ISSN 1682-8356
ansinet.com/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2023.90.98

Research Article
Correlation Between Environmental and Intestinal *Clostridium perfringens* Isolated from Different Chicken Flocks

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Abstract

Background and Objective: *Clostridium perfringens* is a major enteric pathogen of poultry causing necrotic enteritis (NE). Both clinical and subclinical forms of NE are associated with a huge economic loss, so it is very important to detect and study the correlation between environmental and intestinal *C. perfringens* isolated from different origins in poultry farms. **Materials and Methods:** A total of 20 intestinal samples were collected from 20 different commercially diseased poultry flock in Egypt, as well as 10 environmental samples from 10 of the 20 farms that had clinical NE. Statistical package for the social sciences was used for cluster analysis and dendrogram construction. Similarity index between all samples was calculated using the online tool. **Results:** The bacterial susceptibility patterns of both environmental and intestinal isolates showed high resistance index of 100% against streptomycin sulphate, sulfamethoxazole+trimethoprim, tetracycline and spectinomycin. The resistance reached 70 and 100% to ampicillin and cefotaxime: 50 and 80% to amoxicillin: 65 and 70% to bacitracin for intestinal and environmental *C. perfringens* isolates, respectively, while the lowest resistance (27%) was to penicillin V. Using cPCR, all isolates carried α toxin gene, while 60 and 25% of intestinal *C. perfringens* harbored *net*β and β-lactamase (*bla*), meanwhile, 40 and 60% of environmental *C. perfringens* were positive for *net*β and *bla* genes, respectively. **Conclusion:** The results of RAPD analysis, similarity index and dendrograms for 4 environmental and intestinal *C. perfringens* isolates (with *bla* gene) showed high similarity mainly with the same ancestor of environmental origin, which may explained that contamination with *C. perfringens* in the environment acts as a source of horizontal *bla* gene transfer between different *C. perfringens* strains within poultry farms.

Key words: Animal health, antimicrobial resistance, chicken flocks, *Clostridium perfringens*, cPCR, necrotic enteritis, RAPD, toxins

Citation: Bastamy, M., I. Raheel, M. Yehia, M. Hamoud and S. Noseir *et al.*, 2023. Correlation between environmental and tntestinal *Clostridium perfringens* isolated from different chicken flocks. Int. J. Poult. Sci., 22: 90-98.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry all over the world are susceptible to acute clinical or subclinical necrotic enteritis (NE) due to *C. perfringens* infection. In the global poultry sector, NE causes annual economic losses of US $6 billion[1]. Acute NE increases broiler flock mortality by around 1% per day, especially during the last weeks of rearing. On the other side, the subclinical form increases the intestinal mucosal damage leading to significant reduction in digestion, absorption and body weight gain and increased feed conversion ratio (FCR)[2]. Moreover, higher condemnation rates at processing plants are mostly associated with cholangiohepatitis during the subclinical coarse of NE. Also, one of the most frequently isolated bacterial pathogens in foodborne disease outbreaks in humans is *C. perfringens* of poultry origin that poses a risk for transmission to humans through the food chain[3].

There are many sources of contamination associated with *C. perfringens* disease outbreaks such as feed, water, litter or environment of the broiler barns[4]. Not only, the presence of *C. perfringens* in the intestinal tract of broiler chickens or inoculation with high doses of *C. perfringens* are essential for the development of NE, but also one or several predisposing (stress) factors may be involved to elicit its clinical signs and lesions[5]. Among the toxigenic clostridial species (*C. perfringens*, *C. botulinum, C. difficile* and *C. tetani*), the sporulated form of *C. perfringens* is the paradigm species for genetic studies, because of its oxygen tolerance and fast growth rate (8-10 min generation time in optimal conditions). Many of the toxins and enzymes it produces have been studied and most of their structural genes have been cloned and sequenced. Other approaches, such as physical and genetic mapping, have also been used to elucidate the genomic structures of various *C. perfringens* strains[6].

It is necessary to detect major toxins in the gut, blood stream, serous exudates of affected animals and culture supernatant fluids in order to determine the type of *C. perfringens*. In serum neutralization test, the standard assay is performed in guinea pigs or mice which is increasingly undesirable due to expense, complexity, disfavor on humanitarian ground and lack of sensitivity and specificity[7]. Recently, polymerase chain reaction (PCR) based DNA amplifications of the toxin genes, α, β, ϵ, ί and *net*β, were developed for rapid and accurate diagnosis of enterotoxaemia and different types of *C. perfringens*[8].

In literature research, it has been demonstrated that horizontal gene transfer via plasmids and other extra-chromosomal elements can convert nontoxigenic *C. perfringens* strains into toxin producers. Several studies have been conducted using the transformable *C. perfringens* strain to study the genetic regulation of the toxin genes. As compared to other well-studied pathogenic bacteria, the pathogenicity and physiology of *C. perfringens* is still poorly understood[4,6,9]. So, the main goal of the present study was to investigate the correlation between *C. perfringens* isolates from environmental and intestinal origin, the presence of their toxin genes and to test their sensitivity to different antibiotics.

MATERIALS AND METHODS

Ethical approval: All tests and procedures were approved by the local legalization and ethics committee of the institutional animal care and use committee, complying with the general guidelines of Beni-Suef University. All efforts were made to minimize the suffering of animals under study (BSU-IACU/ http://www.bsu.edu.eg).

***Clostridium perfringens* characterization:** Twenty intestinal (duodenal) samples from 20 different commercial diseased chicken flocks suffered from clinical NE in Egypt (10 from broilers, 7 from layers and 3 from breeders) as well as 10 environmental samples (litter n = 6 and ration n = 4) were randomly collected from 10 out of the 20 farms. Complete isolation and identification of *C. perfringens* was carried out on perfringens agar base with tryptose sulphite cycloserine (TSC) supplement[10,11].

Antibiotic resistance profile: The susceptibility of the *C. perfringens* isolates to 16 antimicrobials was demonstrated based on the agar disc diffusion method. Single colonies were inoculated in Robertson's Cooked Meat Medium (RCM) with paraffin liquid seal and kept at 41°C. Three hundred microliter of the supernatant bacterial culture was spread evenly on Mueller-Hinton agar (MHA) (Oxoid, Hampshire, UK) and 16 antibiotic susceptibility discs (Oxoid, Hampshire, UK) were placed on the medium, cultured anaerobically at 41°C for 24 hrs. The antimicrobials used included cefotaxime (30 µg), amoxycillin (10 µg), ampicillin (10 µg), penicillin V (10 UI), erythromycin (15 µg), tylosin (30 µg), kanamycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), lincomycin (10 µg),

Table S1: Types of antimicrobials and the determination criteria for drug resistance

	Size of the zone of inhibition (mm)	
Drugs	Resistant	Sensitive
Cefotaxime	≤22	≥23
Amoxicillin	≤17	≥18
Ampicillin	≤16	≥17
Penicillin V	≤16	≥17
Erythromycin	≤22	≥23
Tylosin	≤19	≥20
kanamycin	≤17	≥18
Streptomycin	≤14	≥15
Tetracycline	≤18	≥19
Lincomycin	≤20	≥21
Spectinomycin	≤20	≥21
Bacitracin	≤12	≥13
Difloxacin	≤20	≥21
Flumequine	≤15	≥16
Metronidazole	≤16	≥17
Sulfamethoxazole+trimethoprim	≤16	≥17

spectinomycin (10 µg), bacitracin (10 UI), difloxacin (5 µg), flumequine (30 µg), metronidazole (5 µg) and sulfamethoxazole+trimethoprim (25 µg). Diameters of the inhibition zones were measured and the determination criteria as described by Wu *et al.*[12] for drug resistance was shown in supplementary Table S1.

Toxins and β-lactamase genes screening using uniplex conventional polymerase chain reaction (cPCR): After DNA extraction of all the 30 samples by boiling[13], Alpha toxin primer sequence F: GTTGATAGCGCAGGACATGTTAAG, R: CATGTAGTCATCTGTTCCAGCATC[14] and *net*β toxin primers sequence F: CGCTTCACATAAAGGTTGGAAGGC, R: TCCAGCACCAGCAGTTTTTCCT[15], bla (β-lactamase resistance genes) primer sequence F: ATGAAAGAAGTTCAAAA ATATTTAGAG, R: TTAGTGCCAATTGTTCATGATGG were used[16]. Bands were fractionated by electrophoresis on a 1.2% agarose gel (2 hrs, 5V cm^{-1}, 0.5XTris-borate buffer) and visualized by ethidium bromide staining.

Random amplification of polymorphic DNA (RAPD) analysis of environmental and intestinal *C. perfringens* isolates: According to Xiao *et al.*[17], RAPD primer sequence: GTGGTGGTGGTGGTG, uniplex PCR was used for studying the molecular relation between 4 environmental and 4 intestinal *C. perfringens* that were carrying bla gene. Enterobacterial repetitive intergenic consensus (ERIC) fingerprinting data were transformed into a binary code depending on the presence or absence of each band. Dendrograms were generated by the unweighted pair group method with arithmetic average (UPGMA) and Ward's hierarchical clustering routine. Dendrogram produced by cluster analysis of ERIC-PCR sequences was used as a simple tool for molecular epidemiology of clostridium isolates. Statistical package for the social sciences (SPSS), version 22 (IBM 2013) was used for cluster analysis and dendrogram construction[18]. Similarity index generated by the unweighted pair group method with arithmetic average (UPGMA) and Ward's hierarchical clustering routine. Dendrogram produced by cluster analysis of ERIC-PCR sequences was used as a simple tool for molecular(Jaccard/Tanimoto Coefficient and number of intersecting elements) between all samples was calculated using the online tool https://planetcalc.com/1664/).

RESULTS

There was a high resistance index of 100% against streptomycin sulphate, sulfamethoxazole+trimethoprim, tetracycline and spectinomycin in the examined intestinal and environmental *C. perfringens* type A isolates. Resistance to lincomycin, erythromycin, flumequine and difloxacin were 100% in all the environmental isolates and the intestinal isolates from breeder chickens only. Regarding ampicillin and cefotaxime, the resistance reached 70, 100%, 50, 80% to amoxicillin and 65, 70% to bacitracin for intestinal and environmental *C. perfringens* isolates, respectively; while for tylosin, the resistance reached 40, 33% for intestinal isolates from broilers and broiler breeders, respectively as compared to 100% for both environmental and intestinal *C. perfringens* isolates from layer chickens. However, the intestinal and most of the environmental isolates were highly sensitive to penicillin V (73%) (n = 22/30) followed by moderate sensitivity to kanamycin (50%) (n = 15/30) and metronidazole (47%) (n = 14/30), (Table 1-3).

On the other hand, the results of toxins and β-lactamase genes screening using uniplex cPCR revealed that 100% of intestinal and environmental *C. perfringens* isolates have α toxin gene, while 60% of intestinal *C. perfringens* carried *net*β (n = 12/20) and 25% had bla gene (n = 5/20). Meanwhile, 40% of environmental *C. perfringens* had harbor *net*β (n = 4/10) and 60% had harbor bla gene (n=6/10) as mentioned in Table 4. Figure 1 and 2 showed high similarity between A/C (broiler origin isolate code 1/ layers origin isolate code 15), B/E (broiler origin isolate code 6/ litter origin isolate code 4) and F/G/H (litter origin isolate code 6/ ration origin isolate code 7/ ration origin isolate code 10), mainly with the same ancestor which originated from environmental source.

Table 1: Antimicrobial profile among intestinal *C. perfringens* isolates

		Antibiotics susceptibility results															
Code	Source	STR	SXT	TE	SPT	L	MTR	K	E	F	DIF	AMP	AMC	PV	CTX	TY	BA
1	Broilers	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
2		R	R	R	R	S	S	S	R	S	R	S	R	S	R	S	S
3		R	R	R	R	S	S	S	R	S	R	S	R	S	R	S	S
4		R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
5		R	R	R	R	R	S	R	S	S	S	R	S	S	S	R	R
6		R	R	R	R	R	R	R	R	R	S	R	R	S	S	R	R
7		R	R	R	R	R	R	S	S	R	R	R	S	S	R	R	R
8		R	R	R	R	S	R	S	S	R	S	R	S	S	R	S	R
9		R	R	R	R	R	R	S	R	R	S	R	S	S	R	S	R
10		R	R	R	R	S	R	R	R	S	S	R	R	S	R	S	R
11	Layers	R	R	R	R	S	S	S	R	S	S	S	R	S	S	R	S
12		R	R	R	R	S	S	S	R	S	R	S	R	S	R	R	S
13		R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
14		R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R
15		R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R
16		R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
17		R	R	R	R	R	R	S	R	R	R	R	S	R	R	R	R
18	Breeders	R	R	R	R	R	R	S	R	R	R	S	S	S	S	S	S
19		R	R	R	R	R	S	R	R	R	R	R	R	S	R	R	R
20		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

STR: Streptomycin sulphate, SXT: Sulfamethoxazole+Trimethoprim , TE: Tetracycline, SPT: Spectinomycin, L: Lincomycin, MTR: Metronidazole, K: Kanamycin, E: Erythromycin, F: flumequine, DIF: Difloxacin, AMP: Ampicillin, AMC: Amoxycillin, PV: Penicillin, V: CTX: Cefotaxime, TY: Tylosin, BA: Bacitracin, S: Sensitive and R: Resistant

Table 2: Antimicrobial profile among environmental *C. perfringens* isolates

		Antibiotics susceptibility results															
Code	Source	STR	SXT	TE	SPT	L	MTR	K	E	F	DIF	AMP	AMC	PV	CTX	TY	BA
1	Litter	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
2		R	R	R	R	R	S	S	R	R	R	R	R	S	R	R	S
3		R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R
4		R	R	R	R	R	S	S	R	R	R	R	S	S	R	R	S
5		R	R	R	R	R	S	R	R	R	R	R	S	S	R	R	S
6		R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
7	Ration	R	R	R	R	R	S	S	R	R	R	R	R	S	R	R	R
8		R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R
9		R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R
10		R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R

STR: Streptomycin sulphate, SXT: Sulfamethoxazole+Trimethoprim, TE: Tetracycline, SPT: Spectinomycin, L: Lincomycin, MTR: Metronidazole, K: Kanamycin, E: Erythromycin, F: flumequine, DIF: Difloxacin, AMP: Ampicillin, AMC: Amoxycillin, PV: Penicillin V, CTX: Cefotaxime, TY: Tylosin, BA: Bacitracin, S: Sensitive and R: Resistant

DISCUSSION

Due to their high economic losses, increased mortalities and the increased risk of contamination, enteric diseases pose a serious threat to poultry production. Necrotic enteritis (NE) was first described and documented in England by Parish[19]. Since then NE has been consistently reported in every continent around the globe. The annual total estimated economic losses due to NE outbreaks in broiler farms were over $2 billion worldwide[20]. The appearance of NE in broiler chickens is always associated with one or several predisposing factors such as coccidia, high protein level in ration and immune-suppressors[21]. Also, *C. perfringens* genome had 3361 predicted coding DNA sequences, harboring numerous virulence genes that are essential for the disease pathogenesis, including 6 for antibiotic and antimicrobial resistance and 3 phage-encoded genes[22].

In the current study, continuous circulation of multidrug resistant *C. perfringens* strains isolated from intestinal and environmental sources were indicated through the high antimicrobial resistance to streptomycin sulphate, sulfamethoxazole+trimethoprim, tetracycline and spectinomycin in both environmental and intestinal *C. perfringens* isolates and to lincomycin, erythromycin, flumequine and difloxacin in all the environmental and the

Table 3: Antimicrobial resistance % among intestinal and environmental *Clostridium perfringens* isolates

		Antibiotics resistance (%)													
Sample origin	Source	STR	SXT	TE	SPT	L	MTR	K	E	F	DIF	AMP	AMC	PV	CTX	TY	BA
Intestinal (n = 20)	Broilers (n = 10)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	6/10 (60%)	6/10 (60%)	4/10 (40%)	6/10 (60%)	5/10 (50%)	4/10 (40%)	7/10 (70%)	5/10 (50%)	1/10 (10%)	7/10 (70%)	4/10 (40%)	7/10 (70%)
	Layers (n = 7)	7/7 (100%)	7/7 (100%)	7/7 (100%)	7/7 (100%)	5/7 (71%)	5/7 (71%)	4/7 (57%)	7/7 (100%)	5/7 (71%)	6/7 (86%)	5/7 (71%)	3/7 (43%)	2/7 (28%)	6/7 (86%)	7/7 (100%)	5/7 (71%)
	Breeders (n = 3)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	2/3 (67%)	2/3 (67%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	2/3 (67%)	2/3 (67%)	1/3 (33%)	2/3 (67%)	2/3 (67%)	2/3 (67%)
Environmental (n = 10)	Litter (n = 6)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	2/6 (33%)	3/6 (50%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	4/6 (67%)	2/6 (33%)	6/6 (100%)	6/6 (100%)	3/6 (50%)
	Ration (n = 4)	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	1/4 (25%)	2/4 (50%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	2/4 (50%)	4/4 (100%)	4/4 (100%)	4/4 (100%)

STR: Streptomycin sulphate; SXT: Sulfamethoxazole+trimethoprim , TE: Tetracycline, SPT: Spectinomycin, L: Lincomycin, MTR: Metronidazole, K: Kanamycin, E: Erythromycin, F: flumequine, DIF: Difloxacin, AMP: Ampicillin, AMC: Amoxycillin, PV: Penicillin V, CTX: Cefotaxime, TY: Tylosin, BA: Bacitracin, S: Sensitive and R: Resistant

breeder chickens' intestinal isolates which may be due to the continuous misuse of these antibiotics leading to rapid acquirement of resistance. These results completely differ from some previous studies conducted by Osman and Elhariri[23], Salah-Eldin *et al.*[24] and Shaaban *et al.*[25] in Egypt who reported that amoxicillin, ampicillin, florfenicol, penicillin, cephradine, fosfomycin and metronidazole, were highly effective antibiotics to treat *C. perfringens* based on antimicrobial sensitivity testing. In USA, Mwangi *et al.*[26] isolated *C. perfringens* toxinotype A carrying *net*β gene in 68% of isolates from apparently healthy birds and 81%, from dead birds with similar multidrug resistant profile to our study (for streptomycin, gentamicin, erythromycin, tetracycline and bacitracin) and the isolates showed a wide genetic relatedness, especially among isolates from the same state with the same antibiotic resistance profile using pulsed-field gel electrophoresis (PFGE) analysis. Using *in vitro* sensitivity testing, we isolated environmental *C. perfringens* carrying *bla* gene (60%) with high antibiotic resistance to amoxicillin and ampicillin (80-100% for environmental and 50-70% for intestinal *C. perfringens* isolates) signaling a danger of horizontal transfer of the bla gene between different strains of *C. perfringens.* On the other hand, the intestinal and most of the environmental *C. perfringens* type A isolates in this study were highly sensitive to penicillin V (73%). To manage NE in broiler chickens, penicillin G potassium (Pot-Pen) was previously administered in drinking water and the results showed significant reduction in the overall mortality due to NE as well as significant improvement in growth performance of the treated chicken[27].

Regarding toxinotyping, all the obtained 30 intestinal and environmental *C. perfringens* type A isolates indicated positive α toxin gene. It is well documented that all types of the *C. perfringens* strains produce α-toxin[27,28]. Regarding *net*β toxin (pore-forming toxin coded by plasmid genomes and belongs to toxinotype G and A) the lower shedding and survival of this *C. perfringens* type may be explained by the higher percentage of *net*β toxin obtained in intestinal *C. perfringens* (60%) compared to environmental *C. perfringens* (40%), as it is mostly recorded with cases of subclinical NE[29-34]. Johansson *et al.*[35], observed the genetic diversity of *C. perfringens* causing mild (subclinical) NE in chickens and recorded the presence of *net*β gene in more than 90% of isolates. Also, Mwangi *et al.*[26], suggested that the presence of *net*β gene in *C. perfringens* isolates from broiler chickens is not enough to cause death unless predisposing proliferation of these virulent *C. perfringens* occurred and NE

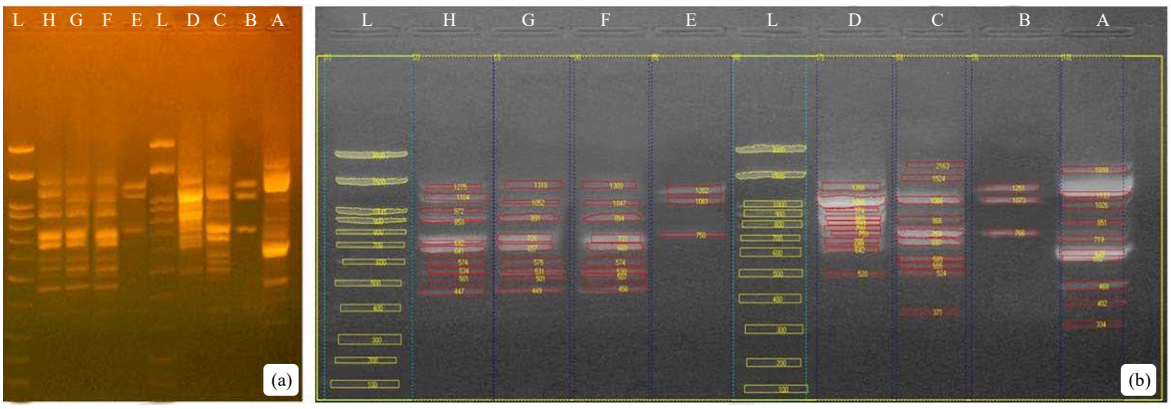

Fig. 1(a-b): Agar gel electrophoresis showing the results of RAPD analysis for 4 environmental and 4 intestinal *C. perfringens* that were carrying bla gene

A: Broiler origin code 1, B: Broiler origin code 8, C: Layer origin code 15, D: Breeder origin code 20, E: Litter origin code 4, F: Litter origin code 6, G: Ration origin code 7, H: Ration origin code 10 and L: 100, 3000 bp. ladder

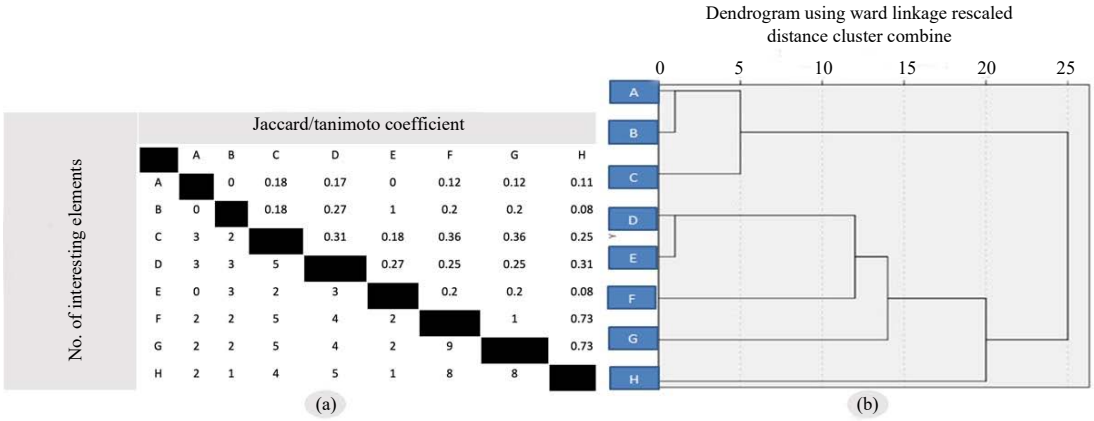

Fig. 2(a-b): Similarity index and dendogram for 4 environmental and 4 intestinal *Clostridium perfringens* that were carrying bla gene

A: Broiler origin code 1, B: Broiler origin code 8, C: Layer origin code 15, D: Breeder origin code 20, E: Litter origin code 4, F: Litter origin code 6, G: Ration origin code 7 and H: Ration origin code 10

developed, leading to higher clostridial shedding. Kiu *et al.*[36] studied the environmental *C. perfringens* isolates (obtained from poultry feed) encoded comparable virulence gene profiles (such as NE-linked *net*β), suggesting that soil and feeds act as potential reservoirs of *net*β-positive *C. perfringens* strains. Profeta *et al.*[37] detected the *C. perfringens* α-toxin gene in 151/167 extracts (90.4%) and *net*β gene in 31/151 (20.5%) from both carcasses and environmental boot socks of broiler chicken and turkey farms. However, Keyburn *et al.*[38], proved that *C. perfringens* strains produce variety of toxins and enzymes that are responsible for the mild to severe myonecrosis lesions.

Random amplification of polymorphic DNA (RAPD) analysis seems to be very promising in tracking of genetic relatedness of *C. perfringens* as mentioned by Afshari *et al.*[39], who used RAPD-PCR to examine the genetic diversity of 49 *C. perfringens* type A isolates from three different sources. They indicated that the most genetic diversity was among poultry isolates (clusters A, C, D and E), while human isolates had lower genetic diversity (clusters B and D). Here, the results of RAPD analysis, similarity index and dendrograms for *bla* positive gene of 4 environmental and 4 intestinal *C. perfringens* isolates showed high similarity between A/C (broiler origin isolate code 1/ layers origin isolate code 15), B/E

Table 4: Occurrence of α, *net*β toxins and bla genes among intestinal and environmental *Clostridium perfringens* isolates

		Intestinal *C. perfringens* (α: 100% n = 20/20, *net*β: 60% n = 12/20, bla 25% n = 5/20)																			
		Source																			
		Broilers										Layers								Breeder	
		Code																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Toxins	α	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	*net*β	+	+	-	-	-	+	-	-	+	+	-	-	+	+	+	+	+	-	+	+
	bla	+	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	+
		Environmental *C. perfringens* (α: 100% n = 10/10, *net*β: 40% n = 4/10, bla 60% n = 6/10)																			
		Source																			
		Litter													Ration						
		Code																			
		1		2		3		4		5		6		7		8		9		10	
Toxins	α	+		+		+		+		+		+		+		+		+		+	
	*net*β	-		-		+		+		+		-		-		-		-		+	
	bla	+		+		-		+		-		+		+		-		-		+	

(broiler origin isolate code 8/litter origin isolate code 4) and F/G/H (litter origin isolate code 6/ration origin isolate code 7/ration origin isolate code 10) originating from the same ancestor of environmental source.

CONCLUSION

The significant prevalence of α and *net*β genes in *C. perfringens* isolates from chickens' intestine and farm environment suggests their critical role in pathogenesis of NE. Also, *bla* gene is commonly detected indicating the higher antibiotic resistance patterns of the isolated *C. perfringens* strains. Penicillin V had the highest *in vitro* sensitivity in the examined *C. perfringens* isolates. Therefore, a continuous surveillance of antibiotic resistant patterns of *C. perfringens* should be considered to ensure proper treatment. Finally, *bla* gene of environmental and intestinal *C. perfringens* isolates showed high similarity using RAPD analysis, similarity index and dendrograms taking into account the importance of environmental origin as a source of horizontal *bla* gene transfer between different *C. perfringens* strains.

ACKNOWLEDGMENT

The authors would like to thank Dr. Moatasem Nawara, chairman of MN trade veterinary pharmaceutical company for antibiotic supply.

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