



Incidence and antibiogram of *Clostridium perfringens* isolated from herbs and spices widely distributed in the Egyptian market.

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ABSTRACT

The present study was designed for isolation and identification of *Clostridium perfringens* (*C. perfringens*) from herbs and spices that are widely distributed in the Egyptian markets; and determination of the drug of choice for its control based on the Antimicrobial Susceptibility Test (AST). A total of 392 samples of herbs and spices were randomly and anonymously selected from the enormous number of samples obtained from the Egyptian markets. An overall occurrence of *C. perfringens* was 8.42% (33 out of 392). Separately, occurrence was higher in herbs than spices being 10.5 and 6.25%, respectively. *C. perfringens* isolates were identified and confirmed with macroscopical, microscopical and biochemical analysis. AST results revealed that isolates of *C. perfringens* from the tested herbs and spices samples were found most susceptible to ampicillin-sulbactam and Penicillin-G with inhibition zones of 19.4 ± 0.98 and 16.6 ± 1.16 mm, respectively. On the other hand, the isolates were found nearly resistant to clindamycin and vancomycin with inhibition zones of 6.28 ± 0.63 and 8.78 ± 0.41 mm, respectively. Meanwhile, the isolates were intermediary susceptible to trimethoprim-sulphamethoxazole combination and chloramphenicol. Data of the present study indicate occurrence of *C. perfringens* in herbs and spices with a considerable rate and the antimicrobials of choice for its control are penicillins and ceftazidime. higher sanitation procedures in herbs and spices industry before its distribution to the Egyptian markets have been recommended.

Keywords: *C. perfringens*, Incidence, Prevalence, Herbs, Spices, AST.

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1. INTRODUCTION

Clostridium perfringens (*C. perfringens*; formerly known as *Clostridium welchii*, or *Bacillus welchii*) is a Gram-positive, rod-shaped, anaerobic, spore-forming pathogenic bacterium of the genus *Clostridium* (Ryan and Ray, 2004). It is ever present in nature and can be found as a normal component of decaying vegetation, marine sediment, the intestinal tract of humans and other vertebrates, insects, and soil. It has the shortest reported generation time of any organism at 6.3 minutes in thioglycollate medium (Bionumbers, 2016). *C. perfringens* is a major cause of human and veterinary enteric disease largely because this bacterium can produce several toxins when present inside the gastrointestinal tract (Smedley Iii et al., 2004). The bacterium causes human gas gangrene and food poisoning as well as several enterotoxemic diseases of animals (Rood, 1998). Spices often act as important vectors for various microorganisms, especially spore formers implicating possible health

problems for consumers; among these microorganisms is *C. perfringens* and its enterotoxin (Banerjee and Sarkar, 2004); (Aguilera et al., 2005). Contaminated spices have been reported to cause foodborne illness and spoilage. Fewer microorganisms are present in spices with higher antimicrobial activity such as sage, cloves, and oregano. Therefore, all spices and herbs should be cleaned and decontaminated with ethylene oxide, irradiation, or other acceptable methods (Giese, 1994). Spices and herbs are considered as a potential source of infection with *C. perfringens* where they may be contaminated because of conditions in which they were grown and harvested. The spores of *C. perfringens* were proved to be present in spices and herbs by (Kneifel and Berger, 1994) and (Pafumi, 1986). A few studies have been tried to assess the incidence of *C. perfringens* in herbs and spices elsewhere worldwide, including South Africa (Baxter and Holzappel (1982), Austria

(Kneifel and Berger (1994), Mexico (Rodríguez-Romo et al. (1998) , India (Banerjee and Sarkar (2003), Turkey (Hampikyan et al. (2009), United Kingdom (Sagoo et al. (2009) , Italy (Vitulo et al. (2011), Lebanon (Debs-Louka et al. (2013), Saudi Arabia (Hassan and Altalhi (2013), However, to our information, no parallel study has been performed in Egypt.

Therefore, the aim of the present study was to investigate the incidence (prevalence) of *C. perfringens* in herbs and spices delivered to and distributed all over the Egyptian markets and to determine the antimicrobial of choice for its control. To fulfil this aim, the following objectives have been investigated: i) isolation of *C. perfringens* from the randomly selected samples; ii) identification of *C. perfringens* in isolates macroscopically, microscopically and biochemically; and iii) antibiotic sensitivity testing of *C. perfringens* in identified isolates.

2. MATERIALS AND METHODS

2.1. Samples

Three hundred and ninety-two samples obtained from the Egyptian markets have been tested for incidence of *C. perfringens* in the present study. Table (1) shows the detailed descriptions of samples used.

2.2. Isolation of *C. perfringens*

2.3. Preparation of test sample:

The samples were prepared in accordance with ISO 6887-2, ISO 6887-3, ISO 6887-4 or ISO 8261.

2.3.1. Test portion, initial suspension and dilutions:

This was done according to ISO 6887 or ISO 8261. Ten g of each sample was taken and diluted with 90 ml maximal recovery diluent (MRD) then mixed well in stomacher and 1 ml was then inoculated into a sterile test tube containing 9.0 ml of MRD. This inoculated suspension was then serially diluted into further tubes containing 9.0 ml MRD.

2.3.2. Inoculation and incubation (Poured-plate technique):

By means of a sterile pipette, 1 ml of the initial prepared suspension (or of the test sample if the initial product is liquid) was transferred, in duplicate, to the centers of sterile petri dishes. Then, 10 to 15 ml of the perfringens agar base with its supplements maintained at 44° C to 47° C in the water bath, were poured into the dish and mixed well with the inoculum by gentle rotation. When the medium solidified, an over-layer of 10 ml of the same perfringens agar base agar was added. The

plates were incubated under anaerobic conditions at 37° C for 20 ± 2 hrs.

2.4. Identification of *C. perfringens*

Identification of *C. perfringens* was accomplished by classical microbiological techniques including, macroscopical appearance and culture characters of grown colonies according to hemolysis (Brett, 1994); microscopical appearance after Gram staining (Oxoid, Code: R40080) of a film prepared from a pure culture according to Beveridge (2001) and by biochemical tests according to schemes of Koneman et al. (1992) using the following tests: Catalase, gelatin hydrolysis, sugar fermentation, indole test, nitrate reduction, urease and lecithinase activity.

2.5. Antimicrobial Susceptibility Test (AST)

Antimicrobial Susceptibility Testing was performed using the Kirby-Bauer Disc Diffusion method (Biemer, 1973). The following discs have been used: Ampicillin 10 µg + sulbactam 10 µg (SAM 20), Chloramphenicol 30 µg (C 30), Ciftazidium + Clavulanic acid (CAL 40), Clindamycin 2 µg (CD 2), Erythromycin 15 µg (E 15), Norfloxacin 10 µg (NOR 10), Penicillin G 10 units (PG 10), Tetracycline 30 µg (T30), Trimethoprim 1.25 µg + Sulphamethoxazole 23.75 µg (TS 25) and Vancomycin 5 µg (VA 5).

2.6. Data management and Statistical analysis

The obtained results were statistically analyzed using EXCEL® software version 2013. The number of positive samples against the total number of examined samples was calculated as %. AST was done in triplicates; and inhibition zones were calculated as Mean ± SEM of three observations.

3. RESULTS

3.1. Incidence of *C. perfringens* in the tested herbs and spices

As shown in Table (2), out of 392 herbs and spices samples obtained from Egyptian markets, totally, 33 isolates were detected with an incidence of 8.42%. The incidence of *C. perfringens* in herbs was 10.5% (21 out of 200 Samples) and in spices was 6.25% (12 out of 192). Table (3) shows the incidence of *C. perfringens* in herb samples while table (4) shows its incidence in spice samples.

3.2. Identification of *C. perfringens* isolates

3.2.1. Culture characters

on blood base agar with 7% sterile blood, *C. perfringens* colonies were 2-3 mm in diameter, rounded, raised, glistening and showed double

zone of haemolysis. On TSC agar, *C. perfringens* colonies were black in colour.

3.2.2. Microscopical examination

Smears were done from characteristic colonial appearance, stained with Gram's stain and examined under oil immersion lens. *C. perfringens* isolates appeared to be Gram positive short plumb bacilli rarely having central oval non-bulging endospores.

3.2.3. Nagler's test

On egg yolk agar medium, the effect of alpha toxin on lecithin gave opalescence on the half of the plate without antitoxin while this was inhibited on the second half with antitoxin. Also all isolates demonstrated positive results in lecithinase activity on TSC agar containing egg-yolk emulsion.

3.2.4. DNase test

DNase test was done for all *C. perfringens* isolates and nearly 69.7% (23 out of 33) gave positive result indicated by pink halo around the streaks.

3.2.5. Biochemical identification

Results of biochemical tests were confirmative to *C. perfringens*; where results of sugar fermentation test revealed that the isolates were positive for glucose, lactose and sucrose sugars while mannitol fermentation was negative. The isolates liquefied gelatin while catalase and indole were negative.

3.2.6. Antimicrobial Susceptibility Testing results

Results of AST are shown in Table (5) with inhibition zones (mm) produced by 10 antibacterials against *C. perfringens* isolates from herbs and spices. *C. perfringens* were highly sensitive to ampicillin + salbactam and Penicillin-G with inhibition zones of 19.4 ± 0.98 and 16.6 ± 1.16 mm, respectively. Meanwhile, the isolates were resistant to clindamycin and vancomycin with inhibition zones of 6.28 ± 0.63 and 8.78 ± 0.41 mm, respectively. Sulphamethoxazol-trimethoprim combination and chloramphenicol gave intermediate efficacy with inhibition zones of 14.6 ± 0.84 and 13.8 ± 0.51 , respectively.

Table (1): List of herbs and spices tested for incidence of *C. Perfringens*

Item name	Item description	Number of samples	Item name	Item description	Number of samples
Anise	Herb	5	Black pepper	Spice	7
Basil	Herb	45	Capsicum	Spice	6
Caraway	Herb	5	Cheese	Spice	18
Chamomile	Herb	5	Cheese + Onion	Spice	18
Calendula	Herb	5	Cilantro	Spice	10
Dill	Herb	10	Coriander	Spice	15
Fennel	Herb	20	Cumin	Spice	6
Henna	Herb	5	Hot chilli	Spice	30
Majoram	Herb	50	Onion	Spice	50
Mint (Dry)	Herb	20	Olive	Spice	15
Parsley	Herb	20	Thyme	Spice	12
Rose Mary	Herb	5	Verbascum	Spice	5
Sage	Herb	5			
Number of Herbs		200	Number of spices		192
Total number of samples				392	

Table (2): Incidence of *C. perfringens* in examined herbs and spices samples

Type Of Samples	Number of Tested samples	Number of +ve Samples	Incidence %
Herbs	200	21	10.5
Spices	192	12	6.25
Overall	392	33	8.42

Table (3): Incidence of *C. perfringens* in examined herb samples

Item Name	No. of Samples	Positive Samples	Incidence %
Anise	5	1	20
Basil	45	4	8.9
Caraway	5	0	0
Chamomile	5	2	40
Calendula	5	1	20
Dill	10	1	10
Fennel	20	1	5
Henna	5	1	20
Majoram	50	6	12
Mint (Dry)	20	1	5
Parsley	20	1	5
Rose Mary	5	0	0
Sage	5	2	40

Table (4): Incidence of *C. perfringens* in examined spice samples

Item Name	No. of Samples	Positive Samples	Incidence %
Black pepper	7	0	0
Capsicum	6	0	0
Cheese	18	0	0
Cheese + Onion	18	1	5.6
Cilantro	10	2	20
Coriander	15	0	0
Cumin	6	1	16.7
Hot chilli	30	1	3.3
Onion	50	5	10
Olive	15	0	0
Thyme	12	1	8.3
Verbascum	5	1	20

Table (5): Inhibition zones (mm) produced by 10 antibacterials against *C. perfringens* isolates from herbs and spices.

Sample	SAM20	PG10	CAL40	E15	CD2	TS25	T30	NOR10	VA5	C30
1	13.33	5.17	4.23	8	5	9	8	10.67	8	11
	±	±	±	±	±	±	±	±	±	±
	0.88	0.44	0.39	0.58	0.58	0.58	0.58	0.67	0.58	0.58
2	11.17	16.27	4.93	10.03	5.23	20.03	7.2	10.1	5.03	20.03
	±	±	±	±	±	±	±	±	±	±
	0.60	0.64	0.35	0.32	0.28	0.32	0.42	0.55	0.20	0.26
3	25	25.2	20.17	10.07	7.33	5.2	10.1	5.23	10.97	10.07
	±	±	±	±	±	±	±	±	±	±
	1.15	0.81	0.38	0.23	0.38	0.47	0.21	0.39	0.32	0.41
4	25.03	25.1	17.5	5.07	5.1	15.07	6.07	9.17	12.1	12.97
	±	±	±	±	±	±	±	±	±	±
	0.260	0.15	0.29	0.12	0.21	0.35	0.23	0.38	0.21	0.26
5	27	20.03	19.87	10.07	15	15.1	8.07	14.9	12.03	15.17
	±	±	±	±	±	±	±	±	±	±
	0.29	0.20	0.24	0.23	0.58	0.38	0.35	0.32	0.26	0.38
6	17.03	20.3	10.27	7.93	5.03	14.93	8.1	10.83	7.07	15.97
	±	±	±	±	±	±	±	±	±	±
	0.43	0.35	0.37	0.23	0.55	0.41	0.49	0.38	0.52	0.32
7	20.07	18.2	14.3	10.03	5.07	15.17	9.93	8.13	7.23	14.03
	±	±	±	±	±	±	±	±	±	±
	0.23	0.25	0.35	0.32	0.23	0.27	0.23	0.47	0.34	0.09
8	NA									
9	20.07	5.13	15.1	5.07	4.03	15	9.03	8.1	5.07	15
	±	±	±	±	±	±	±	±	±	±
	0.35	0.24	0.26	0.52	0.15	0.58	0.55	0.49	0.35	0.58
10	15.17	10	18.03	5	5.17	15	9	9.13	6.07	12.07
	±	±	±	±	±	±	±	±	±	±
	0.44	0.58	0.32	0.58	0.44	0.58	0.58	0.41	0.52	0.23
11	20	18.1	15	10.23	5	10	4.88	5.2	10	14.97
	±	±	±	±	±	±	±	±	±	±
	0.58	0.21	0.58	0.28	0.58	0.58	0.30	0.36	0.58	0.20
12	15	10	5	6.23	5	10.17	6	8.1	5.3	14.93
	±	±	±	±	±	±	±	±	±	±
	0.29	0.58	0.58	0.34	0.29	0.27	0.58	0.15	0.57	0.29
13	15	18	5	11	5	15	13.03	10.03	6.16	13.03
	±	±	±	±	±	±	±	±	±	±
	0.29	0.58	0.58	0.12	0.29	0.58	0.26	0.55	0.55	0.55
14	30.03	23	20.03	13	17.97	8.1	10	15	10.1	16
	±	±	±	±	±	±	±	±	±	±
	0.55	0.58	0.26	0.58	0.26	0.55	0.58	0.17	0.26	0.58
15	23	17.03	8.03	3	3	15	8	10.23	8	11
	±	±	±	±	±	±	±	±	±	±
	0.58	0.26	0.32	0.58	0.29	0.58	0.58	0.34	0.58	0.58
16	20	20.1	16.97	11.97	10	16.97	16.03	15.07	13	15.03
	±	±	±	±	±	±	±	±	±	±
	0.58	0.55	0.32	0.26	0.58	0.15	0.49	0.35	0.58	0.26
17	25.03	27.13	22.4	15.03	10.07	5	8.1	8.03	10.1	5
	±	±	±	±	±	±	±	±	±	±
	0.38	0.52	0.31	0.15	0.52	0.58	0.49	0.26	0.55	0.58
18	12	7.03	6.03	10	5.03	3	3.03	3.03	10.03	5.97
	±	±	±	±	±	±	±	±	±	±
	0.58	0.43	0.55	0.58	0.26	0.58	0.26	0.55	0.61	0.26
19	22.03	21.03	16.03	5	5.03	18	8.06	11.1	10.07	15.1
	±	±	±	±	±	±	±	±	±	±
	0.26	0.15	0.55	0.58	0.43	0.40	0.52	0.55	0.52	0.55
20	17.1	7	20.03	5.1	5	15	4.97	15.03	4.97	15
	±	±	±	±	±	±	±	±	±	±

	0.26	0.58	0.09	0.26	0.06	0.58	0.09	0.49	0.26	0.58
	6	4.97	6.03	2.9	3.07	14.97	7	8.03	7.07	12.03
21	±	±	±	±	±	±	±	±	±	±
	0.58	0.20	0.61	0.21	0.23	0.55	0.58	0.55	0.64	0.55
	30.07	25.03	9	15.07	7	16.03	11.07	6.03	12	15.07
22	±	±	±	±	±	±	±	±	±	±
	0.29	0.55	0.58	0.35	0.58	0.26	0.52	0.26	0.52	0.41
	20.07	20.03	16.1	10.1	5	22.07	10.1	12.1	7	18.03
23	±	±	±	±	±	±	±	±	±	±
	1.10	0.96	0.49	0.496	0.58	0.23	0.55	0.44	0.58	0.32
	20.03	22.07	5.07	12.03	5.03	17.13	11.17	13.17	8.17	15.13
24	±	±	±	±	±	±	±	±	±	±
	0.49	0.52	0.52	0.09	0.32	0.47	0.44	0.44	0.44	0.41
	18.17	16	10.1	12.07	3	15.27	6.97	10.1	8.17	15.43
25	±	±	±	±	±	±	±	±	±	±
	0.27	0.58	0.38	0.29	0.58	0.32	0.26	0.49	0.44	0.34
	14.97	15.1	5	13	4	20.13	10.03	9.17	10.07	15.07
26	±	±	±	±	±	±	±	±	±	±
	0.55	0.26	0.58	0.58	0.58	0.52	0.55	0.44	0.92	0.52
	15	12	6.07	7	4	18.17	15.03	8	12.1	11.13
27	±	±	±	±	±	±	±	±	±	±
	0.58	0.58	0.35	0.58	0.58	0.22	0.09	0.58	0.38	0.47
	15.23	16.33	5.03	14.2	4.97	17.17	9	8.1	7.17	14.43
28	±	±	±	±	±	±	±	±	±	±
	0.45	0.60	0.20	0.36	0.09	0.73	0.58	0.49	0.44	0.54
	27.5	25.47	15	7	5	25.5	8.17	4.97	10.07	13.33
29	±	±	±	±	±	±	±	±	±	±
	0.76	0.52	0.58	0.58	0.29	0.66	0.44	0.15	0.52	0.60
	15.57	12	2.87	10.17	2.9	15.43	15.4	7.17	9.3	14
30	±	±	±	±	±	±	±	±	±	±
	0.72	0.58	0.19	0.44	0.21	0.54	0.67	0.44	0.35	0.58
	15.63	12	5	14.6	5.033	18.23	10.47	10.17	10.27	15.33
31	±	±	±	±	±	±	±	±	±	±
	0.68	0.58	0.58	0.86	0.15	0.39	0.52	0.44	0.43	0.55
	20.3	25.43	16.03	12.3	10.1	16	10.1	8.07	11.07	15.1
32	±	±	±	±	±	±	±	±	±	±
	0.91	0.70	0.55	0.46	0.49	0.58	0.55	0.23	0.52	0.61
	26.5	10	10.1	15.2	15	14.07	20.6	5	10	15.07
33	±	±	±	±	±	±	±	±	±	±
	0.76	0.58	0.21	0.47	0.58	0.52	0.70	0.17	0.58	0.23
	17.43	17.13	6	17.1	5.033	10.03	15.1	9.07	6	13.03
34	±	±	±	±	±	±	±	±	±	±
	0.81	0.70	0.58	0.38	0.55	0.61	0.38	0.52	0.58	0.38
Overall	19.37	16.59	11.40	9.84	6.28	14.57	9.63	9.28	8.78	13.77
Mean	±0.98	±1.16	1.06	±0.66	±0.63	±0.84	±0.63	±0.53	±0.41	±0.51

4. DISCUSSION

Microbiological evaluation studies to determine the prevalence or extent of occurrence of microbes and their strain identification are principal prerequisites to control or reduce safety hazards in food or feed or their additives. The International Standardization Organization (ISO) has regulated requirements for food safety hazards, including microbiology end point with final goal of human and animal safety and welfare (Seagrave, 2007).

Among microbes carrying the risk of foodborne illness is *C. perfringens* on which the present study was focused. *C. perfringens*, formerly *welchii*, has been known as a cause of the serious wound infection, gas gangrene

In the present study, it has been found that occurrence of *C. perfringens* in the tested herbs and spices samples was 8.42% (33 out of 392 samples). This finding may be parallel to Fujisawa et al. (2001) who found occurrence of *C. perfringens* in 7 samples from total of 60 (12%) commercially available curry roux samples in Japan. The present

finding may also comparable to Aguilera et al. (2005) who detected presence of *C. perfringens* in 14 out of 115 spices samples (12.17%) in Argentina. However, the result of the present study may be higher than that reported by Kneifel and Berger (1994) who detected *C. perfringens* only in one caraway sample out of 160 samples of 55 different spices and herbs originating from six different suppliers and retailed at outlets in Vienna; in the same manner Warمیńska-Radyko et al. (2007) detected that none of the examined vegetable salads available at retail stores, supplied by various producers in Poland, contained *C. Perfringens*; and also by Sagoo et al. (2009) who reported incidence value of 0.4% of *C. perfringens* in dried spices and herbs from production and retail premises in the United Kingdom. On the other hand, the result in the current study was much lower than that found by Powers et al. (1975) who detected an incidence of 15% of *C. perfringens* in the spices procured by the Army, Navy, Marines and Air Force in USA especially in oregano samples (53%); and by De Boer and Boot (1983) who found different incidence rates for *C. perfringens* according to the used media being out of 147 samples, 42% were positive when RPM was used, 23% with PEM, 19% with TSC surface plate and 26% with TSC pour plates. Moreover, the data are also lower than incidence rate value of 59% in herbs and spices in India (Banerjee and Sarkar, 2003).

The discrepancy in incidence results may be attributed to various factors including sample sources, sanitation procedures adopted by suppliers, different locations, different materials and methodologies as well as different research environments.

Application of the identification techniques in the present study revealed that on blood base agar with 7% sterile blood, *C. perfringens* colonies were 2-3 mm in diameter, rounded, raised, glistening and showed double zone of haemolysis. While on TSC agar, *C. perfringens* colonies were black in colour. These findings are in agreement with Brett (1994); Cowan et al. (2004) and Abd Al-Tawab et al. (2015) who isolated *C. perfringens* from different sources and purified colonies with the same morphological characters. Reference characters of *C. perfringens* colonies were stated by Markey et al. (2013) as grey, flat and often with irregular edge, 2.0–3.0 mm, with double zone of haemolysis.

Once a pure culture is obtained, the results from a few comparatively simple conventional tests can often identify the bacterium to a generic level, including: a Gram-stained smear from the culture will establish the Gram reaction (Gram-positive or

Gram-negative); and the bacterial cell morphology whether cocci or rods ... etc (Markey et al., 2013). Microscopical examination of Gram stained smears from isolates obtained from herbs and spices in the present study revealed that *C. perfringens* isolates appeared to be Gram positive short plumb bacilli rarely having central oval non-bulging endospores. These findings are in agreement with the reference microscopical characters of *C. perfringens* that were stated by Adams and Moss (2005) as a Gram-positive, rod-shaped anaerobe which forms oval subterminal spores. It differs from most other *Clostridia* in that the relatively large rods (1 x 3-9 pm) are encapsulated and nonmotile. The findings are also consistent with those reported by previous researchers as Cato et al. (1986); Rood and Cole (1991); Heredia and Labbé (2001); McClane (2003); and Labbe and Juneja (2006).

Also, all isolates obtained in the present study demonstrated positive results in lecithinase activity on TSC agar containing egg-yolk emulsion. However, the effect of alpha toxin produced from tested isolates on lecithin gave opalescence only on the half of the plate without *C. perfringens* antitoxin while this was inhibited on the second half with antitoxin. DNase test revealed that nearly 69.69% of the isolates were positive. Further biochemical tests were confirmative to *C. perfringens*; where isolates were positive for glucose, lactose and sucrose sugar fermentation; liquefied gelatin while they were negative for catalase and indole tests. The findings may be consistent with parallel studies carried out by Cowan et al. (2004); ISO (2004); Abd Al-Tawab et al. (2015).

To complete such microbiological study circle, it is not enough to just identify the isolated microorganism. It is also needed to elect what antimicrobial agents the microorganism is most susceptible to (Karlowsky and Richter, 2015). Among the several methods used for AST, the Kirby-Bauer method is best incorporating many of the features recommended by the World Health Organization. It is relatively simple from a technological standpoint and accurate and reproducible; Thus it has been applied in the present study. Isolates of *C. perfringens* from the tested herbs and spices samples were found most susceptible (>15 mm inhibition zone) to ampicillin 10 µg-sulbactam 10 µg (SAM 20) and penicillin G 10 units (PG 10). On the other hand, the isolates were found nearly resistant to clindamycin 2 µg (CD 2) and vancomycin. The isolates were intermediary susceptible to trimethoprim 1.25 µg + sulphamethoxazole 23.75 µg (TS 25) and chloramphenicol. Findings of this study section

may be in partial agreement with Sapico et al. (1972) who found that *C. perfringens* strains were susceptible to penicillin and with poor susceptibility to erythromycin. However, the present findings are not parallel with their results where they found that the strains were susceptible to clindamycin. The AST data is also in agreement with Dutta and Devriese (1980) regarding resistance of *C. perfringens* strains to erythromycin and lincomycin and susceptibility to penicillin. The present data may be in partial parallelism with Marchand-Austin et al. (2014) who found that *C. perfringens* isolates were universally susceptible to penicillin, however, about 14% of isolates were resistant.

5. CONCLUSION

Data of the present study may indicate occurrence of *Clostridium perfringens* in herbs and spices with a rate of about 8% and thus these food additives may act as a source of foodborne disease indicated by conventional microbiological techniques. In addition, the *Clostridium perfringens* isolates were highly susceptible to ampicillin-Salbactam, penicillin and ciftazidium-clavulanic acid, indicated by Antibiotic Susceptibility Test (AST). Accordingly and depending on the data of the present study that may carry biological hazard to both man and animal beings, the authors recommend avoiding of irrational use of herbs and spices as food/feed additives without veterinary supervision; applying the standards of ISO-22000 concerning food safety regulated and labelled by The Egyptian Organization for Standardization and Quality Control (EOS) and International Organization for Standardization (ISO); and developing simple and economic screening field tests to identify different pathogenic bacteria, including *C. perfringens* in food/feed and their additives, including herbs and spices.

6. REFERENCES

- Abd Al-Tawab, A.A., El-Hofy, F.I., Khater, D.F., Kotb, M.A., 2015. Typing of *Clostridium perfringens* isolated from some meat products by using PCR. *Benha Veterinary Medical Journal* 29, 118-123.
- Adams, M., Moss, M., 2005. Bacterial agents of foodborne illness—*Clostridium perfringens*. In, *Food Microbiology*. The Royal Society of Chemistry 2000, Cambridge, UK, pp. 212-217.
- Aguilera, M.O., Stagnitta, P.V., Micalizzi, B., de Guzmán, A.M.S., 2005. Prevalence and characterization of *Clostridium perfringens* from spices in Argentina. *Anaerobe* 11, 327-334.
- Banerjee, M., Sarkar, P.K., 2003. Microbiological quality of some retail spices in India. *Food Research International* 36, 469-474.
- Banerjee, M., Sarkar, P.K., 2004. Growth and enterotoxin production by sporeforming bacterial pathogens from spices. *Food Control* 15, 491-496.
- Baxter, R., Holzapfel, W., 1982. A microbial investigation of selected spices, herbs, and additives in South Africa. *Journal of Food Science* 47, 570-574.
- Beveridge, T.J., 2001. Use of the Gram stain in microbiology. *Biotechnic & Histochemistry* 76, 111-118.
- Biemer, J.J., 1973. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. *Annals of Clinical & Laboratory Science* 3, 135-140.
- Bionumbers, 2016. ID 105474. Available at <http://bionumbers.hms.harvard.edu/bionumber.aspx?id=105474>. In, City.
- Brett, M., 1994. Outbreaks of food-poisoning associated with lecithinase-negative *Clostridium perfringens*. *Journal of medical microbiology* 41, 405-407.
- Cato, E., George, W.L., Finegold, S., 1986. Genus *clostridium*. *Bergey's manual of systematic bacteriology* 2, 1141-1200.
- Cowan, S.T., Barrow, G., Steel, K.J., Feltham, R., 2004. *Cowan and Steel's manual for the identification of medical bacteria*. Cambridge university press.
- De Boer, E., Boot, E., 1983. Comparison of methods for isolation and confirmation of *Clostridium perfringens* from spices and herbs. *Journal of Food Protection* 46, 533-536.
- Debs-Louka, E., El Zouki, J., Dabboussi, F., 2013. Assessment of the Microbiological Quality and Safety of Common Spices and Herbs Sold in Lebanon. *J Food Nutr Disor* 2 4, 2.
- Dutta, G., Devriese, L., 1980. Susceptibility of *Clostridium perfringens* of animal origin to fifteen antimicrobial agents. *Journal of Veterinary Pharmacology and Therapeutics* 3, 227-236.
- Fujisawa, T., Aikawa, K., Takahashi, T., Yamai, S., Ueda, S., 2001. Occurrence of clostridia in commercially available curry roux. *Food Hygiene and Safety Science* 42, 394-397.
- Giese, J., 1994. Spices and seasoning blends: A taste for all seasons. *Food technology*.(USA).

- Hampikyan, H., Bingol, E.B., Colak, H., Aydin, A., 2009. The evaluation of microbiological profile of some spices used in Turkish meat industry. *Journal of Food, Agriculture & Environment* 7, 111-115.
- Hassan, S., Altalhi, A., 2013. Safety Assessment of Spices and Herbs Consumed In Saudi Arabia: Microbiological Quality and Toxin Production. *Life Sci. J* 10, 2819-2827.
- Heredia, N.L., Labbé, R.G., 2001. Clostridium perfringens. *Guide to foodborne pathogens*, 133-142.
- ISO, E., 2004. 7937: Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of Clostridium perfringens. Colony-count technique. European Standard, 3rd ed., BSI, London, UK.
- Karlowsky, J.A., Richter, S.S., 2015. Antimicrobial Susceptibility Testing Systems. In, *Manual of Clinical Microbiology*, Eleventh Edition. American Society of Microbiology, pp. 1274-1285.
- Kneifel, W., Berger, E., 1994. Microbiological criteria of random samples of spices and herbs retailed on the Austrian market. *Journal of Food Protection* 57, 893-901.
- Koneman, E.W., Allen, S.D., Janda, W., Schreckenberger, P., Winn, W., 1992. *Diagnostic microbiology*. Koneman EW, Allen SD. Mycobacteria. Philadelphia: JB Lippincott Company, 703-755.
- Labbe, R.G., Juneja, V., 2006. Clostridium perfringens gastroenteritis. *Foodborne infections and intoxications* 3, 137-184.
- Marchand-Austin, A., Rawte, P., Toye, B., Jamieson, F.B., Farrell, D.J., Patel, S.N., 2014. Antimicrobial susceptibility of clinical isolates of anaerobic bacteria in Ontario, 2010–2011. *Anaerobe* 28, 120-125.
- Markey, B., Leonard, F., Archambault, M., Cullinane, A., Maguire, D., 2013. *Clinical veterinary microbiology*. Elsevier Health Sciences.
- McClane, B.A., 2003. Clostridium perfringens. *Food Science and Technology-New York-Marcel Dekker*, 91-104.
- Pafumi, J., 1986. Assessment of the microbiological quality of spices and herbs. *Journal of Food Protection* 49, 958-963.
- Powers, E.M., Lawyer, R., Masuoka, Y., 1975. Microbiology of processed spices. *Journal of Milk and Food Technology (JMFT)* 38, 683-687.
- Rodríguez-Romo, L.A., Heredia, N.L., Labbé, R.G., García-Alvarado, J.S., 1998. Detection of enterotoxigenic Clostridium perfringens in spices used in Mexico by dot blotting using a DNA probe. *Journal of Food Protection* 61, 201-204.
- Rood, J.I., 1998. Virulence genes of Clostridium perfringens. *Annual Reviews in Microbiology* 52, 333-360.
- Rood, J.I., Cole, S.T., 1991. Molecular genetics and pathogenesis of Clostridium perfringens. *Microbiological Reviews* 55, 621-648.
- Ryan, K., Ray, C., 2004. Sherris medical microbiology: an introduction to infectious diseases. 4th. In. McGraw Hall USA, City.
- Sagoo, S., Little, C., Greenwood, M., Mithani, V., Grant, K., McLauchlin, J., De Pinna, E., Threlfall, E., 2009. Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. *Food microbiology* 26, 39-43.
- Sapico, F.L., Kwok, Y.-Y., Sutter, V.L., Finegold, S.M., 1972. Standardized antimicrobial disc susceptibility testing of anaerobic bacteria: in vitro susceptibility of Clostridium perfringens to nine antibiotics. *Antimicrobial agents and chemotherapy* 2, 320-325.
- Seagrave, P., 2007. ISO 22000: food safety management systems and their related requirements. In: *Sixth world congress on seafood safety, quality and trade*, pp. 153-157.
- Smedley Iii, J., Fisher, D., Sayeed, S., Chakrabarti, G., McClane, B., 2004. The enteric toxins of Clostridium perfringens. In, *Reviews of physiology, biochemistry and pharmacology*. Springer, pp. 183-204.
- Vitullo, M., Ripabelli, G., Fanelli, I., Tamburro, M., Delfino, S., Sammarco, M., 2011. Microbiological and toxicological quality of dried herbs. *Letters in applied microbiology* 52, 573-580.
- Warmińska-Radyko, I., Łaniewska-Trokanheim, Ł., Miś, M., 2007. Microbiological contamination of vegetable salads. *PUBLISHER UWM*, 733.