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PREVALENCE OF CLOSTRIDIUM DIFFICILE ISOLATES IN BROILER CHICKENS - FIRST STUDY IN ROMANIA

PREVALENȚA IZOLATELOR DE CLOSTRIDIUM DIFFICILE LA PUII BROILER - PRIMUL STUDIU DIN ROMÂNIA

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ABSTRACT

*Clostridium diffi*cile has emerged as one of the extensively researched bacteria globally in recent years, owing to its significant impact on human and animal health. Notably, toxigenic *C. difficile* is frequently detected in farm animals and domestic pets, even in the absence of clinical symptoms. The presence of shared *C. difficile* ribotypes (RTs) between humans and animals highlights the potential for zoonotic transmission. Therefore, this study aimed to investigate the prevalence of *Clostridium difficile* in broiler chickens by collecting intestinal content and faecal samples from both the slaughterhouse and farm environment. Out of the 40 samples tested, 6 (15%) were found to be positive for Clostridium difficile, and the majority of these isolates (5 out of 6, 83.33%) were recovered from fecal samples. Furthermore, all 6 isolates were confirmed to be toxigenic (A+, B+, CDT-). This study represents the first analysis conducted in Romania to examine the prevalence of *C. difficile* in broiler chickens. The findings from this study add to the accumulating evidence indicating that poultry can be a potential reservoir for *Clostridium difficile* strains.

Keywords: prevalence, broiler, *C. difficile*, toxin, zoonosis

REZUMAT

Clostridium difficile a devenit una dintre bacteriile cercetate în mod extensiv la nivel global în ultimii ani, datorită impactului său semnificativ asupra sănătății umane și animale. Este de remarcat faptul că *C. difficile* toxigenic este frecvent detectat la animalele de fermă și animalele de companie, chiar și în absența simptomelor clinice. Prezența ribotipurilor comune de *C. difficile* între oameni și animale evidențiază potențialul de transmitere zoonotică. Prin urmare, acest studiu și-a propus să investigheze prevalența *Clostridium difficile* la puii broiler prin colectarea de conținut cecal și mostre de fecale de la abator și de la fermă. Din cele 40 de probe testate, 6 (15%) au fost găsite pozitive pentru *Clostridium difficile*, iar majoritatea acestor izolate (5 din 6, 83,33%) au fost recuperate din probele de fecale. Mai mult, toate cele 6 izolate au fost confirmate ca fiind toxigene (A+, B+, CDT-). Acest studiu reprezintă prima analiză efectuată în România pentru a determina prevalența *C. difficile* la puii broiler. Concluziile acestui studiu contribuie la acumularea de dovezi care indică faptul că puii broiler pot fi un rezervor potențial pentru tulpinile de *Clostridium difficile*.

Cuvinte cheie: *prevalență, broiler, C. difficile*, toxină, zoonoză

Clostridium difficile is a spore-forming, Gram-positive, anaerobic bacillus that is widely distributed in the environment and can be found in the gastrointestinal tracts of both humans and animals. It is considered a significant pathogen and currently holds the position as the primary cause of antimicrobial- and healthcare-associated infectious diarrhoea in humans (9).

C. difficile is capable of producing up to three toxins: toxin A (TcdA), toxin B (TcdB), and the *C. difficile* transferase (CDT) binary toxin (16, 18). Over time, *C. difficile* has been identified in various sources, including food animals such as pigs, cattle,sheep, and poultry. Additionally, it has been detected in retail meat from veal, beef, pork, lamb, chicken, and turkey, as well as in seafood, vegetables, and both household and natural environments (2, 10, 11, 15, 23, 24).

In poultry, *Clostridium difficile* infection (CDI) manifests as necrotizing enteritis, as observed in clinical studies (17, 19). The clinical signs in infected birds typically involve a sudden onset of diarrhoea, followed by rapid deterioration leading to death. Typically, infected poultry succumb to the infection within three days of the appearance of symptoms. The mortality rates associated with CDI in poultry are generally quite high (6). Pathologically, infected birds exhibit distinct gross lesions, including widespread multifocal haemorrhages in the ceca and colon, along with watery faeces observed in the small intestine.

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MATERIALS AND METHODS

Sampling

The samples were collected in April 2023 from a broiler chicken farm and slaughterhouse located in

Cluj County, Romania. A total of 40 samples were collected, following this distribution: 20 faecal samples were obtained from broilers aged between 3 and 6 weeks exhibiting signs of enteritis at the farm, and 20 samples of cecal content from the evisceration area were collected at the slaughterhouse. Both the faecal samples and cecal content were individually collected using sterile gloves and containers to maintain proper hygiene. Subsequently, the samples were promptly transported to the laboratory within a maximum of 4 hours after collection to ensure their integrity and minimise the risk of contamination.

Clostridium difficile isolation

The faecal samples were directly inoculated onto *C. difficile* ChromID™(bioMérieux,Marcy l'Etoile, France), a specialised chromogenic medium designed for the detection and identification of C. difficile strains. This medium contains taurocholate and a mixture of chromogens that enable the selective growth of *C. difficile* and facilitate its differentiation from other microorganisms. After inoculation, all the plates were placed in an anaerobic chamber and incubated at 37°C for duration of 24 hours. The anaerobic chamber provided an atmosphere consisting of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide, creating optimal conditions for growth of *C.difficile*. Following the incubation, the plates were examined for microbial growth and the presence of characteristic *C. difficile* colonies was identified. These colonies typically exhibited collars ranging from grey to black and displayed irregular or smooth borders, aiding in their visual differentiation from other bacterial colonies.

Toxin typing of the isolates

Toxin typing of the isolates was conducted to determine the specific toxin types present. This process involved identifying and characterising the toxins produced by the *Clostridium difficile* isolates. Various methods, such as PCR or enzyme immunoassays, can be employed for toxinotyping, allowing for the detection and classification of specific toxin genes, such as tcdA, tcdB, cdtA, and cdtB. By toxinotyping the isolates, it is possible to gain insights into their virulence potential and better understand their pathogenicity. The DNA extraction was performed using QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The genes responsible for toxin A (tcdA) and toxin B (tcdB), as well as the two components of the binary toxin (CDT) (cdtA and cdtB), were detected using Real-Time PCR (8).

RESULTS AND DISCUSSIONS

Prevalence of C. difficile

The prevalence of *Clostridium difficile* in the 40 samples was determined to be 15% (6 out of 40). Out of these positive samples, 5 isolates were detected from faeces, indicating a prevalence rate of 25% (5 out of 20), while 1 isolate was obtained from the cecum content, representing a prevalence rate of 5% (1 out of 20), as shown in Table 1.

Toxin genes profiling

The findings related to the profiles of virulence genes are displayed in Table 2. All six of the *C. difficile* strains tested positive for toxin tcdA and tcdB and negative for the binary toxin cdtA/B. The prevalence of Clostridium difficile in broiler chickens in Romania has not been previously documented. Therefore, this study aimed to investigate the prevalence and toxin gene

Table 1

Prevalence of *C. difficile*

Location	Farm	Slaughterhouse
Type of sample	Faeces	Cecal content
No. of samples collected	20	20
No. of samples positive	5 (25%)	1 (5%)

Table 2

Toxin genes profiling

Location	Farm	Slaughterhouse
Toxigenic isolates (%) tcdA+, tcdB+, cdtA/B-	5/5 (100%)	1/1 (100%)

Table 3

Prevalence of *C. difficile* in different countries

Country	Sample type	Prevalence	Reference
Austria	Gut/Faeces	3/59 (5%)	(1) 2009
India	Faeces	23/165 (14%)	(7) 2016
Slovenia	Faeces	38/61 (62,3%)	(20) 2008
The Netherlands	Faeces	7/121 (5,8%)	(12) 2012
USA-Texas	Faeces	7/300 (2,3%)	(14) 2011
Zimbabwe	Faeces	20/115 (17,4%)	(4) 2006
Zimbabwe	Faeces	29/100 (29%)	(5) 2008

profiles of *C. difficile* in broiler chickens. The findings of this study contribute to the understanding of *C. difficile* prevalence and its potential as a reservoir for transmission. The detection of *C. difficile* in broiler chickens is significant considering the potential zoonotic transmission and the role of animals in the spread of *C. difficile* infections (13). The presence of shared *C. difficile* ribotypes between humans and animals suggests the possibility of cross-species transmission (21).

In this study, a prevalence rate of 15% was observed, with 25% of the faecal samples and 5% of the cecal samples testing positive for *C. difficile*. Other studies showed a diverse isolation rate of *C. difficile* ranging from 5% up to 62.3% (Table 3).

Furthermore, it has been demonstrated that the prevalence is significantly higher in younger animals and decreases with age (22, 25). The reasons why *Clostridium difficile* infection (CDI) develops in some individuals but not others are still unknown, but it is believed that the composition of the intestinal microbiota likely plays a significant role (3).

Toxin gene profiling revealed that all six isolates were positive for toxin genes tcdA and tcdB, which are associated with the production of toxins A and B. However, none of the isolates tested positive for the binary toxin genes cdtA/B. This toxin gene profile is consistent with toxigenic *C. difficile* strains commonly associated with human infections (21).The small sample size is a limitation of this study, which may affect the generalizability of the findings. However, this study serves as a valuable baseline for future surveillance and characterization of *C. difficile* in broiler chickens.

CONCLUSION

To the best of our knowledge, there is no prior documentation regarding the prevalence of *C. difficile* in broiler chickens in Romania. Therefore, this study represents the first attempt to observe and determine the prevalence and toxin type of *C. difficile* in broiler chickens within the country. One of the limitations of this study is the relatively small sample size. However, the study provides a crucial foundation for future surveillance and characterization of *C. difficile* in food-producing animals. The findings of this study provide significant insights that can be applied by specialists involved in the management and control of *C. difficile* infections. Additionally, these findings serve as a starting point for future research, which may encompass additional investigations such as antimicrobial susceptibility testing and ribotyping analysis of the strains. These analyses can help determine whether broiler chickens can be considered a source of community-associated *C. difficile* infection (CA-CDI) in Romania.

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