Typing of *Clostridium perfringens* isolated from some meat products by using PCR.

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A B S T R A C T

One hundred and twenty random samples of meat products from different supermarkets in El-Gharbia governorate including minced meat, beef burger, sausage, luncheon (30 samples of each) were collected. *C. perfringens*was detected in meat products with an incidence 15%. The incidence of *C.perfringens* in minced meat, sausage, beefburger and luncheon was 16.67%, 23.33%, 16.67% and 3.33%, respectively. Typing of *C.perfringens* isolates revealed that the incidences of toxigenic and non-toxigenic strains were 83.33% and 16.67%, respectively. Typing of toxigenic strains of *C.perfringens* revealed that all isolates were type (A). Polymerase chain reaction (PCR) was applied for detection of toxin genes and enterotoxin of *C.perfringens*. All tested isolates were positive for alpha toxin gene with amplified PCR product of 402 bp and were *cpe* negative. Public health importance of *C.perfringens* isolates was discussed.

Keywords: *Clostridium perfringens*, PCR, Meat products.

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1. INTRODUCTION

Clostridium perfringens is spore-forming anaerobic bacterium widespread in the environment frequently in the intestinal tract of man and animals. It is responsible for gastrointestinal and enterotoxaemic diseases in animals and food-poisoning and gas gangrene in man (Petit et al., 1999). *C. perfringens* has a great effect on the human health causing food poisoning; also *C.perfringens* causes a number of human diseases ranging from necrotic enteritis to wound infection and life threatening gas gangrene. This pathogenicity is associated with lethal extra cellular toxins which have been defined as enzyme activity as collagenase, hyaluronidase and deoxyribonuclease (Norrisand Pettipher, 1987). Usually, *C.perfringens* has been classified into five toxigenic types (A through E) on the basis of its ability to produce major toxins (Yoo et al., 1997). Type A produces only alpha toxin, type B produces alpha, beta and epsilon toxins, type C produces alpha and beta toxins, type D produces alpha and .epsilon toxins and type E produces alpha and iota toxins. Each type of *C.perfringens* can cause different diseases. The most commonly encountered type A strain causes gas gangrene (myonecrosis), diarrhea, and food-borne illness in humans (Hatheway, 1990). Classically, typing of *C. perfringens* is performed with toxin neutralization with mice or guinea pigs (Stern and Batty, 1975 and McDonel, 1986). Because these methods are time consuming and expensive, they have largely been replaced by PCR-based detection methods. In recent years, molecular techniques such as polymerase chain reaction (PCR) have been used for typing of *C.perfringens* (Baums *et al.,* 2004).

2. MATERIAL AND METHODS

2.1. Samples collection:

One hundred and twenty random meat product samplesincluding minced meat,

beef burger, sausage and luncheon (30 of each) were collected from different supermarkets in El-Gharbia governorate. The samples were transferred to the laboratory for bacteriological examination.

2.2. Bacteriological examination:

2.2.1. Isolation and Identification of C.perfringens:

Each 2-3gm of sample was inoculated onto a tube of sterile freshly prepared cooked meat medium, and then the tube was incubated anaerobically in anaerobic jar using anaerobic gas generating kits at 37°C for 24-48 hours. For isolation of *C.perfringens*, a loopful from the previously incubated tube was streaked onto surface of 10% sheep blood agar with neomycin sulphate (200 µg / ml). The plate was incubated anaerobically at 37°C for 24-48 hours. Selected suspected colonies were transferred to tubes of freshly boiled and cooled cooked meat broth and was incubated anaerobically at 37°C for 24hours to have pure culture of isolates for further identification. The plates were examined for thecharacteristic colonies of *C.perfringens*. Subcultures fromthe suspected colonies were identified morphologicallyand biochemically according to Smith and Holdman (1968) and Koneman et al. (1992). Vitek was used as a confirmatory biochemical tool.

2.2.2. Typing of C.perfringens isolates:

Positive strains of *C. perfringens* isolates were typed into toxigenic and non-toxigenic strains using dermonecrotic reaction test and toxin-antitoxin neutralization test (Smith and Holdman, 1968 and Stern and Batty, 1975).

2.3. Determination of toxins and enterotoxin (cpe) genes of C.perfringens by using PCR:

Oligonucleotide primers were used for detection of alpha, beta and epsilon toxins by conventional PCR according to Yoo et al. (1997).

3. RESULTS

3.1. 3.1. Incidence of C.perfringens in meat product samples:

Out of 120 meat products samples collected from different supermarkets in El-Gharbia governorate, 18 *C.perfringens* isolates were detected with an incidence 15%. The incidence of *C.perfringens* in sausage was 23.33% (7/30 samples), in minced meat was 16.67% (5/30 samples), in beef burger was 16.67% (5/30 samples) and in luncheon was 3.33% (1/30 samples).

3.2. Identification of C.perfringens isolates:

3.2.1. Culture characters:

C.perfringens on neomycin sulphate sheep blood agar, colonies were 2- 3 mm in diameter, rounded, raised, glistening and showed double zone of hemolysis. On TSC agar, *C.perfringens* colonies were black in colour.On egg yolk agar medium, the attack of *C.perfringens* alpha toxin on lecithin gave opalescence on the side of the plate without antitoxin while this was inhibited on the other side of the plate with antitoxin.

3.2.2. Biochemical identification:

Results of sugar fermentation test revealed that the isolates were positive for glucose, lactose and sucrose sugars while mannitol fermentation was negative. *C.perfringens* isolates liquefied gelatin while catalase and indole were negative. Vitek 2 compact system confirmed isolates as *C.perfringens*.

3.2.3. Typing of C.perfringens isolates:

Typing of *C. perfringens* by intradermal injection of Guinea pig which revealed that the incidence of toxigenic and non-toxigenic strains were 83.33% (15 out of 18 isolates) and 16.67% (3 out of 18 isolates), respectively. Typing of *C.perfringens* revealed that toxigenic strains of isolated *C.perfringens* were detected in minced meat (100%), sausages (71.4%), beef burger (80%) and luncheon (100%). All isolated *C.perfringens* strains from meat products were type “A”. Action of *C.perfringens*

type "A" alpha toxin appeared as an irregular area of yellowish necrosis and tended to spread downward.

3.3. Genotyping of C.perfringens isolates by using PCR:

PCR was applied by using primers of alpha, beta, epsilon, iota and enterotoxin toxin genes to determine toxin- type of tested strains of *C.perfringens* isolated from sausages, beef burger, minced meat and luncheon. All tested samples were positive for alpha toxin gene with amplification of PCR product at 402 bp in comparison with standard molecular size marker.

Table (1): Oligonucleotide primers used for detection of alpha, beta and epsilon toxins by conventional PCR

Toxin	Primer	Sequence	Amplified product	Reference
Alpha toxin	*cpa (F)*	GTTGATAGCGCAGGACATGTTAAG	402bp	Yoo *et al.,* 1997
	cpa (R)	CATGTAGTCATCTGTTCCAGCATC		
Beta toxin	*cpb(F)*	ACTATACAGACAGATCATTCAACC	236 bp	
	cpb(R)	TTAGGAGCAGTTAGAACTACAGAC		
Epsilon toxin	*cpc (F)*	ACTGCAACTACTACTCATACTGTG	541 bp	
	cpc(R)	CTGGTGCCTTAATAGAAAGACTCC		
Iota toxin	*cpd (F)*	GCGATGAAAAGCCTACACCACTAC	317 bp	
	cpd(R)	GGTATATCCTCCACGCATATAGTC		
Enterotoxin	*cpe (F)*	GGAGATGGTTGGATATTAGG	233 bp	Heikinheimo and Korkeala, 2005
	cpe (R)	GGACCAGCAGTTGTAGATA		

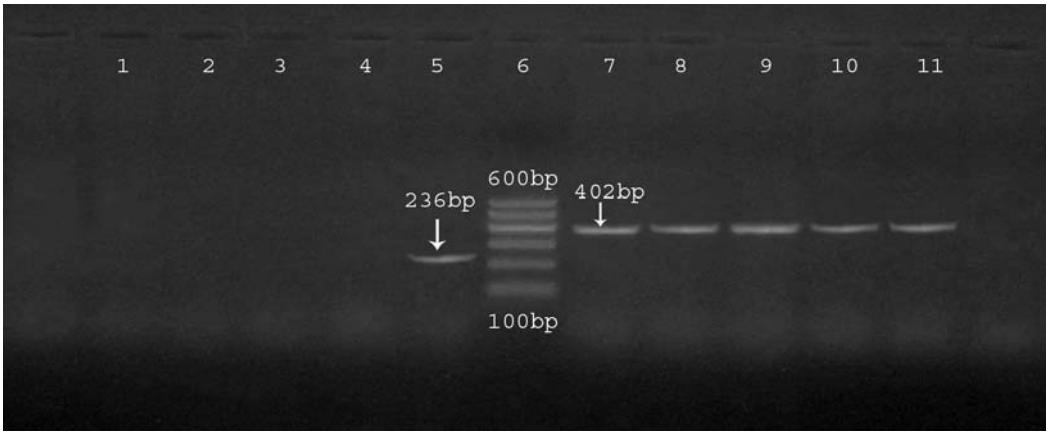

Figure (1): PCR of α and β toxins encoded genes of *C.perfringens*,
PCR products (402bp for alpha and 236bp for beta). Lane 5: positive control for β toxin gene. Lane 6: standard molecular size marker (100bp - 600bp). Lane 7: positive control for α toxin gene. Lane 8, 9, 10, 11 are positive for α toxin gene at 402bp.

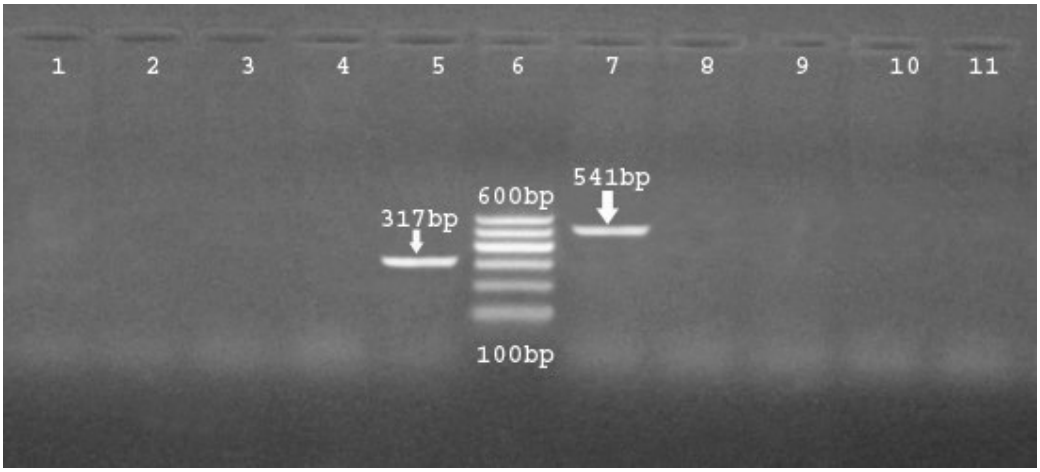

Figure (2): PCR of epsilon and iota toxins encoded genes of *C.perfringens*, PCR products (541bp for ε and 317bp for ι). Lane 5: positive control for ι toxin gene. Lane 6: standard molecular size marker (100bp - 600bp). Lane 7: positive control for ε toxin gene.

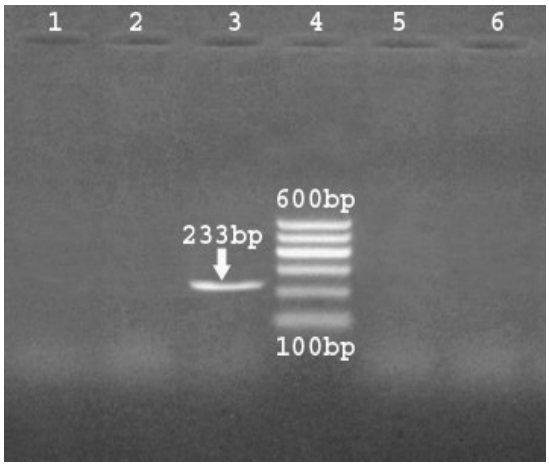

Figure (3): PCR of enterotoxin encoded gene of *C.perfringens*. Lane 3: positive control for enterotoxin gene. Lane4: standard molecular size marker.

4. DISSCUSION

Food illness caused by *C.perfringens* is among the common illnesses resulting from the consumption of contaminated food, the vehicles of infection are typically meat and poultry products. It has been firmly established that an enterotoxin produced in the intestine following sporulation of ingested vegetative cells is responsible for the illness (Duncan, 1973). In the present study, incidence of *C. perfringens* in sausage was 23.33%. This result was completely agreed with (Hassanien, 2004 and El Shater, 2010) who found that incidence of *C. perfringens* in sausage was 24%. Similar results obtained by El – Khateib (1997) who recorded isolation of *C.perfringens* from sausage were in percentage of 26%. In the current study, the incidence of *C.perfringens* in beef burger was 16.67%. This resultwas completely agreed with Fatein (2001) and Elewia (2003) who found the incidence of *C.perfringens* in beef burger was 16%. Nearly similar results were obtained by El-mossalami (2003) who found that incidence in beef burger was 20% but these results vary too much between authors who reported different percentage of isolation,

Edris (1992) stated isolation of *C.perfringens* from beef burger in percentage of 90%. Results showed that isolation of *C.perfringens* from minced meat was 16.67%. A similar result obtained by Hamoda (2012) who stated the incidence of *C.perfringens* in minced meat was 20%. Higher result was obtained by Alkheraije (2013) who recorded that incidence in minced meat was 46% but Lower results were recorded by Herrer (1995) who isolated *C.perfringens* from minced meat with percentage of 7.1%. Results showed that the incidence of *C.perfringens* in luncheon was 3.33%. Similar result was obtained by Khater (2004) who found the incidence of *C.perfringens* in luncheon in plane II was 5%. Higher result was obtained by Khairy (2005) who found the incidence of *C.perfringens* in luncheon was 65%. The incidence of toxigenic and non-toxigenic strains was 83.33% and 16.67%, respectively. These results came in agreement with Hamoda (2012) who recorded that the incidence by intradermal injection of toxigenic and non-toxigenic strains were 81% and 11.9%, respectively while Atwa and Abou EI-Roos (2011) found that Typing of *C. perfringens* revealed that the incidences of toxigenic and non-toxigenic strains were 89.6 and 10.4%.Typing of toxigenic strains of *C.perfringens* was revealed that all isolates were Type "A". Those results completely agreed with Emara (2014). PCR is more accurate and faster than the other normal methods for isolation and identification of causative agents(Aschfalk and Muller, 2002).It has been proved to be a very sensitive and specific technique for detection of the genes encoding alpha and epsilon exotoxin as well as enterotoxin of *C.perfringens* for rapid typing and evaluation of the virulence of the microorganism (Petit *et al.,* 1999). In present study, all tested *C.perfringens* isolates gave characteristic band at 402bp i.e. all isolates were positive for gene encoding alpha toxin as. The results go hand in hand with Augustynowicz et al., (2000) who found that *C.perfringens* alpha toxin gene amplicon was the only one that recorded in all tested isolates and gave a characteristic band at 402bp.

5. CONCLUSION

C.perfringens type A was the most predominant one and it is main cause for food poisoning outbreaks. In this study, PCR was used for diagnostic purpose, as it is much faster and more accurate than conventional microbiological techniques.

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