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Research Article

Profiling of *Bacillus cereus* enterotoxigenic genes from retailed foods and detection of the nhe and hbl toxins with immunological assay

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Abstract
Bacillus cereus produces pore-forming toxins responsible for diarrhoea; therefore, rapidly detecting these toxins in food retailed for consumption is needed. The genomic DNA of 100 *B. cereus* isolates recovered from some retailed foods was extracted and used as a template for enterotoxin detection. The detection of genes of non-haemolyticnonhemolytic enterotoxin (*nheA, nheB, nheC*), hemolysin BL (*hblA, hblC, hblD*), *entFM, cytK* and *bceT* by the isolates was carried out with PCR using primers specific for the targeted genes, while the production of Nhe and Hbl enterotoxins in fifty of the randomly chosen isolates was detected with a Duopath Cereus Enterotoxin kit. Ninety-five percent of the isolates carried one or more components of the NHE complex, while 56% had one or more components of HBL. Sixteen out of the 100 isolates carried all the genes for NHE and HBL complex genes. The *entFM, cytK* and *bceT* genes were detected in 85%, 74% and 60% of *B. cereus* isolates, respectively. Starchy foods had the highest incidence of the HBL complex, while *nheA* and *nheC* occurred mostly in protein foods with 90% and 87% incidence, respectively. The immunological kit was able to detect the production of nonhemolytic enterotoxin (Nhe) in all the *B. cereus* isolates, while 28 *B. cereus* isolates produced hemolysin (hbl). Nineteen isolates that carried one or more genes encoding *hbl* did not produce the toxin. This study clearly showed that retailed foods sold in Ogun State, Nigeria, harbor *B. cereus* enterotoxigenic genes responsible for diarrhoea. These toxins can be rapidly detected in foods using both molecular and immunological methods.

Keywords: *B. cereus*, Duopath cereus kit, Enterotoxins, *hbl, nhe,* PCR, Retailed foods

INTRODUCTION

One important pathogen involved in food poisoning outbreaks is *Bacillus cereus* (Scallan *et al.,* 2011). They can survive and proliferate in diverse environments such as mining soil (Babalola *et al*. 2019, Ayangbenro and Babalola, 2020), food products (Adesetan *et al.,* 2019), plants (Babalola *et al*., 2021, Adeleke *et al.,* 2021), dead or live insects or animals (Lindbäck *et al.,* 2004, Lapidus *et al*., 2008). The contamination of food by *B. cereus* has led to cases of diarrhoea. Virtually all types of food have been involved in *B. cereus* poisoning (EFSA, 2005). Emetic and diarrheal syndromes are the two types of gastrointestinal disorders associated with these bacteria (Stenfors Arnesen *et al.,* 2008; Ehling-Schulz *et al.,* 2019). Foods rich in protein are generally linked with diarrhoeal syndrome, while starchy foods are primarily associated with emetic syndrome (Senesi and Ghelardi, 2010).

The possibility of *B. cereus* poisoning is determined by its defiance to acidic conditions, as the consumption of spores and vegetative cells in food is responsible for diarrhea. After surviving the acidic surroundings of the stomach, they consequently release enterotoxins lead-

ing to diarrhea (Kotiranta *et al.,* 2000). Thus, strains involved in foodborne poisoning are highly enterotoxic (Guinebretière *et al.,* 2002).

Bacillus cereus is known for producing excessive membrane-damaging enterotoxins that have an effect on various mammalian cells and tissues (Senesi *et al.,* 2010). A method for evaluating the probable toxigenicity of *Bacillus* strains is the detection of precise, exceptional DNA orders of genes of emetic and diarrheal toxins. *Bacillus* spp. produce diverse extracellular enzymes, such as phospholipases, metalloproteases, haemolysins, collagenases and beta lactamases (Turnbull *et al.,* 2002); cereolysin O (Clo), enterotoxin S (EntS), sphingomyelinase (SMase), HlyII, InhA1 NprA (Stensfor Arnesen *et al.*, 2008; Cadot *et al.,* 2010), ColA, and ColQ1 (Abfalter *et al.,* 2016, Hoppe *et al.,* 2021) .

Bacillus cereus produces diarrheal toxins during its growth in the small intestine (Ehling-Schulz *et al.,* 2006). It makes five different enterotoxins: nonhemolytic eneterotoxin (NHE), hemolysin BL (HBL), enterotoxin T, cytotoxin K (cytK) and enterotoxin FM which are responsible for diarrheal poisoning and may occur singly or together to cause diarrhea (Lapidus *et al.*, 2008). They are pore-forming enterotoxins. The main component of *B. cereus* virulence related to food poisoning is the enterotoxins NHE and HBL (Stensfor Arnesen *et al.*, 2008). HBL comprises trio protein components: B, a binding component, and L1 and L2, which are lytic components (Beecher *et al.,* 1995). The B component is encoded by the *hblA* gene, and the L_1 and L_2 components are encoded by the *hblD* and *hblC* genes respectively and are organized into one operon (Granum *et al.,* 1999) along with a fourth gene, *hblB* (encoding the B' protein) (Lund *et al.*, 2000).

Nhe is also made up of three proteins, A, B and C and are encoded by the genes *nheA*, *nheB* and *nheC* respectively, and organized into one operon (Granum *et al*., 1999). Sastalla *et al.* (2013) reported that Hbl and Nhe have exceptional attributes. There must be a stepwise, consecutive binding of the three toxin components on the cellular membrane for lysis to occur. Illness due to *B. cereus* is widely underreported because it is self-limiting in a healthy individual and lasts for no more than 24 h, but sometimes, both illnesses have become serious leading to hospitalization and/or death (Ankolekar *et al.*, 2009). Many authors have reported illnesses or death due to *B. cereus* (Ghelardi *et al*., 2002, Dierick *et al*., 2005; Naranjo *et al*., 2011; Martinelli *et al*., 2013; Powell, 2014; Delbrassine *et al*., 2015; Lopez *et al*., 2015; Carroll *et al*., 2019; Chen *et al.,* 2019; Thirkell *et al*., 2019 and Schreiber *et al*., 2022).

Many PCR-based methods are employed in the detection of *B. cereus* enterotoxigenic genes, such as standard PCR and multiplex PCR. For the detection of *B. cereus* toxins, two immunological assays are frequently used: the *B. cereus* Enterotoxin–Reversed Passive Latex Agglutination (BCET-RPLA) kit manufactured by OXOID, which detects the *Hbl* gene and the *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay (BDE-VIA) by TECRA which detects the *NheA* gene. BCET-RPLA detects toxins by polystyrene latex particles sensitized with purified antiserum, while enzyme-labelled antibodies are employed in BDE-VIA (Beecher and Wong, 1994). Additionally, the Duopath cereus enterotoxin kit produced by MERCK is a fast and sensitive assay for the detection of NheB and L_2 components of *Nhe* and *Hbl,* respectively (Krause *et al.,* 2010). The immunological kit uses gold-labelled monoclonal antibodies (Dietrich *et al.,* 2005). It is a fast method for detecting food contaminated with enterotoxigenic *B. cereus*.

Since *B. cereus* is known for the production of excessive enterotoxins, which cause gastrointestinal disturbances and sometimes death, the aim of the present study was to rapidly detect the virulence genes encoding enterotoxin production in retailed foods with PCR-based methods using primers specific for the genes and the production of Nhe and Hbl enterotoxins with immunological assays.

MATERIALS AND METHODS

Test organism

Retail foods are foods sold to consumers to be eaten at home with or without further preparation. One hundred (100) *B. cereus* isolates recovered from some retailed foods such as fried meat, smoked fish, smoked hide, raw vegetables, cooked rice, jollof rice and cooked pasta were used in this study. They have been previously characterized by biochemical tests specific for *B. cereus* (Adesetan *et al*., 2019).

Genomic DNA extraction

The extraction of the genomic DNA of the *B. cereus* isolates was reported earlier by Adesetan *et al.* (2020). The extracted DNA was refrigerated at - 20°C in Eppendorf tubes.

Primer pairs used in detecting *B. cereus* enterotoxigenic genes

The primer pairs synthesized by Integrated DNA Technology (IDT) USA were employed for the detection of enterotoxigenic genes of *B. cereus* and the amplified fragment is presented in Table 1, while the PCR conditions used for the detection of enterotoxigenic genes of the *B. cereus* strains are presented in table 2.

Enterotoxin assay for *B. cereus*

The assay was carried out using a Duopath Cereus Enterotoxins kit (Merck, Germany). It is a gold-labelled immunosorbent assay for the qualitative detection of *B. cereus* enterotoxins. The method was as described by the manufacturer (Merck, Germany). Two colonies of *B.*

Table 1. List of primers used for the identification of *B. cereus* and its enterotoxins

Target gene	Primer	Primer sequence 5'------- 3'	Amplified fragment (bp)	Reference
hblA	HBLA1 HBLA2	GCTAATGTAGTTTCACCTGTACGAAC AATCATGCCACTGCGTGGACATATAA	834	Mantynen and Lindstrom (1998)
hblD	L1A L1B	AATCAAGAGCTGTCACGAAT CACCAATTGACCATGCTAAT	410	Ryan *et al*. (1997)
hblC	L2A L2B	AATGGTCATCGGAACTCTAT CTCGCTGTTCTGCTGTTAAT	749	Ryan *et al*. (1997)
nheA	nheA 344 S nheA 843 A	TACGCTAAGGAGGGGCA GTTTTTATTGCTTCATCGGCT	499	Granum *et al*. (1999)
nheB	nheB 1500 S nheB 2269 A	CTATCAGCACTTATGGCAG ACTCCTAGCGGTGTTCC	769	Granum *et al*. (1999)
nheC	nheC 2820 S nheC 3401 A	CGGTAGTGATTGCTGGG CAGCATTCGTACTTGCCAA	581	Granum *et al*. (1999)
bceT	BCET1 BCET3	CGT ATC GGT CGT TCA CTC GG AGC TTG GAG CGG AGC AGA	661	Hansen and Hendriksen (2001)
cytK	Cyt K F Cyt K R	CGA CGT CAC AAG TTG TAA CA CGT GTG TAA ATA CCC CAG TT	565	Ngamwongsatit *et al*. (2008)
entFM	ent A ent B	ATG AAA AAA GTA ATT TGC AGG TTA GTA TGC TTT TGT GTA ACC	1269	Asano *et al.* (1997)
ces	CES BF CES BR	CAA GTG AAA ATT CGT GGA TTC C CCC CTA AGG AGT GGC CAC C	838	Oltusak-Walczak and Walczak (2007)

Table 2. PCR conditions used for the detection of enterotoxigenic genes of *B. cereus* strains from retailed foods

Target gene	PCR conditions					PCR Volume (25 µL)
	Denaturation	Primer annealing	No of cycles	Extension	Final extension	
hblA	94°C for 1 min	55°C for 45 Secs.	30	72°C for 2 mins.	72°C for 2 mins.	12.5 µl Master mix, 11 µl nuclease free water, 0.5µl primer and 1 µl template DNA
hblD			30			
hblC			30			
nheB			30			
nheC			30			
bceT			30			
nheA	94°C for 2 mins.	56°C for 1 min.	35			
cytK	94°C for 45 secs.	54°C for 1 min.	35			
entFM	94°C for 45 secs.	52°C for 45 secs	30			
ces	94°C for 1 min.	50°C for 1 min.	35			

cereus on Luria-Bertani agar were picked and dissolved in 1 ml casein glucose yeast extract (CGY) broth. It was incubated for 4 hrs at 37°C. Then, it was cooled to room temperature. A micro pipette was used to drop 150 µl into the circular sample port on the test device. After 30 minutes of applying the sample to the device, the result was observed.

Electrophoresis

Five (5) µl of gel loading dye was added to 5 µl of each PCR product, and 10 µl of the obtained mixture was analysed in 2.0% agarose gel (LASEC) containing SYBR safe stain (Life Technologies, Thermo Fisher), immersed in Tris/Acetic acid/EDTA (TAE) (BioRad, USA) buffer, and run at 80 V for 90 minutes. A molecular ladder (Thermo Fisher) of 1 kb base pairs was used as a molecular weight marker. After migration of DNA bands, the gel was photographed on a Gel Doc 2000 Image analyser (BioRad, USA).

RESULTS

Toxigenic profile of *B. cereus*

Table 3 summarizes the toxigenic profile of *B. cereus* isolates from the retailed foods. The gene with the highest incidence was *entFM* (85%) followed by *nheA* (83%). *hblC* and *hblD* had the same incidence of 41% each, while *hblA* had 36%.

Fig. 2 shows the distribution of toxins among the different groups of foods. Starchy food had the highest inci-

Table 3. Toxigenic profile of *Bacillus cereus* genes in the sampled foods

Code/Gene	*nheA*	*nheB*	*nheC*	*hblA*	*hblC*	*hblD*	*entFM*	*cytK*	*bceT*	*ces*
SG_5	+	+	+	-	+	+	+	+	-	-
SP_6	-	-	+	-	-	-	+	-	-	-
SG_7	+	+	-	-	-	-	+	+	-	-
RB_8	+	+	-	+	+	+	+	+	-	-
CR_{10}	+	+	+	+	+	-	+	+	+	-
TT_{11}	+	+	-	-	-	-	+	+	+	-
WR_{13}	+	+	-	+	+	+	+	+	+	-
SG_{17}	+	+	+	+	+	+	+	+	+	-
MT_{22}	+	+	+	-	+	+	+	+	-	-
CB_{26}	+	+	+	-	-	-	+	+	-	-
GP_{27}	-	+	+	-	-	-	+	+	-	-
RB_{29}	+	+	+	+	+	+	+	+	+	-
WR_{30}	+	+	-	-	-	-	+	+	+	-
RB_{31}	+	+	+	+	-	+	+	+	+	-
PN_{32}	+	+	+	-	-	-	+	+	-	-
MP_{34}	+	+	+	+	-	-	+	+	-	-
TT_{35}	+	+	+	-	-	-	+	+	+	-
WR_{36}	-	+	-	-	-	-	+	+	-	-
SG_{38}	-	+	+	-	-	-	+	+	-	-
WR_{39}	+	+	+	-	-	-	+	+	-	-
SW_{46}	+	+	+	-	-	-	+	+	-	-
MT_{51}	+	+	+	+	+	+	-	+	-	-
CB_{59}	+	-	+	-	-	-	+	+	+	-
PM_{60}	+	-	+	-	-	-	-	+	-	-
SG_{61}	+	+	-	-	-	-	+	+	+	-
PM_{62}	+	-	+	-	-	-	+	+	-	-
SW_{68}	+	+	-	-	-	-	+	-	+	-
WR_{71}	-	+	+	-	-	-	+	+	+	-
CB_{72}	-	+	+	-	-	-	+	+	+	-
MP_{75}	+	+	+	-	-	-	+	+	+	-
SW_{79}	-	-	-	-	-	-	-	-	+	-
RB_{84}	+	+	+	+	+	+	+	-	-	-
SW_{86}	-	+	+	-	-	-	+	+	+	-
GP_{87}	+	+	+	-	+	+	+	+	+	-
WR_{88}	+	+	-	+	+	+	-	+	+	-
SW_{89}	+	+	-	+	-	-	+	-	+	-
WR_{90}	+	+	+	-	-	-	+	+	+	-
WR_{91}	-	-	-	-	-	-	+	-	+	-
SG_{92}	+	+	+	+	+	+	+	+	+	-
SG_{93}	+	+	+	+	+	+	-	-	+	-
WR_{94}	+	+	+	-	-	-	+	+	+	-
SP_{96}	+	-	-	-	-	-	+	+	+	-
SW_{98}	+	-	+	-	-	-	+	+	-	-
SG_{99}	+	+	-	-	-	+	+	-	-	-
SP_{100}	+	+	-	-	+	-	+	+	-	-
SP_{102}	+	-	+	-	+	-	+	+	+	-

Contd…..

Table 3. Contd….

Code/Gene	*nheA*	*nheB*	*nheC*	*hblA*	*hblC*	*hblD*	*entFM*	*cytK*	*bceT*	*ces*
MP_{104}	+	+	+	-	+	-	+	+	+	-
RB_{105}	-	-	-	-	-	-	-	-	+	-
TT_{106}	+	+	+	+	+	+	+	+	+	-
MP_{111}	+	+	+	+	+	+	+	+	+	-
PM_{112}	+	-	+	-	+	-	-	+	+	-
MP_{113}	+	-	+	-	-	-	+	+	-	-
GP_{114}	+	+	+	-	-	-	+	+	+	-
MP_{117}	+	+	+	-	+	+	-	+	+	-
SG_{118}	+	+	+	+	-	+	+	+	+	-
WR_{119}	+	-	+	+	-	+	+	+	+	-
TT_{120}	+	-	+	+	-	+	+	-	-	-
CR_{122}	+	+	+	+	-	+	+	+	+	-
WR_{123}	+	-	+	-	-	-	+	+	-	-
SW_{124}	-	+	+	-	-	-	+	+	+	-
WR_{126}	+	+	+	+	-	+	+	+	+	-
SG_{127}	+	+	+	-	+	-	-	+	+	-
SG_{129}	-	-	+	+	-	+	+	+	+	-
WR_{130}	+	-	+	-	-	-	-	-	-	-
SW_{131}	+	+	+	-	-	-	+	+	-	-
SW_{134}	+	-	+	-	-	-	+	+	+	-
CB_{135}	+	+	+	-	-	-	+	+	+	-
SW_{136}	+	-	+	-	-	-	+	+	+	-
GP_{137}	+	-	-	-	-	-	+	+	+	-
SG_{139}	-	+	+	+	-	+	+	+	+	-
WR_{140}	+	+	+	+	-	+	-	+	+	-
PN_{142}	+	+	+	-	-	-	+	+	+	-
TT_{143}	+	-	+	-	+	-	+	+	+	-
MT_{146}	+	+	+	+	-	-	+	+	+	-
WR_{147}	+	+	+	-	+	-	+	-	+	-
MP_{149}	+	-	+	-	+	-	+	+	-	-
WR_{150}	-	-	+	-	-	-	+	-	+	-
SW_{151}	+	-	+	-	-	-	+	-	+	-
GP_{153}	+	-	+	-	+	+	-	+	+	-
MT_{154}	+	-	+	-	+	+	+	+	-	-
RB_{155}	-	-	-	-	-	-	-	-	+	-
RB_{157}	+	+	+	+	+	+	+	+	-	-
TT_{158}	+	+	+	+	+	+	-	-	+	-
MT_{160}	+	+	+	-	-	-	+	+	+	-
WR_{163}	+	-	-	-	-	-	+	-	-	-
WR_{165}	+	-	-	+	+	-	+	-	-	-
MT_{167}	+	+	+	-	+	+	+	+	+	-
JR_{168}	+	+	-	+	+	+	+	+	-	-
SG_{169}	-	-	+	-	+	-	+	-	-	-
JR_{170}	+	+	+	+	+	+	+	-	-	-
WR_{176}	+	+	+	+	+	+	+	-	-	-
MT_{181}	+	+	+	+	+	+	-	+	-	-
WR_{186}	+	+	+	-	+	+	+	+	+	-
SG_{187}	+	+	+	+	+	+	+	-	-	-
JR_{189}	+	+	+	+	+	+	+	-	-	-
MT_{191}	+	-	+	-	+	+	+	+	-	-
SG_{192}	-	-	-	-	-	-	+	+	+	-
WR_{193}	+	-	+	+	-	+	+	-	-	-
SG_{199}	+	+	-	+	+	+	+	-	-	-
MT_{200}	+	+	+	+	+	+	+	+	+	-

Key: + present; - absent

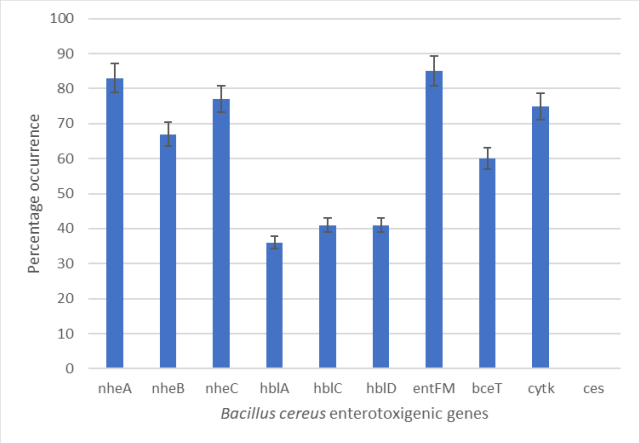

Fig. 1. *Incidence of B. cereus enterotoxigenic genes in the food samples*

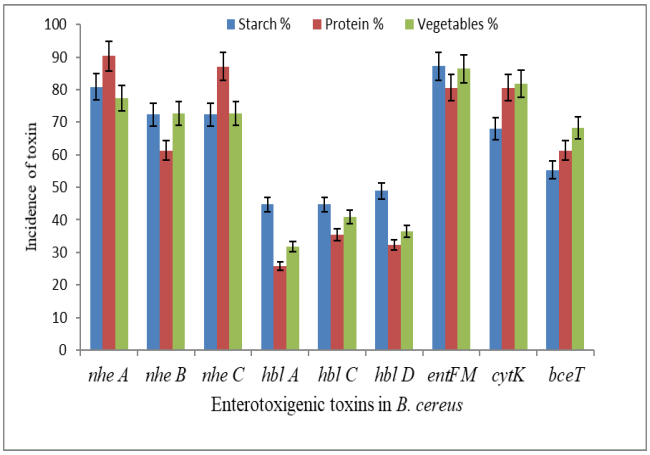

Fig. 2. *Chart showing the distribution of toxins of B. cereus among food groups.*

dence of the HBL complex: *hblA* – 45%, *hblC* – 45%, *hblD* – 49% and *entFM* – 87%. *cytK* (82%) and *bceT* (68%) occurred mostly in vegetables while *nheA* and *nheC* occurred mainly in protein, with incidences of 90% and 87%, respectively.

Enterotoxin assay

Fig. 3 shows the results of the enterotoxin assay performed on the isolates. The result correlates with the PCR performed on the isolates. All the isolates that carried the *Nhe* and *Hbl* genes also produced the toxin except for five of the isolates that possessed the *hbl* genes but the toxin was not detected with the kit.

Enterotoxigenic genes of *B. cereus*

Plates 1-9 show the amplified genes and the size of the gene, while Fig. 1 shows the incidence of each gene among the 100 *B. cereus* isolates.

DISCUSSION

Bacillus cereus has become a threat in food due to the production of several toxins involved in food poisoning. Hansen and Hendriksen (2001) and Ngamwongsatit *et al*. (2008) reported that the existence of any one component of the NHE or HBL genes might initiate food poisoning. In line with this, that is, occurrence based on the existence of any of the triple complexes of NHE and HBL responsible for food poisoning, ninety-five (95%) of the isolates carried one or more components of the NHE complex in this study (Plates 1-3, Fig. 1). This is supported by the findings of Hwang and Park (2015), who stated that 100% of their *B. cereus* food isolates harbor *nhe*. On the other hand, Gao *et al.* (2018) and Yu *et al*. (2020) detected the NHE complex in 93% and 83% of their *B. cereus* strains, respectively. The *nheA, nheB* and *nheC* genes were detected in 83%, 67% and 77% of *B. cereus* strains, respectively, in this work. Keisam *et al*. (2019) and Gdoura-Ben Amor (2019) re-

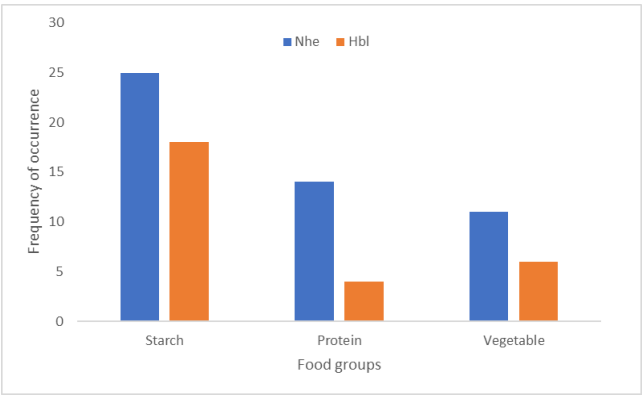

Fig. 3. *Frequency of enterotoxin production by B. cereus using Duopath immunological kits*

ported the *nheA, nheB* and *nheC* genes in 93%, 77% and 100% and 98.9%, 86.8% and 97.7% of their *B. cereus* isolates, respectively. However, Li *et al*. (2016), Ranjbar and Shahreza (2017) and Frentzel *et al.* (2018) reported that 92%, 88.8% and 100% of the *B. cereus* isolates harbored the *nheA* gene, respectively. The NHE complex was not seen in five strains, while there was no amplification in one or two components of NHE. This conforms with the study of Ranjbar and Shahreza (2017) and Keisam *et al.* (2019). Guinebretière *et al*. (2010) stated that NHE is an incessant part of the *B. cereus* group and is prevalent among isolates from food -poisoning cases and the environment. It was reported by Stensfor Arnesen *et al*. (2008) that cytotoxicity is mainly due to the pore-forming ability of NHEs.

In this study, only 56% of the isolates harbored one or more components of the HBL complex (Plates 4-6, Fig. 1), which is lower than the work of other authors such as Hwang and Park (2015) and Keisam *et al.* (2019). Twenty-one (21%) of the *B. cereus* strains carried all the three components of the HBL genes in this finding. This is lower than the 45% and 39% detection rates of the *hblACD* complex reported by Gao *et al.* (2018) and Yu *et al.* (2020). However, Schreiber *et al.* (2022) did not detect the *hbl* genes in any of their isolates. In this

research, the *hbl* genes were not detected in 44 *B. cereus* strains, while 33 strains carried either one or two genes. This is supported by the work of Ranjbar and Shahreza (2017), Keisam *et al*. (2019) and Yu *et al*. (2020) who also reported nondetection of one, two or all components of the HBL complex in some of their *B. cereus*. Some *B. cereus* strains that showed no amplification of the *hbl* genes during PCR were detected using Southern blotting by Guinebretière *et al*. (2002). According to them, this may be a result of sequence polymorphism rather than the absence of one of the genes of the HBL complex.

In a previous study by Adesetan *et al.* (2019), all *B. cereus* were β-hemolytic, but the present work revealed that not all of them possessed the hemolytic gene (*hbl*). Ouoba *et al*. (2008) could not find any collaborative results between the *hbl* genes and haemolysis. According to Schoeni and Wong (2005), the presence of haemolysis in *B. cereus* is not limited only to strains having the *hbl* genes. Additionally, Oda *et al*. (2010) reported that strains that are negative for genes encoding HBL displayed β-hemolysis, implying that other products, such as sphingomyelinase, with hemolytic activity can be produced. *hbl* genes are generally not as common as *nhe* genes. The occurrence of *cytK* and *hbl* genes varies with the phylogenetic group (Guinebretière *et al*., 2010).

entFM was the predominant gene detected in this work, with 85% of strains carrying the gene (Plate 7, Fig. 1). This is slightly lower than the 99%, 100%, 96% and 100% occurrence reported by Li *et al.* (2016), Keisam *et al.* (2019), Gao *et al.* (2018) and Yu *et al*. (2020), respectively. However, Ranjbar and Shahreza (2017) detected the gene in 55.5% of *B cereus* strains in milk-based infant foods. The most predominant enterotoxin gene in *B. cereus* of foodborne origin was discovered to be *entFM*. It has been shown to be cytotoxic to Vero cells, and the degree of its cytotoxicity depends on the bacterial strain (Yang *et al*., 2007, Boonchai *et al*., 2008).

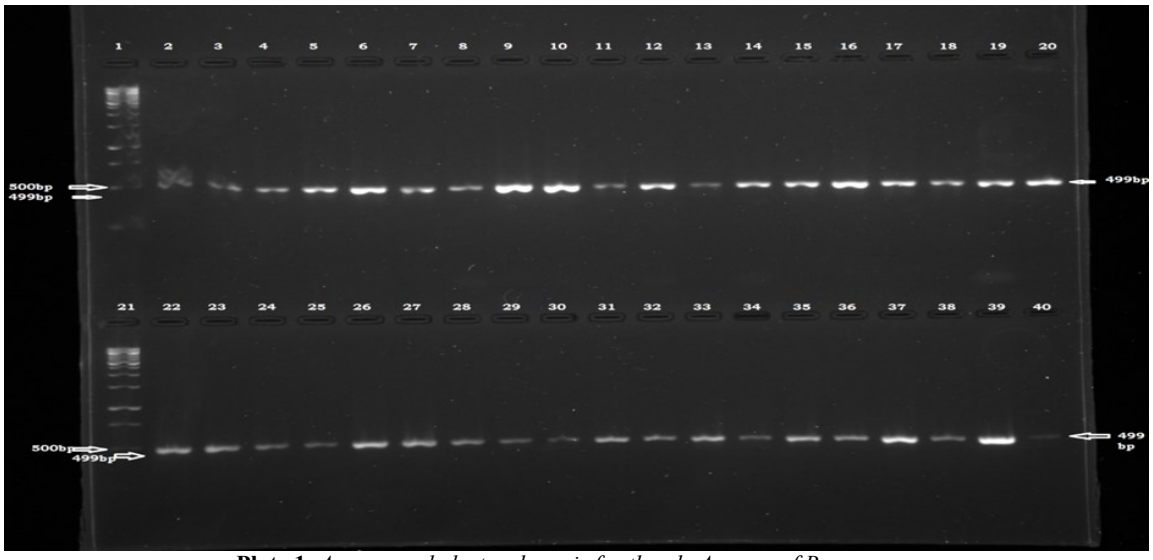

Plate 1. *Agarose gel electrophoresis for the nheA genes of B. cereus*

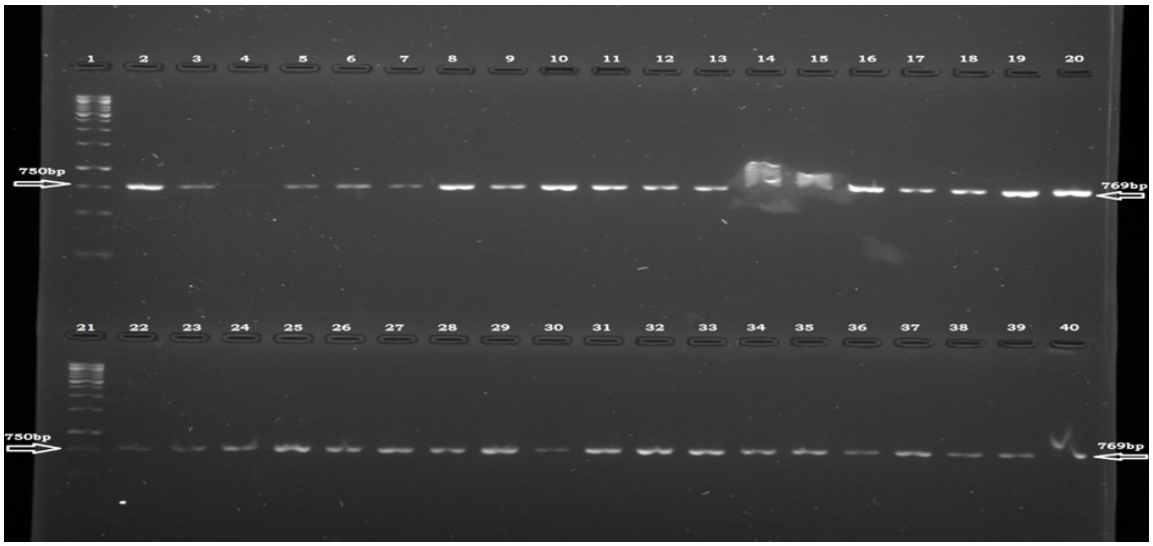

Plate 2. *Agarose gel electrophoresis for the nheB genes of B. cereus*

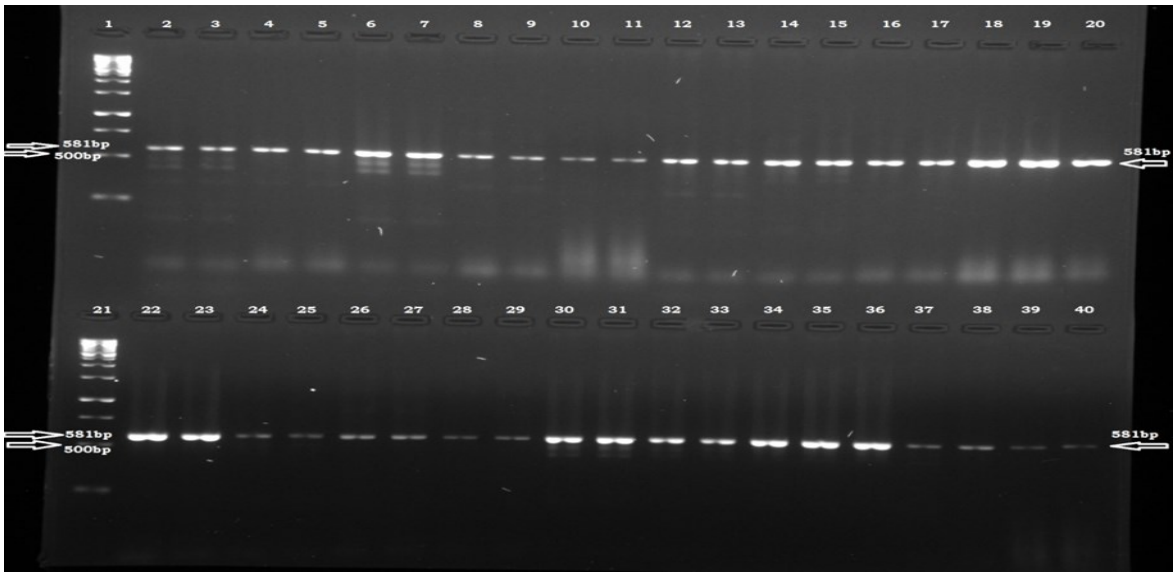

Plate 3. *Agarose gel electrophoresis for the nheC genes of B. cereus*

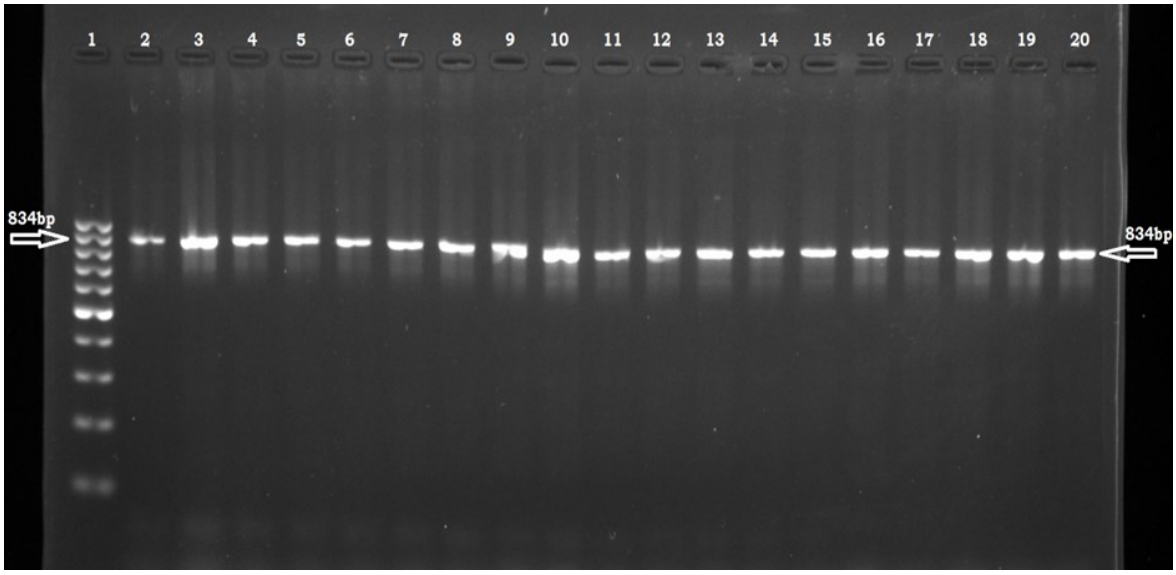

Plate 4. *Agarose gel electrophoresis for the hblA genes of B. cereus*

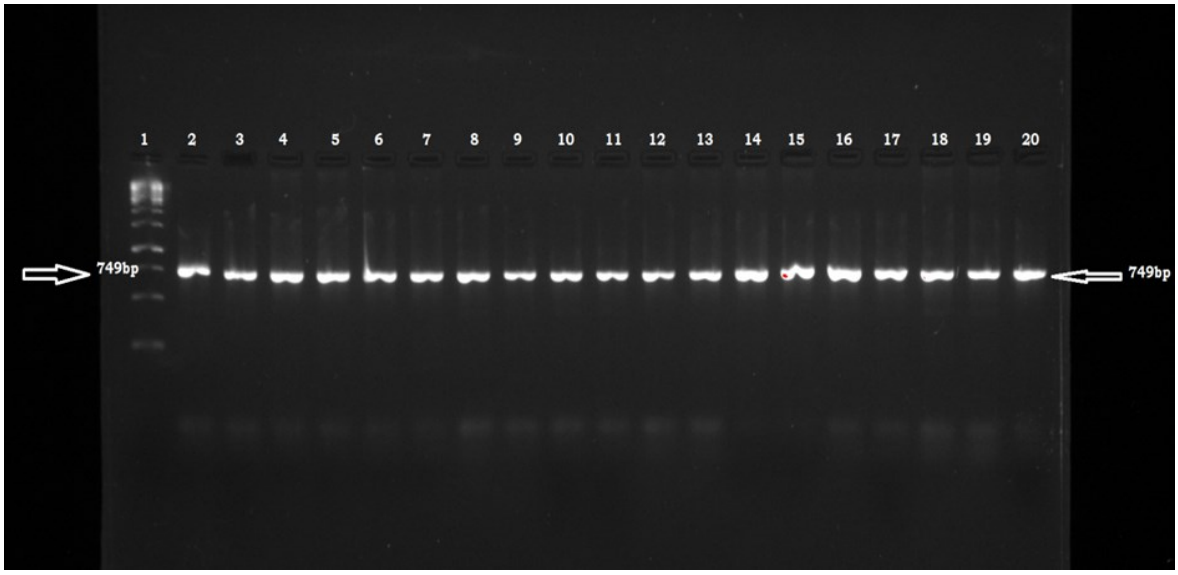

Plate 5. *Agarose gel electrophoresis for the hblC genes of B. cereus*

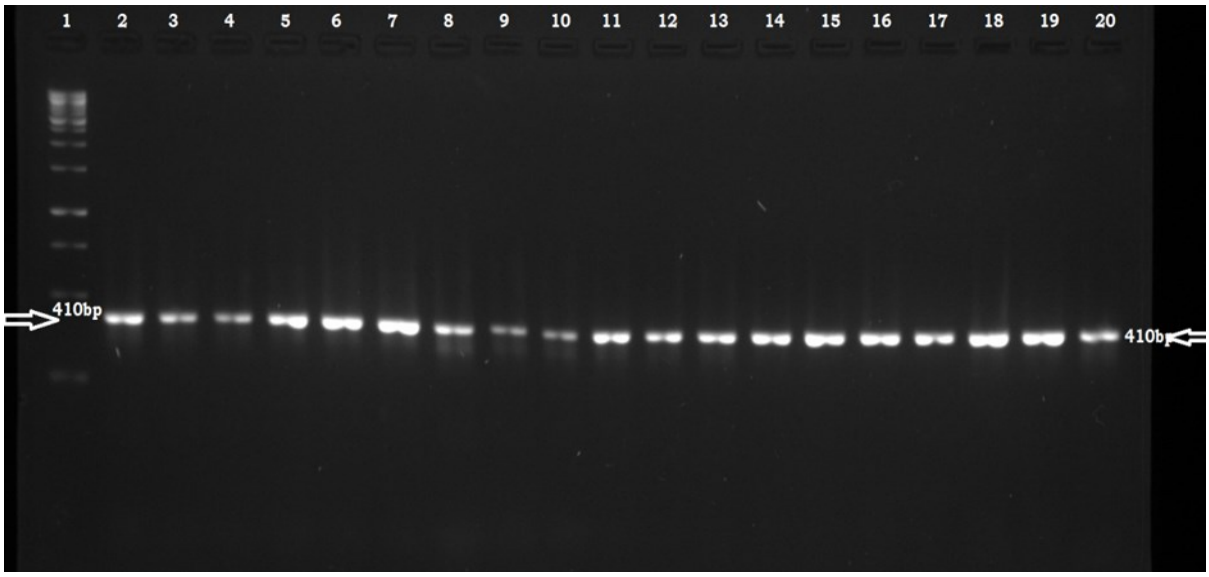

Plate 6. *Agarose gel electrophoresis for the hblD genes of B. cereus*

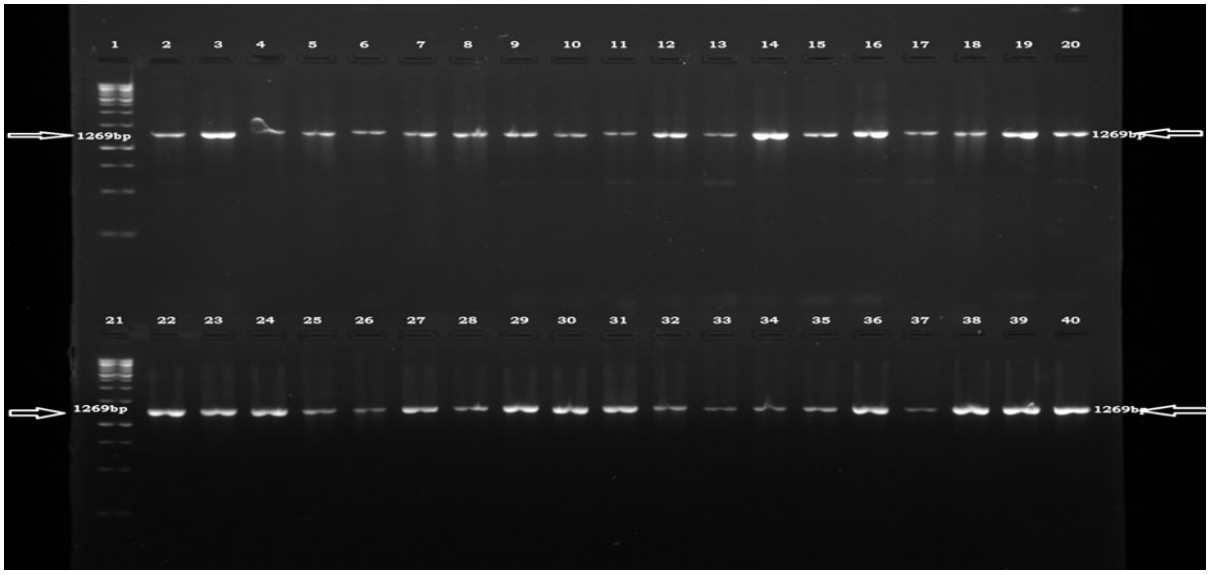

Plate 7. *Agarose gel electrophoresis for the entFM genes of B. cereus*

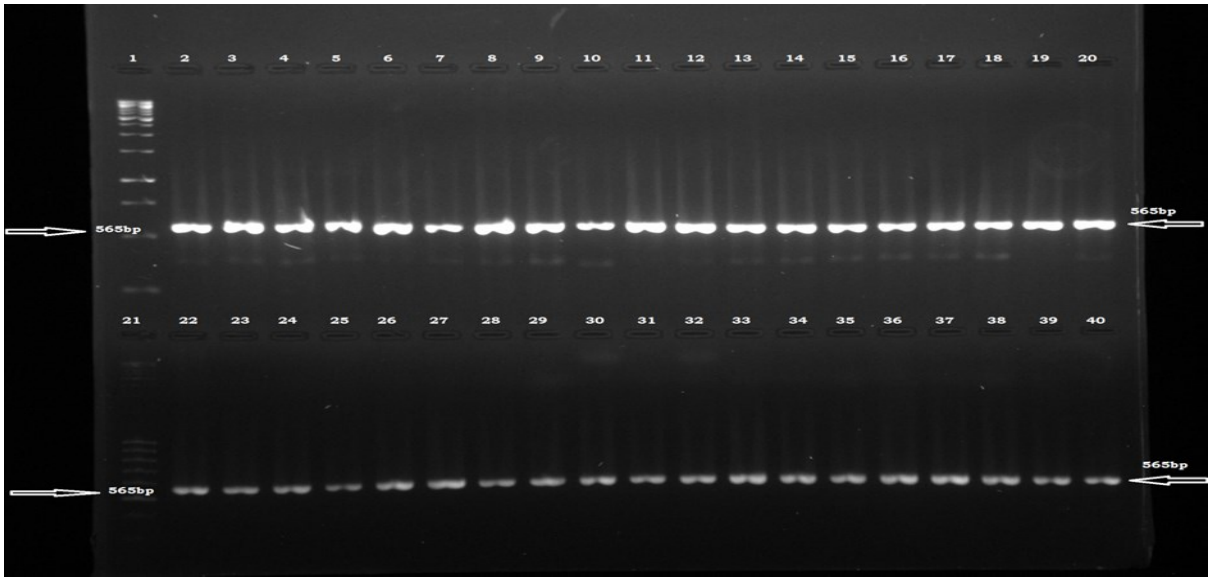

Plate 8. *Agarose gel electrophoresis for the cytK genes of B. cereus*

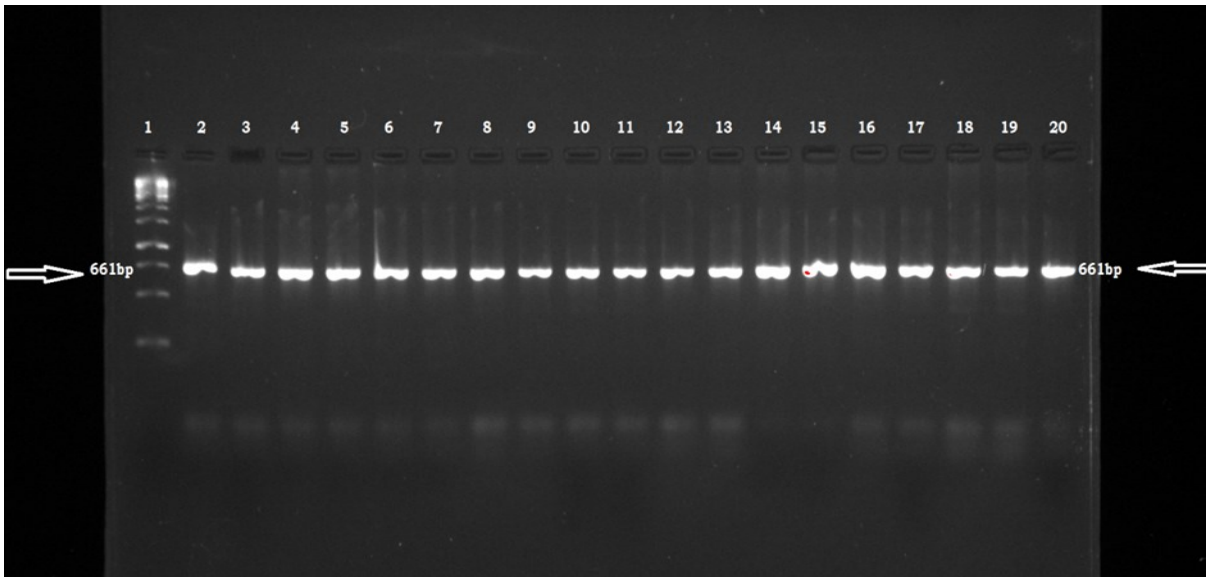

Plate 9. *Agarose gel electrophoresis for the bceT genes of B. cereus*

Cytk was detected in 74% of the strains in this study (Plate 8, Fig. 1). This compares well with the work of Li *et al.* (2016) and Gao *et al.* (2018). However, Yu *et al*. (2020) detected the gene in 68% of their *B. cereus* isolates, while Frentzel *et al.* (2018) and Schreiber *et al*. (2022) did not detect the *cytk* genes in any of their isolates. Heini *et al.* (2018) detected the gene in 7 out of 21 *B. cereus* isolates. The *cytK* protein is extremely cytotoxic. The gene was found to be abundant among food-borne isolates (Rosenquist *et al*., 2005). *cytK* is incriminated as the prime virulence factor in *B. cereus* diarrhoea, marked by necrosis and hemolysis. The toxin was first recovered from the *B. cereus* NVH 391-98 strain, which resulted in the death of three persons after consuming food containing the organism (Lund *et al*., 2000).

The *bceT* gene was detected in 60% of the strains in the present study (Plate 9, Fig. 1), which is higher than the 50.6% reported by Gdoura-Ben Amor *et al.* (2019). Ranjbar and Shahreza (2017) did not detect this gene in any of their strains. The primer pair synthesized by Mantynen and Lindstrom (1998) used by Das *et al*. (2009) for the detection of the *bceT* gene was also used in this research but there was no amplification. Hansen and Hendriksen (2001) employed different primer pairs to detect the *bceT* gene and concluded that the gene differs in sequence among strains.

In this study, the *ces* (cereulide) gene was not detected in any of the strains. This finding corroborates the work of Thirkell *et al.* (2019), who also did not detect the *ces* gene in any of their strains. Frentzel *et al.* (2018) and Heini *et al.* (2018) detected the gene in only one strain. Gao *et al.* (2018), Gdoura-Ben Amor *et al.* (2019) and Yu *et al.* (2020) detected the gene in 5%, 4% and 7% of their isolates, respectively. However, Schreiber *et al*. (2022) detected the *ces* genes in all their isolates. Ehling-Schulz *et al*. (2004, 2005) inferred that emetic toxin -producing strains emerged recently from enterotoxin-producing strains by acquiring the *ces* gene. They are rare compared to enterotoxin-producing strains.

All the *B. cereus* isolates in this study possessed at least one of the diarrheal genes. Seventeen (17) strains possessed the entire components of the HBL and NHE complex, while only six (6) harbored all of the genes (Table 1). Stensfor Arnesen *et al*. (2008) reported that the enterotoxins HBL and NHE are the core virulence factors in *B. cereus*.

In the present study, the distribution of the toxins among the group of foods showed that the occurrence of the HBL complex was very high in starchy foods (Fig. 2). Twelve (12) of the isolates that possessed the three components of the HBL complex were recovered from starchy foods. On the other hand, the NHE complex is prevalent in protein and *entFM* in starchy foods, while *cytk* and *bceT* occur more frequently in vegetables. According to Schoeni and Wong (2005) and Senesi and Ghelardi (2010), starchy foods such as pasta and rice have been implicated in emetic syndrome, while foods rich in protein such as meat and meat products are associated with diarrheal toxins. However, it is evident from this research that those starchy foods can also cause diarrhea.

The Duopath enterotoxin kit was able to detect the respective Nhe and Hbl toxins in the isolates. The kit detects the *nheB* of the enterotoxin Nhe and the L_2 component of Hbl. Ten isolates for which the *nheB* gene was not detected by PCR were positive for the Nhe toxins. Ankolekar *et al*. (2009), also reported that seven of their strains that were negative for the primer were detected with a TECRA-VIA kit. Additionally, Hansen and Hendriksen (2001) and Rahmati and Labbe (2008) had similar results where NHE was produced in the

absence of the *nheA* gene. Moravek *et al*. (2006) submission is that the ability to produce Nhe among *B. cereus* strains varies, more than 10 µg NheB per ml of supernatant are released by high producing strains, while low producers may be below 0.1 µg concentration.

Additionally, twelve isolates possessed the *hblC* (L_2) gene but did not produce the toxin in the present study. Those that possessed the gene but did not produce toxin may be the result of enterotoxin production in low quantities that the Duopath kit could not detect. According to Krause *et al.* (2010), the detection limits of the assay for NheB and L2 components are 6 and 20 ng/ml, respectively.

Conclusion

This study has showed that *B. cereus* in retailed foods in Ogun State, Nigeria, harbored numerous enterotoxigenic genes and also produced enterotoxins which are associated with gastrointestinal disease, with diarrhea and abdominal cramp as symptoms. If such foods are not well processed before consumption, they can result in foodborne disease. Therefore, consumers should practice strict personal hygiene during the processing or further processing of foods.

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Conflict of interest

The authors declare that they have no conflict of interest.

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