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Detection of Clostridium perfringens in Uncooked Meat and Butchers' Tools in Basrah: A Public Health Concern

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Abstract

C. perfringens is an important anaerobic pathogen that causes foodborne gastrointestinal illness in humans and animals. The study was aimed at detecting *Clostridium perfringens* (*C. perfringens*) in raw meat and tools of butcher shops in local markets of Basrah Governorate. Seventy-five swab samples were collected from butcher shops in Basrah Governorate, southern Iraq, including 50 swab samples from sheep carcasses and 25 swab samples from knives and butchers' tools, during the period from June to August 2024. The samples were transferred directly to the Central Laboratory at the College of Veterinary Medicine, University of Basrah, and were subjected to isolation and diagnosis using conventional methods, culture on chromogenic agar, and molecular methods based on the 16S rRNA gene, as well as detection of the *cpa* gene. The results revealed the presence of *C. perfringens* in 20% of the total samples studied (75), distributed between 20% of the total meat swabs (50) and 20% of the tool swabs (25). The *cpa* gene was also detected in all *C. perfringens* positive samples, which confirms that the isolates belong to type A of *C. perfringens*. The results of this study indicate that the source of this contamination arises from poor sanitation in slaughter shops and/or to diseases of slaughtered animals, which requires the imposition of strict health measures and public health awareness to reduce the occurrence of meat contamination.

Keywords: Basra Governorate, *Clostridium perfringens,* Iraq, Meat, *16S rRNA*

Introduction

Raw meat is regarded as an ideal medium for the growth of various types of microbes, including fungi and bacteria. During the slaughtering process, the digestive systems of slaughtered animals are the most important source of contaminating microbes (1). The public health system is exposed to various virulent bacteria due to unauthorized slaughter in the open air, poor hygiene in slaughterhouses, and improper processing in food sites. Meat's microbiological characteristics are an indirect indicator of its level of poor sanitation. Inefficient carcass handling can lead to higher total bacterial colonies in meat (2).

In recent years, foodborne pathogens have become a major public health concern worldwide, resulting in high incidence and mortality (3). *C. perfringens* is an important anaerobic pathogen that causes foodborne gastrointestinal illness in humans and animals, and meat products are the most common vectors of *C. perfringens* type A food poisoning (4). *C. perfringens* is known for its rapid growth and produces more than 15 toxins that cause a range of different diseases in humans and animals. Of the highly toxic toxins that commonly cause damaging effects are Alpha toxin, Beta toxin, Epsilon toxin, Enterotoxin, Beta2 toxin, and Theta toxin. These are known for their variety and wide characteristic features which together hamper the control and treatment efforts (5). Furthermore, *C. perfringens* food poisoning ranks among the most common foodborne illnesses worldwide (6). *C. perfringens* affects both animals and humans through toxic production, which results in more than 20 different types of toxins (7).

A system can differentiate *C. perfringens* strains by identifying specific toxins as identification markers. The toxinogenic character of *C. perfringens* strains extend their classification into seven toxinotypes (A, B, C, D, E, F, G) through toxin-encoding gene detection, which includes CPA, CPB, ETX, ITX, CPE, and NetB (8). *C. perfringens* Type A infection has been identified as a common cause of food-borne disease in industrialized countries and is a leading cause of food poisoning cases in the USA, responsible for approximately 14% of food poisoning cases (9). Local studies on the quality of meat production chains, especially about C. *perfringens* and toxinotyping, are limited in the Basrah governorate. The study aimed to detect *C. perfringens* in raw meat and tools in butcher shops in local markets of Basra Governorate.

Materials and Methods

Sample collection: From June to August 2024, 75 samples were collected from butchers' markets. Using sterile swabs, equivalent areas (2.5 CM) of meat surface (located on the carcasses) and similarly the butcher’s tools were rubbed evenly, and then the swabs were inoculated into a thioglycolate medium containing tubes. The cultures were transported to the College of veterinary medicine central laboratory and cultivated under anaerobic conditions for 48 hrs. at 37°C using an anaerobic jar and gas

bags. The samples included 50 samples of slaughtered sheep meat and 25 samples of tools, distributed as 15 samples of knives and 10 samples from the butcher's table. The samples were selected from different areas of the Local markets of Basrah Governorate. Within two hours after slaughtering, the samples were collected and brought to the laboratory in sterile tubes containing thioglycolate broth to create anaerobic conditions (10)

Culture and growth conditions: Using an anaerobic jar and anaerobic CO2 gas bags (CampyGen™, Thermo Scientific USA), the thioglycolate broth samples were incubated at 37°C for 24 to 48 hours under anaerobic conditions. Thioglycolate samples were then spread out on chromogenic agar medium for *C. perfringens* (CHROMagar™, France) using sterile cotton swabs, put in the anaerobic jar with anaerobic bags, and incubated for 48 to 72 hours at 37°C. Additional phenotypic analysis, such as colony morphology, Gram staining, spore staining, and growth on blood agar, was performed on selected colonies (11,12,13)

DNA extraction: In accordance with the manufacturer's instructions, the Wizard Genomic isolation kit from Promega™, USA, was used to perform the genomic DNA extraction. Following DNA extraction, a nanodrop spectrophotometer was used to assess the isolated DNA's concentration and purity. The isolated DNA was kept at -20 °C till use.

Molecular characterization: *C. perfringens* species-specific primers targeting the *16S rRNA* gene were used to perform PCR on *C. perfringens* (13). In addition, the toxin gene *cpa* was detected using PCR primers targeting the *cpa* gene (14). The primer sequences and corresponding lengths of the amplicons are shown in Table 1. `PCR for the two targets was performed in a total reaction volume of 25 µl, including 12.5 µl of GoTaq® Green Master Mix (Promega Corporation, USA), 3 µl of purified genomic DNA (100–150 ng total), 1.5 µl of each primer (0.3 µmol), and 6.5 µl of sterile distilled water. Thirty-five amplification cycles (cycle steps are listed in Table 1) were performed after initial denaturation at 95 °C for 5 min. There was a final extension step of five minutes at 72°C.

Gel electrophoresis: To prepare 1.5% gel electrophoresis, 1.5 g of agar beads were dissolved in 100 ml of TBE solution. Following sample loading and gel electrophoresis, the gel was placed under a UV light source to examine the final product. A digital camera was then used to take pictures. The DNA marker (100-3000 base pairs) was used to visualize the PCR bands.

Table 1,2: The genes, primers, and PCR program used in this study.

Gene	Primers	Sequence (5'-3')	Amplification (35 cycles)			size	Ref.
			Denaturation	Annealing	Extension		
16S rRNA	C._perfring16S-F C._perfring16S-R	AAAGATGGCATCATCATTCAAC TACCGTCATTATCTTCCCCAAA	94°C 1min	50°C 1min	72°C 1min	279bp	(14)

Gene	Primers	Sequence (5'-3')	Amplification (35 cycles)			size	Ref.
			Denaturation	Annealing	Extension		
cpa	CPA-F CPA-R	GTTGATAGCGCAGGACATGTTA CATGTAGTCATCTGTTCCAGCATC	95°C 1min	56.2°C 20s	72°C 1min	402bp	(15)

Results

Growth on chromogenic agar

The results showed that 15 samples out of a total of 75 samples, at a rate of 20%, were positive for *C. perfringens*. Ten meat surface samples and five butcher's tool samples, including three knives as well as two butcher tables, showed *C. perfringens* presence. The positive samples showed *C. perfringens* colonies on the chromogenic agar and had rod-shaped Gram-positive bacteria when looked at under a microscope (Figures 1 and 2). The positive results from the analyzed samples composed 20% of the full set of tested specimens.

Molecular Identification of C. perfringens Using *16S rRNA*: Conventional PCR testing of the *16S rRNA* gene showed that of the 15 samples analyzed through PCR testing, 15 (100%) generated specific band patterns of 279 base pairs in length. The observed band matched the DNA ladder positioning at the expected region, confirming the expected results as shown in Figure 3 (Table 2).

Detection of the *cpa* gene: PCR amplification of the *cpa* gene showed a 402 base pair PCR product in all the positive *C. perfringens* isolates 15, 100%) (Figure 4) (Table 2).

Figure 1: Red-colored colonies CHROM agar *C. perfringens*

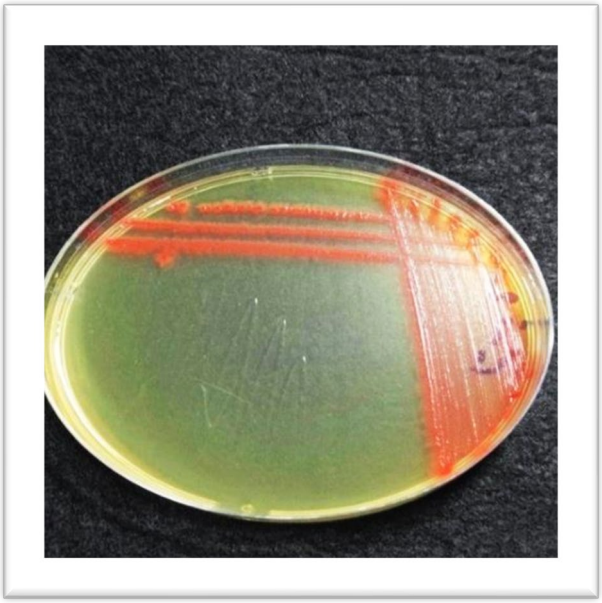

Figure 2: Gram-staining demonstrating Rod-shaped (bacilli) organisms with purple coloration.

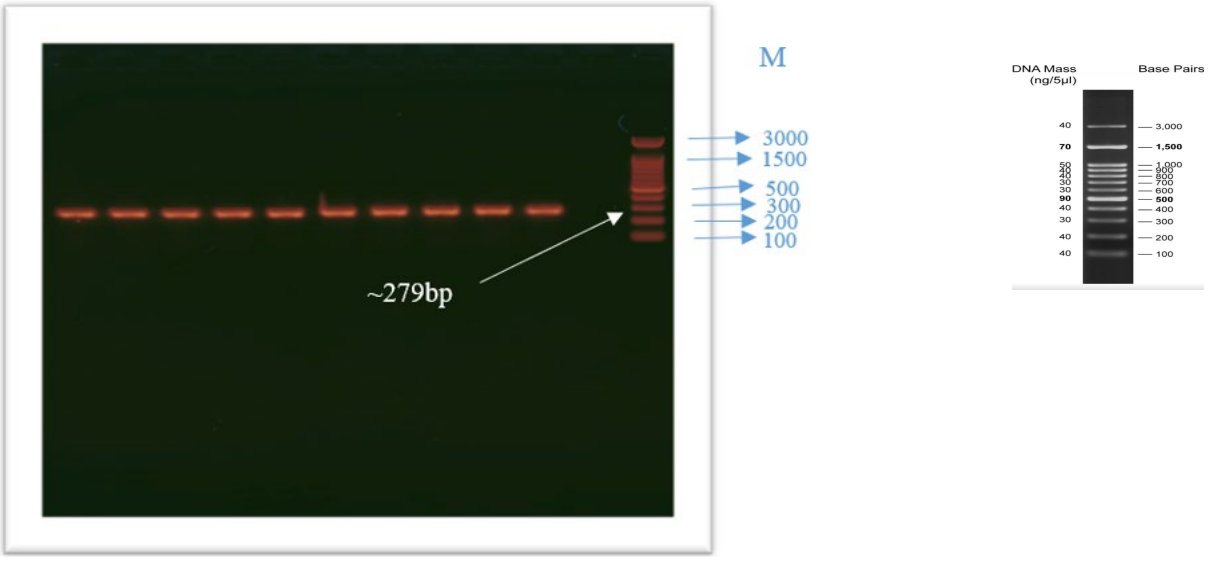

Figure 3: PCR amplification product of *16S rRNA* gene showing at 279 bp. Lane (M) shows a DNA marker 100 to 3000 bp ladder.

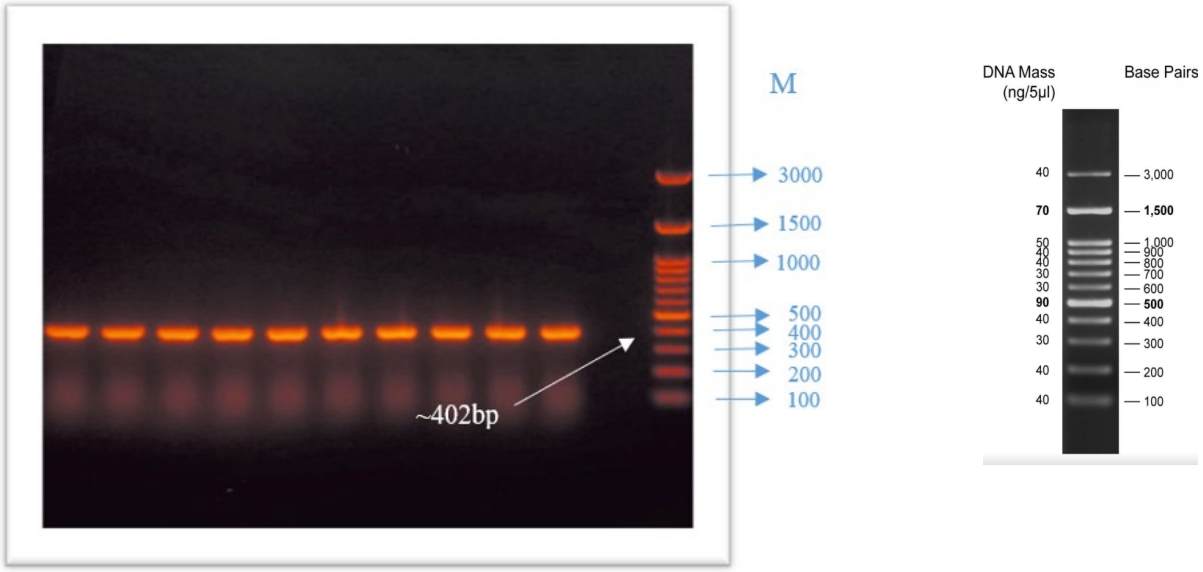

Figure 4: PCR amplification product of *cpa* gene showing at 402 bp. Lane (M) shows a DNA marker 100 to 3000 bp ladder.

Table 3: Number and percentage of *16S rRNA* and *cpa*. genes of different samples using PCR

gene	samples	
	meat	Butchers' tools
16S rRNA	10/50 (20%)	5/25 (20%)
cpa	10/50 (20%)	5/25 (20%)

Discussion

C. perfringens is regarded as one of the human food poisoning and enteric-disease causing pathogens in animals. In addition, the ability to form spores has complicated the control measures and posed additional public health alerts. Meat contamination in *C. perfringens* is one of the indicators of potential infection or food poisoning in animals. This study aimed to understand the degree of contamination of meat and butcher's tools in *C. perfringens* and the dominant toxinotyping among the contaminating isolates. The isolation method we used ensured the screening of a similar surface area across all samples. This is important to solidify our results and prevent any source of sampling errors. *C. perfringens* isolates obtained in this study were screened for the toxin gene *cpa* linked to human infection. The results showed a high level of *C. perfringens* contamination in the samples. This is alarming to public health. Our results were similar to those of previous studies. Conducted by Jang et al. (16) found that 10% of beef meat in Seoul, Korea, was contaminated with *C. Perfringens* and only tested positive for the *cpa* gene. Others have demonstrated the presence of this gene in beef from four different types of meat markets in Seoul, Korea (12.20%) (17).*C. perfringens* has been studied in other animals such as birds. The results of the current study are also in agreement with (18), who analyzed 53 isolates of *C. perfringens* from poultry of different parts of Sweden by PCR for toxin typing and reported that all the isolates belonged to Toxinotype A of *C. perfringens* with the gene coding for alpha-toxin production. Also, Dar et al. (19) found that all isolates were *C. perfringens* type A by a multiplex PCR, and the *cpa* gene was the dominant gene.

In Iraq, we only found a limited number of studies about *C. perfringens* and meat contamination. During an investigation, for abomasal lesions in slaughtered animals (Al-Qasim, Babylon, Iraq), the results revealed that *C. perfringens* was identified in 14% of cases, mostly from ulcers (42.8%) and nodules (57.14%) (20). Several pathogenic bacteria were detected in another study of bacterial contamination in frozen buffalo meat (Hama, Syria, and Mosul, Iraq). The samples comprised 60 specimens from meat shops in Hama City, Syria, and a second batch of 40 specimens from shops in Mosul City, Iraq. The detected pathogens were *E. coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *C. perfringens*, among others (21). In Basrah, there were limited

studies that did not tackle the toxinotyping of isolates, as they indicated the presence of a number of *C. perfringens* in sheep and beef meat by PCR (1). To our knowledge, our study is the first to link *C. perfringens* meat pollution to toxinotyping in the Basrah governorate.

Our findings align with those of (22) who identified *C. perfringens* in meat and fast food in Duhok, Iraq. Type A was the predominant strain, although Type D and Type F strains were identified in beef kebabs and chicken shawarma, respectively. The contamination in *C. perfringens* is complicated by the fact that it can survive in canned food under anaerobic conditions and develop spores that can spread to other forms of food. A study about bacteria in canned foods (Ibrahim Khalil Border, Iraq) indicated that *Bacillus cereus, Clostridium sporogenes, and Clostridium perfringens* were commonly identified (23).

The limitations of our study include the limited number of samples and the fact that other Clostridial species were not included, such as *Clostridium difficile*, and *Clostridium septicum*. Furthermore, the anerobic nature of *C. perfringens* has limited our ability to extend the geographical area for sample collection. Future studies should consider a larger sample size and a broader range of Clostridial species to provide a more comprehensive understanding of the topic. Collectively, this study's findings necessitate further analysis of other types of anaerobic contaminants. In addition, there is a need for continual surveillance systems in slaughterhouses.

Conclusion:

The widespread occurrence of type A of *C. perfringens* in meat and butchers' tools underscores the necessity for more stringent hygiene protocols, improved storage conditions, and heightened public health awareness to avert contamination and foodborne diseases**.**

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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الكشف عن *Clostridium perfringens* في اللحوم غير المطهية وأدوات الجزارين في البصرة: قضية صحية عامة

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الخلاصة

يُعد *Clostridium perfringens* من مسببات الأمراض اللاهوائية المهمة التي تسبب أمراض الجهاز الهضمي المنقولة بالغذاء في البشر والحيوانات. هدفت هذه الدراسة إلى الكشف عن *C. perfringens* في اللحوم النيئة والأدوات المستخدمة في محلات الجزارة بالأسواق المحلية في محافظة البصرة. تم جمع خمس وسبعين عينة مسحة من محلات الجزارة في محافظة البصرة، جنوب العراق، شملت 50 عينة مسحة من ذبائح الأغنام و25 عينة مسحة من السكاكين وأدوات الجزارة، وذلك خلال الفترة من يونيو إلى أغسطس 2024. نُقلت العينات مباشرة إلى المختبر المركزي في كلية الطب البيطري، جامعة البصرة، وخضعت للعزل والتشخيص باستخدام الطرق التقليدية، وزُرعت على الأوساط الكروموجينية، كما تم إجراء التشخيص الجزيئي بناءً على جين *16S rRNA* ، بالإضافة إلى الكشف عن جين*cpa*. أظهرت النتائج وجود *C. perfringens*في 20% من إجمالي العينات المدروسة (75 عينة) ، حيث وُجدت البكتيريا في 20% من مسحات اللحوم (50 عينة) و20% من مسحات الأدوات (25 عينة). كما تم الكشف عن جين *cpa* في جميع العينات الإيجابية لـ *C. perfringens*، مما يؤكد أن العزلات تنتمي إلى النمط A من *C. perfringens*. تشير نتائج هذه الدراسة إلى أن مصدر هذا التلوث يرجع إلى ضعف إجراءات النظافة في محلات الذبح و/أو إصابة الحيوانات المذبوحة بأمراض، مما يستلزم فرض تدابير صحية صارمة وتعزيز الوعي بالصحة العامة للحد من تلوث اللحوم.

الكلمات المفتاحية: محافظة البصرة، *Clostridium perfringens* ، العراق، اللحوم، *16S rRNA*.