2015) have not found any evidence of these toxin genes. Contamination rates have ranged from 1.5% to 33.6%, indicating a widespread presence of *C. difficile* in the global meat supply chain. The persistent nature of *C. difficile* spores, which can endure harsh environmental conditions for extended periods, facilitates their transmission from personnel, equipment, and contaminated surfaces to food due to improper hygiene practices and posing a significant public health risk (EFSA, 2013; Hampikyan et al., 2018). The body of research investigating *C. difficile* contamination in vegetables and ready-to-eat salads remains limited. It has been observed that compost

fertilizers derived from farm animals have the potential to harbor *C. difficile* spores (Jöbstl et al., 2010; Quesada-Gómez et al., 2013). Hence, there is a potential risk of *C. difficile* entering the food chain due to the use of fertilizers on the land. The potential for contamination of vegetables and fruits arises from the utilization of contaminated water during irrigation or washing processes (Rupnik and Songer, 2010). Furthermore, even if good agricultural procedures are implemented, there is still a plausible possibility of spore transfer to fresh produce via fertilizer (Quesada-Gómez et al., 2013). The ingestion of minimally processed or uncooked vegetables or fruits has the potential to function as a means of transmission for CDI. Research conducted in Canada (Metcalf et al., 2010), South Wales (al Saif and Brazier, 1996), and Scotland (Bakri et al., 2009) has demonstrated raw vegetables contamination rates ranging from 2.3% to 7.5%, highlighting the potential for non-animal food sources to contribute to the spread of *C. difficile*, including antibiotic-resistant strains.

Although the studies have focused more on animal -derived foods and their different foodstuff, there is a scarcity of data on the prevalence and characteristics of *C. difficile* contamination in seafood. Several studies have reported the presence of *C. difficile* in various marine creatures, such as edible bivalve molluscs (e.g., scallops), shellfish (e.g., shrimp, clam, cockle, mussel, oyster), finfish, and fishes (e.g., perch, salmon), from different regions of the world. The prevalence of *C. difficile* contamination in seafood varies widely, ranging from 3.17% to 66.6%, depending on the location, species, and source of the samples. Italy (Pasquale et al., 2012; Troiano et al., 2015), Canada (Metcalf et al., 2011), United States (Montazeri et al., 2015; Norman et al., 2014), and Iran (Nayebpour and Rahimi, 2019) are some of the areas where *C. difficile* has been detected in seafood. These studies reveal that seafood, like other food products, can be contaminated by *C. difficile* and that there may be geographical differences in the contamination levels of this bacterium.

Presence of *C. difficile* in poultry meat and products

The detection of *C. difficile* in poultry raises concerns that poultry may be a possible reservoir for CDI. Studies demonstrate an association between *C. difficile* and its human pathogenic ribotypes in chicken carcasses, implicating chickens as a likely source of bacterial contamination and potentially contributing to the transmission of *C. difficile* to humans (Pasquale et al., 2012; Rahimi et al., 2015; Hampikyan et al., 2018).
The presence and characterization of *C. difficile* in poultry meat are summarized in Table 1. It has been observed that the presence of *C. difficile* in poultry products was detected within a range of 1% to 44.4%. Upon examination of these studies, it becomes apparent that the region of North America has exhibited the highest recorded prevalence rate, standing at an impressive 44.4% (Songer et al., 2009). Subsequently, Türkiye has been documented to possess a prevalence rate of 37.3% (Bingol et al., 2020), while Iran follows suit with a prevalence rate of 24.4% (Barezi et al., 2023). It is evident that the prevalence of *C. difficile* in meat collected in Asia is lower than that in meat collected in Europe, and the prevalence of *C. difficile* in Europe's meat is lower than that in United States-origined meat. In addition, the binary toxin (cdtA/B) was found in isolates originating more often from Canada and the United States (Songer et al., 2009; Weese et al., 2010; Varshney et al., 2014). Similarly, several investigations conducted in Europe have reported the presence of dual toxin genes (Bingol et al., 2020; Tkalec et al., 2020). In contrast, certain studies have documented the absence of binary toxin genes in isolates obtained from chicken meat (De Boer et al., 2011; Guran and Ilhak, 2015; Heise et al., 2021).

Recently, there has been increasing concern about the uncontrolled use of antibiotics in poultry. Different countries ban the use of antibiotics, yet the poultry industry uses various types of antibiotics to promote growth, treat diseases, and prevent disease. Because of this use, there is ongoing concern about the development of antibiotic resistance in *C. difficile* in poultry (Bingol et al., 2020). The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommends vancomycin and metronidazole as the top choices for treating *C. difficile* infections in humans (Cho et al., 2020). Studies have shown that vancomycin and metronidazole are the antibiotics to which *C. difficile* strains are most frequently susceptible on all continents (Harvey et al., 2011; Quesada-Gómez et al., 2013; Varsheney et al., 2014; Ersöz and Coşansu, 2018; Lee et al., 2018; Usui et al., 2020; Attia et al., 2021; Barezi et al., 2023). This demonstrates their continued efficacy in treating *C. difficile* infections. On the other hand, resistance to clindamycin ranged from 2.2% to 50.0% (Lee et al., 2018; Usui et al., 2020), and some strains showed intermediate resistance (Lee et al., 2018; Attia et al., 2021; Filabadi et al., 2022). In addition to that, Harvey et al. (2011) and Bingol et al. (2020) both found that cefotaxime was not effective against many strains of C. *difficile*, with 100% (7/7) and 97.1% (67/69) of these strains being resistant. These results raise concerns that clindamycin and cefotaxime may show a higher frequency of resistance, limiting treatment options.

Table 1. Review of the presence and characterization of *Clostridioides difficile* in poultry meats from investigations conducted in countries on the European, Asian and American continents.

Reference	Study period	Country	Raw / Cooked	Sample material	Samples (n)	Positive samples (n^p)	Percentage of positive samples (%)	Toxin type (n^t)	PCR ribotype (n^r)
Von Abercron et al. (2009)	April - September 2008	Sweden	R	poultry meat	4	0	0.0	-	-
	April - September 2008	Sweden	C	poultry sausages					
Indra et al. (2009)	February 2008 - April 2008	Austria	R	chicken meat	6	0	0.0	-	-
De Boer et al. (2011)	October 2008 - March 2009	Netherlands	R	chicken meat	257	7	2.7	tcdA+ tcdB+ cdtAB- (4) tcdA- tcdB- cdtAB- (3)	001, 003 (2), 071, 087, NT** (2)
Guran and Ilhak (2015)	October 2012 - April 2013	Türkiye	R	chicken carcass (leg quarters, breast, wings, drumsticks, livers)	310	25	8.1	tcdA+ tcdB- (8) tcdA- tcdB+ (5)	-
Ersöz and Coşansu (2018)	April 2013 - February 2014	Türkiye	R	chicken (breast)	27	0	0.0	-	-
Bingol et al. (2020)	US*	Türkiye	R	chicken carcass	185	69	37.3	tcdA+ tcdB+ cdtAB+ (17) tcdA+ tcdB+ cdtAB- (14) tcdA+ tcdB- cdtAB+ (1) tcdA+ tcdB- cdtAB- (3) tcdA- tcdB+ cdtAB+ (13) tcdA- tcdB+ cdtAB- (11) tcdA- tcdB- cdtAB+ (0) tcdA- tcdB- cdtAB- (10)	003 (1), 010 (1), 020 (2), 027 (6), ML#027 (6), 085 (4), 087 (4), 470 (4), 456 (2), NT** (39)
Tkalec et al. (2020)	April 2015 - December 2015 March 2016 - December 2016 March 2016 - December 2017	Slovenia	R	chicken meat	60	3	5.0	-	001, 014/020, 015
				chicken meat preparations	120	5	4.2	-	001, SLO 052, 078
Heise et al. (2021)	July 2017 January 2018 - June 2018 June 2019 - July 2019	Germany	R	poultry meat (skin-out) (chicken and turkey)	42	0	0.0	-	-
				poultry meat (skin-on) (chicken and turkey)	322	51	15.8	tcdA+ tcdB+ (43)	002/2 (10); 001 (9); 005 (5); 014 (5); NT** (4); 087 (2); 049 (1); 020 (1); 464 (1); 503 (1); 212 (1); 220/1 (1); 625 (1); AI-29 (1); 205 (3); 701 (2); 010 (1); 578 (1); 629 (1)
Songer et al. (2009)	January 2007 - April 2007	USA	R	ground turkey	9	4	44.4	toxinotype V NAP7 tcdA+ tcdB+ cdtAB+ (4)	078
Weese et al. (2010)	November 2008 - June 2009	Canada	R	chicken carcasses (thigh, wing, and leg)	203	26	12.8	-	078 (26)
Harvey et al. (2011)	July 2010	USA	R	chicken meat	96	7	7.3	toxinotype V NAP7 (3) or NAP7-variant (4)	-
Varshney et al. (2014)	October 2011 - September 2012	USA	R	chicken meat (thighs)	77	6	7.8	tcdA+ tcdB+ cdtAB+ (4) tcdA- tcdB- cdtAB+ (1) tcdA- tcdB- cdtAB- (1)	-
				ground turkey	76	11	14.5	tcdA+ tcdB+ cdtAB+ (3) tcdA+ tcdB+ cdtAB- (1) tcdA+ tcdB- cdtAB+ (1) tcdA- tcdB- cdtAB+ (4) tcdA- tcdB- cdtAB- (2)	027 (1), 078 (2)
Mooyottu et al. (2015)	US*	USA	R	chicken meat (wing)	100	0	0.0	-	-
Quesada-Gómez et al. (2013)	November 2009 - April 2010	Costa Rica	R	chicken meat	67	1	1.5	tcdA+ tcdB+ cdtAB-	029

Continuation of Table 1

Hasanzade and Rahimi (2013)	US*	Iran	R	turkey meat	120	14	11.7	-	-
Hasanzadeh and Rahimi (2013)	US*	Iran	R	chicken meat	120	19	15.8	-	-
Rahimi and Khaksar (2015)	April - October 2012	Iran	C	chicken nugget	150	0	0.0	-	-
Razmyar et al. (2017)	2014	Iran	R	packed chicken parts (necks, thighs, wings)	65	10	15.4	tcdA+ tcdB+ (5) tcdA+ tcdB- (1) tcdA- tcdB- (2) tcdA+ tcdB+ cdtAB+ (2)	-
Lee et al. (2018)	April 2013 - March 2014	South Korea	R	chicken meat	149	25	16.8	tcdA+ tcdB+ (2)	-
Usui et al. (2020)	March 2015 - March 2016	Japan	R	chicken (liver)	28	1	3.6	-	-
				chicken meat	89	6	6.7	-	-
Attia (2021)	October 2019 - November 2019	Saudi Arabia	R	chicken carcass (legs, thighs, wings, breasts)	250	11	4.4	-	-
Ghorbani Filabadi et al. (2022)	July 2018 - July 2019	Iran	R	chicken meat	100	1	1.0	tcdA+ tcdB+	-
Ansarian Barezi et al. (2023)	July 2018 - July 2019	Iran	R	quail meat	60	1	1.7	-	-
				duck meat	60	12	20.0	-	-
				chicken meat	90	22	24,4	-	-
Hazarika et al. (2023)	July 2019 - De cember 2020	India	R	chicken meat	28	4	14.81	tcdA+ tcdB+ (2)	-
			C	chicken sausage	10	0	0.0	-	-
			C	chicken salami	10	0	0.0	-	-

ML#: most likely; np: number of positive samples; nr: number of ribotypes; nt: number of toxin types; NT**: Non-typable by the National Reference Laboratory for *Clostridium difficile*; US*: unspecified

***Prevalence of C. difficile in poultry meat in Europe*:** Research conducted in Europe has revealed that the presence of *C. difficile* is frequently observed in chicken meat with a range of 2.7 % to 37.3 % (Table 1). The ribotypes RT 001, 014, 027, and 078 identified in research conducted in Germany (Heise et al., 2021), the Netherlands (De Boer et al., 2011), Slovenia (Tkalec et al., 2020), and Türkiye (Bingol et al., 2020), are commonly detected in human that have been linked to human CDI.

Prevalence of C. difficile in poultry meat in America: *C. difficile* has been isolated from a variety of poultry and its products in America and Canada, including uncooked ground turkey, chicken meat, and chicken thigh, wing, and leg. The prevalence of *C. difficile* in these products ranges from 7.3 % to 44.4 % (Table 1). Several studies have also found that some of the *C. difficile* strains isolated from poultry and its products are toxigenic. These strains are responsible for the production of toxins that cause CDI. Additionally, some of these strains have been identified as ribotypes 027 and 078, which are two of the most virulent strains of *C. difficile* and are associated with significant CDI in humans (Songer et al., 2009; Weese et al., 2010; Varshney et al., 2014).

Prevalence of C. difficile in poultry meat in Asia: Numerous investigations have made contributions towards conducting a thorough examination of the occurrence of *C. difficile* in diverse reservoirs of poultry meat inside the Asian region. The observed prevalence of *C. difficile* in poultry meat exhibits considerable heterogeneity, ranging from a minimum of 1% to a maximum of 24.4% (Table 1). A greater number of research have been undertaken in Iran compared to other nations within the Asian continent (Hasanzadeh and Rahimi, 2013; Razmyar et al., 2017; Ghorbani Filabadi et al., 2022; Barezi et al., 2023). These studies have revealed a range of *C. difficile* prevalence rates, spanning from 1 % to 15.8 %, focusing on diverse poultry meat varieties such as chicken, turkey, quail, duck, and partridge (Barezi et al., 2023). Moreover, *C. difficile* has also been identified in South Korea (Lee et al., 2018), Japan (Usui et al., 2020), Saudi Arabia (Attia, 2021), and India (Hazarika et al., 2023).

In contrast to the prevailing trend, there exist studies that have documented an inability to identify the *C. difficile* strain within endeavors carried out in both the United States (Mooyottu et al., 2015) and the European (Indra et al., 2009; Von Abercron et al., 2009; Ersöz and Coşansu, 2018) continents. In the Asian continent, Rahimi and Khaksar (2015) investigated the heat-treated food (chicken nuggets), in contrast to earlier research, and indicated that no evidence of the presence of this pathogen was found. Additionally, upon closer examination of the African continent, it is worth noting that thus far only a solitary study has been documented (Abdel-Glil et al., 2018), wherein the existence of *C. difficile* was regrettably not ascertained.

Factors contributing to discrepancies in isolation rates across continents: The observed variabilities in *C. difficile* isolation rates across continents can perhaps be attributed to variations in the procedures employed for the isolation and identification of *C. difficile* within each continent. The absence of universally accepted ISO protocol for the identification of *C. difficile* in food products makes it difficult to compare the results of different studies, resulting in data inconsistencies. (Blanco et al., 2013) reported that the method used to isolate *C. difficile* may have a substantial effect on the prevalence statistics for this pathogen. The observed discrepancies in prevalence can also be ascribed to disparities in the biological material, collection techniques, hygiene practices, sampling methodologies, process size, and cultural practices utilized at each individual site.

The prevalence rates of *C. difficile* may be subject to variation due to factors such as geographical location and seasonal fluctuations. Research conducted at hospitals in Taiwan and Australia revealed that the prevalence of CDI exhibited a peak in the month of March, while the lowest incidence was observed during the last quarter of the year (Lee et al., 2016; Worth et al., 2016). Furuya-Kanamori et al. (2015) emphasized that the infection peaks in spring and is seen at a lower frequency in summer and autumn.

Rodriguez-Palacios et al. (2009) identified the prevalence of *Clostridioides difficile* in retail meat products, while Rodriguez et al. (2019) investigated the distribution of this pathogen in environmental soil samples. The results of both investigations indicate that there is a notable prevalence of *C. difficile* during the winter season. The observation that the detection of *C. difficile* in poultry was predominantly reported throughout the winter and spring seasons, particularly in the months of November to March, indicates a noteworthy correlation with the mentioned investigations.

Additionally, it is imperative to consider the age, breed, and other relevant characteristics of the animals that were included in the sample (Varshney et al., 2014; Ersöz and Coşansu, 2018; Hampikyan et al., 2018). Knight et al. (2013) observed that the prevalence of *C. difficile* decreases with the age of production animals, so meat from older animals poses much less risk. Colonization of *C. difficile* in chickens occurs predominantly during the initial two weeks following hatching, followed by a gradual decline as the poultry age.

Presence of *C. difficile* in heat-treated products

C. difficile is currently not recognized as a foodborne pathogen. Therefore, the available data regarding the viability of this strain in food is comparatively limited when compared to other pathogenic species within the Clostridium genus. In comparison to other pathogens, the most notable characteristic of *C. difficile* is the great resilience of its spores to a range of physical conditions, including heat and chemicals. Given the capacity of spores to endure the acidic environment of the stomach and the elevated temperatures encountered during cooking procedures, it is conceivable that these microorganisms may endure in food items even after being cooked (Rodriguez-Palacios et al., 2010; Rodriguez et al., 2013). Moreover, this persistence of spores presents a formidable obstacle in the thorough eradication of spores during the culinary preparation of food and the sanitation of food processing equipment and surfaces (Esfandiari et al., 2014).

The process of heating can lead to a decrease in the oxygen content within cooked food, which can result the creation of anaerobic conditions that can trigger the germination and growth of spores (Kouassi et al., 2014). In addition, heat treatments have the potential to enhance the resilience of some pathogens that can produce heat-shock proteins, hence leading to the possibility of pathogen selection during the process of heat treatments (Cowen and Lindquist, 2005). This selection increases the pathogenic properties of microorganisms exposed to heated foods, leading to their widespread presence in the food supply and increasing the risk of foodborne infections resulting from the consumption of these foods.

Flock et al. (2022) revealed the survival of *C. difficile* in fermented pork summer sausage even after exposure to a pH below 5 and cooking at 66.5°C for 45 minutes. In a study conducted by Rodriguez-Palacios et al. (2010), it was shown that vegetative cells of the bacterium C. difficile were able to withstand the recommended cooking temperatures for beef set by USDA, which is 71°C, for a duration of 2 hours. However, it was found that subjecting the food to a

reheating process at a temperature of 85°C resulted in the elimination of 90% of *C. difficile* spores within a span of 10 minutes. Similarly, it has been observed that *C. difficile* spores can withstand temperatures ranging from 60°C to 75°C for extended periods, with significant reduction in spore count only occurring at temperatures exceeding 85°C (Lawley et al., 2009). It was also demonstrated that the inhibition of *C. difficile* spore increased by subjecting them to a heat shock at a temperature of 96°C for a duration of 15 minutes. However, heat treatment is not always effective in killing spores, as some spores may exhibit persistence or regenerative ability even after exposure to high temperatures (Rodriguez-Palacios and LeJeune, 2011). Research on thermal inactivation kinetics further supports this notion, indicating that heating *C. difficile* spores to 100°C for 30 seconds resulted in a 3.75 log reduction, while a temperature of 105°C for the same duration achieved a 4.29 log reduction (Saad et al., 2023).

The heat resistance of *C. difficile* spores has been extensively documented in various studies. Songer et al. (2009) detected *C. difficile* in 14.3% ready-to-eat summer sausage and 62.5% ready-to-eat pork braunschweiger, highlighting its persistence after cooking processes. Ribotype 027 was identified in sausage, while both ribotype 078 and ribotype 027 were detected in braunschweiger. The prevalence of ribotype 078 in food sources indicates a potential for greater heat resistance compared to several other ribotypes, such as RT027 (Rodriguez-Palacios et al., 2016). This strain, possessing the ability to endure temperatures as high as 96°C, has been linked to instances of *C. difficile* infections (Brown and Wilson, 2018).

The fact that spores usually survive at temperatures recommended for cooking indicates the potential role of food in the transmission of the disease (Rodriguez-Palacios and LeJeune, 2011; Deng et al., 2015). Therefore, the current cooking recommendations ought to be revised to incorporate *C. difficile* and it is crucial to adopt more efficacious intervention measures aimed at mitigating spore contamination in food products. Moreover, the presence of *C. difficile* in ready-to-eat meat products underscores the potential role of food in the transmission of this pathogen, necessitating stricter control measures throughout the food chain. These combined efforts are crucial to mitigate the risk of foodborne *C. difficile* infections and safeguard public health.

Protection

Safeguarding consumer health throughout the food production continuum is paramount, necessitating strict adherence to prescribed food safety standards at every stage, from production and processing to storage, shipment, and consumption. In the realm of poultry meat and its products, sanitation protocols must be meticulously executed throughout the entirety of the poultry rearing process, following the farm-to-table methodology. During production, the utmost care must be exercised when extracting internal organs and removing feathers, ensuring no contamination of the carcass. This precautionary measure aims to avert potential contamination. Additionally, cleanliness of materials, machinery, and personnel hygiene must be maintained, and animal waste disposal must be handled with caution and attentiveness (Akkaya and Hampikyan, 2019).

The spores of *C. difficile* demonstrate an exceptional degree of resistance when confronted with adverse physical circumstances. The spores of *C. difficile* may survive in the environment for a period longer than five months (Kramer et al., 2006). A multitude of methodologies like subzero temperatures as low as -80°C, elevating temperatures to a maximum of 85°C, desiccation, exposure to UV radiation, utilization of alcohol gel, and application of various disinfectants, have been ascertained to be ineffective in eradicating said pathogenic bacterium (Deng et al., 2015; Connor et al., 2017).

Due to the ability of spores to survive cooking temperatures, *C. difficile* spores must be heated to a temperature above 85°C to ensure food safety (Rodriguez-Palacios and LeJeune, 2011; Deng et al., 2015). Nevertheless, the current cooking recommendations ought to be revised to incorporate *C. difficile*, and it is crucial to adopt more efficacious intervention measures aimed at mitigating spore contamination in food products. Furthermore, it is important to raise awareness among food handlers and consumers about proper food handling and cooking practices to prevent *C. difficile* contamination. These comprehensive measures will help ensure a safer food supply and protect individuals from potential infections.

Conclusions

The present review has provided an overview of the current understanding of *Clostridioides difficile* contamination in poultry meat and its products. While the role of contaminated poultry in human illness remains a subject of debate, the growing body of evidence suggests that poultry may serve as a potential reservoir for *C. difficile* infections within communities. The detection of identical *C. difficile* ribotypes, including RT001, RT014, RT027 and RT078,

in both human illnesses and poultry further supports this notion.

Nonetheless, rigorous hygiene management practices throughout the poultry production and handling process are crucial in minimizing the risk of *C. difficile* contamination and potential human exposure. Although there is limited research conducted to date on heat-treated poultry meat products, *C. difficile* exhibit resistance to heat treatment and have been identified in meat products derived from various animal sources, including pigs and cattle, suggests a potential concern regarding the potential presence of this bacterium in poultry meat products.

In conclusion, even though poultry is one of the agents that may play a role in the transmission of *C. difficile* infections, its potential role in foodborne transmission warrants further investigation and proactive intervention. The development and implementation of effective control strategies for the prevention of *C. difficile* cases related to poultry meat may be essential to protect public health and reduce the prevalence of *C. difficile* infections.

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