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Molecular characterization of enterotoxigenic *Bacillus cereus* isolated from meat products and human in Kaliobia, Egypt

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Abstract: The present study was performed on 210 random samples of meat products (beef burger, kofta, luncheon, minced meat, sausage) and diarrheic human stool of patients suffering from vomition and diarrhoea (35 for each), were collected from different shops and hospitals at Kaliobia Governorate, Egypt, for bacteriological examination for detection the prevalence of enterotoxigenic *B. cereus* strains in these samples, beside the phenotypic characterization and detection of some virulence genes in them. Bacteriological examination of the collected samples resulted in, isolation of 51 (24.3%) isolates of *B. cereus* from 210 samples, (11/31.4%) from koftasamples; (13/37.1%) minced meat; (9/25.7%) sausage; (7/20.0%) beef burger; (6 /17.1%) luncheon and (5/14.3%) from human stool samples. The isolated *B. cereus* strains were enterotoxigenic ones, as they had haemolytic; amylase; proteolytic; lipolytic and Lecithinase activities. The PCR results cleared that, the diagnostic, phylogenetic marker gene of *B. cereus* (*gro*EL) was amplified in all 10 studied *B. cereus* isolates and *cyt*K; *hbl; nhe*; and *ces* enterotoxigenic virulence genes were detected in 9; 7; 8 and 6, respectively, out of 10 studied isolates. Moreover, the *gro*EL gene in isolated *B. cereus* sequences was seem to be identical by 95.80 % identity with the strains of *B. cereus* with Gene Bank sequences. So, it was concluded that, *B. cereus* strains are enterotoxigenic ones and they may be the causative agents in patients suffering from vomition and diarrhoea, as they are meat-borne pathogens of public health importance.

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Key words: Human stool; Meat products, enterotoxins, virulence genes

1. Introduction

Enterotoxigenic *Bacilluscereus* strain considered as one of the most important foodborne pathogen through consuming contaminated foods and a known human pathogens causing emetic and diarrheal syndromes (**Ceuppens *et al.* 2013** and **Sánchez-Jennifer, *et al.*, 2014)**. This bacterium is a Gram-positive**,** motile**,** rod shaped endospore-forming bacteria, aerobic that also grows well anaerobically and characterized as mesophilic or psychrotrophic; mesophilic strains have a growth range of 15-55^{O}C and their spores tend to be more heat resistant. Whereas, psychrotrophic ones have a growth range of 4-35^{O}C and their spores tend to be less heat resistant **(Organji *et al*., 2015).** Although *B. cereus* is implicated in many foodborne illness outbreaks in many countries worldwide, however only a few cases are reported because the symptoms are mostly similar to *Staphylococcus aureus* and *Clostridiumperfringens* food poisoning (**Stenfors- Arnesen *et al*., 2008; Bottone, 2010** and **Bennett *et al*., 2013**). The pathogenicity of *B. cereus* could be attributed to large number of secreted cytotoxins that may contribute to diarrhoeal disease, that is elicited by three pore-forming heat-labile enterotoxins; the two enterotoxin-complexes *nhe* (non-hemolytic enterotoxin) and *hbl* (haemolysin BL), each consist of three different protein components, named *nhe*A, *nhe*B, *nhe*C and L2, L1, respectively, beside single-component toxin *cyt*K "cytotoxin K" (**Stenfors Arnesen *et al*., 2008**; **Fagerlund *et al.*, 2010** and **Pfrunder *et al*., 2016**). In addition to these proteins, *B. cereus* produces degradative enzymes (proteases, sphingomyelinase, phosphatidylinositol- and phosphatidylcholine-specifc phospholipases "PIPLC and PC-PLC") which are either secreted or directed to the cell surface (**Abostate *et al*., 2006** and **Gohar *et al*., 2008**). The gastrointestinal manifestation of the disease caused by *B. cereus* is connected to two clinical pictures: diarrhoea and emesis. In general, both types of food poisoning are relatively mild and self-limiting, and symptoms usually disappear within 24 h.

Nevertheless, during the last few years, severe forms of both types of disease have occasionally involved hospitalization or even deaths (**Dierick *et al.*, 2005**). The diarrhoeal toxicoinfection, type of *B. cereus* food poisoning is elicited by heat-labile enterotoxins produced by bacteria in the small intestine of the host; the two enterotoxin-complexes *nhe* (non-hemolytic enterotoxin) and *hbl* (hemolysin BL) and the single protein *cyt*K "cytotoxin K" (**Neil *et al.*, 2003** and **Stenfors Arnesen *et al.*, 2008**). Symptoms are abdominal pain and diarrhoea, appears 8 to 16 hours after ingestion of contaminated food, and normally disappears within 12 to 24 hours. Nevertheless more severe cases requiring hospitalization have been described; endocarditis; meningitis and fatal cases were recorded (**Lund *et al.*, 2000** and **Logan and Rodrigez-Diaz, 2006**). In contrast, the emetic syndrome is caused by intoxication that is caused by a heat- and acid stable emetic toxin cereulide, produced by *B. cereus* within a food matrix prior to consumption, and probably elicits emesis by stimulating the vagus afferent through binding to the 5-HT3 receptor (**Agata *et al.*, 1995** and **Ehling-Schulz *et al.*, 2004**). Sometimes both types of symptoms are produced probably due to the synergistic effects of one or more enterotoxin (s), *B. cereus* produces emetic toxin and four other enterotoxins: hemolysin BL, non-hemolytic enterotoxin; cytotoxin K (**Lindback *et al.*, 2004).** PCR-based techniques are used increasingly in food-microbiology research as they are well developed and when applied as culture confirmation tests, they are reliable, fast and sensitive. PCR methods offer a sensitive and specific detection of pathogens and can discriminate virulent bacteria from a virulent member of the same species as well (**Malorny *et al.*, 2003** and **Oltuszak-walczak and Walczak, 2007**). As *B. cereus* induced food poisoning symptoms in human and the level of contamination of meat products with *B. cereus* constitutes serious problems for consumers, so, the present study was conducted to throw light over *B. cereus* isolates specially enterotoxigenic in common meat products (beef burger; kofta; luncheon; minced meat and sausage) beside diarrheic human stool of patients suffering from vomition and diarrhoea at Kaliobia Governorate, Egypt, beside the phenotypic characterization of the isolate and determination of virulence genes in them.

2. Materials and Methods

2.1. Samples

A total of 210 random samples of meat products (beef burger, kofta, luncheon, minced meat, sausage) and diarrheic human stool of patients suffering from vomition and diarrhoea (35 for each), were collected from different shops and hospitals at Kaliobia Governorate, Egypt, for detection the prevalence of enterotoxigenic *B. cereus* strains in these samples, beside the phenotypic characterization and detection of some virulence genes in them.

2.2. Bacteriological examination

A total of 25 grams of each meat product sample under examination were prepared for bacteriological examination following **APHA (2001**). Beside two grams of each stool sample was homogenized in 18 mL of sterile pure water then 1.0 mL was added to a universal bottle containing 9.0 mL of 0.1% peptone water and incubated at 37ºC for 24 h for primary enrichment. Observation of turbidity in enrichment cultures was considered as a presumptive positive result **(Organji *et al.*, 2015)**.

2.2.1. Isolation and identification of *B. cereus* strains following Markey *et al.* (2013):

Typical *B. cereus* colonies (blue colonies that surrounded by a blue zone of egg yolk precipitation against greenish yellow background on *Bacillus cereus* agar base with Polymyxin B and Egg yolk supplements and whitish colonies with a zone of precipitation and red media on *Bacillus cereus* medium with Polymyxin B and Egg yolk supplements) were picked up for identification morphologically by Gram stain and biochemical tests following **De Vos *et al.* (2009)** and **Markey *et al.* (2013).**

2.2.2. Detection of virulence factors of *Bacillus cereus* strains

A. Phenotypic virulence factors of *Bacillus cereus* strains: The haemolytic; amylase; proteolytic (caseinase); lipolytic and lecithinase activities for isolated *B. cereus* strains, tests were performed as described by **Yang and Fang (2003).**

B. Genotypic identification and detection of virulence genes in *Bacillus cereus* strains by PCR.

Genotypic identification of10 random *B. cereus* isolates using diagnostic, phylogenetic marker gene of *B. cereus* (*gro*EL) beside genotyping detection of cytotoxic K gene (*cyt*K); hemolysin BL **(*hbl*)** gene; non-hemolytic enterotoxin (*nhe***)** gene and emetic toxin cereulide, cereulide synthetase gene (*ces*) in these isolates using polymerase chain reaction, following QIAamp® DNA Mini Kit instructions (Qiagen, Germany, GmbH), Emerald Amp GT PCR master mix (Takara, Japan) and 1. 5% agarose gel electrophoreses **(Sambrook *et al.*, 1989)** using the Primers sequences, target genes, amplicons sizes and cycling conditions showed in Table (1).

2.2.3. Sequence of *gro*EL of *B. cereus*

The purified PCR product was sequenced using Sanger Dideoxy method (**Sanger *et al.*, 1977**). The sequences of the gene fragment of the isolates were compared with other bacterial sequences by using NCBI GenBank database using the BLAST program, available at website

http://blast.ncbi.nlm.nih.gov/Blast.cgi phylogenetic tree was performed by using MEGA 6 program. Sequence of *gro*EL of *B. cereus* Bankit2294277 Bacillus with accession numbers MN845929.

3-Results

The results of bacteriological examination of examined meat product and human stool samples; phenotypic virulence factors; genotyping identification; genotyping detection of virulence genes and phylogenetic tree for the isolated *B. cereus* strains were tabulated in Tables (2-3) and Figures (1-6).

Table (1): Primers sequences, target genes, amplicons sizes and cycling conditions

Target gene		Primer sequence (5'-3')	Amplified segment (bp.)	Primary denaturation	Amplification (35 cycles)			Final extension	References
					Secondary denaturation	**Annealing**	**Extension**		
groEL	F	TGCAACTGTATTAGCACAAGC T	533bp.	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Das *et al*., 2013**
	R	TACCACGAAGTTTGTTCACTACT							
cytK	F	ACA GAT ATC GGI CAA AAT GC	421 bp.	94˚C 5 min.	94˚C 30 sec	49˚C 40 sec	72˚C 45 sec.	72˚C 10 min.	**Ehling-Schulz*et al*., 2006**
	R	CAA GTI ACT TGA CCI GTT GC							
hbl	F	GTAAGCGAACCTGTCTGTAACAACA	1091bp.	94˚C 5 min.	94˚C 30 sec	49˚C 40 sec.	72˚C 1 min.	72˚C 10min	
	R	GTA AAT TAI GAT GAI CAA TTTC							
nhe	F	AAG CIG CTC TTC GIA TTC	766 bp.	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
	R	ITI GTT GAA ATA AGC TGT GG							
ces	F	GGTGACACATTATCATATAAGGTG	1271 bp.	94˚C 5 min.	94˚C 30 sec	49˚C 40 sec.	72˚C 1.2 min.	72˚C 12 min.	
	R	GTAAGCGAACCTGTCTGTAACAACA							

Table (2): Prevalence of *B. cereus* strains isolated from examined samples

Samples	Number of sample	Negative samples		Positive samples	
		No.	%	No.	%
Beef burger	35	28	80.0	7	20.0
kofta	35	22	62.9	13	37.1
luncheon	35	29	82.9	6	17.1
Minced meat	35	24	68.6	11	31.4
Sausage	35	26	74.3	9	25.7
Stool	35	30	85.7	5	14.3
Total	210	159	75.7	51	24.3

Percentage in relation to total number of each sample in each row (35 for each sample and 210 for total).

Table (3): Phenotypic virulence factors of *B. cereus* isolates

Phenotypic virulence activities	*B. cereus* strains	
	No.	%
Haemolytic activity (β- haemolysis)	50	98.0
Starch hydrolysis (amylase activity)	50	98.0
Proteolytic (caseinase) activity	49	96.1
Lipolytic activity	48	94.1
Lecithinase activity	51	100.0

%: Percentage in relation to total number of isolates (51)

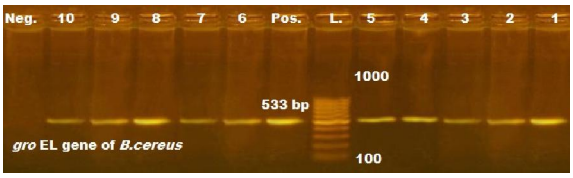

Fig (1): Diagnostic, phylogenetic marker gene of *B. cereus* (*gro*EL)
Lane L: 100-1000 bp. DNA Ladder. Neg.: Negative control (*E. coli* AJ413986)
Pos.: Positive control (*B.cereus* form Ahri. at 533 bp.)
Lane 1-10: *B. cereus* (Positive at 533 bp.)

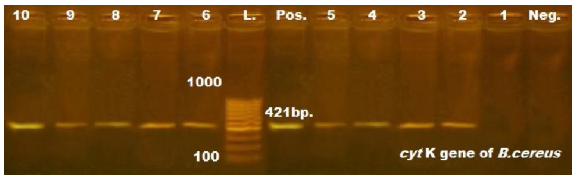

Fig. (2): Cytotoxic K (*cyt*K) gene
Lane L: 100-1000 bp. DNA Ladder. Neg.: Negative control (*E. coli* AJ413986)
Pos.: Positive control (*B.cereus* form Ahri. at 421 bp.)
Lane 2- 10: *B.cereus* (Positive at 421 bp.)
Lane 1: *B.cereus* (Negative)

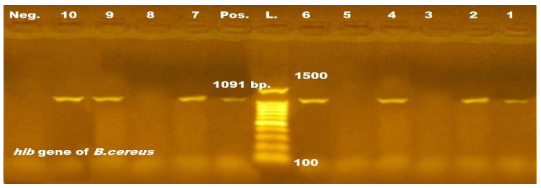

Fig. (3): Hemolysin BL (*hlb*) gene
Lane L: 100-1500 bp. DNA Ladder. Neg.: Negative control (*E. coli* AJ413986)
Pos.: Positive control (*B.cereus* form Ahri. at 1091 bp.)
Lane 1; 2; 4; 6; 7; 9 & 10: *B.cereus* (Positive at 1091 bp.) Lane 3; 5 & 8: *B.cereus* (Negative)

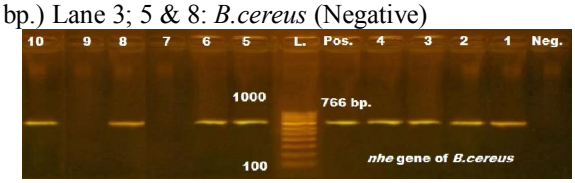

Fig. (4): Non-hemolytic enterotoxin (*nhe*) gene
Lane L: 100-1000 bp. DNA Ladder. Neg.: Negative control (*E. coli* AJ413986)
Pos.: Positive control (*B.cereus* form Ahri. at 766 bp.)
Lane 1- 6; 8 & 10: *B.cereus* (Positive at 766 bp.) Lane 7 & 9: *B.cereus* (Negative)

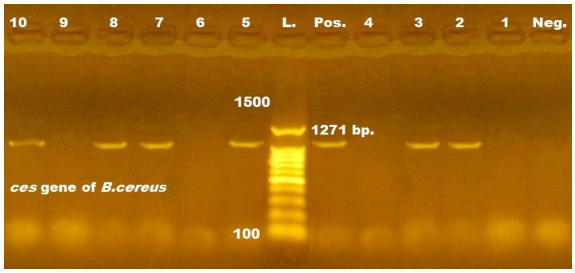

Fig. (5): Cereulide synthetase (*ces*) gene
Lane L: 100-1500 bp. DNA Ladder. Neg.: Negative control (*E. coli* AJ413986)
Pos.: Positive control (*B.cereus* form Ahri. at 1271 bp.)
Lane 2; 3; 5; 7; 8 & 10: *B.cereus* (Positive at 1271 bp.)
Lane 1; 4; 6 & 9: *B.cereus* (Negative)

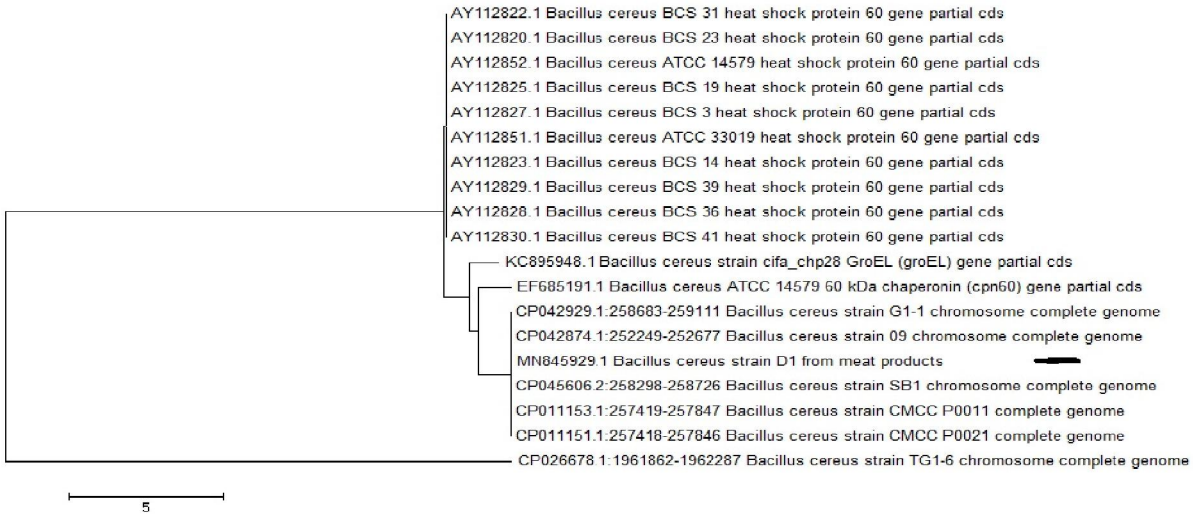

Fig. (6): The phylogenetic tree for the strains related to the isolated *B.cereus*

4- Discussion

Enterotoxigenic *B. cereus* strains is considered as an important foodborne pathogenin meat products that has been isolated from the stools of adults and children at many countries (**Stenfors Arnesen *et al.*, 2008; Humphries and Linscott, 2015** and **Soleimani *et al.*, 2017).** Meanwhile, the data concerning the occurrence of this pathogen in meat products and its involvements in food poisoning with diarrheal cases in Egypt are sparse.

The results of bacteriological examination of examined samples (Table, 2) revealed that, a total of 51 (24.3%) isolates of *B. cereus* were recovered from 210 samples; where they were mostly isolated from koftasamples (13/37.1%) followed by minced meat (11/31.4%) then sausage (9/25.7%); beef burger (7/20.0%); luncheon (6 /17.1%) and stool samples (5/14.3%). The results of *B. cereus* isolation from meat products were nearly similar to those obtained by **Abd El-Tawab *et al.* (2015); Salim, Dalia *et al.* (2015); Shawish and Tarabees (2017); Soleimani *et al.* (2017)** and **Abd El-Wahaab, Shimaa *et al.* (2018)**. But disagree with those obtained by **Rather *et al.* (2011)**; **Tewari *et al.* (2012)** and **El-Sayed (2019)**

who isolated *B. cereus* from meat products with lower incidence and with those of **Ghanaym (2014)** who recorded higher incidence. Moreover, the highest isolation of *B. cereus* from sausage and minced meat samples, this may be due to the added additives and spices, which are considered a potential risk factor that can increase the number of Bacillus spores and hence magnitude the incidence of food poisoning. The results of *B. cereus* isolation from human stool, conceded those recorded by **Banerjee *et al.* (2011)**; **Martinelli *et al.* (2013**) and **Organji *et al.* (2015**) who isolated *B. cereus* strains from stool samples of diarrheic patients. Moreover, the surveillance systems for foodborne disease differ between countries, and so it is difficult to compare data and obtain true incidence estimates. Several factors contribute to underreporting of most outbreaks of foodborne *B. cereus* disease. The clinical course is generally short and mild, so patients rarely seek medical attention, and when diagnosed, the disease is not always reportable (**Stenfors Arnesen *et al.*, 2008; Al-Abri *et al.*, 2011 and Organji *et al.*, 2015**). As the diagnosis of diarrhea stool samples in clinical laboratories in Egypt is solely based on the detection of Salmonella, Shigella, *E. coli* and Entamoeba. Thus, diarrhea caused by other pathogens, Campylobacter and *B. cereus* may not be reported and usually if stool cultures were negative for the sought after pathogens, then the diarrhea case could be reported as "unknown etiology" and/or "viral infection". So, detection of *B. cereus* must be considered in these cases.

The results of phenotypic virulence factors of isolated *B. cereus* strains (Table, 3) showed that, 50 *B. cereus* isolated strains (98.0%) had haemolytic activity, as they showed large grayish circular, smooth, glistening colonies and surround by β-haemolysis (β- Hemolytic activity will show lysis and complete digestion of red blood cell contents surrounding colony). These results came in harmony with those recorded by **Wu *et al.* (2008)**; **Chon *et al.* (2012)**; **Kumari and Sarkar (2014)** and **Visiello *et. al.* (2016).** For starch hydrolysis (amylase activity), 50 *B. cereus* isolated strains (98.0%) showed positive, the colonies are surrounded by clear zone around them, as they hydrolyzed starch on starch agar and detected by logul iodine due to amylase enzyme. Similar results were recorded by **Chon *et al.* (2012)**; **Kumari and Sarkar (2014)** and **Ozdemir and Arslan (2019).** For proteolytic (caseinase) activity, 49 *B. cereus* isolated strains (96.1%) had protease enzyme that was shown by the formation of a clear zone on milk agar media due to proteolysis of milk casein. Similar results were obtained by **Chon *et al.* (2012); Kumari and Sarkar (2014)**; **Sharaf, Eman *et al.* (2014)** and **Ozdemir and Arslan (2019).** The lipolytic activity appeared that, 48 *B. cereus* isolated strains (94.1%) presented lipolytic activity on agar supplemented with tributyrin and were detected by a transparent zone surrounding the colony on an opaque background. Similar results were recorded by **Chon *et al.* (2012)**; **Kumari and Sarkar (2014)** and **Ozdemir and Arslan (2019).** Also**,** all isolated *B. cereus* strains (100.0%) had Lecithinase activity that was clearly marked by an opaque zone extending from the edge of the colony. These results were agreed with those of **Chon *et al.* (2012)**;**Sharaf - Eman *et al.* (2014)** and **Kumari and Sarkar (2014)**. So, isolated *B. cereus* strains were enterotoxigenic ones, as they had haemolytic; amylase; proteolytic; lipolytic and Lecithinase activities.

The PCR technique is capable of identifying the enterotoxigenic *B. cereus* isolates. Based on the fact that virulence genes varies not only among different species but also among strains of the same species. Thus, numerous studies have been conducted to identify virulence factors genes of isolated *B. cereus* strains (**Kim *et al.*, 2010; Savic *et al.*, 2015 and Rather *et al.*, 2016).** So, the present study was directed mainly for identification of 10 *B. cereus* isolates besides recognizing 4 virulence genes that may play a role in pathogenicity of these isolates by using one of the recent developments molecular biological techniques (PCR). These genes were diagnostic, phylogenetic marker gene of *B. cereus* (*gro*EL); cytotoxic K gene (*cyt*K); hemolysin BL **(*hbl*)** gene; non-hemolytic enterotoxin (*nhe***)** gene andemetic toxin cereulide, cereulide synthetase gene (*ces*)**.**

Bacillus cereus strains were identified genotypically through detection the diagnostic, phylogenetic marker gene of *B. cereus* (*gro*EL) and the PCR results showed that, it was amplified in all 10 studied *B. cereus* isolates giving product of 533 bp. as shown in Fig. (1), so, all of them were *B. cereus*. Similar detection of *gro*EL gene, as a phylogenetic marker for identification of *B. cereus* strains from other *B. cereus* group strains isolated from food and human, was recorded by **Park *et al.* (2007)**; **Yushan *et al.* (2010)**; **Lim *et al.* (2011)** and **Kim *et al.* (2013)**.

The diarrheal syndrome due to *B. cereus* is caused by enterotoxins produced by the bacteria in the small intestine, which act on the epithelial cells, causing massive secretion of fluid into the intestinal lumen leading to diarrhea **(Madigan *et al.*, 2003)**. *Bacillus cereus* produces three different enterotoxins that are believed to be involved in food poisoning, (*cyt*K) cytotoxin K; (*hbl*) hemolysin BL and (*nhe*) non-hemolytic enterotoxin **(Granum, 2001 and Moravek *et al.*, 2006).** The result of PCR amplification of the cytotoxic K **(*cyt*K)** gene in *B. cereus* isolates (Fig.,2) showed that, the *cyt*K gene was amplified in 9 out of 10 studied *B. cereus* isolates giving product of 421 bp. The results came in harmony with those of **Sánchez *et al.* (2014); Tewari**

***et al.* (2015)**; **Tewari and Singh (2015); Rather *et al.* (2016); Zhang *et al.* (2016); Jung *et al.* (2017)** and **Abd El-Wahaab, Shimaa *et al.* (2018)**. Meanwhile, **Ozdemir and Arslan (2019)** failed to detect *cyt*K gene in *B. cereus* strains. The result of PCR amplification of the hemolysin BL **(*hbl*)** in *B. cereus* isolates (Fig., 3) revealed that, the *hlb* gene was amplified in 7 out of 10 studied *B. cereus* strains giving product of 1091 bp. These results came in accordance with those recorded by **Sánchez *et al.* (2014)**; **Tewari *et al.* (2015)**; **Tewari and Singh (2015); Kohneshahri *et al.* (2016); Rather *et al.* (2016); Zhang *et al.* (2016)**; **Jung *et al.* (2017)** and **Ozdemir and Arslan (2019).** The result of PCR amplification of the non-hemolytic enterotoxin **(*nhe*)** gene in *B. cereus* isolates (Fig., 4) showed that, the *nhe* gene was amplified in 8 out of 10 studied *B. cereus* strains giving product of 766 bp. These results were agreed with those obtained by **Lee *et al.* (2012); Sánchez- Jennifer, (2014); Tewari *et al.* (2015)**; **Tewari and Singh (2015); Rather *et al.* (2016); Jung *et al.* (2017)**; **El-Shora, Heba (2019)** and **Ozdemir and Arslan (2019).**

The emetic syndrome is caused by a cyclic dodecadepsipeptide, cereulide which produced in food during vegetative growth, no treatment can destroy this stable molecule, including stomach acid and the proteolytic enzymes of the intestinal tract **(Granum, 2001)**. After release from the stomach into the duodenum, cereulide is bound to a 5-HT3 receptor **(Agata *et al.*, 1995)**, and stimulation of the vagus afferent causes emesis (vomiting). The result of PCR amplification of the cereulide synthetase **(*ces*)** in *B. cereus* isolates (Fig., 5) showed that, the *ces* gene was amplified in 6 out of 10 studied *B. cereus* strains giving product of 1271 bp. Similar findings were recorded by **Chon *et al.* (2012); Lee *et al.* (2012); Kim *et al.* (2013); Salim- Dalia *et al.* (2015); Savic *et al.* (2015); Jung *et al.* (2017)** and **El-Sayed (2019)**. Meanwhile, **Ahaotu *et al.* (2013)**; **El-Shora, Heba (2019)** and **Ozdemir and Arslan (2019)** failed to detect *ces* gene in *B. cereus* strains.

Regarding the sequence detection of *gro*EL gene in isolated *B. cereus* strains, the sequences obtained for *B. cereus* with provided Gene Bank accession number MN845929 (phylogenetic tree, Fig., 6), it was seem to be identical by 95.80 % identity with the strains of *B. cereus* with the following Gene Bank sequences, EF685191.1. for **Hill *et al.* (2013)**; KC895948.1. for **Tripathy *et al.* (2013);** AY112825.1, AY112822.1, AY112827.1, AY112829.1 and AY112851.1 for **Chang *et al.* (2016)**;. CP011153.1 and CP011151.1 for **Wang *et al.* (2016)**; CP026678.1 for **Vilchez (2018)**; CP045606.2 for **Batinovic and Petrovski (2019);** CP042929.1 for **Wang (2019)** and CP042874.1 for **Wang and Liu (2019).**

Finally, each *B. cereus* isolates harbored at least one of the enterotoxin genes indicating their pathogenic nature, which must be considered as serious health hazard and it is high probability of the potential transmission of enterotoxigenic studied strains to humans from the food chain, more particularly through contamination of meat products. So, PCR is a rapid and highly sensitive diagnostic method for detection of *B. cereus* virulence genes, therefore, PCR amplification using specific primers would facilitate direct detection of these isolates in meat products and diarrheic human stool. Moreover, the recorded results showed a relatively high rate of *B. cereus* pathogen, this may be due to mishandling; the negligence of hygienic aspects and consumption of contaminated meat products. Therefore, it was concluded that, *B. cereus* strains are enterotoxigenic ones and they may be the causative agents in patients suffering from vomition and diarrhoea, as they are meat-borne pathogens of public health importance.

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