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Clostridium Perfringens Toxin Types Associated with Meat: Review in Iran

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ABSTRACT

Food poisoning due to *Clostridium perfringens* (*C. perfringens)* is a major food health problem, particularly in terms of meat consumption. Due to human's susceptibility to this pathogen, detection methods and prevention measures should be implemented to reduce its incidence. Several pathogenic strains of *C. perfringens* have been identified so far. One of the potential concerns about this bacterium is its toxin-producing characteristic that causes food poisoning. It has seven toxin types (A-G) according to the existence of four unique toxin genes. This study aimed to assess the prevalence of food poisoning caused by *C. perfringens* in meat and meat-derived products in Iran. We collected and categorized all the available data on this issue in Iran. Moreover, we summarized some methods used to detect toxins and genes and finally placed a prevention section for clarifying how to prevent such events. The best method for preventing such an organism's growth is by keeping foods in their normal state (hot and cold criteria) and chilling prepared foods in shallow containers as soon as possible.

Keywords: *Clostridium perfringens*, Foodborne diseases, Poisoning, Toxin, Meat

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Introduction

Food poisoning is one of the most common health problems all over the world. It has been reported more in underdeveloped and third world countries due to their low levels of hygiene. Some bacteria cause food poisoning by producing toxins in food **(1)**. Among all bacteria, *Clostridium perfringens* is one of the most important agents causing food poisoning due to its toxin production ability, short incubation time, and survivability in harsh environments. The clinical signs and symptoms can vary, but the most common signs are abdominal cramps, diarrhea, and to a less extent vomiting **(2)**. Since *C. perfringens* cannot synthesize 13 required amino acids (out of the total 20), protein-rich food constitutes a favorable medium for this bacterium **(3)**. Raw meat and chicken are the most common infection sources; however, this bacterial infection can also be transmitted from legumes **(4)**. Thus, we focused on meat poisoning to produce more impor-

tant and precise results. Indeed, *C. perfringens* is among the anaerobic, gram-positive, spore-forming bacteria that are environmentally widespread **(5, 6)**.

Genes of *C. perfringens* encode more than 17 unique toxins, which can be categorized into five types of toxin (A-E) according to the existence of four different toxin genes: α (alpha), β (beta), ε (epsilon), and ι (iota) toxins. Alpha-toxin is encoded by the gene of *cpa* and all types of *C. perfringens* produce this toxin. Enterotoxin, encoded by *cpe* gene, is the main virulence factor implicated in food poisoning in humans **(7,8)**. It was not a long time ago when it was suggested that this typing design should include types F and G, which encompass the *Clostridium perfringens* enterotoxin (CPE) and NetB toxin of *C. perfringens*, respectively. However, further studies are required before formally accepting this design. Even though the gene encoding α toxin is located on chromosomes, one can find *cpe* gene in both plasmid and chromosome. In compareson, the genes of the remaining toxins are found on various plasmids of different size. The vehicles of food poisoning by *C. perfringens* are typically meat and its products **(9-13)**.

Approximately 2–5% of all the isolates of *C. perfringens*, most of which belong to type A, generate *cpe* **(14)**. One of the most frequently reported food poisoning pathogens in Europe, the United States, and Turkey is *cpe*-positive *C. perfringens* type A **(13,15,16)**. Therefore, for a better comprehension of the epidemiology of *C. perfringens* infections, the identification of the toxin types of *C. perfringens* is vital, which can also help in a better development of preventive measures in practice. It is likely that contamination of meat products or meat dishes with insufficient cooking and high *C. perfringens* counts is the main reason for outbreaks. Meat products can be contaminated through various routes. The most common way is the internal route in animals after slaughtering, which manifests itself as a post mortem invasion. Besides, external sources like dirty hands, soil, water, animal skin, and processing equipment can be important sources of infection **(16, 17)**. The test of neutralization of toxin is commonly employed in guinea pigs or mice for the typing of *C. perfringens* **(18,19)**. Nonetheless, this detection technique is costly and time-consuming; therefore, as an alternative, the molecular techniques, such as polymerase chain reaction (PCR), have often been used most recently **(20, 21)**.

This paper reviews the incidence of *C. perfringens* meat poisoning in Iran, considering the toxin types and their encoding genes. Moreover, detection methods, food safety concerns and prevention strategies are discussed.

Risk Factors for Food Poisoning

Meat and meat products are among the most popular foods worldwide, and food poisonings are sometimes accompanied by meat poisoning. *C. perfringens* is an obligate anaerobic bacterium, and hence it prefers to grow at a deficient level or under oxygen-free conditions. It is found in deep musculature due to this trait. Since humans are susceptible to this bacterium's food poisoning, risk factors that compromise food safety should be discussed and established. Symptoms can vary from diarrhea to even death, but fatalities are rare, occurring in <0.03% of cases **(22)**. Death is usually caused by dehydration in age extremities, i.e., very young or very older people, and in immunocompromised people **(3)**. Based on the prevalence, the risk of contaminated and illness-causing food can be categorized as high or low. High-risk sources include beef and poultry, and they account for most of the outbreaks. Low-risk sources include seafood and sausage **(23)**. Although preventive measures have already been taken against this pathogenic agent, *C. perfringens* is still a significant cause of Iran's food-borne infections.

Enterotoxaemia

C. perfringens enterotoxin (CPE) is the most vital virulence factor causing human gastrointestinal (GI) diseases among the isolates type A. However, a very small percentage (<5%) of all the *C. perfringens* generate this toxin **(24)**. The role that *C. perfringens* enterotoxin plays in food poisoning has been entrenched. *C. perfringens* food poisoning symptoms comprise severe cramps of the abdomen and watery diarrhea. The onset of these signs commonly starts 6 to 24 hours after eating contaminated foods with C. *perfringens* at large numbers. Usually, the disease does not last long and diminishes in less than 24 hours. Symptoms of less severance may persist for 1 or 2 weeks. However, *C. perfringens* enterotoxin production is related to the process of sporulation, which happens in the small intestine after consuming a large number of temperature-abused foods **(25)**. Numerous surveys of *C. perfringens* incidence have been reported in foods **(26)**, but not many of them included fish **(27,28)**, that means most of the outbreaks are due to meat products. Few non-outbreak isolates contain the *cpe* enterotoxin gene of *C. perfringens* **(29,30)**. Between 1983 and 2002, this organism was ranked second and third in terms of confirmed cases and foodborne outbreaks of bacterial cause in the United States, respectively **(31)**. In addition, Lund et al. (2002) reported a single-component enterotoxin **(38)**. The necrotic enteritis that it caused is similar to that caused by the toxin of *C. perfringens*, but it is rarely reported.

Materials and Methods

To detect six toxin genes: *cpa* (alphatoxin), *cpb* (beta toxin), *etx* (epsilon toxin), *cpiA* (iota toxin), *cpe* (enterotoxin), and *netB* (NetB) with PCR, the DNA is extracted from isolates by the boiling method **(32, 33)**. The lethality assay for mouse and skin test for guinea pig, which are conventionally used for the typing of *C. perfringens*, are time-consuming and costly and raise ethical concerns du to use of laboratory animals. Nowadays, researchers usually adopt molecular methods, including microarray and PCR, especially real-time PCR **(34-37)**. More to the point, various protocols of PCR have been evolved for the identification of the *cpa*, *cpb*, *etx*, *iA*, *cpe*, *cpb2*, and *netB* genes that encode the generation of toxins, including α, β, ε, ι, enterotoxin, β2, and NetB **(19-34)**. Multiplex PCR, one of these protocols, enables the rapid, unlabored and simultaneous detection of multiple genes at lower costs. By virtue of these advantages, multiplex PCR is among the typically employed molecular approaches for *C. perfringens* typing, and some primers are used for the detection of these toxins (Table 1).

Table 1. Nucleotide sequences of commonly used multiplex PCR primers for detecting the toxin gene of *C. perfringens* (8,14,41).

Toxin/gene	Primer	Sequence (5'-3')	Fragment length
α/*cpa*	CPALPHATOX-F	GCTAATGTTACTGCCGTTGA	324 bp
	CPALPHATOX-R	CCTCTGATACATCGTGTAAG	
β/*cpb*	CPBETATOX-F	GCGAATATGCTGAATCATCTA	196 bp
	CPBETATOX-R	GCAGGAACATTAGTATATCTTC	
ε/*etx*	CPETOXIN-F	GCGGTGATATCCATCTATTC	655 bp
	CPETOXIN-R	CCACTTACTTGTCCTACTAAC	
ι/*iA*	CPIOTA-F	ACTACTCTCAGACAAGACAG	446 bp
	CPIOTA-R	CTTTCCTTCTATTACTATACG	
CPE/*cpe*	CPENTEROTOK-F	GGAGATGGTTGGATATTAGG	233 bp
	CPENTEROTOK-R	GGACCAGCAGTTGTAGATA	
β2/*cpb2*	CPBETA2TOK-F	AGATTTTAAATATGATCCTAACC	567 bp
	CPBETA2TOK-R	CAATACCCTTCACCAAATACTC	
NetB/*netB*	JRP6656	CTTCTAGTGATACCGCTTCAC	738 bp
	JRP6655	CGTTATATTCACTTGTTGACGAAAG	

There are commercially available assay kits to detect the toxins; however, they determine only one component of each complex and positive isolates can be considered only potentially enterotoxigenic. An overview of the toxins detection methods is shown in Table 2. Besides, PCR primers specific for the enterotoxin genes and the cereulide gene (*ces*) have been developed recently **(39)**. Furthermore, multiplex PCR assay provides a rapid and straightforward method for genotyping *C. perfringens* isolates **(40)**. An overview of the toxins and their prevalence is shown in Tables 3-5.

Table 2. Overview of *C. perfringens* toxins detection methods

Method	Advantage	Limitation	Reference
ELISA	* High sensitivity * High specificity * Rapid detection * Easily adaptable	* Some may take several days * Fecal material inhibits sensitivity * serological cross-reaction	42,43
Nucleic acid amplification	* High sensitivity * High specificity	* Cannot replace traditional reference standards as a single method	44
Immunochromatographic assay	* High sensitivity * Rapid detection (20 minutes)	Not described	45
^{18}F labelling	* Sufficient stability in plasma	* Being subject to liver uptake * Rapid metabolic degradation	46
Electrochemiluminescence	* High selectivity * High sensitivity	* Inaccurate at high temperatures	47

Table 3. Overview of *C. perfringens* types, toxins and genes that cause diseases in humans and animals (8, 48)

C. *perfringens* type	Toxin	*C. perfringens* toxin gene	Diseases	
			Human	Animal
A	α	*cpa* *cpa, cpb* *cpa, cpe* *cpa, cpe, cpb2*	Gangrene Food poisoning Antibody associated diarrhea, sporadic diarrhea	Diarrhea (dogs, pigs, etc.) Necrotic enteritis (Fowl)
B	α,β,ε	*cpa, cpb, etx* *cpa, cpb, etx, cpb2*	-	Dysentery (lambs) Enterotoxaemia (sheep)
C	α,β	*cpa, cpb* *cpa, cpb, cpb2* *cpa, cpb, cpb2, cpe* *cpa, cpb, cpe*	Enteritis necroticans (pigbel)	Necrotic enteritis (piglets, foals, etc.) Acute enterotoxaemia (adult sheep)
D	α, ε	*cpa, etx* *cpa, etx, cpb2* *cpa, etx, cpb2, cpe* *cpa, etx, cpe*	-	Enterotoxaemia (goats, sheep, etc.)
E	α, ι	*cpa, iA*	-	Enterotoxaemia (calves and rabbits)
F	α, CPE	*cpa, cpe*	Food poisoning, Antibody associated diarrhea	-
G	α, NetB	*cpa, netB*	-	Necrotic enteritis (chickens)

Table 4. Prevalence of different C. *perfringens* toxinotypes in food (by type) (%) in Iran

Province	Meat type	Toxinotypes				Year of publication	Ref
		Type A	Type B	Type C	Type D		
		α	α, β, ε	α, β	α, ε		
Chaharmahal and Bakhtiari	Chicken	42	-	-	-	2017	49
Kerman	Ostrich	100	0	0	0	2014	50
Razavi Khorasan	Beef	81	4	4	4	2015	51
Alborz	Mutton	63.6	25	21.4	53.3	2016	52
Razavi Khorasan	Chicken	29.03	-	70.96	-	2015	53

Table 5. Prevalence of different C. *perfringens* toxinotypes in food (by gene) (%) in Iran

Province	Meat type	Gene							Year of publication	Ref
		cpa	*cpb*	*cpe*	*cpi*	*etx*	*cpb2*	*netB*		
Chaharmahal and Bakhtiari	Beef	75.5	50	62	37.5	25	-	-	2017	54
Razavi Khorasan	Chicken	100	100	-	-	-	-	83.33	2014	55
Kerman	Chicken	-	-	-	-	-	-	17.78	2016	56
Razavi Khorasan	Beef	81	18	-	-	-	-	-	2015	53
Alborz	Mutton	-	-	38.3	-	-	-	-	2016	52
Razavi Khorasan	Chicken	-	-	25	-	-	-	-	2015	51

Discussion

Recently, there have been some significant developments in illuminating the spore germination mechanism of *C. perfringens*, which led to the detection and delineation of appropriate germinants and their receptors of *C. perfringens* FP and NFB strains' spores **(57, 58)**. Despite the variations in the inclination of germinants among the strains, still in some germinants such as AK or l-cysteine, the germination of spores can be induced in a broad extent of *C. perfringens* strains **(57, 60)**. Such insights have been the cause of evolving innovative strategies concerning the spore germination induction followed by destroying the germinated spores with mild treatments afterwards **(60-63)**. Some examples are as follows. (i) When AK germinant was used in meat products before high hydrostatic pressure (HHP) treatment (586 MPa)

at high temperature (73°C for 10 min), the procedure significantly destroyed the spores of C*. perfringens* in meat-contained feed **(62)**. (ii) Chemical preservatives, e.g., nisin, sorbate, and benzoate, at permissive levels efficiently halted the proliferation of germinated *C. perfringens* spores in rich environment. Nevertheless, to achieve significant inhibitory effects against the spores of *C. perfringens*, higher levels of chemicals were needed to be inoculated into chicken meat **(60, 64)**. (iii) Provoking spore germination significantly increased the sporicidal activity of typical disinfectants against *C. perfringens* FP spores attached to stainless steel chips **(57)**. This inactivation strategy based on germination induction was also efficient in destroying spores from other *Clostridium* species **(65.66)**. Collectively, provoking spore germination before inactivation treatment renders a unique strategy to improve the sporicidal power for *Clostridium* spores.

Moreover, other strategies are available for the control and inactivation of the *Clostridium* toxins, includeing physical approaches, which consist of thermal and pressure treatments and chemical agents, e.g., nitrate, nitrite, and organic acids **(67)**. The latter consists of lactic acid, acetic acid, and phosphates **(67)**. Vegetative cells of *C. perfringens* can be killed via devastating physical conditions. Still the difficult part of removing *C. perfringens* from food is their spores, which can be eliminated by adding environmental stress factors including ozone **(69)**, ultrasound **(70)**, and gamma radiation **(71)**.

In addition, two types of vaccines have been established to be employed against this bacterium, which are the gas gangrene vaccine and epsilon toxin vaccine **(68)**.

Conclusion

C. perfringens is considered one of the most common food poisoning agents, especially in the meat industry. There are some published reports every year indicating the outbreaks of the *C. perfringens* food poisoning that have even caused death in some cases. Therefore, effective methods should be used to detect and prevent the food poisoning caused by such bacterium. PCR-based techniques can be a very reliable tool for detecting the pathogen, and there also exist several helpful strategies such as germina-tion-induced inactivation, training the consumer about the correct handling of food, proper prepara-tion of food, and food storage in order to avoid this pathogenic agent. Besides, surveillance plays a key role in the effectiveness of the prevention strategies before food is delivered to the consumer.

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Conflict of Interest

The authors declared no conflict of interest.

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