Detection of alpha toxin and enterotoxins of *Clostridium perfringens* isolated from minced meat by real time polymerase chain reaction (PCR)

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*Clostridium perfringens* is one of the most widespread pathogen producing toxins related to variable pathogenic conditions, particularly food poisoning in humans. Thus, this study described the prevalence, enumeration, toxigenic types and antibiotic susceptibility of *C. perfringens* strains isolated from minced meat in Egypt as well as the validation of a real-time polymerase chain reaction (PCR) test for the identification of *C. perfringens* toxin genes. A high prevalence of *C. perfringens* (98/105, 93.3%) was detected in minced meat samples. The total count of viable *C. perfringens* in 23 samples was 2.0 × 10² to 4.5 × 10² with a mean value 3.7 × 10² ± 1.07 × 10² CFU/g. The toxin typing of *C. perfringens* based on lecithinase activity and dermonecrotic reactions in albino guinea pig exhibited 33 (33.7%) as toxigenic strains of *C. perfringens* type A and 65 (66.3%) as non-toxigenic strains. Antibiotic susceptibility testing of isolates against 15 different antimicrobial agents indicated that *C. perfringens* was extremely sensitive to penicillin, followed by erythromycin, tetracycline, doxycycline and amoxicillin. All the other drugs were relatively less effective against the isolates. The real time PCR (RT-PCR) was performed for screening of alpha (*cpa*), beta, epsilon, iota toxins and enterotoxin (*cpe*) genes in toxigenic isolates of *C. perfringens* type A. All toxigenic strains of *C. perfringens* type A (33, 33.7%) were positive for alpha toxin (*cpa*) and enterotoxin (*cpe*) genes, while none of these isolates carried beta, epsilon and iota toxin genes. To the best of the researchers’ knowledge, this is one of the studies that used RT-PCR for the determination of toxigenic strains of *C. perfringens* in Egypt. It is suggested that RT-PCR could be used instead of the conventional culture procedures for identification of *C. perfringens* in minced meat in Egypt.

**Key words:** *Clostridium perfringens*, minced meat, alpha toxin (*cpa*) gene, enterotoxin (*cpe*) gene, real time-polymerase chain reaction (RT-PCR).

**INTRODUCTION**

Meat and meat products as a source of vital nutrients represent an essential part of the human food because...
humans cannot easily get these nutrients through vegetables and their derived products (Byers et al., 2002; Basyoni, 2003). The microbiological quality and safety of commercially processed meat and poultry products have a special concern for producers, consumers and public health officials all over the world (Okolocha and Ellerbroek, 2005). Clostridium perfringens, as a Gram-positive spore producing and non-motile bacilli, inhabits the environment (soil) and intestinal tract of humans and animals (Hayes, 1992; Labbe and Juneja, 2006).

C. perfringens is one of the most common clostridia genus isolated from minced meat and related to food poisoning in humans. The pathogenicity of C. perfringens organisms is connected to numerous toxins that are also evaluated for bacterial toxin typing, within them, all toxigenic isolates of the bacteria yield alpha (α) toxin coded by cpa gene. The other major lethal toxin formed by the organisms are beta (β), epsilon (ε) and iota (ι) toxins which are thoroughly associated with the virulence of bacterium (Hattheway, 1990; Titball et al., 1999). Besides these major lethal toxins, some isolates with a percentage of 0 to 5% have an ability of forming enterotoxin coded by cpe gene which is the major reason for public C. perfringens type A food poisoning (Mcclane, 2007; Juneja et al., 2010).

The exposure of meat dishes or meat products to insufficient cooking with the presence of high counts of C. perfringens in them is responsible for food outbreaks. The meat and meat products can be contaminated with C. perfringens through various sources, mostly internally from animals after slaughtering as post mortem invasion or externally from polluted hands, animals skin, soil, water and processing equipments (Satia, 1990). The toxin neutralization test is classically utilized in mice or guinea pigs for typing of C. perfringens (Stern and Batty, 1975; McDonel, 1986). This method is time consuming and costly; thus, it has mainly been substituted by molecular techniques for example, polymerase chain reaction (PCR), especially the real time PCR for typing of C. perfringens in the last years (Baums et al., 2004; Chon et al., 2012). Therefore, the present study was undertaken to throw light on the occurrence, enumeration, typing, chemotherapeutic agents susceptibility and determination of the toxin profile (alpha, beta, epsilon, iota and enterotoxin) of Clostridium perfringens strains in minced meat via real time-PCR (RT-PCR) technique in Egypt.

MATERIALS AND METHODS

Samples collection

In total, 105 samples from minced meat were obtained from large supermarket, butcher shops and retail meat shops distributed in different geographic areas in Mansoura province, Egypt during September to December, 2016. The samples were immediately transferred to the laboratory in sterile polyethylene bags placed inside an icebox and subjected to required investigation without delay.

Isolation and identification of C. perfringens

The samples were enriched in freshly prepared cooked meat media (CMM), and then incubated anaerobically using anaerobic jar at 37°C for 24 h. A loopful from the inoculated medium was subcultured onto 10% sheep blood agar supplemented with neomycin sulphate (200 µg/ml) and incubated anaerobically at 37°C for 48 h. The presumptive colonies were picked and subjected to standard morphological and biochemical identification (nitrile reductive, sugar fermentation, gelatin liquefaction, indole, methyl red and Vogus Proskauer tests) (Koneman et al., 1992).

Enumeration of viable C. perfringens in minced meat

The counting of C. perfringens was performed based on FAO (1992) Briefly, twenty-five grams of each samples were aseptically taken and homogenated in stomacher 400 (Seward, UK) with 25 ml of 0.1% peptone water to provide original dilution 1/10, followed by serial two fold dilutions. The pour plate method was performed using tryptose sulphite cycloserine (TSC) agar followed by incubation of the plates anaerobically at 37°C for 20 h. Next, the number of suspected (black) colonies was calculated for the plate having an optimal counting more than 20 colonies. Not regarding the count, a maximum of ten colonies were picked up for verification from each sample. The interpretation of results occurred as colony forming units (CFU) per gram of the sample.

Test of Nagler’s reaction (lecithinase activity)

This test was applied on the positive C. perfringens isolates as described by Smith and Holdeman (1986).

Typing of C. perfringens isolates

The typing of C. perfringens isolates was done for toxigenic and non-toxigenic strains by dermonecrotic test in albino guinea pigs as recommended by Stern and Batty (1975).

Sensitivity of C. perfringens isolates to chemotherapeutic agents

The disc diffusion assay was employed on a pure subculture from isolates of C. perfringens based on the guidelines of the British Society for Antimicrobial Chemotherapy (BSAC, 2011). The 15 most effective antibiotics (Oxoid) frequently utilized for treatment of C. perfringens infections were examined. In brief, the antibiotic discs were placed on the surface of seeded Muller Hinton agar (Oxoid) plates, followed by their incubation anaerobically at 37°C for 24 h. C. perfringens ATCC 13124 was used as a control strain. The sensitivity was judged as stated by BSAC approaches for antimicrobial susceptibility testing (2011). The isolates categorized as intermediate were regarded as sensitive to simplify the data analysis.

Real-time polymerase chain reaction (RT-PCR) for C. perfringens toxin genes determination

The real-time PCR was applied for screening of alpha (cpa), beta (cpb), epsilon, iota (cpi) toxin genes and enterotoxin (cpe) gene in toxigenic isolates of C. perfringens type A. The bacterial DNA was extracted from isolates by the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with some changes of the manufacturer’s recommendation. Specific primers and cycling were used in this
Table 1. Oligonucleotide primers, sequences, target genes, amplicon sizes and cycling conditions for SYBR Green RT-PCR.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence (5'-3')</th>
<th>Length of amplified product (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha toxin (cpa)</td>
<td>GTTGATAGCGGAGACATGTTAAG CATGTAGTCATCTGTTCCAGCATC</td>
<td>402</td>
<td></td>
</tr>
<tr>
<td>Beta toxin (cpb)</td>
<td>ACTATACAGACAGATCATTCAACC TTAGGAGCAGTTAGAACTACAGAC</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>Epsilon toxin</td>
<td>ACTGCAACTACTACTACTACTGTG CTGGTCCTTAATAGAAAGACTCC</td>
<td>541</td>
<td>Yoo et al. (1997)</td>
</tr>
<tr>
<td>Iota toxin (cpi)</td>
<td>GCGATGAAAAGCTACACCACCTAC GGTATATCCCTCCACGCATATAGTC</td>
<td>317</td>
<td></td>
</tr>
<tr>
<td>Enterotoxin (cpe)</td>
<td>ACATCTGCAATAGCCTAGGAAAT CCAGTAGCTGTAATTGTTAAGTGT</td>
<td>247</td>
<td>Kaneko et al. (2011)</td>
</tr>
</tbody>
</table>

**Figure 1.** *C. perfringens* isolates showing double zone of haemolysis on sheep blood agar.

assay as described by Yoo et al. (1997) and Kaneko et al. (2011) (Table 1). Primers were utilized in 25 ml reaction, comprising of 12.5 ml of the 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.5 ml of each primer of 20 pmol concentration, 6.5 ml of water and 5 ml of DNA template. The reaction was achieved in a stratagene MX3005P real time PCR machine with the following program: one cycle for 5 min at 94°C, after that, 40 cycles consisting of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; and then one cycle for 1 min at 95°C, 1 min at 55°C and 30 s at 95°C as the dissociation curve assay.

**Statistical analysis**

The data obtained were evaluated using statistical package for social science, version 17 (SPSS Software, SPSS Inc., Chicago, USA) and stated as means ± standard deviation (SD).

**RESULTS**

**The prevalence of *C. perfringens* in minced meat**

*C. perfringens* was isolated from 98 (93.3%) out of 105 different minced meat samples with regards to traditional methods (Table 2). *C. perfringens* isolates were Gram positive short plump rarely sporulated and non motile bacilli. *C. perfringens* isolates revealed double zone of haemolysis on sheep blood agar (Figure 1) and black zone on TSC (Figure 2).
Table 2. Prevalence and typing of *C. perfringens* isolated from minced meat samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive sample</th>
<th>Lecithinase positive isolates</th>
<th>Type of toxigenic isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Minced meat (105)</td>
<td>98 (93.3%)</td>
<td>33 (33.7%)</td>
<td>33</td>
</tr>
</tbody>
</table>

Figure 2. *C. perfringens* isolates showing black zone on tryptose sulphite cycloserine agar.

Enumeration of *C. perfringens* isolates

Total count of viable *C. perfringens* in the examined minced meat (98) were less than 10 CFU/g in 75 samples and more than 20 colonies in the remaining (23) samples. These 23 samples showed total count of $2.0 \times 10^2$ to $4.5 \times 10^2$ with a mean value $3.7 \times 10^2 \pm 1.07 \times 10^2$ CFU/g (Table 3).

Toxin typing of *C. perfringens* isolated from minced meat

Nagler’s test (Lecithinase activity) represented the action of *C. perfringens* alpha toxin on Lecithin of egg yolk which appeared as pearly opalescence zone surrounding the colonies, while this reaction was inhibited by *C. perfringens* alpha toxin antiserum (Figure 3). The lecithinase activity was detected in 33 (33.7%) strains of *C. perfringens* in the examined minced meat.

Moreover, toxin typing of lecithinase positive *C. perfringens* based on dermonecrotic reactions in albino guinea pig showed that 33 (33.7%) strains were determined as *C. perfringens* type A, while other strains were regarded as non-toxigenic strains (65, 66.3%) (Table 2 and Figure 4).

Antibiotic susceptibility of *C. perfringens* isolates

The sensitivity of *C. perfringens* isolates derived from minced meat to 15 different antibiotics was determined. The *C. perfringens* isolates were highly sensitive to penicillin (88, 89.8%), followed by erythromycin, tetracycline (65, 66.3%, each), then doxycycline and amoxicillin (64, 65.3% each). In contrast, *C. perfringens* isolates were resistant to ofloxacin (96, 97.95%), streptomycin, cloxacillin (94, 95.9% each), amikacin (90, 91.8%), trimethoprim sulphamethazole (85, 86.7%), oxytetracycline (83, 84.7%), cephalothin, cefepime (80,
Table 3. Total viable count of *C. perfringens* isolated from minced meat (n=98).

<table>
<thead>
<tr>
<th>Count of <em>C. Perfringens</em> (CFU/g)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5×10^2</td>
<td>6</td>
</tr>
<tr>
<td>4.1×10^2</td>
<td>4</td>
</tr>
<tr>
<td>2.4×10^2</td>
<td>5</td>
</tr>
<tr>
<td>2.3×10^2</td>
<td>3</td>
</tr>
<tr>
<td>2.2×10^2</td>
<td>2</td>
</tr>
<tr>
<td>2.0×10^2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
</tr>
</tbody>
</table>

*Mean ± SD* 3.2×10^2 ± 1.07 ×10^2

Less than 10 CFU/g 75

Overall total 98

*The mean of bacterial count ± standard deviation (SD).*

![Image of Lecithinase activity](image)

**Figure 3.** Lecithinase activity of *C. perfringens*alpha toxin on lecithin of egg yolk agar appearing as pearly opalescence zone surrounding the colonies

81.6% each) and kanamycin (78,79.6%) (Table 4).

**RT-PCR for toxin produced by *C. perfringens* determination**

The RT-PCR showed that all tested *C. perfringens* type A (33, 100%) harbored alpha toxin gene (*cpa*) (Figure 5) and enterotoxin gene (*cpe*) (Figure 6). On the other hand, they were negative to beta, epsilon and iota toxin genes.

**DISCUSSION**

Food illness related to *C. perfringens* is one of the major diseases associated with the ingestion of contaminated food, particularly meat and poultry products. It has been severely developed that the production of enterotoxins in the intestine from ingested vegetative cells is related to these diseases (Duncan, 1973). In the last years, many investigations were established on the prevalence of *C. perfringens* in raw and processed meat as well as poultry.
Table 4. Antimicrobial sensitivity test of C. perfringens isolates (n=98).

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Code</th>
<th>R</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>OFX5</td>
<td>96</td>
<td>97.95</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S10</td>
<td>94</td>
<td>95.9</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>Cx1</td>
<td>94</td>
<td>95.9</td>
</tr>
<tr>
<td>Amikacin</td>
<td>AK30</td>
<td>90</td>
<td>91.8</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Amp10</td>
<td>87</td>
<td>88.8</td>
</tr>
<tr>
<td>Trimethoprine-sulphamethazole</td>
<td>SXT25</td>
<td>85</td>
<td>86.7</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>T30</td>
<td>83</td>
<td>84.7</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>V1f30</td>
<td>80</td>
<td>81.6</td>
</tr>
<tr>
<td>Cefeprime</td>
<td>Fep30</td>
<td>80</td>
<td>81.6</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>K30</td>
<td>78</td>
<td>79.6</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>DO30</td>
<td>34</td>
<td>34.7</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Ax25</td>
<td>34</td>
<td>34.7</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>E15</td>
<td>33</td>
<td>33.7</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TE30</td>
<td>33</td>
<td>33.7</td>
</tr>
<tr>
<td>Penicillin</td>
<td>P10</td>
<td>10</td>
<td>10.2</td>
</tr>
</tbody>
</table>

S: Sensitive; R: Resistant; %: Percentage of sensitive isolates.

These documents indicated wide spread of C. perfringens in raw and processed meat and poultry (Labbe and Doyle, 1989; Labbe et al., 2000). Therefore, the high prevalence of C. perfringens (93.3%) in minced meat in the present study is not surprising. These results confirmed the findings of the previous studies obtained by Miwa et al. (1998), Mcclane (2007) and Grass et al. (2013) who noted the high prevalence of C. perfringens in market meats. Similarly, Guran et al. (2014) found that 96 and 88% of the ground beef and sheep meat samples were contaminated with C. perfringens in Turkey, respectively. Also, Wen and Mcclane (2004) isolated C. perfringens from 66% ground meat samples. Additionally, Kamber et al. (2007) isolated C. perfringens from 55% of minced meat samples in Turkey. On the other hand, a lower prevalence of C. perfringens in minced meat was
Figure 5. Representative real-time PCR amplification plots for alpha toxin (cpa) gene in toxigenic strains of *C. perfringens* isolated from minced meat samples that show threshold amplification cycle at 14 to 28 cycles.

Figure 6. Representative real-time PCR amplification plots for enterotoxin (cpe) gene in toxigenic strains of *C. perfringens* isolated from minced meat samples that show threshold amplification cycle at 13 to 26 cycles.

recorded as 12.5% by Afshari et al. (2015), 16.67% by Abd El-Tawab et al. (2015), 20% by Hamoda (2012) and 40% by Alkheraije (2013). The lowest prevalence was found by Herrer (1995) as 7.1%. The variation in the incidence of *C. perfringens* in minced meat may be related to the original contamination of minced meat and poor hygienic measures in processing factors. The cutting, handling and wrapping procedures could
separately be associated with the adding of *C. perfringens* spores and vegetative cells.

Food poisoning occurs due to ingestion of foods containing large populations of viable vegetative cells of *C. perfringens* and the subsequent production of toxins in the intestine. The presence of about 1 million organisms/gram of food was necessary to produce food poisoning after ingestion of such food (Johnson et al., 2007). Thus, enumeration of *C. perfringens* in food is usually done to investigate the suspected involvement of this bacterium in food poisoning. The current study revealed that the total count of viable *C. perfringens* in 75 examined minced meat samples were less than 10 CFU/g. Thus, the count of such samples was neglected because the anaerobic counts of the examined samples were within the permissible limits requested by the Egyptian Standard Specification and not enough to create food poisoning in humans, and millions of viable *C. perfringens*/g may be needed for induction of food poisoning in humans. However, the current study showed total count of viable *C. perfringens* in the remaining 23 samples with a mean value of $3.7 \times 10^5 \pm 1.07 \times 10^5$ CFU/g that might pose hazards. These results were consistent with Kamber et al. (2007) who stated that the average value of *C. perfringens* recovered from minced meat were $2.75 \times 10^5$ and $6.82 \times 10^5$ CFU/g from local markets and butcher’s shops, respectively. Also, Ali (2009) documented $1.7 \times 10^5 \pm 3.5 \times 10^5$ and $2.1 \times 10^5 \pm 1.1 \times 10^5$ CFU/g as the average of *C. perfringens* count of fore and hind quarters of raw cattle meat, respectively. In addition, El-Atwa and Abou El-Roos (2011) recorded a lower mean count of $1.2 \times 10^5$ of *C. perfringens* in minced meat. However, other studies by Abo Zaied (1998) and El-Melegy (2015) showed a higher mean count of *C. perfringens* with an average of $2.2 \times 10^5 \pm 3.8 \times 10^3$ CFU/g in meat samples. Shaltout et al. (2017) enumerated the total count of vegetative form of *C. perfringens* in the tested raw beef samples as $1.7 \times 10^5$ to $2.50 \times 10^5$ with an average of $6.22 \times 10^5 \pm 2.35 \times 10^5$ CFU/g. It is likely that a high prevalence of *C. perfringens* in minced meat is indicative of neglect of sanitary measures during production and handling of this product. Furthermore, presence of this bacterium in large numbers could constitute a public health hazard.

Toxin typing of *C. perfringens* strains revealed that the prevalence of *C. perfringens* type A was 33.7% in minced meat samples, whereas *C. perfringens* type B, C and D were not identified. Similarly, Shaltout et al. (2017) detected the high incidence of *C. perfringens* type A (50%) amongst strains isolated from raw beef samples with the absence of other toxin types in Egypt. El-Jakee et al. (2013) demonstrated that *C. perfringens* belonging to type A was the most dominant ones in poultry. On the other hand, this result was higher than that obtained by Emara (2014) who documented the occurrence of *C. perfringens* type A in meat samples as 16.73% in Egypt. Additionally, this result was lower than literature reports that indicated the prevalence of *C. perfringens* type A in 77.4% of the ground beef and sheep meat samples in Turkey (Guran et al., 2014) and 81% of minced meat in Iran (Afshari et al., 2015). Also, Kamber et al. (2007) determined 12, 1, 4 and 2% of minced meat samples contaminated with *C. perfringens* types A, B, C and D in Turkey, respectively.

In the last decades, the development of antimicrobial resistance among pathogenic bacteria is widespread. Hence, the *C. perfringens* isolates were tested for their antibiotic sensitivity to 15 frequently used antibiotics belonging to different antimicrobial classes to assess the most appropriate antibiotic for *C. perfringens* infection. This study reveals the high resistance of *C. perfringens* isolates from minced meat to the most examined antibiotics. Ofloxacin, streptomycin, cloxacillin, amikacin and trimethoprim sulphamethazole were the least effective antibiotic as most of the strains were resistant to these agents followed by oxytetracycline, cephalothin, cefepime and kanamycin. These results are compatible to previous studies documented by Johansson et al. (2004) and Silva et al. (2009). This resistance of *C. perfringens* to these antibiotics is due to the excessive use of these agents either as therapeutic agent or growth promoter in the food of farm animals. However, a higher sensitivity of *C. perfringens* isolates to penicillin was noticed, followed by erythromycin, tetracycline, doxycycline and amoxicillin. The observations were similarly detected by Skariyachan et al. (2010), Abd El-Rhman (2015) and Khan et al. (2015) where *C. perfringens* recovered from meat exhibiting susceptibility to penicillin, ampicillin and tetracycline while the organisms were moderately sensitive to erythromycin and vancomycin. Consequently, these antibiotic agents were proved to be most effective drugs against these isolates based on their high rate of sensitivity.

*C. perfringens* is still a common cause of food borne diseases through its ability to produce toxins particularly alpha toxin (*cpe*) and enterotoxins (*cpe*) which are responsible for food poisoning (Schalch et al., 1999). The present investigation showed that all tested *C. perfringens* type A (33, 100%) harbored alpha toxin (*cpe*) gene and *cpe* gene by RT-PCR. In contrast, these isolates were negative to beta, epsilon and iota toxin genes. The application of RT-PCR showed specificity of the oligonucleotide primers that was verified by positive amplification of 402 bp fragments for *C. perfringens* alpha toxin genes (*cpe*) and 247 bp fragments for *C. perfringens* enterotoxin genes (*cpe*) from DNAs extracts of all tested isolates from minced meat. These results were compatible with Guran et al. (2014) and Abd Eltwab et al. (2016) who found alpha toxin (*cpe*) gene in all *C. perfringens* type A isolates. Hence, it is clarified that the results obtained by RT-PCR provided a good compatibility with the results obtained by conventional culture means. Also, in contrast to conventional culture approach, the RT-PCR assay is a rapid and specific tool
and has probable practice as an identifying method for enterotoxigenic C. perfringens in food samples, considering its detection ability and time-saving efficiency (Singh, 2005; Albini et al., 2008; Yang et al., 2010; Chon et al., 2012). A lot of studies have used RT-PCR for detection of C. perfringens and their toxin genes in different samples (Wu et al., 2011; Chon et al., 2012). Albini et al. (2008) identified toxigenic strains of C. perfringens in animal isolates using RT-PCR. Mizher et al. (2016) revealed alpha (cpl) toxin genes of C. perfringens in 40 and 70% of cattle and sheep, respectively using real time PCR. In previous studies, the C. perfringens enterotoxin genes (cpe) were identified in 2.2 and 28.57% of examined isolates using multiplex PCR (Guran et al., 2014; Abd Eltwab et al., 2016), while, Razmyar et al. (2014) found that all isolates of C. perfringens obtained from ostrich flocks carried alpha toxin gene (cpl) and absence of enterotoxin gene (cpe) by multiplex PCR. Also, Afshari et al. (2015) detected 81% of alpha toxin (cpl) gene and absence of cpe gene in minced meat isolates by multiplex PCR. However, few studies are available on the use of such technique (RT-PCR) for estimation of C. perfringens and their toxin genes in minced meat in Egypt.

Conclusion

This investigation was concluded on the high prevalence of C. perfringens, particularly type A in minced meat, which is regarded as a public health hazard to consumers in Egypt. In this respect, strict hygienic measures and suitable regulations should be imposed for production, handling and distribution of minced meat to safeguard consumers. Moreover, the real time PCR is a promising molecular method for the rapid determination of toxigenic strains of C. perfringens instead of conventional microbiological techniques as it is much faster and more accurate.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES


