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Determination of Antimicrobial Susceptibility of *Clostridium Perfringens* Strains Isolated from Healthy and Diseased Ostriches (*Struthio camelus*)

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A B S T R A C T

Gastrointestinal diseases are considered the most prevalent and economically considerable diseases in the ostrich breeding industry, in which necrotic enteritis caused by *C. perfringens* induces high mortality, especially in ostrich chicks. Several antimicrobial agents are used to prevent enteric diseases, enhancing growth rate and increasing feed conversion ratio. This procedure results in a high prevalence of resistance among enteric bacteria with the possibility of a consequent emergence of antibiotic resistance in zoonotic enteropathogens. This study determined the susceptibility of *C. perfringens* strains isolated from the intestine and faeces of disease and healthy ostriches in southeast Iran to 8 antimicrobial agents. A total of 40 *C. perfringens* isolates were collected from several ostrich flocks and were tested using the broth microdilution method. The susceptibility of obtained isolates to antibiotics was as follows: ceftriaxone (80%), cefazolin (77.5%), florfenicol (72.5%), tetracycline (62.5%), penicillin (47.5%), sulfadiazine (20%), sulfadimidine (7.5%) and neomycin (7.5%). In conclusion, *C. perfringens* strains isolated from ostriches should be tested and monitored for antibacterial susceptibility patterns. The present study is the first to determine the antimicrobial susceptibility of *C. perfringens* isolated from ostrich.

Keywords: *Ostrich, C. perfringens, necrotic enteritis, Broth Microdilution, Antimicrobial Susceptibility.*

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1 Introduction

Clostridium perfringens (*C. perfringens*) is a common environmental Gram-positive spore form in anaerobic bacterium, and normal gastrointestinal tract flora is the causal agent of a broad range of diseases in humans and animals (1-3). This bacterial species is the most significant cause of clostridial enteric disease in domestic animals (4, 5). Necrotic enteritis (NE) is one of the most economically crucial enteric poultry diseases, especially in broilers and turkeys, which induces more generally determined fulminant infection, which can result in outbreaks with various mortality rates (6-8). In addition to the economic significance of *C. perfringens* in poultry, it poses a risk to public health via the food chain (2, 9-11). Gastrointestinal diseases are considered the most prevalent and economically considerable diseases in the ostrich-rearing industry. The predisposing factors contributing to disease development are poor management, stress, lack of environmental hygiene, and other similar diseases primarily caused by different bacterial infections (2, 12, 13). NE with different types and species of *Clostridium* as causative agents, such as *C. perfringens*, *C. difficile*, and *C. sordellii*, have been often associated with necrotising enterocolitis in ostrich in which *C. perfringens* is the most common agent involved in ostrich enteritis among this pathogenic microbial population (14-17). Several antibiotic drug combinations called Antibiotic Growth Promoters (AGPs) are added to poultry feed at sub-therapeutic levels to enhance growth rate, improve feed conversion ratio, and decrease the outbreak of different diseases (8, 18-23). AGPs have been used worldwide in poultry production since their beneficial outcomes were demonstrated for the first time (22, 24-26). Over the years, the widespread use of antibiotics in human and animal medicine has induced a significant microflora pressure and, subsequently, manifestation of antibiotic resistance phenomenon among pathogenic bacteria (27-29). In Iran, all poultry-producing areas and most of the broiler-producing countries of Europe, avian clostridial diseases such as NE in ostrich are controlled by the routine use of a combination of antimicrobial agents and ionophore anticoccidial agents, both of which possess antibiotic effects on Gram-positive bacteria such as *C. perfringens* (15, 21, 23, 30, 31). Hence, antibiotic resistance has significantly increased in different groups of bacteria in recent years and has become a global issue with significant repercussions for public health. So, the scientific community has noted the necessity for antibiotic susceptibility evaluation in indicator bacteria from various sources to measure antibiotic resistance's evolution. Subsequently, much attention is being focused on this worldwide problem, which has led to the banning and regulation of AGP usage by some countries.

There are various recommended methods for antibiotic susceptibility testing based on the Clinical and Laboratory Standards Institute (CLSI) (32) in which the agar dilution method has been mentioned as the reference method to which all other methods should be compared according to the National Committee for Clinical Laboratory Standards (NCCLS) (33). Minimum Inhibitory Concentrations (MICs) are considered the gold standard method for specifying the susceptibility of organisms to antibiotics and, hence, are used to judge the performance of all other susceptibility testing methods. MICs are used in diagnostic laboratories to confirm uncommon resistance and give a decisive answer when a borderline result is obtained by different testing methods (34). In the ostrich breeding industry of Iran, NE, mainly caused by *C. perfringens*, is one of the most important fatal diseases in which antimicrobial agents are used extensively to prevent diseases; therefore, assessing potential increases in antibiotic resistance is a concerning subject. Since there is no information about the antibiotic susceptibility of *C. perfringens* strains in ostriches, this is the first study to determine the *in-vitro* resistance of *C. perfringens* to eight common antibiotics relevant to ostrich breeding.

2 Methods and Materials

2.1 Source of isolates

A total of 40 *C. perfringens* isolates were collected from 40 ostrich flocks located in different parts of Iran, which have been obtained and archived in a previous field study from 2010 to 2014 (35). Twenty bacterial isolates with the acute form of NE were obtained from twenty ostrich flocks, and the remaining isolates were selected from twenty healthy flocks. In the history of these farms, the commonly used antibiotics include oxytetracycline, neomycin, enrofloxacin and trimetprim+sulfadimethoxin. Ostriches from healthy flocks were subjected to the autopsy, and isolates of *C. perfringens* were collected aseptically with sterilised cotton swabs from intestinal samples. Also, all isolates obtained from diseased ostrich flocks with high mortality due to NE were confirmed by autopsy, and sampling was performed by scrubbing the intestinal wall of affected birds.

2.2 Bacterial identification and growth conditions

All intestinal samples were inoculated on blood agar containing 7% defibrinated sheep blood and incubated anaerobically at 37ºC for 48 hr. Colonies which showed characteristic double hemolysis zones were selected and sub-cultured on selective culture media of *C. perfringens*, including Tryptose Sulfite Cycloserine agar (TSC) and Tryptose Sulfite Neomycin agar (TSN), to obtain purified bacterial isolates. The identity of all isolates was verified by their colonial and microscopical morphology, hemolytic pattern, Gram staining of faecal specimens and intestinal scraping and biochemical tests as described previously (36). Gram staining of tissue specimens from field cases of NE was also performed. All culture media used in this study were purchased from Merck® (Germany). All confirmed *C. perfringens* isolates were stored at -70°C in 20% glycerol in brain heart infusion (BHI) until further usage. The reference strain of *C. perfringens* ATCC 13124 was used as a positive control and included in every test batch (Pasteur Institute Collection, Paris, France). This strain was stored under conditions similar to those of the clinical isolates. All treatments of birds were conducted according to Animal Care Guidelines of the Research Committee, Faculty of Veterinary Medicine Ferdowsi University of Mashhad.

2.3 Antimicrobial susceptibility testing

All the procedures, interpretive criteria and breakpoints were according to Clinical and Laboratory Standards Institute guidelines (32). The anti-clostridial activity of eight different antimicrobials was evaluated in the susceptibility testing technique using broth microdilution (Table). Antimicrobial dilution ranges were established based on previously published studies (37, 38). The broth microdilution plates were purchased in a frozen flat bottom tissue culture 96-well format (BIOFIL®, Guangzhou, China). Each well contained 50 µl of 2 × antimicrobial dilution in supplemented brucella broth for anaerobes. Serial two-fold dilutions of antibiotics were made. For MIC determination, after *C. perfringens* growth at 37°C on blood agar plates under anaerobic conditions for 24 h, 2 to 3 confirmed colonies were resuspended into 5 ml of buffered saline to achieve a 0.5 McFarland turbidity. This suspension was diluted 20-fold, and approximately 1×10^5 colony-forming units of each bacterial strain were inoculated using a Denley multipoint inoculator. One hundred microliters of this suspension were then transferred to 11 ml of supplemented brucella broth. This suspension was thoroughly mixed, and 50 µl aliquots were dispensed into each of the wells on MIC microplates for a final volume of 100 µl. The microplates were sealed with perforated sealers and incubated overnight at 37°C in square anaerobic jars (Merck®, Germany).

A reference strain of *C. perfringens* ATCC 13124 was included as a control with every batch tested. Two different MICs were tested for this strain in triplicate on three other days, yielding 18 test replications and readings. A fresh bacterial suspension was prepared for each replicate in 5 ml of distilled water from overnight bacterial growth on blood agar plates. Bacterial counts were performed to distinguish the bacterial numbers in the plate wells. Following the susceptibility test, panels were inoculated with the bacterial suspension,100 ml was withdrawn from antimicrobial-free growth control wells and serially diluted in phosphate-buffered saline solution. Aliquots of these dilution series were plated on blood agar plates, and bacterial counts were registered after 24 h incubation in an anaerobic condition at 37°C. Meanwhile, a sterility test was done by inoculating a panel with supplemented brucella broth only, followed by incubation. The MIC50 and MIC90 were read randomly using a lightbox (Sensititre, Trek Diagnostic systems), and they were determined as the lowest concentration of the antimicrobial agent, which inhibited at least 50% and 90% of the visible bacterial growth.

3 Results

The reproducibility of MICs with reference *C. perfringens* was established by 18 independent experiments for each antimicrobial agent within one doubling dilution. Identical MIC values were obtained with all replicates for each antimicrobial agent. The average colony forming units of *C. perfringens* ATCC 13124 per ml of growth media was 1×10^5, as evaluated in each experiment. The lowest value was 9.2×104 CFU/ml, and the highest was 1.1×10^5 CFU/ml. The isolates were categorised as susceptible or resistant according to the microbiological criteria based on MIC distributions of the National Committee for Clinical Laboratory Standards guidelines (32). According to the CLSI instructions for reading the MIC of antimicrobial agents, ceftriaxone and cefazolin showed the lowest MIC50 (1 µg/ml) against ostrich isolates with susceptibility of 80% and 77.5%, respectively. Penicillin and florfenicol were in second place with MIC50 of 4 µg/ml MIC and susceptibility levels of 47.5% and 72.5%, respectively. Tetracycline and neomycin were placed in the third and fourth rank with the

MIC50 of 16 µg/ml and 512 µg/ml, and the susceptibility level of *C. perfringens* isolates against them was 62.5% and 7.5%. Sulfadimidine and sulfadiazine showed the highest MIC50 (>1024 µg/ml) and lowest susceptibility levels of 7.5% and 20%. MIC90 was most insufficient for penicillin, florfenicol and tetracycline (256 mg/ml), followed by ceftriaxone, cefazolin and neomycin (512mg/ml), sulfadimidine and sulfadimidine (>1024 µg/ml). The MIC values, MIC50 and MIC90 values for each antimicrobial agent against obtained *C. perfringens* isolates are summarised in Table 1.

Table 1. Frequency distribution of MICs of eight antimicrobial agents obtained by broth microdilution method on 40 C. perfringens strains isolated from ostriches.

	MIC (µg\ml)																	
Antibiotic	<0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024	MIC_{50}	MIC_{90}	MIC_{bp}[1]
CTR	0	0	16	6	2	4	2	0	2	0	0	0	8	0	0	1	512	≤16
TCN	0	7	1	1	3	3	3	7	2	4	1	8	0	0	0	16	256	≤4
FFC	0	5	2	3	8	6	1	2	2	1	1	9	0	0	0	4	256	≤8
NEO	0	1	1	1	0	1	1	1	2	5	0	0	27	0	0	512	512	---
SFD	0	0	0	0	1	0	0	1	0	6	2	2	0	3	25	>1024	>1024	---
SDM	0	0	0	0	1	0	0	0	0	2	2	3	2	6	25	>1024	>1024	---
CEZ	0	0	0	21	3	3	1	3	0	0	0	0	9	0	0	1	512	≤16
PEN	12	5	1	1	0	2	2	2	1	0	0	14	0	0	0	4	256	≤0.05

(CTR: Ceftriaxone, TCN: Tetracycline, FFC: Florfenicol, NEO: Neomycin, SFD: Sulfadiazine; SDM: Sulfadimidine, CEZ: Cefazolin, PEN: Penicillin).
MIC_{bp}: Minimal Inhibitory Concentration Break-point (Susceptibility level)
[1] All MIC breakpoints were adopted from CLSI 2018. Methods for antimicrobial susceptibility testing of anaerobic bacteria**.** Twenty-eight edition. Approved Standard M100. *National Committee for Clinical Laboratory Standards*, Wayne, P.A.)

The resistance percentage of *C. perfringens* strains to sulfadimidine, sulfadiazine and neomycin in both healthy and diseased groups was high. Despite the high susceptibility (73.6%) of isolates to cefazolin in diseased ostriches, interestingly, *C. perfringens* strains belonging to the healthy group showed low susceptibility. Furthermore, in both groups, the susceptibility of isolates to penicillin, florfenicol, tetracycline and ceftriaxone was high. The antibiotic susceptibility pattern of *C. perfringens* strains obtained from healthy and diseased ostriches is presented in Table 2. Biochemical properties of *C. perfringens* strains in this study are shown in Table 3.

Table 2. Antibiogram profile of C. perfringens isolates obtained from healthy (H) and diseased (D) ostriches.

Frequency Percentage								
	PEN	CEZ	SDM	SFD	NEO	FFC	TCN	CTR
4.76 (H)	S	S	S	S	S	S	R	S
4.76 (H)	S	S	S	S	R	S	S	S
23.81 (H)	S	S	R	R	R	S	S	S
9.52 (H)	R	S	R	R	R	R	R	S
14.29 (H)	R	S	R	R	R	S	R	S
9.52 (H)	S	S	R	S	R	S	S	S
9.52 (H)	R	R	R	R	R	R	R	R
4.76 (H)	R	R	S	S	S	R	S	S
4.76 (H)	R	R	R	R	R	S	S	S
4.76 (H)	R	S	R	R	R	S	S	S
4.76 (H)	S	S	R	R	R	S	S	R
4.76 (H)	R	S	R	R	R	R	S	S
15.79 (D)	R	S	R	R	R	S	S	S
26.32 (D)	S	S	R	R	R	S	S	S
26.32 (D)	R	R	R	R	R	R	R	R
10.53 (D)	R	S	R	R	R	S	R	S
5.26 (D)	S	S	R	R	S	S	S	S
15.79 (D)	S	S	R	S	R	S	S	S

CTR: Ceftriaxone, TCN: Tetracycline, FFC: Florfenicol, NEO: Neomycin
SFD: Sulfadiazine, SDM: Sulfadimidine, CEZ: Cefazolin, PEN: Penicillin

Table 3. Biochemical properties of C. perfringens strains isolated in this study

Basic Characteristics		Properties
Gram staining	+/_	/ 40 / 0
Hemolysis	α / β / Double Zone	0 / 0 / 40
Motility	Motile / Non-motile	0 / 40
Shape		Straight Rods with Blunt Ends
Capsule	+/_	40 / 0
Gas	+/_	40 / 0
Spore	+/_	40 / 0
Indole	+/_	0 / 40
Glucose	+/_	38 / 2
Maltose	+/_	36 / 4
Lactose	+/_	39 / 1
Sucrose	+/_	39 / 1
Manitol	+/_	29 / 11
Oxidase	+/_	0 / 40
Catalase	+/_	0 / 40
Fructose	+/_	40 / 0
Gelatin Hydrolysis	+/_	40 / 0
Lecithinase	+/_	40 / 0
Lipase	+/_	0 / 40
Amylase	+/_	40 / 0
Elastase	+/_	40 / 0

4 Discussion

Antibiotic feed additives in poultry nutrition lead to high outbreaks of resistance between their intestinal bacterial flora, resulting in antibiotic resistance in zoonotic enteropathogens. Despite increasing concerns about the emergence of antimicrobial-resistant strains, which indicates diverse spread in different geographic regions, more work needs to be done to investigate this issue in ostrich with NE.

To the best of our knowledge, the present study is the first report about the determination of antimicrobial susceptibility of *C. perfringens* strains collected from ostriches with faecal and intestinal origin. In this study, we tried to obtain widespread data on the resistance situation of *C. perfringens* on ostrich farms in Iran. The purpose of providing specimens from many different flocks and farms rather than including many strains from a few farms is to evaluate the *C. perfringens* reference strain. The broth microdilution method used in this study is a reproducible way of specifying MICs for different antimicrobial agents. Although there is no data about the antimicrobial susceptibility of *C. perfringens* isolated from ostriches' intestines and faeces, several similar studies on the poultry field exist. In Iran, the most common antimicrobial agents used in the ostrich industry during NE and gastrointestinal diseases include tetracycline, neomycin, sulfadiazine and sulfadimidine, respectively. Nevertheless, the *C. perfringens* isolates obtained from both groups were still susceptible to tetracycline in the third rank, suggesting relative susceptibility to this common antibiotic among the *C. perfringens* bacterial population. In contrast, there is a high resistance against neomycin, sulfadimidine and sulfadiazine, which are used in the ostrich industry in both groups, which shows the necessity to restrict the application of these antibiotics.

On the other hand, *C. perfringens* isolates had high susceptibility to ceftriaxone and cefazolin as human medicine antibiotics and their use is forbidden in veterinary medicine. The high resistance of isolates obtained from healthy ostrich flock to sulfadimidine, sulfadiazine and neomycin would be due to their widespread usage and transfer of plasmids containing resistance genes between *C. perfringens* strains.

In recent studies, the researchers stated that all *C. perfringens* isolated from broiler chickens with NE were susceptible to penicillin, which is consistent with the present research on the susceptibility of ostrich-origin isolates to this antibiotic (39, 40). In several studies, the researchers reported higher levels of tetracycline resistance in Swedish C. perfringens strains isolated from different broiler farms (40-42). In contrast, despite the extensive use of tetracycline in cases of ostrich with NE in Iran, little resistance against *C. perfringens* isolates has been found in the current study. In Egypt, the antimicrobial susceptibility of *C. perfringens* strains in broiler chickens revealed that florfenicol is one of the helpful antimicrobial agents similar to our research with

high susceptibility to ostrich isolates (43). In keeping with our results, an investigation in Belgian broilers showed that all tested isolates were sensitive to florfenicol, which shows complete susceptibility of obtained strains against *C. perfringens* with NE (44). Meanwhile, they stated that most of the isolates showed acquired resistance to tetracycline, which this finding was in contrast with the present study in which susceptibility of tetracycline against *C. perfringens* isolates was demonstrated. In another investigation, the researchers recommended that *C. perfringens* infections in broilers of Jordan could be treated with either penicillin or tetracycline. Their finding was similar to ours but with different poultry origins (45).

In the current study, MIC50 of penicillin and tetracycline were 4 µg/ml and 16 µg/ml, respectively. Still, in an analysis from Ontario, Canada, to evaluate antimicrobial susceptibility in various animal species, the MIC of penicillin and tetracycline were lower and higher in chickens than in this study. Hence, they stated that higher susceptibility of penicillin against chicken *C. perfringens* strains and resistance to tetracycline was spread across all species, such as chicken origin, in contrast to the present investigation (38).

A surprising finding in this study was the decreased susceptibility of penicillin against *C. perfringens* isolates compared to the results of other studies in broilers and turkeys (38, 40, 43, 46, 47). On the other hand, several studies in human medicine reported resistance to beta-lactam antibiotics mediated by the decreased affinity of essential penicillin-binding protein (48-52), and this report is in agreement with our results. Hence, the lower susceptibility of ostrich isolates compared to broiler strains could be due to decreased affinity of the mentioned protein or increased usage of human medicine drugs in studied ostrich farms without our knowledge. Meanwhile, in a study, the researchers reported high penicillin susceptibility against *C. perfringens* strains obtained from broilers. However, they increased the MIC of this antibiotic compared to a similar study several years ago, indicating resistance formation over several years (45). Although *C. perfringens* is innately resistant to neomycin, we have found 7.5% susceptibility of *C. perfringens* strains against this antibiotic in this study. This finding is inconsistent with previous studies in broilers and turkeys, which reported poor efficacy of this antibiotic to *C. perfringens* isolates with 93% and 94% resistance levels, respectively (43, 46). According to the antimicrobial susceptibility testing of *C. perfringens* isolates in canine species, the researchers reported the high resistance of bacterial strains against tetracycline, which this antibiotic should be avoided for the treatment of *C. perfringens-associated* diarrhea in dogs (53-55), and this finding is I contrast with result of our study that we reported susceptibility of ostrich *C. perfringens* isolates to this antibiotic.

Due to the relatively high prevalence of resistance and the potential for the outbreak of antimicrobial resistance, this agrees with the other studies. However, there was still relative susceptibility of *C. perfringens* against (49, 56). A decreased susceptibility to some antimicrobial agents of practical relevance for therapy and prevention of ostrich enteric diseases induced by *C. perfringens*, the most prevalent antibiotics, was observed. As explained, decreased susceptibilities were distributed less regularly between the investigated poultry species. They indicated a potential correlation with the particular antimicrobial use practices in each of these poultry species, so various distributions of several antimicrobial agents were observed in ostrich isolates similar to other poultry species, as described before. These findings agree with the published reports on the distribution of MIC values of tetracycline used in enteric diseases of poultry (38, 41, 44) but in contrast with the susceptibility of this antibiotic to the *C. perfringens* of ostrich origin.

In summary, for the first time, this study creates a baseline for antimicrobial agent susceptibility of *C. perfringens* isolated from ostrich in southeast Iran. The results indicate widespread resistance of *C. perfringens* strains to antimicrobial agents. Some decreased susceptibility to multiple antimicrobials customarily used in enteric diseases of the ostrich was observed in this study, thus suggesting the potential for therapeutic challenges in the future if the necessary attention is not given to avoid the selection of multi-resistant organisms.

5 Conclusion

In conclusion, based on the results of the current study, monitoring of antibacterial susceptibility patterns of *C. perfringens* strains isolated from infected ostriches should be routine to investigate the tendencies in susceptibility of *C. perfringens* isolates, considering the possibility of occurring antibiotic resistance between animal and human pathogenic bacterial population. In addition, *C. perfringens* infections diagnosed and isolated in ostriches of Iran should be treated with tetracycline and florfenicol, for which resistant isolates are rare.

Conflict of Interest

Authors declare there is no conflict of interest.

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Author Contributions

AA participated in coordinating the study. All authors contributed in design and manuscript writing. All authors have read and agreed to published version of the manuscript.

Data Availability Statement

Data are available from the first author upon reasonable request.

References

1. Borriello S. Clostridial diseases of the gastrointestinal tract in animals. Clostridia in gastrointestinal disease: CRC Press; 2018. p. 195-215. [DOI]
2. Immerseel FV, Buck JD, Pasmans F, Huyghebaert G, Haesebrouck F, Ducatelle R. Clostridium perfringens in poultry: an emerging threat for animal and public health. Avian pathology. 2004;33(6):537-49. [PMID: 15763720] [DOI]
3. Uzal FA, Freedman JC, Shrestha A, Theoret JR, Garcia J, Awad MM, et al. Towards an understanding of the role of Clostridium perfringens toxins in human and animal disease. Future microbiology. 2014;9(3):361-77. [PMID: 24762309] [PMCID: PMC4155746] [DOI]
4. Silva ROS, Lobato FCF. Clostridium perfringens: A review of enteric diseases in dogs, cats and wild animals. Anaerobe. 2015;33:14-7. [PMID: 25644183] [DOI]
5. Songer JG. Clostridial enteric diseases of domestic animals. Clinical microbiology reviews. 1996;9(2):216-34. [PMID: 8964036] [PMCID: PMC172891] [DOI]
6. McDevitt R, Brooker J, Acamovic T, Sparks N. Necrotic enteritis; a continuing challenge for the poultry industry. World's Poultry Science Journal. 2006;62(2):221-47. [DOI]
7. Paiva D, McElroy A. Necrotic enteritis: Applications for the poultry industry. Journal of Applied Poultry Research. 2014;23(3):557-66. [DOI]
8. Tsiouris V. Poultry management: a useful tool for the control of necrotic enteritis in poultry. Avian Pathology. 2016;45(3):323-5. [PMID: 26950433] [DOI]
9. Engström B, Fermer C, Lindberg A, Saarinen E, Båverud V, Gunnarsson A. Molecular typing of isolates of Clostridium perfringens from healthy and diseased poultry. Veterinary microbiology. 2003;94(3):225-35. [PMID: 12814890] [DOI]
10. Ghoneim N, Hamza D. Epidemiological studies on Clostridium perfringens food poisoning in retail foods. Rev Sci Tech. 2017;36(3):1025-32. [PMID: 30160688] [DOI]
11. Grass JE, Gould LH, Mahon BE. Epidemiology of foodborne disease outbreaks caused by Clostridium perfringens, United States, 1998–2010. Foodborne pathogens and disease. 2013;10(2):131-6. [PMID: 23379281] [PMCID: PMC4595929] [DOI]
12. Allaart JG, van Asten AJ, Gröne A. Predisposing factors and prevention of Clostridium perfringens-associated enteritis. Comparative immunology, microbiology and infectious diseases. 2013;36(5):449-64. [PMID: 23790636] [DOI]
13. Moore RJ. Necrotic enteritis predisposing factors in broiler chickens. Avian Pathology. 2016;45(3):275-81. [PMID: 26926926] [DOI]
14. Frazier KS, Herron AJ, Hines ME, Gaskin JM, Altman NH. Diagnosis of enteritis and enterotoxemia due to Clostridium difficile in captive ostriches (Struthio camelus). Journal of Veterinary Diagnostic Investigation. 1993;5(4):623-5. [PMID: 8286467] [DOI]
15. Keokilwe L, Olivier A, Burger W, Joubert H, Venter EH, Morar-Leather D. Bacterial enteritis in ostrich (Struthio camelus) chicks in the western cape province, South Africa. Poultry Science. 2015;94(6):1177-83. [PMID: 25840967] [DOI]
16. Razmyar J, Kalidari GA, Tolooe A, Rad M, Movassaghi AR. Genotyping of Clostridium perfringens isolated from healthy and diseased ostriches (Struthio camelus). Iranian journal of microbiology. 2014;6(1):31.
17. Shivaprasad H. Hepatitis associated with Clostridium difficile in an ostrich chick. Avian Pathology. 2003;32(1):57-62. [PMID: 12745381] [DOI]
18. Bedford M. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. World's Poultry Science Journal. 2000;56(4):347-65. [DOI]
19. Calik A, Omara II, White MB, Evans NP, Karnezos TP, Dalloul RA. Dietary non-drug feed additive as an alternative for antibiotic growth promoters for broilers during a necrotic enteritis challenge. Microorganisms. 2019;7(8):257. [PMID: 31412542] [PMCID: PMC6723652] [DOI]
20. Dahiya J, Wilkie D, Van Kessel A, Drew M. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Animal Feed Science and Technology. 2006;129(1-2):60-88. [DOI]
21. Hussein EO, Ahmed SH, Abudabos AM, Suliman GM, Abd El-Hack ME, Swelum AA, N. Alowaimer A. Ameliorative effects of antibiotic-, probiotic-and phytobiotic-supplemented diets on the performance, intestinal health, carcass traits, and meat quality of Clostridium perfringens-infected broilers. Animals. 2020;10(4):669. [PMID: 32290578] [PMCID: PMC7222811] [DOI]
22. M'Sadeq SA, Wu S, Swick RA, Choct M. Towards the control of necrotic enteritis in broiler chickens with in-feed antibiotics phasing-out worldwide. Animal Nutrition. 2015;1(1):1-11. [PMID: 29766984] [PMCID: PMC5884463] [DOI]
23. Pham VH, Abbas W, Huang J, He Q, Zhen W, Guo Y, Wang Z. Effect of blending encapsulated essential oils and organic acids as an antibiotic growth promoter alternative on growth performance and intestinal health in broilers with necrotic enteritis. Poultry Science. 2022;101(1):101563. [PMID: 34823183] [PMCID: PMC8628017] [DOI]

24. Costa MC, Bessegatto JA, Alfieri AA, Weese JS, Filho JA, Oba A. Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. PLoS One. 2017;12(2):e0171642. [PMID: 28222110] [PMCID: PMC5319738] [DOI]
25. Huyghebaert G, Ducatelle R, Van Immerseel F. An update on alternatives to antimicrobial growth promoters for broilers. The Veterinary Journal. 2011;187(2):182-8. [PMID: 20382054] [DOI]
26. Upadhayay U, Vishwa PCV. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: the trends and advances-a review. Int J Pharmacol. 2014;10(3):129-59. [DOI]
27. Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipsitch M. Antibiotic resistance—the interplay between antibiotic use in animals and human beings. The Lancet infectious diseases. 2003;3(1):47-51. [PMID: 12505035] [DOI]
28. Sørum H, Sunde M. Resistance to antibiotics in the normal flora of animals. Veterinary research. 2001;32(3-4):227-41. [PMID: 11432415] [DOI]
29. van den Bogaard AE, Stobberingh EE. Epidemiology of resistance to antibiotics: links between animals and humans. International journal of antimicrobial agents. 2000;14(4):327-35. [PMID: 10794955] [DOI]
30. Butaye P, Devriese LA, Haesebrouck F. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clinical microbiology reviews. 2003;16(2):175-88. [PMID: 12692092] [PMCID: PMC153145] [DOI]
31. Elbadawy M, Aboubakr M. Efficacy of colimoxŪ (a new combination of amoxicillin and colistin) in the control of experimentally induced necrotic enteritis in broiler chickens. Int J Pharmacol Toxicol. 2017;5:51-6. [DOI]
32. Weinstein MP, Lewis JS. The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. Journal of clinical microbiology. 2020;58(3):10.1128/jcm. 01864-19. [PMID: 31915289] [PMCID: PMC7041576] [DOI]
33. Kiehlbauch JA, Hannett GE, Salfinger M, Archinal W, Monserrat C, Carlyn C. Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. Journal of clinical microbiology. 2000;38(9):3341-8. [PMID: 10970381] [PMCID: PMC87384] [DOI]
34. Nhung NT, Chansiripornchai N, Carrique-Mas JJ. Antimicrobial resistance in bacterial poultry pathogens: a review. Frontiers in veterinary science. 2017;4:126. [PMID: 28848739] [PMCID: PMC5554362] [DOI]
35. Jafari SM, Kalidari G, Peighambari SM, Razmyar J. Ostrich as a possible source of pathogenic strains of Clostridium difficile. The Thai Journal of Veterinary Medicine. 2017;47(4):537-41. [DOI]
36. Maldonado-Barragán A, Caballero-Guerrero B, Lucena-Padrós H, Ruiz-Barba JL. Induction of bacteriocin production by coculture is widespread among plantaricin-producing Lactobacillus plantarum strains with different regulatory operons. Food microbiology. 2013;33(1):40-7. [PMID: 23122499] [DOI]
37. Marks SL, Kather EJ. Antimicrobial susceptibilities of canine Clostridium difficile and Clostridium perfringens isolates to commonly utilized antimicrobial drugs. Veterinary microbiology. 2003;94(1):39-45. [PMID: 12742714] [DOI]
38. Slavić Đ, Boerlin P, Fabri M, Klotins KC, Zoethout JK, Weir PE, Bateman D. Antimicrobial susceptibility of Clostridium perfringens isolates of bovine, chicken, porcine, and turkey origin from Ontario. Canadian Journal of Veterinary Research. 2011;75(2):89-97. [PMID: 21731178] [PMCID: PMC3062930] [DOI]
39. Llanco L, Nakano V, Ferreira A, Avila-Campos MJ. Toxinotyping and antimicrobial susceptibility of Clostridium perfringens isolated from broiler chickens with necrotic enteritis. International Journal of Microbiology Research. 2012;4(7):290. [DOI]
40. Silva R, Salvarani F, Assis R, Martins N, Pires P, Lobato F. Antimicrobial susceptibility of Clostridium perfringens strains isolated from broiler chickens. Brazilian Journal of Microbiology. 2009;40:262-4. [PMID: 24031355] [PMCID: PMC3769726] [DOI]
41. Johansson A, Greko C, Engström B, Karlsson M. Antimicrobial susceptibility of Swedish, Norwegian and Danish isolates of Clostridium perfringens from poultry, and distribution of tetracycline resistance genes. Veterinary microbiology. 2004;99(3-4):251-7. [PMID: 15066727] [DOI]
42. Sasaki Y, Yamamoto K, Tamura Y, Takahashi T. Tetracycline-resistance genes of Clostridium perfringens, Clostridium septicum and Clostridium sordellii isolated from cattle affected with malignant edema. Veterinary microbiology. 2001;83(1):61-9. [PMID: 11524166] [DOI]
43. Osman K, Elhariri M. Antibiotic resistance of Clostridium perfringens isolates from broiler chickens in Egypt. Rev Sci Tech. 2013;32(3):841-50. [PMID: 24761735] [DOI]
44. Gholamiandehkordi A, Eeckhaut V, Lanckriet A, Timbermont L, Bjerrum L, Ducatelle R, et al. Antimicrobial resistance in Clostridium perfringens isolates from broilers in Belgium. Veterinary research communications. 2009;33:1031-7. [PMID: 19597952] [DOI]
45. Gharaibeh S, Al Rifai R, Al-Majali A. Molecular typing and antimicrobial susceptibility of Clostridium perfringens from broiler chickens. Anaerobe. 2010;16(6):586-9. [PMID: 20969969] [DOI]
46. Gad W, Hauck R, Krüger M, Hafez H. Determination of antibiotic sensitivities of Clostridium perfringens isolates from commercial turkeys in Germany in vitro. Archiv für Geflügelkunde. 2011;75(2):80-3.
47. Watkins K, Shryock T, Dearth R, Saif Y. In-vitro antimicrobial susceptibility of Clostridium perfringens from commercial turkey and broiler chicken origin. Veterinary Microbiology. 1997;54(2):195-200. [PMID: 9057262] [DOI]
48. Alshammari MK, AlKhulaifi MM, Al Farraj DA, Somily AM, Albarrag AM. Incidence of Clostridium perfringens and its toxin genes in the gut of children with autism spectrum disorder. Anaerobe. 2020;61:102114. [PMID: 31704282] [DOI]
49. Anju K, Karthik K, Divya V, Priyadharshini MLM, Sharma RK, Manoharan S. Toxinotyping and molecular characterization of antimicrobial resistance in Clostridium perfringens isolated from different sources of livestock and poultry. Anaerobe. 2021;67:102298. [PMID: 33220406] [DOI]
50. Forbes JD, Kus JV, Patel SN. Antimicrobial susceptibility profiles of invasive isolates of anaerobic bacteria from a large Canadian reference laboratory: 2012–2019. Anaerobe. 2021;70:102386. [PMID: 34044100] [DOI]
51. Li J, Zhou Y, Yang D, Zhang S, Sun Z, Wang Y, et al. Prevalence and antimicrobial susceptibility of Clostridium perfringens in chickens and pigs from Beijing and Shanxi, China. Veterinary Microbiology. 2021;252:108932. [PMID: 33316633] [DOI]
52. Xiaoting W, Chengcheng N, Chunhui J, Yan L, Jing L, Qingling M, et al. Antimicrobial resistance profiling and molecular typing of ruminant-borne isolates of Clostridium perfringens from Xinjiang, China. Journal of Global Antimicrobial Resistance. 2021;27:41-5. [PMID: 34438107] [DOI]

53. Park M, Rafii F. The prevalence of plasmid-coded cpe enterotoxin, β2 toxin, tpeL toxin, and tetracycline resistance in Clostridium perfringens strains isolated from different sources. Anaerobe. 2019;56:124-9. [PMID: 30802555] [DOI]
54. Viegas FM, Ramos CP, Xavier RGC, Lopes EO, Júnior CAO, Bagno RM, et al. Fecal shedding of Salmonella spp., Clostridium perfringens, and Clostridioides difficile in dogs fed raw meat-based diets in Brazil and their owners' motivation. PLoS One. 2020;15(4):e0231275. [PMID: 32287295] [PMCID: PMC7156072] [DOI]
55. Yadav JP, Kaur S, Dhaka P, Vijay D, Bedi JS. Prevalence, molecular characterization, and antimicrobial resistance profile of Clostridium perfringens from India: A scoping review. Anaerobe. 2022;77:102639. [PMID: 36108893] [DOI]
56. Jubeh B, Breijyeh Z, Karaman R. Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches. Molecules. 2020;25(12):2888. [PMID: 32586045] [PMCID: PMC7356343] [DOI]