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Abstract. *Bacillus cereus* isolated from different food sources. The diarrheal toxin genes such as *cytK*, hemolytic enterotoxin (*hblA*, *hblC* and *hblD*), non-hemolytic enterotoxin (*nheA*, *nheB* and *nheC*), *bceT* and *entFM* in addition to emetic toxin gene were detected by PCR. The *cyt K* gene was observed in 94.87 % of the isolates. *entFM* and emetic toxin gene were found very rare in all food samples at the percentage 2.56% and 7.69% respectively. Uncooked rice which has a highest number of bacterial isolation, also showed relatively high percentage of the *cytK* and *bceT* genes (90%). These two genes present in 100% of *Bacillus cereus* isolates in most food samples. Bacteria isolated from burger meat contain all investigated genes.

Keywords. *Bacillus cereus*, diarrheal toxin, emetic toxin.

1. Introduction

Bacillus cereus is a Gram-positive, facultative anaerobic, spore-producing, motile, bacterium in the form of a rod. Enterotoxins produced by bacteria in intestine can cause diarrhea. 10^5–10^7 cells or spores if ingested with food, heat labile toxins are produced. Incubation period of this bacterium is range between 8-16 hours. The sign of toxicity of *B. cereus* toxins appears as nausea, vomiting, diarrhea and abdominal pain [1, 2]. Three toxins produced during the vegetative cell growth of *B. cereus* in the intestine that caused diarrheal syndrome, Cyt K, NHE and HBL [4, 5, 6]. The first enterotoxin-hemolysin BL (HBL) was discovered by [7]. NHE toxin on three molecular types A, B, and C. Bacteria need these proteins for cytotoxicity of multi-component enterotoxins [5]. The HBL complex is encoded by genes on a single operon. The B, L1 and L2 proteins are encoded by the *hblA*, *hblD* and *hblC* genes [8, 9]. NHE toxin is a major cause of cytotoxicity in *B. cereus* [10]. The emetic disease is caused by a toxin produced by *B. cereus* called cereulide. This toxin showed stability against heat, acid, and trypsin. Several studies have been done previously for bacterial food contamination [11, 12 13, 14, 15]. This study was done to investigate the *B. cereus* that responsible for food poisoning by detecting the presence of diarrheal and emetic toxin genes.

2. Materials and Methods

2.1. Bacterial strains

Bacillus cereus isolates from different food sources were previously described [16]. The DNA of all isolates was extracted by genomic DNA kit (company and origin). The identification of the isolates was done by DNA sequencing using 16S *rRNA* gene [16].

2.2. Detection of diarrheal and emetic toxin genes by polymerase chain reaction

The primers used in this study for the detection of diarrheal and emetic toxin genes with the anticipated size of the amplified product were listed in Table 1.

Table 1. Primers of diarrheal and emetic toxins genes.

Genes	Primers 5`-3`	Program cycling	Product (bp)	References
entFM	**F;** ATGAAAAAA GTA ATT TGC AGG **R;** CGT GCA TCT GTT TCA TGAAA	Initial denaturation 94°C 5min, denaturation 94°C 30sec, annealing 50°C 45 sec, extension 72°C 45 sec , final extension 72°C 5 min , 35 cycles	1269	[17]
hblA	**F;** AAG CAA TGG AAT ACA ATG GG **R;** AGA ATC TAA ATCATGCCA CTG C	Initial denaturation 95°C 15min, denaturation 95°C 30sec, annealing 60°C 30 sec, extension 72°C 1min, final extension 72°C 5 min , 40 cycles	1154	[18]
hblC	**F;** GAT AC(T,C) AAT GTG GCA ACT GC **R;** TTG AGA CTG CTC G(T,C)T AGT TG	Initial denaturation 94°C 2min, denaturation 94°C 15sec, annealing 55°C 45 sec, extension 72°C 2min, final extension 72°C 5 min , 35 cycles	740	[18]
hblD	**F;** ACC GGT AAC ACT ATT CAT GC **R;** GAG TCC ATA TGC TTA GATGC	Initial denaturation 94°C 2min, denaturation 94°C 15sec, annealing 55°C 45 sec, extension 72°C 2min, final extension 72°C 5 min , 35 cycles	829	[18]
nheA	**F;** TAC GCT AAG GAG GGG CA **R;** GTT TTT ATT GCT TCA TCG GCT	Initial denaturation 94°C 2min, denaturation 94°C 15sec, annealing 55°C 45 sec, extension 72°C 2min, final extension 72°C 5 min , 35 cycles	499	[19]
nheB	**F;** CTA TCA GCA CTT ATG GCA G **R;** ACT CCT AGC GGT GTT CC	Initial denaturation 94°C 2min, denaturation 94°C 15sec, annealing 55°C 45 sec, extension 72°C 2min, final extension 72°C 5 min , 35 cycles	769	[19]
nheC	**F;** CGG TAG TGA TTG CTG GG **R;** CAG CAT TCG TAC TTG CCA A	Initial denaturation 94°C 2min, denaturation 94°C 15sec, annealing 55°C 45 sec, extension 72°C 2min, final extension 72°C 5 min , 35 cycles	581	[19]
bceT	**F;** CGT ATC GGT CGT TCA CTC GG **R;** TTT CTT TCC CGC TTG CCT TT	Initial denaturation 94°C 2min, denaturation 94°C 15sec, annealing 56°C 45 sec, extension 72°C 2min, final extension 72°C 5 min , 35 cycles	924	[19]
cytK	**F;** CGA CGT CAC AAG TTG TAA CA **R;** CGT GTG TAA ATA CCC CAG TT	Initial denaturation 94°C 1min, denaturation 94°C 45sec, annealing 54°C 1 min, extension 72°C 2min, final extension 72°C 5 min , 35 cycles	565	[20]
Emetic Toxin gene	**F;** GAC AAG AGA AAT TTC TAC GAG CAA GTA CAA T **R;** GCA GCC TTC CAA TTA CTC CTT CTG CCA CAG T	Initial denaturation 95°C 15min, denaturation 95°C 30sec, annealing 60°C 30 sec, extension 72°C 1min, final extension 72°C 5 min , 40 cycles.	635	[21]

3. Results

3.1. Detection of diarrheal toxin genes

3.1.1. Detection of cyt K gene

cyt K gene was found in 94.87% of the isolated bacteria. No significant differences (P > 0.05) in rate of detection of this gene in the investigated samples (Table 2, Figure 1).

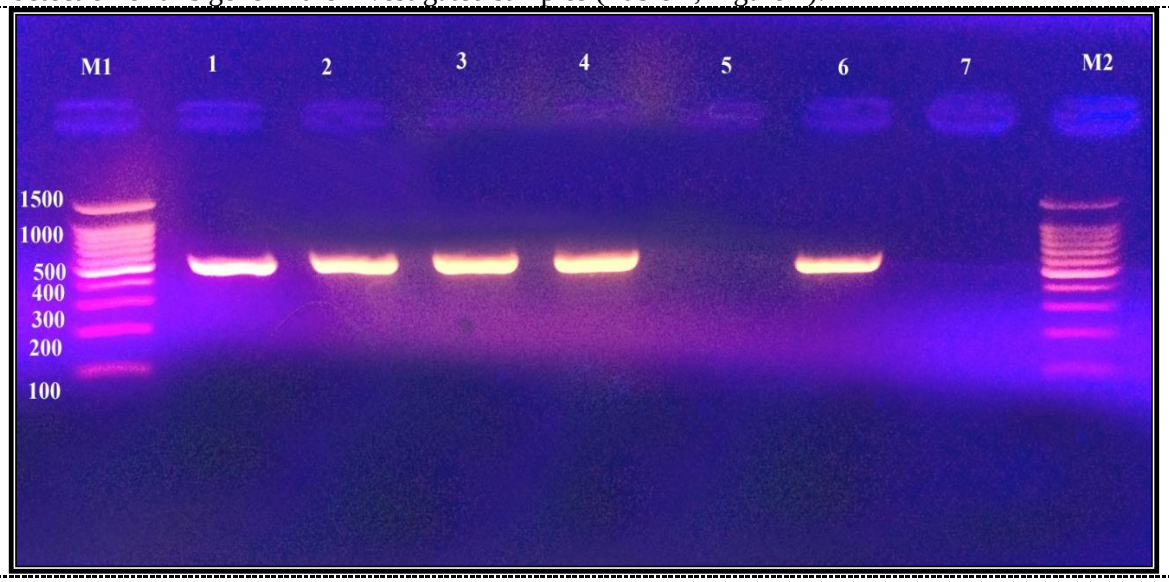

Figure 1. PCR amplification of *cytK* gene of *Bacillus cereus* isolates. M1 = Ladder; 1-4 & 6 = *cyt K* gene approximately 565 bp.; 5 = negative for *cyt K* gene; 7 = control negative.

3.1.2. Detection of (hbl and nhe) genes by multiplex PCR

Bacillus cereus isolated from different food were tested for presence of genes code for enterotoxin such as *hblA, hblC, hblD, nheA, nheB,* and *nheC* by multiplex PCR (Table 2, Figure 2). In cream isolates, the genes were detected in 6 isolates. *hblC* gene had the highest percentage 100.00 % followed by *hblA, hblD, nheA, nheB* and *nheC* genes were found in 50.00, 16.66, 50.00, 66.66 and 50.00%, respectively. In beef isolates, the genes were detected in 6 isolates. *hblC* gene had the highest percentage 66.66 % followed by *nheB* 50% and *hblD, nheA* and *nheC* genes were found as 33.33% for each one. The gene *hblA* was not found. In frozen beef isolates, the genes were detected in 5 isolates. *hblC* and *nheB* genes had the highest percentage 60.00 % followed by *nheA* and *nheC* genes were found in 40.00 % . The lowest percentage was found in *hblA* gene. The *hblD* gene was not found in all of the isolates. In burger isolates, the genes were detected in 7 isolates. *hblC* and *nheB* genes had the highest percentage 57.14 % followed by *hblD, nheC* genes were found in 42.85%. The lowest percentage was found in hblA and nheA in 28.57%. In cooked rice isolates, the genes were detected in 5 isolates. hblC, hblD and nheC genes had the highest percentage 40.00 % followed by *hblA, nheA* and *nheB* genes were found in 20.00 % for each one. In uncooked rice isolates, the genes was detected in 10 isolates. *hblC, hblD* and *nheC* genes had the highest percentage 40.00 % followed by *hblA, nheA* and *nheB* genes were found in 20.00 % for each one.

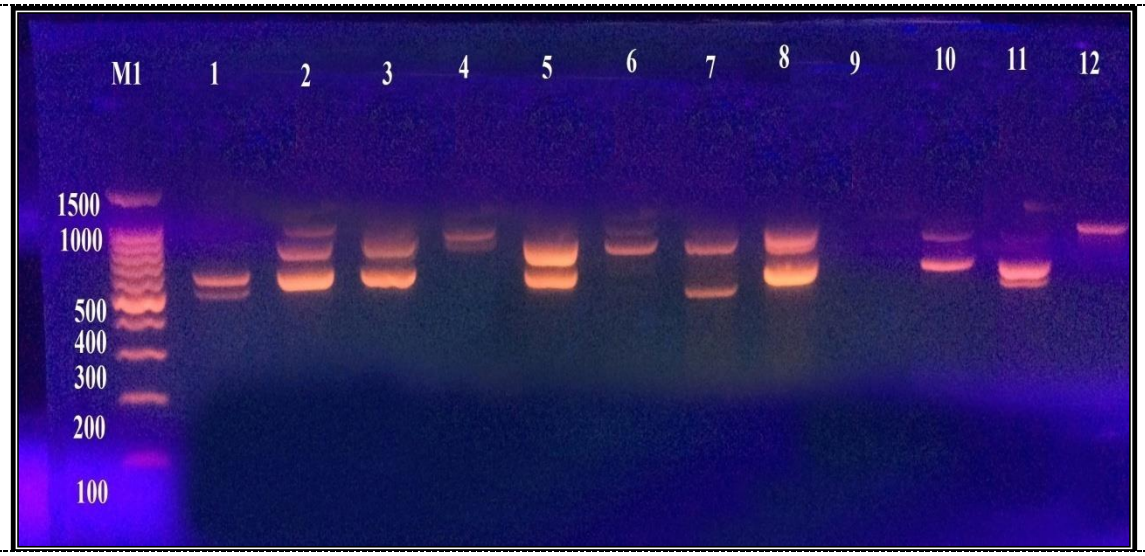

Figure 2. PCR amplification of *hblACD* and *nheABC* genes of *Bacillus cereus* isolates. Lane (M1)= Ladder, Lane 1 =*nheB, nhe* (769 and 581) bp, Lane 2 = *hblA*, hblD and *nheB*, (1154, 829 and 769) bp, Lane 3 = *hblA, hblD, nheB* and *hblC* (1154, 829, 769 and 740) bp, Lane 4 = *hblD* and *nheB* (829 and 769) bp, Lane 5 = *hblA, nheB* and *hblC* (1154, 769 and 740) bp, Lane 6 = *hblA, hblD, nheB* and *hblC* (1154, 829, 769 and 740) bp, Lane 7 = *nheB* and *nheC* (769 and 581) bp, Lane 8 = *nheB* and *hblC* (769 and 740) bp, Lane 9 = negative for genes. Lane 10 = *hblD, nheB* and *hblC* (829, 769 and 740) bp, Lane 11 = *nheB, hblC* and *nheC* (769, 740 and 581) bp, Lane 12 = *nheB* (769 bp).

3.1.3. Detection of bce T gene

The *bce* T gene was detected in 37 isolates (94.87 %) out of 39 isolates. The high rate (100%) found in beef, burger, frozen beef, and cooked rice. The lowest percentage was found in cream 83.33%. Results showed differ significantly ($P < 0.05$) in the detection of this gene in tested samples (Table 2, Figure 3).

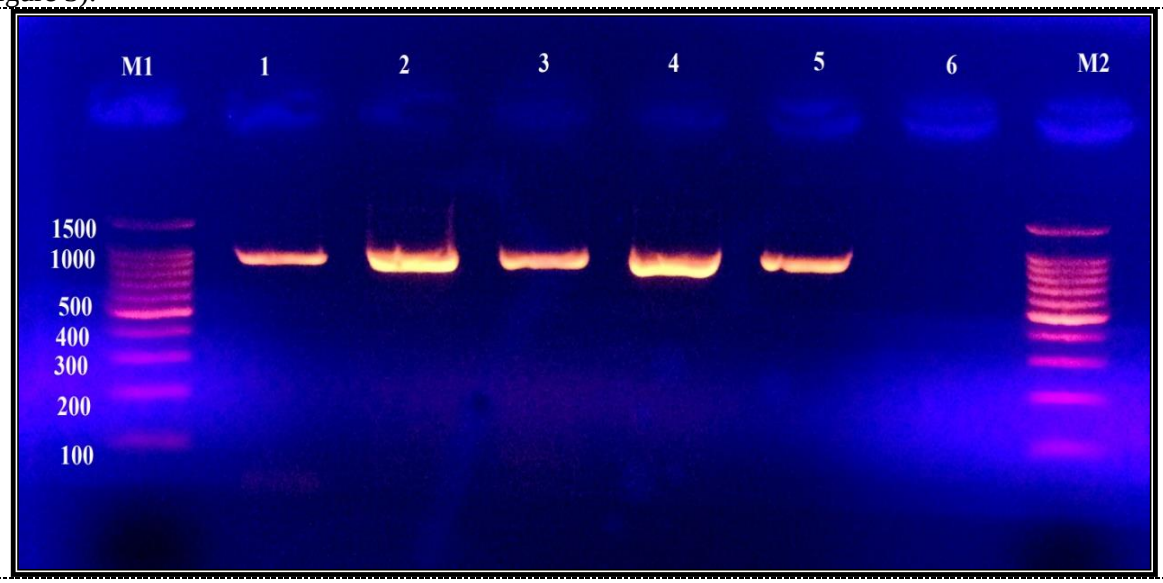

Figure 3. PCR detection of enterotoxin *bceT* gene M =Ladder; 1- 5 =positive for enterotoxin *bceT* gene approximately 924 bp.; 6= negative control.

3.1.4. Detection of enterotoxin (ent FM) gene

The *entFM* gene was detected in 1 isolate (2.5%) out of 39 isolates, it was found in burger 16.66%. The difference is non-significant (P > 0.05) in the detection of this gene in the tested samples (Table 2, Figure 4).

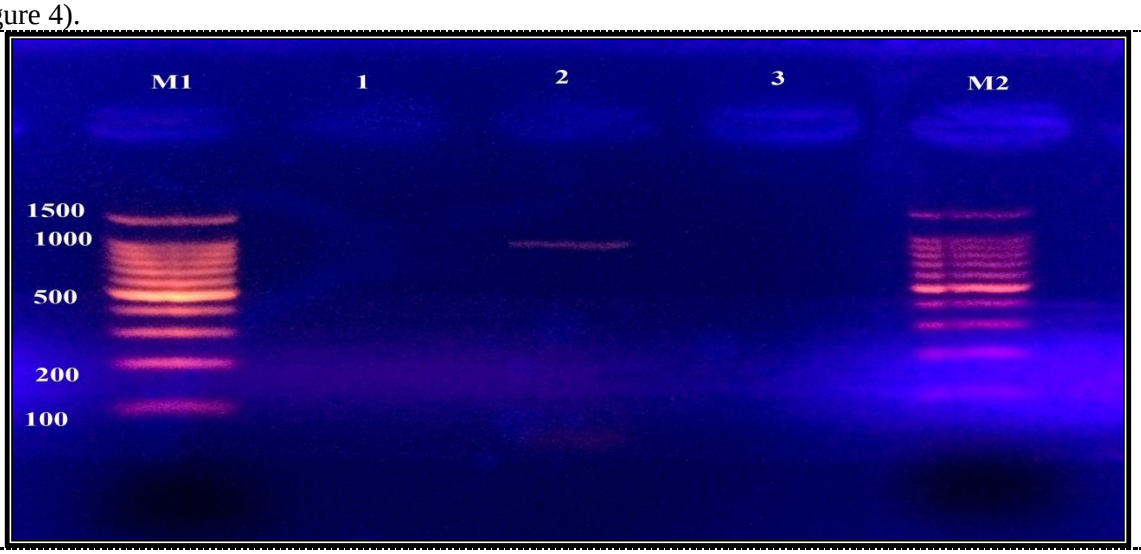

Figure 4. Detection of enterotoxin ent FM gene. M1, M2=Ladder; Lane 2=positive for *ent (FM)* gene approximately 1269 bp.

3.1.5. Detection of emetic toxin gene in Bacillus cereus

The emetic gene was detected in 3 isolates (7.6 %), distributed in cream and frozen beef and burger in 16.66, 20 and 14.28 %, respectively but was not detected in beef, cooked rice, and uncooked rice. The differences is non-significant found in the detection of emetic gene in the tested samples at (P > 0.05), (Table 2, Figure 5).

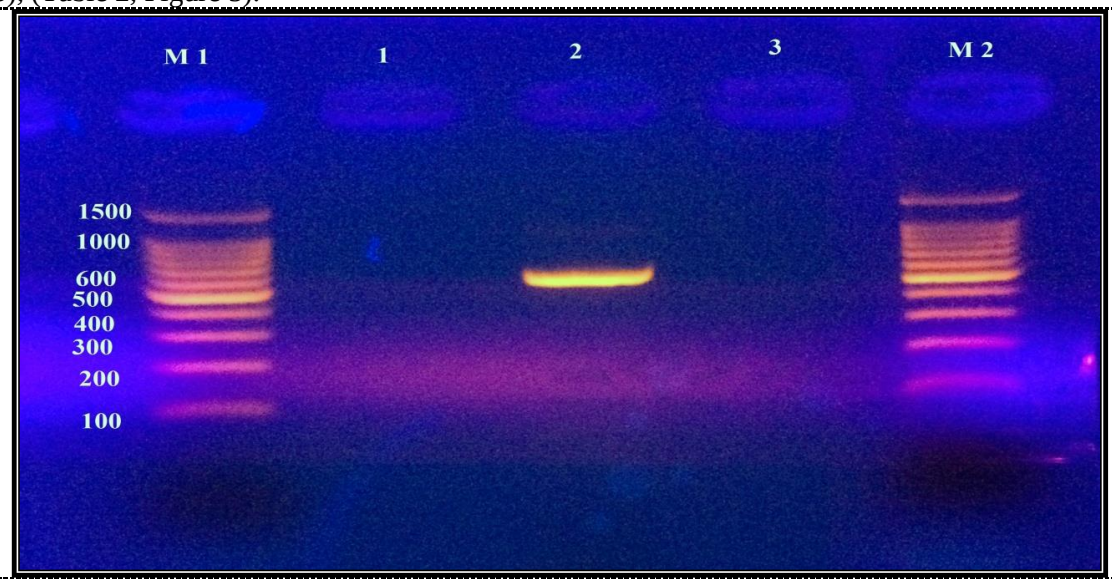

Figure 5. PCR Amplification of emetic toxin gene. M= Ladder; 2 positive for emetic gene approximately 635 bp.; 1 negative for emetic toxin gene; 3 control negative

3.2. The occurrence of diarrheal and emetic toxins genes in Bacillus cereus isolates

The presence of diarrheal and emetic toxins genes in *B. cereus* isolated from food sources are shown in (Table 2).

Table 2. The occurrence of diarrheal enterotoxin and emetic toxins genes in *Bacillus cereus* isolates.

Sample No. of isolates	*hblA* No.(%)	*hblC* No. (%)	*hblD* No. (%)	*nheA* No.(%)	*nheB* No.(%)	*nheC* No. (%)	*cytK* No. (%)	*bceT* No. (%)	*entFM* No.(%)	Emetic toxin No. (%)
Cream(6)	3 (50)	6 (100)	1 (16.66)	3 (50)	4 (66.66)	3 (50)	5 (83.33)	5 (83.33)	0(0)	1(16.66)
Beef (6)	0 (0)	4 (66.66)	2 (33.33)	2 (33.33)	3 (50)	2 (33.33)	6 (100)	6 (100)	0(0)	0(0)
Frozen beef (5)	1 (20)	3 (60)	0 (0)	2 (40)	3 (60)	2 (40)	5 (100)	5 (100)	0 (0)	1 (20)
Burger(7)	2 (28.57)	4 (57.14)	3 (42.85)	2 (28.57)	4 (57.14)	3 (42.85)	7 (100)	7 (100)	1 (14.28)	1 (14.28)
Cooked rice(5)	1 (20)	2 (40)	2 (40)	1 (20)	1 (20)	2 (40)	5 (100)	5 (100)	0 (0)	0 (0)
Uncooked rice(10)	2 (20)	7 (70)	5 (50)	2 (20)	6 (60)	5 (50)	9 (90)	9 (90)	0 (0)	0 (0)
Total (39) (%)	9 (23.07)	26 (66.6)	13 (33.3)	12 (30.7)	21 (53.8)	17 (43.5)	37 (94.87)	37 (94.87)	1 (2.5)	3 (7.6)

4. Discussion

The *Bacillus cereus* spoilage depends on two main factors: bacterial concentration in dairy products and cytotoxicity of isolates [22]. Several studies reported isolates produced cytotoxins and caused spoilage of milk, while others reported that cytotoxin production was not required for spoilage. As noted earlier, several factors affected toxin production, including environment and temperature signals [22]. The *cyt K* gene was detected in 37 isolates (94.87 %) out of 39 isolates. In cream sample, *hblC* gene had the highest percentage 100% while in beef samples, *hblC* gene had the highest percentage (66.66). On the other hand, the gene *hblA* was not found in beef. In frozen beef samples, hblC and *nheB* genes have the highest percentage of 60% (for each) followed by *nheA* and *nheC* genes which were found in 40% for each. In burger samples, each *hblC* and *nheB* genes had the highest percentage (57.14%) followed by *hblD, nheC* genes were found in 42.85% for each. The lowest percentage (28.57%) was found in each of *hblA* and *nheA*. In cooked rice samples, each of *hblC, hblD* and *nheC* genes had the highest percentage (40%) followed by each of *hblA, nheA* and *nheB* genes which were found in 20% of samples isolates. In uncooked rice samples, *hblC, hblD* and *nheC* genes had the highest percentage 40% for each, followed by *hblA, nheA* and *nheB* genes which were found in 20% for each one. [20, 23, 24, 25] detected the gene *cytK* in 50, 88, 70.40 and 65.98% of their isolates. [26] found the *cytK* gene in 27 (87.09%) out of 31 isolates. *nhe, hbl, ceses, ctyK* and *cry* genes also reported in the 92 isolates of *B. cereus* which were isolated in the previous study [16]. The rate is higher than the presence of nhe and hbl in *B. cereus* from Korea's food [27]. The positive rates for *nhe* and *hbl* in twenty raw milk samples were higher than those found for fifty four milk samples in China. In the study of [28], the positive rates of *nhe* and *hbl* were 62.0% and 37.0%, respectively.[29] found that the haemolysin gene *hblA, hblC* and *hblD* found in in most isolates. Haemolytic enterotoxin gene was isolated from vegetables [30, 31]. While haemolytic enterotoxin gene of *B. cereus* not reported from milk [30, 32]. Enterotoxin gene *bceT* gene was detected in 37 isolates (94.87%). This agreed with other studies [33, 34]. Whereas [31] found that all isolates were negative for this gene. *entFM* gene was found in one bacterial isolate (2.5%) out of 39 isolates, it was found in burger. Other studies were indicated that this gene was specific for entotoxigenic *B. cereus* [17, 35, 36]. Whereas other workers noticed that the *entFM* gene was found in 27 (93 %) *B. cereus* isolates [37]. In this study, 3 isolates (7.6%) detected the emetic toxin gene. While [38] found the emetic gene at percentage of

41.6%. Analysis of PCR is quick and easy to identify foods suspected of causing enterotoxigenic *Bacillus cereus* food poisoning.

5. Conclusion

It can be concluded that most investigated food have *Bacillus cereus* bacteria and these bacteria harboring different types of genes that can be harmful for man when ingested with food. The genes are relatively highly occurred in studied samples especially *cytK* and *bceT*.

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