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-
tand 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 *cpe* 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 comparison, 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 severity 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 (alpha toxin), 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 due 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 alpha, beta, epsilon, iota, enterotoxin, beta2, and NetB (19-34). Multiplex PCR, one of these protocols, enables the rapid, unlabeled 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 |
|------------|--------|------------------|----------------|
| alpha/cpa  | CPALPHATOX-F | GCTAATGTTACTGCCGTTGA | 324 bp |
|            | CPALPHATOX-R | CTCCTGACATCTGTGTAAG | |
| beta/cpb   | CPBETATOX-F | CGAAATATGCTGAGATATCTA | 196 bp |
|            | CPBETATOX-R | GAGGAAACTTAGTATATCTTC | |
| epsilon/etx| CPETOXIN-F | GCTGGATATCCATCTATTC | 655 bp |
|            | CPETOXIN-R | CTCTTCTTATTACTATACG | |
| iota/iA    | CPIOTA-F | ACTACTCTCAGAAGACAG | 446 bp |
|            | CPIOTA-R | CTCTTCTTATTACTATACG | |
| CPE/cpe    | CPENTEROTOK-F | GGAGATGGTTGGATATTAGG | 233 bp |
|            | CPENTEROTOK-R | GGACCAGCAGTGTGTAAG | |
| beta2/cpb2 | CPBETA2TOK-F | AGATTTAAAAATGATCCTAACC | 567 bp |
|            | CPBETA2TOK-R | CAATACCCCTTCACCAAATATC | |
| NetB/netB  | JRP6656 | CTCTGATGAACTCCGTTGAC | 738 bp |
|            | JRP6655 | CGTTATTTATCCTCTTGGT | |

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               | * Some may take several days                    | 42,43     |
|                            | * High specificity               | * Fecal material inhibits sensitivity           |           |
|                            | * Rapid detection                | * serological cross-reaction                    |           |
|                            | * Easily adaptable               |                                                 |           |
| Nucleic acid amplification | * High sensitivity               | * Cannot replace traditional reference standards as a single method | 44        |
|                            | * High specificity               |                                                 |           |
| Immunochromatographic assay| * High sensitivity               |                                                 | 45        |
|                            | * Rapid detection (20 minutes)   | * Being subject to liver uptake                 | 46        |
|                            |                                  | * Rapid metabolic degradation                   |           |
| ^18F labelling              | * Sufficient stability in plasma |                                                 | 47        |
| Electrochemiluminescence    | * High selectivity               | * Inaccurate at high temperatures               |           |
|                            | * High sensitivity               |                                                 |           |
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, cbp             | Gangrene | Diarrhea (dogs, pigs, etc.)      |
|                     |       | cpa, cbp, cpe             | Food poisoning | Necrotic enteritis (Fowl) |
|                     |       | cpa, cbp, cbp2            | Antibody associated diarrhea, sporadic diarrhea |
| B                   | α, β, ε| cpa, cbp, etx, cbp        | -        | Dysentery (lambs) Enterotoxaemia (sheep) |
|                     |       | cpa, cbp, etx, cbp2       |          | Necrotic enteritis (piglets, foals, etc.) |
|                     |       | cpa, cbp, cbp2, cpe, cbp, cpe| Enteritis necroticans (pigbel) |
| C                   | α, β  | cpa, cbp, cbp             | Necrotic enteritis (piglets, foals, etc.) |
|                     |       | cbp, cbp, cbp2, cpe, cbp, cpe| Acute enterotoxaemia (adult sheep) |
| D                   | α, ε  | cpa, etx, cbp2, cpe, cbp, cbp2| -        | 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 |
|---------------------------------|-----------|-------------|---------------------|-----|
| Chaharmahal and Bakhtiari       | Chicken   | Type A      | 2017                | 49  |
|                                 |           | α           |                     |     |
|                                 |           | α, β, ε     |                     |     |
|                                 |           | α, β, ε     |                     |     |
|                                 |           | α, ε        |                     |     |
| Kerman                          | Ostrich   | Type A      | 2014                | 50  |
|                                 |           | α           |                     |     |
|                                 |           | α, β, ε     |                     |     |
|                                 |           | α, β        |                     |     |
|                                 |           | α, ε        |                     |     |
| Razavi Khorasan                 | Beef      | Type A      | 2015                | 51  |
|                                 |           | α           |                     |     |
|                                 |           | α, β        |                     |     |
|                                 |           | α, ε        |                     |     |
|                                 |           | α, β, ε     |                     |     |
|                                 |           | α, β, ε     |                     |     |
|                                 |           | α, ε        |                     |     |
| Alborz                          | Mutton    | Type D      | 2016                | 52  |
|                                 |           | α           |                     |     |
|                                 |           | α           |                     |     |
|                                 |           | α, ε        |                     |     |
|                                 |           | α, β, ε     |                     |     |
|                                 |           | α, β, ε     |                     |     |
|                                 |           | α, ε        |                     |     |
| Razavi Khorasan                 | Chicken   | Type A      | 2015                | 53  |
|                                 |           | α           |                     |     |
|                                 |           | α           |                     |     |
|                                 |           | α, ε        |                     |     |
|                                 |           | α, β        |                     |     |

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

| Province                        | Meat type | Gene | Year of publication | Ref |
|---------------------------------|-----------|------|---------------------|-----|
| Chaharmahal and Bakhtiari       | Beef      | cpa  | 2017                | 54  |
|                                 |           | cbp  |                     |     |
|                                 |           | cpe  |                     |     |
|                                 |           | cpi  |                     |     |
|                                 |           | etx  |                     |     |
|                                 |           | cbp2 |                     |     |
|                                 |           | netB |                     |     |
| Razavi Khorasan                 | Chicken   | cpa  | 2014                | 55  |
|                                 |           | cbp  |                     |     |
|                                 |           | cpe  |                     |     |
|                                 |           | cpi  |                     |     |
|                                 |           | etx  |                     |     |
|                                 |           | cbp2 |                     |     |
|                                 |           | netB |                     |     |
| Kerman                          | Chicken   | cpa  | 2016                | 56  |
|                                 |           | cbp  |                     |     |
|                                 |           | cpe  |                     |     |
|                                 |           | cpi  |                     |     |
|                                 |           | etx  |                     |     |
|                                 |           | cbp2 |                     |     |
|                                 |           | netB |                     |     |
| Razavi Khorasan                 | Beef      | cpa  | 2015                | 53  |
|                                 |           | cbp  |                     |     |
|                                 |           | cpe  |                     |     |
|                                 |           | cpi  |                     |     |
|                                 |           | etx  |                     |     |
|                                 |           | cbp2 |                     |     |
|                                 |           | netB |                     |     |
| Alborz                          | Mutton    | cpa  | 2016                | 52  |
|                                 |           | cbp  |                     |     |
|                                 |           | cpe  |                     |     |
|                                 |           | cpi  |                     |     |
|                                 |           | etx  |                     |     |
|                                 |           | cbp2 |                     |     |
|                                 |           | netB |                     |     |
| Razavi Khorasan                 | Chicken   | cpa  | 2015                | 51  |
|                                 |           | cbp  |                     |     |
|                                 |           | cpe  |                     |     |
|                                 |           | cpi  |                     |     |
|                                 |           | etx  |                     |     |
|                                 |           | cbp2 |                     |     |
|                                 |           | netB |                     |     |

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 germin-
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 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, including 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 germination-induced inactivation, training the consumer about the correct handling of food, proper preparation 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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