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Toxinotyping and Protein-Protein Interaction (PPI) Networks and Functional Annotation of the Detected Toxin Genes of *Clostridium perfringens* Isolated from food samples in Duhok Province, Iraq

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*C*lostridium perfringens* is a zoonotic pathogen causing health problems like gas gangrene, food poisoning, and other enteric infections, especially in livestock, causing global economic losses. In the present article, *C. perfringens* isolates were obtained from 94 food samples, including chicken shawerma, meat shawerma, chicken kabab, meat kabab; and canned fish and chickens collected from 6 markets and stores, and 34 restaurants within Duhok province, Iraq during the period from June 1st, to August 31, 2022. The strains were isolated on CHROMagar™ *C. perfringens* agar and blood agar, and confirmed by PCR depending on 16 rRNA, and toxin typing based on genes encoding alpha (CPA), beta (CPB), epsilon (ETX), iota (IAP), enterotoxin (CPE), and necrotic beta-like (NetB) toxins. From the 62 samples collected from the restaurants, 44 were grown on CHROMagar™ *C. perfringens* as orange colonies, representing 71%, and confirmed with PCR. All the samples collected from canned foods were negative for *C. perfringens*. Out of 28 isolates from meat kabab, 26 (92.9%) carried alpha toxin gene, while only 2 (7.15%) samples carried both alpha and epsilon toxin genes, which represent type D strains. From the 8 isolates isolated from chicken shawerma, only alpha (CPA) gene was detected in 6 (75%) samples, while alpha toxin and enterotoxin genes were detected in 2 (25%) isolates which refer to type F type. All the other samples were diagnosed as type A as they carried only the alpha toxin gene from the six toxins investigated.

Protein-Protein Interaction (PPI) Networks and Functional Annotation of the Detected Toxin Genes revealed that the top Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were glycerophospholipid metabolism; activation of this pathway led to induction of the toxin production in *C. perfringens* which in turn causes gangrene related diseases in humans and animals.

To conclude that the prevalence of *C. perfringens* was very high in food samples from restaurants, especially in meat kabab and chicken kabab, followed by a relatively lower rate in meat shawerma and chicken shawerma. However, the vast majority of the isolates from food samples were *C. perfringens* type A, but D and F types were also detected, as besides CPA toxin, they also produce ETX and CPE toxins. None of the investigated imported canned foods (tuna, sardine, and processed chicken) carried *C. perfringens*.

Keywords: *Clostridium perfringens*, Alpha toxin, Epsilon, Enterotoxin, KEGG pathways.

Introduction

The widespread pathogen *Clostridium perfringens* is a considerably versatile pathogen in domesticated animals and humans, causing various health issues, such as clostridial myonecrosis "gas gangrene" in infected wounds, food poisoning, and enteric necroticans, which is a severe enteric infection (also called darmbrand or pigbel). In animal husbandry, *C. perfringens* causes significant economic losses due to several animal diseases, including lamb dysentery (necrohemorrhagic enteritis) in sheep, and necrotic enteritis of poultry and some newborn animals from various species

(including cattle, sheep, and pigs), depending on the types of toxins produced by different strains [1].

Clostridium perfringens is isolated from different environments, including soil, sewage, water, and intestinal content (present in both spore and vegetative forms as a part of the microbiota). The most frequent type isolated from the intestinal tract (of healthy and diseased animals and humans) and the environment is type A. The resident strains of *C. perfringens* in the intestinal tract alter their pathogenicity when displaced by virulent strains from the environment or get plasmids carrying genes encoding for toxins. Many predisposing factors play roles in outbreaks, including environmental contamination, gastric and intestinal pH, intestinal microflora, and nutrition [2], and prevent ingestion of strains with high virulence, which can be considerably prophylactic [3].

Clostridium perfringens is a rod spore-forming, Gram-positive, anaerobic, and negative for oxidase, catalase, indole, urease, Voges-Proskauer, and indole test [4]. For cultural and growth properties investigation, this bacterium has been cultivated on different culture media, including Brain Heart Infusion (BHI) agar, Nutrient agar, Egg Meat agar, Thioglycollate agar, and Reinforced Clostridial RCM agar, anaerobically at 37°C for 24 hours [5]. The colonies obtained from different culture media under anaerobic conditions can be more thoroughly investigated by colony morphology, Gram staining, in which pure colonies show Gram-positive single or pair arranged rod-shape cells, with thick and straight sides [6], biochemical, serological tests like the ELISA technique, and molecular techniques using 16S rRNA, rpoB, gyrB, dnaK, and recA genes for the identification [7]–[9].

According to a recent toxinotyping system, out of more than 20 toxins and hydrolytic enzymes are produced by *C. perfringens*, six major potent toxins (CPA, CPB, ETX, ITX, CPE, and NetB) are used to classify it into seven toxinotypes (from A to G). Based on this classification, type A strains of *C. perfringens* are mainly the causative agent of gas gangrene and do not cause food poisoning in humans, while type B, C, and D strains have clinical importance in the veterinary field by causing enteric diseases. Type F strains, which produce CPE toxin, are associated with food poisoning and antibiotic-associated diarrhea in humans. Type G represents isolates that release NetB toxin and lead to necrotic enteritis in poultry [10].

Since the sequencing of *C. perfringens* using a whole-genome shotgun strategy, many researchers have discovered genes that are responsible for coding various virulence factors, apart from toxins used for genotyping, such as toxins and enzymes including Tpel, Theta toxin or Perfringolysin O (PFO), Binary enterotoxin of *C. perfringens* (BEC), Beta2 toxin (CBP2), degradative enzymes like sialidases (neuriaminidases), and other potential virulence factors [11]. Alpha-toxin is produced by all strains of *C. perfringens*, which hydrolyze the phospholipid of the cell membrane, and as a consequence, cell necrosis is followed by immune-mediated pathological alterations [12].

Predisposing factors enhancing *C. perfringens* ability to cause health issues could be vary starting from environmental contamination, changes in gastric and intestinal pH, dietary alteration, concurrent infections, and the reverse impact of the administration of antibiotics. However, minimizing the risk caused by *C. perfringens* can be achieved through the use of probiotics, prebiotics, plant extracts and essential oils, organic acids, bacteriophages, lysozymes, and antimicrobial peptides [2].

In order to identify the potential source of *C. perfringens* which represent a public health concern causing health issues including food poisoning, implement measures to ensure food safety, understand the virulence potential for different strains, evaluate the risk, and develop the targeted prevention and control strategies. This study aims to isolate and genotype *C. perfringens* from food samples collected from restaurants and supermarkets in Duhok province/Iraq and to identify PPI network construction and gene ontology analysis of the detected toxin genes.

Material and Methods

Sample Collection and Isolation of *C. Perfringens*

During the period from June 1st, to August 31, 2022, 94 food samples were collected from 6 markets and stores, and 34 restaurants within Duhok province, Iraq. The samples were collected from the canned fish and chickens, chicken shawerma, meat shawerma, chicken kabab, and meat kabab; for this purpose, 1-2 grams of each sample were taken under aseptic conditions. All the samples were put into tubes containing 10 ml of thioglycollate broth (HiMedia, Mumbai, India), which was used as transportation and enrichment media, at the site of collection it was transferred directly to the laboratory and incubated overnight at 37°C for enrichment before inoculation onto CHROMagar™ *C. perfringens* (CHROMagar, France), which is a novel chromogenic selective culture medium. This media was prepared according to the manufacturer's instructions, while blood agar was incubated anaerobically prepared, using gas packs (Thermo Scientific™ Oxoid™ AnaeroGen™ 2.5L Sachet) and anaerobic jar (The 2.5-litre Oxoid AnaeroJar) for 24 hours at 37°C [13]. Suspected isolates were kept in

25% glycerol in brain heart infusion broth (BHIB) (v:v) , and stored at -20°C for further analysis [14].

DNA Extraction

DNA extraction was performed by the boiling method according to [15], with several steps: two to three of the suspected pure culture colonies of *C. perfringens* were collected from CHROMagar™ *C. perfringens* agar plates, and suspended in 100 µL of nuclease-free water placed into a microfuge tube. The suspension was incubated at 100 °C for 10 minutes and immediately cooled on ice for 5 minutes. The bacteria debris was eliminated by centrifugation (10000 rpm, 5 min.), the supernatant was transferred into a new tube, and used as a bacterial DNA template., then stored at −20 °C until ready for use. The quantification and purity of DNA were measured using a DS-11FX+ spectrophotometer (DeNovix Inc., Wilmington, DE, USA).

16S rRNA-Based Molecular Identification of *Clostridium perfringens*

Molecular identification of *C. perfringens* was performed by using specific primers for the detection of 16S rRNA gene (Table 1). To obtain a 50 µL volume product, all the PCR reactions were performed using 25 µL of 2x AddStart Taq Master (Addbio, Korea), 2.5 µL of each forward and reverse primers (10 µM) (Macrogen – Korea), and 2 µL of template DNA (concentration of ~10 ng/µl). The PCR cycling conditions were as follows: 2 mins. at 94°C followed by (35) cycles of 94 °C for 30 secs., annealing 53 for 30 secs., and extension at 72 °C for 30 secs., followed by 5 mins. at 72°C for the final extension [16] .

TABLE 1. Primers used for detection and amplification of specific 16S rRNA gene for *C. perfringens*

Target genes	Primer Sequence5'-3'	Annealing temp.	Amplicon size bp	References
16S rRNA	F-TAACCTGCCTCATAGAGT R-TTTCACATCCCACTTAATC	53	481	[17]

Toxin-typing Based on Six Major Toxins

For the toxinotyping of *C. perfringens*, uniplex and multiplex PCR were used to detect six toxin genes including alpha, beta, epsilon, iota, enterotoxin, and necrotic beta-like toxin (Table 2). A summary of the latest toxin-typing scheme is presented in table 3. The designed primers were blasted using (BLASTN, version 2.9.0+) https://www.ebi.ac.uk/Tools/sss/ncbiblast/nucleotide.html website to recognize the reported BLAST sequences for the detected genes and the presence of significant homology with *C. perfringens* sequences. To obtain a 50 µL volume product, all the PCR reactions were performed using 25 µL of 2x AddStart Taq Master (Addbio, Korea), 2.5 µL of each forward and reverse primers (10 µM), and 2 µL of template DNA (concentration of ~10 ng/µL). PCR cycling conditions were as follows; 2 mins. at 94ºC followed by 35 cycles of 94 ºC for 30 secs., (annealing, according to table (3.13) for each primer) for 30 secs., extension at 72 ºC for 30 secs., and followed by 5 mins. at 72 ºC for the final extension [16].

TABLE 2. List of primers used for toxin typing of *Clostridium perfringens* by PCR amplification

Toxin (Target genes)	Primer Sequence5'-3'	Amplicon size bp	References
Alpha (CPA)	**F:** GCTAATGTTACTGCCGTTGA **R:**CCTCTGATACATCGTGTAAGAATC	325	[18]
Beta (CPB)	**F:** GCGAATATGCTGAATCATCTA **R:** GCAGGAACATTAGTATATCTTC	196	[10]
epsilon (ETX)	F: TGGGAACTTCGATACAAGCA R: AACTGCACTATAATTTCCTTTTCC	376	[19]
Iota	F: AATGGTCCTTTAAATAATCC R: TTAGCAAATGCACTCATATT	272	[19]
Enterotoxin (CPE)	F:GGAGATGGTTGGATATTAGG R: GGACCAGCAGTTGTAGATA	233	[20]
NETB	F: CAGGCCATTTCATTTTTCCGT R: ACCGCTTCACATAAAGGTTGGA	157	[16]

TABLE 3. The 2018 *Clostridium perfringens* toxin-based typing scheme [10].

Genotype	CPA	CPB	ETX	ITX	CPE	NetB
A	+	-	-	-	-	-
B	+	+	+	-	-	-
C	+	+	-	-	+/-	-
D	+	-	+	-	+/-	-
E	+	-	-	+	+/-	-
F	+	-	-	-	+	-
G	+	-	-	-	-	+

Gel electrophoresis

The PCR product was analysed using a 2% (w/v) agarose gel prepared in 1X tris acetate ethylenediamineacetate (TAE) buffer containing Prime Safe Dye (GeNet Bio, Korea). A 100bp DNA marker (GeNet Bio, Korea) was used as ladder. The gel was allowed to run at 100 volts, 300 amperes for 40 mins using (Cleaver Scientific – UK) and was visualized under the UV transilluminator[16].

Protein-Protein Interaction (PPI) Networks and Functional Annotation of Toxin Genes

The protein-protein interaction (PPI) networks of the toxin genes (CPA and CPE) were achieved by String based on physical interactions. A string database is an online predicated gene function tool, with several networks obtained from different genomic or proteomic datasets [21,22]. A string database was utilized to display the gene ontology annotation and KEGG pathway analysis of the detected toxin genes. The current study used P<0.01 as a statistically significant. The bar graphs were generated and observed using GraphPad Prism v8.0.4.

Results

Microbiological and Molecular Identification of *Clostridium perfringens*

From the 62 food samples (6 meat shawerma, 24 chicken shawerma, 28 meat kabab, and 4 chicken kabab) collected from restaurants, 44 were grown on CHROMagarTM *C. perfringens* as orange colonies (Figure 1a), while on blood agar, the colonies were typically surrounded by a "double zone of hemolysis" (Figure 1b), representing 71%. Whereas, the 32 samples collected from canned foods (28 tuna and sardine fish, and 4 processed chicken) were negative for *C. perfringens* after culturing on CHROMagar[TM] *C. perfringens*. All 44 isolates recovered from food samples were confirmed positive with the PCR technique (Figure 2).
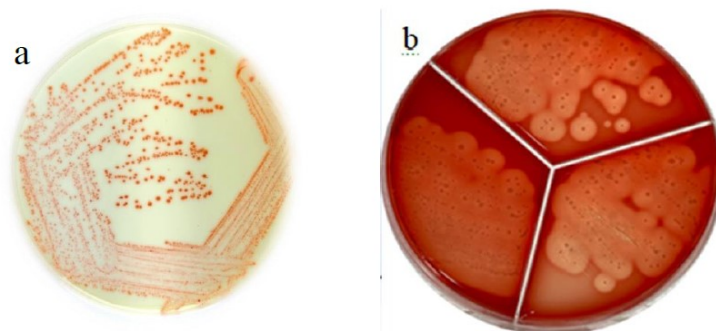

Fig. 1. (a) Orange colonies of *Clostridium perfringens* on CHROMagar[TM] *C. perfringens* agar, (b) *Clostridium perfringens* colonies on blood agar demonstrating double zone of hemolysis.
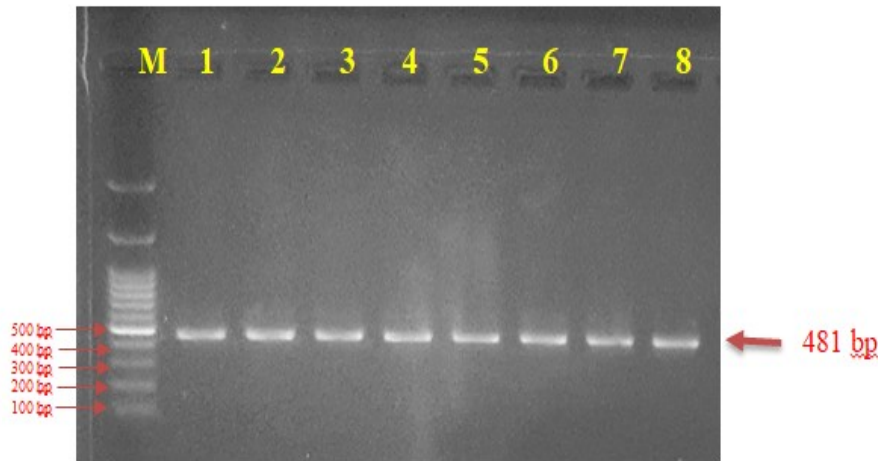
Fig. 2. Simplex PCR confirmation of *Clostridium perfringens* using 16S rRNA gene.
Lane M: marker (DNA ladder, 100 bp); lane 1: positive control for 16S rRNA; lanes 2-8: a 481 bp positive 16S rRNA gene from samples.

Toxin-Genotyping Based on Six Major Toxins

Toxin typing of 44 isolates showed the presence CPA, ETX and CPE genes (Figure 3), representing type A, type D, and type F strains of *C. perfringens*, none of other types were detected, as shown in Table 4.

TABLE 4. Distribution of *Clostridium perfringens* genotypes among different samples.

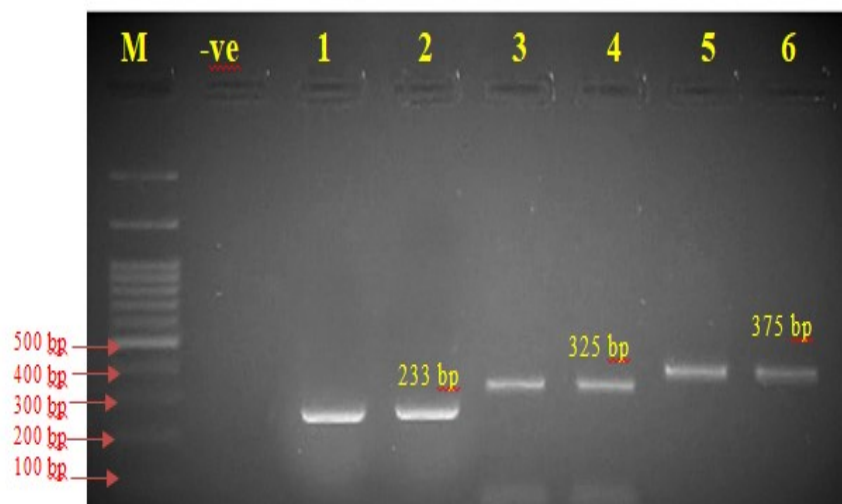| Source of sample	Number of isolates	Type A	Type D	Type F	Other types
Meat kabab	28	26 (92.85%)	2 (7.15%)	0	0
Chicken shawerma	8	6 (75%)	0	2 (25%)	0
Chicken kabab	4	4 (100%)	0	0	0
Meat shawerma	4	4 (100%)	0	0	0

Fig. 3. Simplex PCR for the detection of *Clostridium perfringens* toxin encoding genes.
M: marker (DNA ladder, 100 bp); (-ve) negative control, lanes 1-2: CPE toxin encoding gene; lanes 3-4: CPA toxin encoding gene; lanes 5-6: ETX toxin encoding gene.

Identification of PPI Network Construction and Gene Ontology Analysis

A total of 55 edges and 23 nodes were achieved, as shown in figure 4. The PPI network revealed that CPA is likely to have a relationship with CPE via several genes, such as CPE1329, cisD and psdD. The nodes in the interactive network are represented as genes, while edges form protein-protein associations.
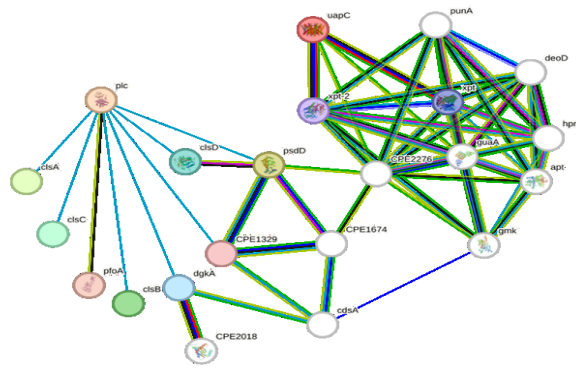
Fig. 4. Protein-protein interaction network representing interactions among (CPA and CPE) genes. The nodes and edges were constructed using the STRING tool.

String v11 was applied for subsequent gene ontology and KEGG pathway analysis of detected toxin genes. 28 biological process (BP), and 10 molecular functions (MF) terms were engaged as shown in Figures (5a, 5b) The enriched KEGG items of the (CPA and CPE) toxin genes are revealed in figure 5c, including glycerophospholipid metabolism, metabolic pathway, purine pathway and biosynthesis of secondary metabolites.
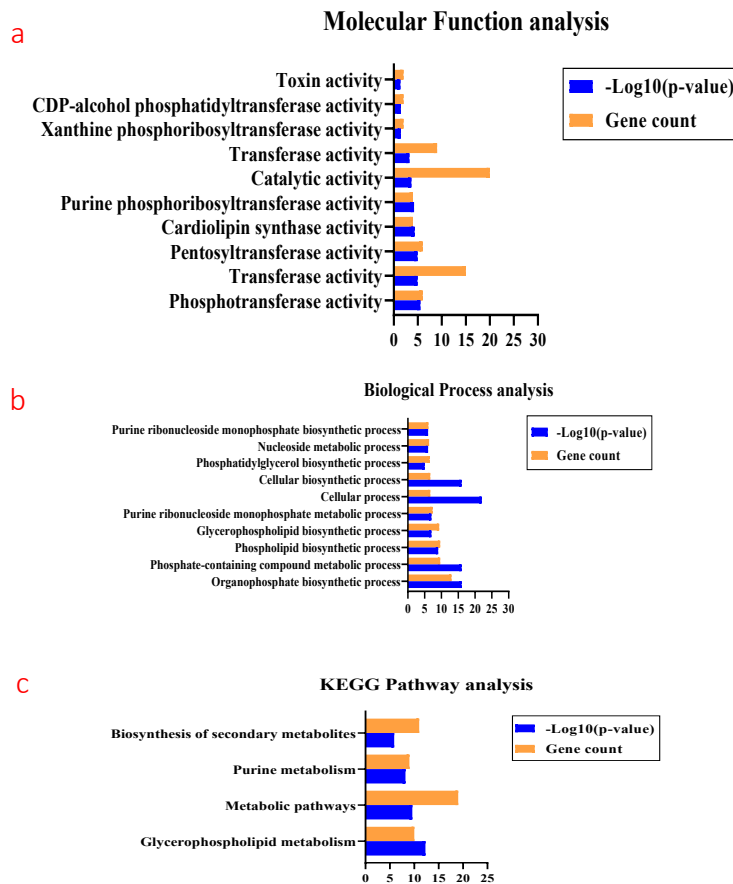

Fig. 5. Functional annotation analysis enriched for detected toxin genes (CPA and CPE) using String software.

The bars represent p-value and gene counts, the GO term enrichment significance, $p \leq 0.01$. Terms are connected based on shared genes. (a) Biological processes (BP), (b) molecular function (MF) and (c) KEGG pathways. The bar graphs were generated by GraphPad prism v8.0.4.

Discussion

Under certain situations, *Clostridium perfringens* can transform from a commensal bacterium into a formidable pathogen, producing an arsenal of toxins involved in its pathogenicity leading to health issues and significant economic losses [23], therefore, understanding the prevalence and characteristics of this bacterium will help identifying the potential sources of contamination, their toxinotyping will facilitate understanding the pathogenesis process as the toxins are responsible for the virulence potential of different strains. Aiming to detect and genotype of *Clostridium perfringens* will give help to obtain crucial information for risk assessment and the development of targeted prevention and control strategies.

In this study, CHROMagar™ *C. perfringens*, which is a novel chromogenic selective culture medium, was used. As the addition of certain antibiotics to selective culture media is commonly used to make them more selective to allow the growth of certain strains of *C. perfringens*, the recovery of this bacterium pure colonies from CHROMagar™ *C. perfringens* was considerably easier, taking into account that the added supplements to inhibit the growth of the unwanted bacteria showed no impact on *C. perfringens* growth.

The studies on *C. perfringens* has been conducted on isolates recovered from different sources including humans, sheep, goat, cattle, chickens, swine, turkey, and camels [24]. In current study all obtained orange colonies on CHROMagar™ which were taken from restaurant were confirmed by PCR as *C. perfringens* illustrating that the sensitivity of the CHROMagar™ was very high. Moreover , *C. perfringens* was not detected from the analysis of 32 samples collected from imported canned foods, while in other studies *C. perfringens* has been isolated from canned food [25] the absence of this bacterium in the canned food tested could be due to processing techniques including physical methods and chemical food preservatives added to such foods.

Clostridium perfringens has been isolated from different sources including animals' feces [16], raw chicken, raw beef [26], and even cooked foods [27], including meat shawerma [28] and chicken shawerma [29]. Previous studies conducted in Turkey showed only (10%) [30], (6%) of the cooked chicken shawerma [29] and (0%) cooked chicken shawerma [28] contained *C. perfringens*, compared to current results, (33%) for the chicken shawerma, and (66.6%) for the meat shawerma, these numbers are very low.

The presence of *C. perfringens* in high rates in shawerma (meat and chicken) samples is linked to different causes, including the cooking process in which the center close to the shawerma skewer creates an anaerobic condition with the optimum temperature for the growth of bacteria ranging from 25°C to 45°C, which is stable for a relatively long time depending on the size and shape of the shawerma, and this environment creates a favorable temperature for the bacterial growth. The temperature of uncooked parts cannot be controlled and when the sliced parts get cooked and served, they may not receive sufficient cooking [30]. In a previous study conducted on food samples in China [31], the results showed that 66.7% of the isolates recovered from raw mutton carried CPA toxin and diagnosed as type A strains, while 6.7% carried CPE toxin, and the other isolates were type B. In another study by Anwar and collegues [32], samples were collected from diverse food sources including meat samples (lamb, beef, and pork), poultry samples (turkey and chicken), seafood samples (salmon, cod, snapper, shellfish, squid, flounder, tuna, tilapia, and other fishes), and dairy products. The obtained results for genotypes rate were close to the current study results as (91.2%) were type A as they carried CPA toxin encoding genes, (5.9%) contained CPA and ETX genes refereeing to type D, while only 2.9% carried CPA, CPB and ETX genes, and diagnosed as type B. But the prevalence rate of *C. perfringens* isolates in samples investigated in this study was higher for meat and chicken samples, as in their samples the isolates recovered from were 21% and 28% for beef and chicken meat, respectively, while the *C. perfringens* were not detected in both studies [32].

In the present study, a string database was used to obtain experimentally validated interactions between the defined toxin genes as well as KEGG and GO enrichment analyses in *C. perfringens*. The current study showed an interaction between CPA and CPE toxin genes, as reported in previous studies [33].

Gene enrichment analysis documented that the top biological processes and molecular functions (GO terms) were organophosphate biosynthetic processes and phosphotransferase activity, respectively. Moreover, the detected toxin genes in these GO terms were engaged at high levels in the phosphorylation and transfer of the phosphoryl group to the regulatory proteins which in turn activate several toxin and virulence genes in clostridium species [34]. Interestingly, the identified genes in the top KEGG terms were notably enriched in glycerophospholipid metabolism pathways. The genes in the glycerophospholipid metabolism

pathway play key roles in metabolic toxin production in the Clostridium species [35]. Consistent with these gene ontology findings, several studies have shown the biological and molecular functions of the CPA and CPE toxin isolated from different specimens of domesticated species including sheep, goat, and cattle [36]. These results can provide insight into certain molecular mechanisms that may be involved in the stimulatory effects of these toxin genes in regulating the cytotoxic activity of the bacteria.

Conclusion

The prevalence of *C. perfringens* was very high in food samples from restaurants, especially in meat kabab and chicken kabab as it reached 100%, followed by a relatively lower rate in meat shawerma and chicken shawerma. Making these foods potential health risks for consumers. However, the vast majority of the isolates from food samples were *C. perfringens* type A, but D and F types were also detected, which increases the risk, as besides CPA toxin, they also produce ETX and CPE toxins. None of the tested imported canned foods (tuna, sardine, and processed chicken) from Duhok markets carried *C. perfringens*.

The top KEGG pathways were glycerophospholipid metabolism; activation of this pathway led to the induction of toxin production in *C. perfringens,* which in turn causes gangrene-related diseases in humans and animals.

Conflicts of interest

The authors declare no conflict of interest.

Author`s contributions

Conceptualization, sample collection, laboratory studies, and writing (the original draft): Ayaz M. Mamsin; supervision, data curation, and writing (review and editing): Khadija Kh. Barzani; bioinformatics analysis and software: Bushra T. Mohammed. All authors read and approved the final manuscript.

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النمط الجيني و شبكات التفاعل بين البروتين والبروتين والتوضيحات الوظيفية للجينات المسئولة عن صنع السموم التي تم ايجادها في عزلات المطثية الحاطمة *Clostridium perfringens* التي تم عزلها من عينات الطعام من محافظة دهوك- العراق

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المطثية الحاطمة (كلوستريديوم بيرفرينجنز) *Clostridium perfringens* هي إحدى مسببات الأمراض حيوانية المنشأ التي تسبب مشاكل صحية مختلفة ، مثل الغرغرينا الغازية ، و التسمم الغذائي ، و الالتهابات المعوية الأخرى، خاصة في الحيوانات الداجنة، مما يؤدي إلى خسائر اقتصادية عالمية كبيرة. في هذا البحث، تم عزل المطثية الحاطمة (كلوستريديوم بيرفرينجنز) *C. perfringens* من 94 عينات غذائية من مصادر مختلفة بما في ذلك (شاورما الدجاج، و شاورما اللحم، و كباب اللحم، و كباب الدجاج) و الأطعمة المعلبة من (التونة، و السردين، و الدجاج المصنع) من 6 اسواق تجارية و 34 مطعم من محافظة دهوك في فترة (من 1 حزيران الى 31 آب 2022). تم عزل السلالات باستخدام الوسط الزراعي كروماغار كلوستريديوم بيرفرينجنز .CHROMagar™ C (*perfringens*) و تأكيدها بطريقة البيولوجيا الجزيئية باستخدام جين 16S rRNA ، والتنميط الجيني باستخدام الجينات المسؤولة عن إنتاج السموم ألفا ، و بيتا ، و إبسيلون ، و أيوتا ، و إنتروتوكسين ، و نت بي(NetB).

ومن بين 62 عينة طعام تم جمعها من المطاعم، تمت زراعة 44 منها في الوسط الزراعي كروماغار كلوستريديوم بيرفرينجنز (CHROMagar™ *C. perfringens*)ظهرت على هيئة مستعمرات برتقالية و التي كانت تمثل 71% و تم تأكيدها بتقنية ال PCR. كانت جميع العينات التي تم جمعها من الأطعمة المعلبة (التونة ،والسردين ، والدجاج المصنع) سلبية بالنسبة لبكتيريا المطثية الحاطمة كلوستريديوم بيرفرينجنز(*C. perfringens*) بعد زراعتها في الوسط الزراعي كروماغار كلوستريديوم بيرفرينجنز (CHROMagar™ *C. perfringens*).

الدراسة اظهرت ان من أصل 28 عزلة من عينات كباب اللحم، أن 26 (92.9%) تحمل فقط CPA، في حين أن عزلتين فقط (7.15%) تحملان كلاً من CPA و ETX اللذين يمثلان سلالات النوع D، ومن الـ 8 سلالات المعزولة من شاورما الدجاج، تم اكتشاف CPA فقط في 6 عينات (75%)، بينما تم اكتشاف CPA وCPE في عزلتين (25%)، واللذين يشيران إلى سلالات النوع . F و جميع العزلات الاخرى كانت تمتلك CPA فقط من مجموع الجينات المسئولة عن صنع السموم التي تم دراستها، و التي تحدد النمط الجيني A.

نتيجة شبكات التفاعل بين البروتين والبروتين والتوضيحات الوظيفية ل جينات المسئولة عن صنع السموم التي تم ايجادها اوضحت ان أهم مسار في موسوعة كيوتو للجينات و الجينومات في عملية ايض ال glycerophospholipid، و تفعيل هذا المسار يؤدي الى انتاج السموم و بالتالي الى الامراض المتعلقة بالغرغرينة في الشر و الحيوانات.

نستنتج من هذه الدراسة ان انتشار المطثية الحاطمة (كلوستريديوم بيرفرينجنز) *C. perfringens* عالي جدا في العينات التي تم جكعها من المطاعم و خاصة كاب اللحم و كباب الدجاج و بنسب اقل في شاورما اللحم و شاورما الدجاج. رغم ان الغالبية العظمى من العزلات كانت تنتمي للنمط الجيني A و لكن الانماط D و F تم تشخيصهم ايضا، كون مع وجود ال CPA ، كانت هناك عزلات تتوفر فيها ال ETX و CPE. و جميع العينات التي تم جمعها من الأطعمة المعلبة (التونة ،والسردين ، والدجاج المصنع) سلبية بالنسبة لبكتيريا المطثية الحاطمة (كلوستريديوم بيرفرينجنز) *C. perfringens*.

الكلمات المفتاحية: المطثية الحاطمة ، سم الالفا، ابسيلون، انتيروتوكسين ، مسارات KEGG