DE GRUYTER
OPEN

J Vet Res 61, 53-58, 2017
DOI:10.1515/jvetres-2017-0007

Toxinotyping and antimicrobial resistance of *Clostridium perfringens* isolated from processed chicken meat products

Dalia Hamza[1], Sohad Dorgham[2], Ashraf Hakim[2]

[1]Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Giza 11221, Egypt
[2]Department of Microbiology and Immunology, National Research Centre, Giza 12622, Egypt
daliahamza@cu.edu.eg

Received: September 26, 2016 *Accepted: March 8, 2017*

Abstract

Introduction: The toxinotyping and antimicrobial susceptibility of *Clostridium perfringens* strains isolated from processed chicken meat were determined. **Material and Methods:** Two hundred processed chicken meat samples from luncheon meats, nuggets, burgers, and sausages were screened for *Clostridium perfringens* by multiplex PCR assay for the presence of *alpha* (*cpa*), *beta* (*cpb*), *epsilon* (*etx*), *iota* (*ia*), and enterotoxin toxin (*cpe*) genes. The *C. perfringens* isolates were examined *in vitro* against eight antibiotics (streptomycin, amoxicillin, ampicillin, ciprofloxacin, lincomycin, cefotaxime, rifampicin, and trimethoprim-sulfamethoxazole) **Results:** An overall of 32 *C. perfringens* strains (16%) were isolated from 200 processed chicken meat samples tested. The prevalence of *C. perfringens* was significantly dependent on the type of toxin genes detected (P = 0.0), being the highest in sausages (32%), followed by luncheon meats (24%), burgers (6%), and nuggets (2%). *C. perfringens* type A was the most frequently present toxinotype (24/32; 75%), followed by type D (21.9 %) and type E (3.1%). Of the 32 *C. perfringens* strains tested, only 9 (28%) were enterotoxin gene carriers, with most representing type A (n = 6). *C. perfringens* strains differed in their resistance/susceptibility to commonly used antibiotics. Most of the strains tested were sensitive to ampicillin (97%) and amoxicillin (94%), with 100% of the strains being resistant to streptomycin and lincomycin. It is noteworthy that the nine isolates with enterotoxigenic potential had a higher resistance than the non-enterotoxigenic ones. **Conclusion**: The considerably high *C. perfringens* isolation rates from processed chicken meat samples and resistance to some of the commonly used antibiotics indicate a potential public health risk. Recent information about the isolation of enterotoxigenic *C. perfringens* type E from chicken sausage has been reported.

Keywords: processed chicken meat, *Clostridium perfringens*, enterotoxin, antimicrobial resistance.

Introduction

The microbiological quality and safety of meat are significant for the health of consumers around the world (25). Anaerobic bacteria constitute an important group of microorganisms that are responsible for many public health hazards. *C. perfringens* is a member of this group that is more widely spread than others, since its principal habitats are the soil and intestinal contents of humans and animals (18). Disease flare-up due to *C. perfringens* can have various origins, one of which is poultry (15).

In poultry, *C. perfringens* constitutes a human health hazard through the food chain and is one of the most frequently isolated bacterial pathogens from chicken meat, constituting up to 70%–98% of the cases (7, 9). *C. perfringens* is an omnipresent pathogen of the intestinal tract of poultry, associated with different phases of poultry growth and production. Chicken carcasses and meat parts may likewise be contaminated with *C. perfringens* during evisceration in the slaughterhouse (29).

C. perfringens causes a number of human diseases ranging from necrotic enteritis to wound infection and life-threatening gas gangrene (21). *C. perfringens* has been grouped into five types (A-E) based on toxins produced (*alpha*, *beta*, *epsilon*, and *iota*). The *alpha* toxin is produced by all the types of *Clostridium perfringens*. The toxin is a necrotising toxin which is believed to be a virulence marker (4, 22). In addition, *C. perfringens* produces other minor toxins, such as enterotoxins, which are not associated with a specific strain and are responsible for causing the gastrointestinal disorders that may be food- or non-food-borne.

© 2017 D. Hamza et al. This is an open access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/)

Unauthenticated
Download Date | 7/27/17 4:48 PM

The enterotoxin gene (*cpe*) exists on either a chromosome in food-poisoning isolates or a large conjugative plasmid in non-food-borne gastrointestinal disease (12).

Our study aimed to detect the presence of *C. perfringens* in processed chicken meat samples (luncheon, nuggets, burger, and sausage) and characterise the isolates for the existence of enterotoxin gene. In addition, the *in vitro* resistance of *C. perfringens* isolates against certain antibiotics was determined.

Material and Methods

Samples. In total, 200 processed chicken meat samples of luncheon, nuggets, burger, and sausage (n = 50 each) were collected from different markets in Cairo during the period from March to April 2015. When collected, the samples were directly transferred in iceboxes to the laboratory.

***C. perfringens* isolation and identification.** Ten grams of each sample was diluted in 90 mL of sterile 0.1% peptone water and homogenised in a blender at 2000 rpm for 1–2 min. About 1 mL of each homogenised food suspension was inoculated into cooked meat broth tubes and incubated anaerobically at 37°C for 48 h. A loopful form each tube was streaked on neomycin sulphate sheep blood agar plates and incubated anaerobically for a further 48 h. Suspected colonies were examined microscopically and biochemically (9).

DNA extraction and PCR assay. DNA was extracted from pure colonies of *C. perfringens* that showed a double zone of haemolysis on blood agar by using an extraction kit (QIA amp Mini Kit, Qiagen, Germany). Specific oligonucleotide primers for the *alpha* (*cpa*), *beta* (*cpb*), *epsilon* (*etx*) , *iota* (*ia*), and enterotoxin toxin (*cpe*) genes of *C. perfringens* were used as described in Table 1. Multiplex PCR assay was carried out in a reaction mixture (25 μL) containing 1 μL of template DNA, 12.5 μL of Dream Taq Green PCR Master mix, 0.5 μL of each primer (10 pmol/μL), and 10.5 μL of DNase-free water. PCR amplification was carried out in a Biometra PCR thermal cycler. Following initial denaturation for 3 min at 94°C, the samples were subjected to 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension for 10 min at 72°C (28). The PCR reaction mixtures were analysed by electrophoresis using 1.5% (w/v) agarose gel in the presence of 100 bp DNA ladder (Fermentas Life Science, EU) (14). Types D and E, and positive-control of national *C. perfringens* strains were also included in the PCR.

Antimicrobial sensitivity test. Thirty-two *C. perfringens* isolates were examined *in vitro* against eight antibiotics, which included streptomycin (10 μg), amoxicillin (10 μg), ampicillin (10 μg), ciprofloxacin (5 μg), lincomycin (30 μg), cefotaxime (30 μg), rifampicin (5 μg), and trimethoprim-sulfamethoxazole (1.25 + 23.75 μg) (Oxoid, UK). The test was carried out using the agar disc diffusion test, (17, 26). The isolates were cultured anaerobically at 37° for 24 h in 10% neomycin sheep blood agar, then suspended in 0.9% NaCl to a 0.5 McFarland standard. Every isolate was inoculated onto Mueller-Hinton agar plates (Remel, USA) and the antibiotic discs were applied. The plates were incubated anaerobically at 37° for 24 h. The interpretation of the results was performed according to CLSI, 2012 (8).

Statistical analysis. PASW Statistics, Version 18.0. software (SPSS Inc., USA) was used to analyse the data. The Chi square (x^2) test was applied (provided that at least 80% of the cells had an expected frequency of five or greater, and that no cell had an expected frequency smaller than 1.0). Otherwise, the Fisher-Freeman-Halton Exact test was used (*i.e.* the Fisher's Exact test for contingency tables larger than 2x2). A value of $P < 5$ was considered as significant.

Results

Overall, 32 *C. perfringens* isolates were obtained from 200 processed chicken meat samples (luncheon, nuggets, burger, and sausage; 50 of each with an incidence of 16%, Table 2). There was a relationship between the kind of meat and the occurrence of *C. perfrigens.* A high isolation rate was observed in sausage samples (16/50), followed by luncheon (12/50), while nuggets showed the lowest rate (1/50). The results in Table 2 show that the rate of *C. perfringens* type A was high (24/32), followed by that of type D (7/32), and then the unprecedented isolation of type E in sausage (1/32).

Fig. 1A shows the amplification of *alpha* toxin gene at 324 bp, representing *C. perfringens* type A, while amplifications of *epsilon* toxin gene at 655 bp and *alpha* toxin gene represented *C. perfringens* type D. In addition, Fig. 1A shows the presence of enterotoxigenic *C. perfringens* gene (*cpe*) at 233 bp. In Fig. 1B, type C was used as a positive control revealing two bands, one at 324 bp for the *alpha* gene and another at 196 bp for the *beta* gene. Fig. 1B also shows the amplification of *iota* toxin gene at 446 bp and *alpha* toxin gene representing the isolated *C. perfringens* type E. The *cpe* gene was represented in Fig. 1 by amplification at 233 bp for enterotoxigenic *C. perfringens* type A (Fig. 1A) and *C. perfringens* enterotoxin type E (Fig. 1B). According to Table 3, the enterotoxigenic isolates which carried *cpe* gene amounted to 9 out of 32 *C. perfringens* isolates with an incidence of 28 %. Enterotoxigenic *C. perfringens* type A was most frequently isolated (6/24), followed by type D (2/7), and then type E (1/1).

Antibiotic sensitivity test. The occurrence of antibiotic resistance among 32 *C. perfringens* isolates was as follows: streptomycin (100%), lincomycin (100%), trimethoprim-sulfamethoxazole (94%), ciprofloxacin (41%), cefotaxime (34%), and rifampicin (31%). On the other hand, the isolates showed high sensitivity to amoxicillin (94%) and ampicillin (97%). Out of 32 *C. perfringens* isolates, the nine that harboured the enterotoxin gene, demonstrated a higher resistance to the antibiotics compared to non-enterotoxigenic isolates (Table 4).

Unauthenticated
Download Date | 7/27/17 4:48 PM

Table 1. Multiplex PCR primers for five toxins genes of *C. perfringens*

Gene	Primer Sequence	Amplified size (bp)	Reference
cpa	GCTAATGTTACTGCCGTTGA CCTCTGATACATCGTGTAAG	324	(14)
cpb	GCGAATATGCTGAATCATCTA GCAGGAACATTAGTATATCTTC	196	
etx	GCGGTGATATCCATCTATTC CCACTTACTTGTCCTACTAAC	655	
ia	ACTACTCTCAGACAAGACAG CTTTCCTTCTATTACTATACG`	446	
cpe	ACTACTCTCAGACAAGACAG CTTTCCTTCTATTACTATACG`	233	

Table 2. Occurrence of *C. perfringens* among examined samples and typing of *C. perfringens* isolates by using multiplex PCR

Sample	Number of samples	Positive samples		*C. perfringens* type						P-value
				A		D		E		
		Number	%	Number	%	Number	%	Number	%	
Luncheon	50	12	24	9	75	3	25	0	0	0.0 *
Nuggets	50	1	2	1	100	0	0	0	0	1.0
Burger	50	3	6	3	100	0	0	0	0	0.035 *
Sausage	50	16	32	11	69	4	25	1	6	0.0 *
Total	200	32	16	24	75	7	21.9	1	3.1%	0.0 *
P-value		0.000 *		0.885		0.999		1.000		

* a significant difference

Table 3. Occurrence of *C. perfringens* enterotoxin in different toxin types

Sample	*C. perfringens* enterotoxin type					
	A		D		E	
	Number	%	Number	%	Number	%
Luncheon	2(9)	22	1(3)	33	0(0)	0
Nuggets	0(1)	0	0(0)	0	0(0)	0
Burger	1(3)	33	0(0)	0	0(0)	0
Sausage	3(11)	27	1(4)	25	1(1)	100
Total	6	25	2	28	1	100
P-value	0.999		0.999		1.000	
	9 out of 32 (28%)					

Table 4. Antibiogram of *Clostridium perfringens* strains isolated from processed chicken meat products

Antimicrobials	Antibiotic profile											
	Resistant						Sensitive					
	*[a] CPE+ (9)		*[b] CPE- (23)		Total (32)		CPE+ (9)		CPE- (23)		Total (32)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Streptomycin	9	100	23	100	32	100	0	0	0	0	0	0
Lincomycin	9	100	23	100	32	100	0	0	0	0	0	0
Trimethoprim-sulfamethoxazole	9	100	21	91	30	94	0	0	2	9	2	6
Ciprofloxacin	6	67	7	30	13	41	3	33	16	70	19	59
Cefotaxime	4	44	7	30	11	34	5	56	16	70	21	66
Rifampicin	5	56	5	22	10	31	4	44	18	78	22	69
Amoxicillin	1	11	1	4	2	6	8	89	22	96	30	94
Ampicillin	1	11	0	0	1	3	8	89	23	100	31	97

*[a] CPE+ – *C. perfringens* enterotoxin positive; *[b] CPE- – *C. perfringens* enterotoxin negative

Unauthenticated
Download Date | 7/27/17 4:48 PM

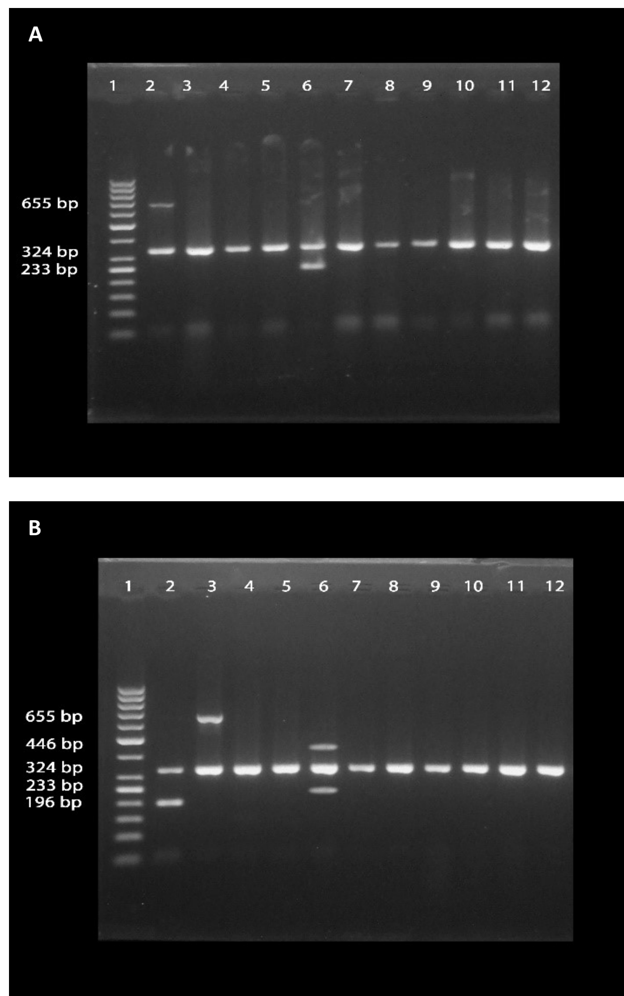

Fig. 1. Agarose gel electrophoresis of multiplex PCR products of *C. perfringens* strains isolated from selected chicken meat products and a positive control

A) Lane 1 – 50bp DNA ladder. Lane 2 – *C. perfringens* type D (positive control). Lane 6 – *C. perfringens* type A, enterotoxin. Lanes 3, 4, 5, and 7 to 12 – *C. perfringens* type A

B) Lane 1 – 50bp DNA ladder. Lane 2 – *C. perfringens* type C (positive control). Lane 3 – *C. perfringens* type D. Lane 6 – *C. perfringens* type E, enterotoxin. Lanes 4, 5, and 7 to 12 – *C. perfringens* type A

Discussion

Food can be a source of different human ailments, therefore identification and control of food pathogens are a fundamental part of nourishment microbiology. *C. perfringens* was responsible for food poisoning in the 1940s; ever since, cases of food poisoning due to *C. perfringens* contamination have been reported (6).

According to our results, *C. perfringens* was isolated in 16% of 200 processed chicken meat samples collected from the Egyptian markets. This result is similar to that of Nasr *et al*. (23) who reported an isolation rate of 14.3%, while in another study, a high 37% prevalence was noted (20). In our study, the occurrence of *C. perfringens* may be possibly attributed to the method adapted in preparing chicken meat products. Moreover, the occurrence of *C. perfringens* may be ascribed to a high protein content in sampled products (13).

As shown in Table 2 and Figs 1A and 1B, *C. perfringens* type A was the most predominant type among *C. perfringens* isolates. The percentages of isolation of that type from luncheon, nuggets, burger, and sausage were 75, 100, 100, and 69, respectively. *C. perfringens* type D was isolated in 25%, 0%, 0%, and 25% of the cases, respectively. *C. perfringens* type E was only isolated from sausage samples, constituting 6% of isolates. These results are in accordance with results of Nasr *et al*. (23) who found that 70.8% of toxigenic isolates from processed chicken meat products proved to be *C. perfringens* type A, while 12.9% of the isolates belonged to type D.

A small percentage of *C. perfringens* produces the enterotoxin (CPE), which is responsible for food poisoning. CPE has been also associated with sporadic diarrhoea and in some cases with sudden infant death syndrome (5).

Table 3 shows that out of 32 *C. perfringens* strains, six of enterotoxigenic strains belonged to type A, two to type D, and one to type E. This is similar to observations of Khan *et al*. (16) who toxinotyped six *C. perfringens* isolates from raw chicken meat and found that three of them were of type A and one each of the remaining three belonged to types B, C, and D. Likewise, the recognition of enterotoxin genes in type A isolates collected from raw and processed chicken meat products was reported by Guran and Oksuztepe (13). Moreover, the prevalence of *C. perfringens* exotoxin and enterotoxin was not related to the type of product ($P > 0.05$) (Tables 2 and 3). In fact, *C. perfringens* type A was isolated in almost all studies in which poultry meat was examined (24). Additionally, *cpe* gene was identified in *C. perfringens* isolates of all types (A-E), but type A was unusually common (18).

Interestingly, our results (Fig. 1B) provided some novel information about the isolation of *C. perfringens* enterotoxin type E from chicken sausage. This observation may account for several foodborne *C. perfringens* outbreaks that could be linked mainly to chromosomal *cpe* type A strains with heat-resistant spores, or occasionally less heat-resistant spore-forming plasmid *cpe* type A strains; both can be found in retail foods (21).

Furthermore, we examined the occurrence of resistance of 32 *C. perfringens* isolates to eight antibiotics which are widely used in poultry farms (Table 4). It was found that antibiotic sensitivity was highly related to the type of antibiotic used, either in *C. perfringens* enterotoxin-positive or -negative strains. The resistance to streptomycin, lincomycin, and trimethoprim-sulfamethoxazole was 100%, 100%, and 94% respectively, while appropriate percentages for ciprofloxacin, cefotaxime, and rifampicin were 41, 34, and 31, respectively. On the other hand, *C. perfringens* isolates showed high sensitivity to amoxicillin (94%) and ampicillin (97%).

Unauthenticated
Download Date | 7/27/17 4:48 PM

These results are in agreement with those of Osman and Elhariri (26) who mentioned that *C. perfringens* isolates showed high resistance to streptomycin (100%), lincomycin (100%), and trimethoprim-sulfamethoxazole (98%). Furthermore, previously reported results (27, 2) showed intermediate sensitivity of *C. perfringens* to cefotaxime, ciprofloxacin, and low sensitivity to lincomycin. Abd-El Gwad and Abd El-Kader (1) demonstrated that *C. perfringens* isolates were highly sensitive to ampicillin, ciprofloxacin, and amoxicillin, which is consistent with our results suggesting that ampicillin and amoxicillin may be the drugs of choice for *C. perfringens* infection (3). Moreover, we found that *C. perfringens* isolates which carried enterotoxin gene showed a higher resistance to the antibiotics than non-enterotoxigenic isolates.

The frequent isolation of *C. perfringens* from processed chicken meat makes a public health risk. Its prevalence may be due to unhygienic measures not only during chicken rearing, but also during industrial processing. *C. perfringens* in poultry seems to occur very early and can be transmitted within the broiler chicken production, starting from the hatchery (10). Notably, *C. perfringens* is found in eggshell cracks, chicken fluff, and paper pads present in the hatchery (11).

Our study affirmed that there is a noticeable increase in the occurrence of enterotoxigenic *C. perfringens* in processed chicken meat, especially sausage. Moreover, we identificated *C. perfringens* enterotoxin type E in chicken meat. The study also contends that enterotoxigenic isolates of *C. perfringens* are more resistant to antibiotics than non-enterotoxigenic isolates.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This work was not funded by any organisation.

Animal Rights Statement: None required.

Acknowledgements: We would like to express our deepest thanks to Dr. Elshaimaa Ismael, Faculty of Veterinary Medicine, Cairo University, Egypt, for her kind help in statistical analysis.

References

1. Abd-El Gwad A.M., Abd El-Kader H.A.: The occurrence of *Clostridium perfringens* in the intestine of broiler chickens Assiut governorate. Ass Univ Bull Environ Res 2001, 4, 13–22.
2. Abd El–Hamid H.S., Ellakany H.F., Aboelmagd B.A., Elbestawy A.R., Bedawy Sh.: Clinical and laboratory studies on chicken isolates of *Clostridium perfringens* in El–Behera, Egypt. J World's Poult Res 2015, 5, 21–28.
3. Agunos A., Léger D., Carson C.: Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada. Can Vet J 2012, 53, 1289–300.
4. Ata N., Khairy E.A., Dorgham S.M., Zaki M.S.: *Clostridium perfringens* disease. Life Sci J 2013, 10, 1599–1602.
5. Bos J., Smithee L., McClane B., Distefano R.F., Uzal F.: Fatal necrotizing colitis following a foodborne outbreak of enterotoxigenic *Clostridium perfringens* type A infection. Clin Infect Dis 2005, 40, 78–83.
6. Brynestad S., Granum P.E.: *Clostridium perfringens* and foodborne infections. Int J Food Microbiol 2002, 74, 195–202.
7. Cakmak O., Ormanc F.S.D., Tayfur M., Erol I.: Presence and contamination level of *Clostridium perfringens* in raw frozen ground poultry and poultry burgers. Turk J Vet Anim Sci 2006, 30, 101–105.
8. CLSI: Performance standards for antimicrobial susceptibility testing, Twenty-Fourth Informational supplement. 2012, M100–S24. Villanova, USA.
9. Collee J.G., Fraser A.G., Marmion B.P., Simmons A.: Practical medical microbiology. 14th education, Churchill Living Stone, New York 1996.
10. Craven S.E., Cox N.A., Bailey J.S., Cosby D.E.: Incidence and tracking of *Clostridium perfringens* through an integrated broiler chicken operation. Avian Dis 2003, 47, 707–711.
11. Craven S.E., Cox N.A., Stern N.J., Mauldin J.M.: Prevalence of *Clostridium perfringens* in commercial broiler hatcheries. Avian Dis 2001, 45, 1050–1053.
12. Freedman C.J., Archana S., Bruce A., McClane B.A.: *Clostridium perfringens* enterotoxin: action, genetics, and translational applications. Toxins 2016, 8, 73.
13. Guran H.S., Oksuztepe G.: Detection and typing of *Clostridium perfringens* from retail chicken meat parts. Lett Appl Microbiol 2013, 57, 77–82.
14. Hamza D.A., Ghoneim N.H.: Epidemiological studies on *Clostridium perfringens* food poisoning, Scholar press. Omniscriptum Marketing DEU, Germany 2015.
15. Hook D., Jalaludin B., Fitzsimmons G.: *Clostridium perfringens* food-borne-outbreak: an epidemiological investigation. Aust NZ J Public Health 1996, 20, 119–122.
16. Khan M., Nazir J., Anjum A.A., Ahmad M.U., Nawaz M.: Toxinotyping and antimicrobial susceptibility of enterotoxigenic *Clostridium perfringens* isolates from mutton, beef and chicken meat. J Food Sci Technol 2015, 52, 5323–5328.
17. Martel A., Devriese L.A., Cauwerts K., De-Gussem K., Decostere A.: Susceptibility of *Clostridium perfringens* isolates from broiler chickens to antibiotics and anti-coccidials. Avian Pathol 2004, 31, 3–7.
18. McClane B.A.: *Clostridium perfringens*, In: *Food Microbiology*, edited by Doyle M.P., Beuchat L.R., ASM press, Washington 2007, pp. 423–444.
19. Miki Y., Miyamoto K., Kaneko-Hirano I., Fujiuchi K., Akimoto S.: Prevalence and characterization of enterotoxin gene carrying *Clostridium perfringens* from retail meat products in Japan. Appl Environ Microbiol 2008, 74, 5366–5372.
20. Miwa N., Nishina T., Kubo S., Atsumi M, Honda H.: Amount of enterotoxigenic *Clostridium perfringens* in meat detected by nested PCR. Int J Food Microbiol 1998, 42, 195–200.
21. Miyamoto K., Li J., McClane B.A.: Enterotoxigenic *Clostridium perfringens*: detection and identification. Microbes Environ 2012, 27, 343–349.
22. Murray P.R., Baron E.J.O., Pfaller M.A.J., Orgensen J.H., Yolken R.H.: Clostridium, Manual of Clinical Microbiology, Vol. 1, ASM Press, Washington 2003, pp. 940–966.
23. Nasr E.M., Shehta A.A., Amer A.H.: Enterotoxigenicity and typing of *Clostridium perfringens* isolates from some poultry products in Egypt. J Appl Sci Res 2007, 3, 1804–1808.
24. Nowell V.J., Poppe C., Parreira V.R., Jiang Y.F., Reid-Smith R.: *Clostridium perfringens* in retail chicken. Anaerobe 2010, 16, 314–315.
25. Okolocha E.C., Ellerbroek L.: The influence of acid and alkaline treatments on pathogens and the shelf life of poultry meat. Food Control 2005, 16, 217–225.

Unauthenticated
Download Date | 7/27/17 4:48 PM

26. Osman K.M., Elhariri M.: Antibiotic resistance of *Clostridium perfringens* isolates from broiler chickens in Egypt. Rev Sci Tech Off Int Epiz 2013, 32, 841–850.
27. Silva R.O.S., Francisco C.F.J., Marcus V.R.M., Carlos A.O.J., Nelson R.M.: Genotyping and antimicrobial susceptibility of *Clostridium perfringens* isolated from *Tinamidae*, *Cracidae* and *Ramphastidae* species. Brazil Cienc Rural 2014, 44, 486–491.
28. van Asten A.J., van der Wiel C.W., Nikolaou G., Houwers D.J., Grone A.A.: Multiplex PCR for toxin typing of *C. perfringens*. Vet Microbiol 2009, 136, 411–412.
29. Voidarou C., Vassos D., Rozos G., Alexopoulos A., Plessas A.: Microbial challenges of poultry meat production. Anaerobe 2011, 17, 341–343.

Unauthenticated
Download Date | 7/27/17 4:48 PM