Research Article

The Effect of *Bacillus cereus* Organism on Fish and Its Effect on Human Health

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Abstract | Four hundred samples of Tilapia and Mullet, were screened for the frequency of *B. cereus*. It was identified by molecular methods based on sequencing of genes, the potential of the *groEL* gene as a phylogenetic marker, and identified *(hbl*,*(nhe), (cytK),* and *(ces)* enterotoxigenic genes. *B. cereus* isolates were analyzed for antibiotic susceptibility. A lab trial was conducted for two weeks using 60 Tilapia fish were divided into three equal groups, (1): kept as control negative, (2): infected intraperitoneally with (0.1ml) 8×10^7 (CFU/ ml/ fish) *B. cereus* on 1st day, (3) infected I/P intraperitoneally with (0.1ml) 8×10^7 (CFU/ml/fish) *B. cereus* on the first day and treated with erythromycin (sensitive antibiotic) on at day 5 (100 mg /kg food) for 10 days. Isolates were (22%) from Tilapia, 16% from Mullet. Gene sequences were determined for the *groEL* PCR products generated from 28 references *B. cereus* group strains and our isolates. Comparison of sequences showed that our strain groups were identical to others in nucleotide sequence similarity, ranging from 98% to 100%. The topology of the *groEL*- based trees was comparable to that of the phylogenetic tree from *B. cereus* group strains. Three subclusters could be identified, (*nhe*) and (*cytK*) could be detected in all of the *B. cereus* isolates, (*hbl*) could be detected in 50% , and (*ces*) gene could be detected only in 25%. During the experimental period, the high mortality rate was (80%) in the group (G2) - Reisolation of *B. cereus* in G2 was 83.3% and 91.6%, while in G3, it was 16.6% and 8.3% on the first and second week, respectively. The results of selected blood parameters and enzymes proved that *B. cereus* infection exhibit high levels of AST, ALT, ALP, and creatinine with reduced total protein, albumin, globulin, and albumin: globulin ratio. While in the treated group, reversible changes occurred.

Keywords | *B. cereus,* Enterotoxigenic genes, PCR, Phylogenetic tree, Fish

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INTRODUCTION

Bacillus cereus is a pathogen linked to foodborne sickness all over the world. It is widespread due to its basic dietary requirements and the formation of spores that are resistant to both harsh environmental conditions and cleaning processes. *B. cereus* is a diverse group of bacteria that is particularly interesting because of its ability to cause disease. It is widely found in food. The allowed rate of *B. cereus* in food is less than 1000 cfu/g. Food poisoning can be caused by as few as 1000 cfu/g (Stenfors Arnesen et al., 2008). In humans, the most prevalent cause of food poisoning is *B. cereus*. It causes vomiting with or without diarrhea (Lund and Granum, 1997). Diarrhea is due to enterotoxin generation in the small intestine, which can be identified in the mucus layer with or without attachment of it to the intestinal epithelium of the host (Granum, 1994).

According to (Lund et al., 2000), 3- ingredients heat-labile enterotoxins hemolysin BL (*HBL*) and nonhemolytic

enterotoxin (*NHE*), as well as cytotoxin K (*Cyt* K) were produced by food poisoning strains. As the main reason for diarrhea is due to increasing intestinal permeability and suppressing epithelial cells, where these enterotoxins are secreted in the small intestine (Logan et al., 2011). Most foods typically involved in diarrheal syndrome include fish, some animal, and milk products, the cereulide is a toxin with a low molecular weight that is encoded by the *ces* gene. It causes emetic sickness, the cyclic dodecadepsipeptide toxin cereulide, which is not affected by the surrounding conditions, causes nausea or vomiting, during growth of vegetative form, insufficient chilled meals cause cereulide to be formed (Drobniewski, 1993). The *B. cereus* from the psychrotrophic phylogenetic group's II and VI can grow at less than -7°C, posing a hazard to pasteurized goods kept in cold storage (Jan et al., 2011).

Marine fishes can create bioactive substances with antibacterial activity to protect themselves from harmful microorganisms. Fish get bacteria from the aquatic environment (water and food) which is populated with this bacteria (Kanagasabapathy et al., 2012). The disease has become a big issue in the fish farming sector as the industry has become more intensive and commercialized (Bondad et al., 2005). Human food poisoning and illnesses have also been related to *B. cereus* and different *Bacillus* species (Logan et al., 2011). Recognition of foodborne pathogens, including *Bacillus* species, is hard (Kwon et al., 2009). Because of the health risks associated with food safety and quality and human health, *B. cereus* and its toxins are of very critical importance.

This research aimed to investigate the incidence of *B. cereus* in fish, depending on molecular methods with gene sequencing, determine the toxin gene profiles of isolates, and assess their antimicrobial resistance pattern to selected antibiotics, with the study of the effect of infection with this microbe on Tilapia fish and some of its blood parameters and enzymes.

MATERIALS AND METHODS

Sampling

After immediate transfer to the laboratory in cool boxes, 400 samples were collected from 100 fish (farm source), 50 Tilapia, 50 Mullet, and 4 samples from each fish (from gills, liver, spleen, and muscles). Sampling was done using sterile swabs from these organs.

Bacillus cereus isolation and identification

Mannitol egg yolk-phenol red-polymyxin-agar (MYP). According to the procedure described by Shinagawa (1990). In a brain heart infusion (BHIB), Polymyxin (100 units/ml) was administered into the samples. The BHIB tubes were incubated for 24 hours at 30°C. Cultivation on MYP plates (Oxoid, Basingstoke, England), then placed in an incubator for twenty-four hours at thirty-seven degrees Celsius. Colony appearance (rough and bright pink colonies which are surrounded by precipitated zone due to lecithinase production), microscopy (APHA, 1992), and biochemical characterization (positive for catalase test, motile and citrate utilization test) on sheep blood agar, *B. cereus* hydrolyzes starch and causes beta-hemolysis. According to the researchers, *B. cereus* was positive for both nitrate reduction and Voges-Proskauer, but negative for the oxidase test (Logan and De Vos, 2009).

DNA extraction

To extract DNA from isolates, the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was employed, certain modifications according to their instructions, each sample was processed in proteinase K (10 μl) and lysis buffer (200 μl) in a total volume of 200 μl for 10 minutes at 56°C. The lysate was given 200 µl of hundred percent ethanol after incubation, washing, and centrifugation of each sample by following the manufacturer's instructions. Elution of nucleic acid using a kit and hundred μl of elution buffer. Metabion (Germany) contributed the primers, which are shown in Table 1.

The PCR amplification of genes *groEL, hbl, nhe, cyt* K, and *ces* gene: In a 25-μl reaction, 12.5 μl of EmeraldAmp (Takara, Japan), one μl of each primer at a concentration of twenty pmol, 5.5 μl of H_2o, and 5 μl of DNA template were added. The 2720 thermal cycler was used to carry out the reaction (applied biosystems).

The PCR products were analyzed

They were separated electrophoretically, on a 1.5 percent agarose gel, 5V/cm gradients in 1x TBE buffer at ambient temperature, Applichem, Germany, GmbH. For analysis, 15 μl was put into every gel slot. A Generuler 100 base pair ladder, Fermentas, Germany. The gel was photographed by Alpha Innotech, Biometra, and we evaluated our data using computer software.

Phylogenetic analysis of *groEL* gene

Biosystems 3130 of the genetic analyzer by (HITACHI, Japan) was used to gather DNA sequences, and their identity to GenBank was determined using a BLAST® analysis (Altschul et al., 1990). MegAlign module of Lasergene DNAStar issuance 12.1 was used to produce the phylogenetic tree (Thompson et al., 1994). Phylogenetic studies were done in MEGA6 by (maximum parsimony and likelihood) and neighbor-joining (Tamura et al., 2013).

Antimicrobial susceptibility testing

The *B.cereus* isolates were routinely tested on Mueller

Hinton Agar plates by disc diffusion assay for their sensitivity to a panel of antimicrobials (Oxoid, Milano, Italy). Antimicrobial agents that were tested included: Ciprofloxacin (CIP 5 μg), ampicillin (A 10 μg), chloramphenicol (CHL 30 μg), gentamycin (G 10 μg), vancomycin (V15 μg), cephalosporin (CN 30 μg), enrofloxacin (5 μg), erythromycin (E 15 μg), amikacin (Ak 30μg), oxytetracycline (30 μg), streptomycin (S 10 μg), and rifampicin (5 μg), (CLSI, 2013).

Bacterial strain

The most dangerous isolate has (*hbl), (nhe), (cyt* K), and *(ces)* virulence genes of enterotoxigenic strains of *B. cereus*. This culture was diluted to get an inoculum level of about 8×10^7 CFU/mL.

Experimental design

Tilapia fish (*Oreochromis niloticus*) with normal behavioral reactions and free from any skin lesions (70±5 g) which were collected from a farm in Kafr El-Sheikh belonging to the Gharbia governorate, Egypt. After a period of acclimatization, ten randomly selected fish were examined bacteriologically to ensure that they were free from *B. cereus*. We divided (60 fish) into three groups in three glass aquaria (60 x 40 x 40 cm) (20 fish in each group) with three replicates. These aquaria were saved in aerated, dechlorinated water from the tap. The temperature was set at 26°C as well as continuous oxygen supply by an air pump (Innes, 1966). Tilapia fish were fed twice daily on a fixed diet of three percent of the weight of the fish under experiment (Eurell et al., 1978). We adjusted the amount of feed based on aquarium fish weight by weighing them weekly in the morning before feeding, and we determined the daily amount of food after recording the death rate. Once a day, feces were drained out, and 30 percent of the aquarium water was changed to maintain its high quality.

The groups were divided as follows

The first group: Kept as a negative control, which was a non-infected group. Injected intraperitoneally (0.1 mL of 0.85% sterile solution).

The second group: The fish was infected with (0.1mL) 8×10^7 (CFU/mL/fish) *B. cereus* intraperitoneally (I/P) on the first day which represents the control positive group.

The third group: The fish were infected with (0.1mL) 8×10^7 (CFU/mL/fish) *B. cereus* (I/P) on the first day and treated with Erythromycin (our isolates were highly sensitive to erythromycin) on day 5 (100mg /kg food) for 10 days (Annarita et al., 2007) (the period of the experiment was two weeks).

The clinical symptoms appearing on the tested infectious fish were recorded daily for a 15 days trial period.

Reisolation of *B. cereus*

During the experiment, we collected 72 samples (72 samples from 18 fish by 4 samples from each fish) from Tilapia fish muscles, gills, spleens, and livers at two times in the first and second week for *B. cereus* reisolation. Bacterial culture and identification of *B. cereus* (Shinagawa, 1990), microscopical examination (APHA, 1992), and biochemical properties of the isolates were performed according to the criteria of (Logan and De Vos, 2009).

Blood collection

On the 15th day, (fish without feeding for twenty four hours before blood samples were taken). We randomly collected blood samples from the caudal vein without heparin from fish (n=5 from each group) following anesthesia with 50

Table 1: The genes with sequences of their primers and cycling conditions steps

Target gene	sequences of Primers	Amplified segment (bp)	Primary Denaturation 94°C for 5 minute	Amplification by (35 cycles)			Final extension 72°C for 10 minute	Reference
				Secondary denaturation 94°C for 30 second	Annealing for 40 second	Extension		
B. cereus groEL	F.TGCAACTGTATTAGCACAAGC T	533			55°C	72°C for 45 second		(Das *et al.*, 2013)
	R.TACCACGAAGTTTGTTCACTACT							
hbl	F. GTA AAT TAI GAT GAI CAA TTTC	1091			49°C	72°C for 60 second		(Ehling-Schulz *et al.*, 2006)
	R.AGA ATA GGC ATT CAT AGA TT							
nhe	F.AAG CIG CTC TTC GIA TTC	766			49°C	72°C for 45 second		
	R.ITI GTT GAA ATA AGC TGT GG							
cytK	F.ACA GAT ATC GGI CAA AAT GC	421			49°C	72°C for 45 second		
	R.CAA GTI ACT TGA CCI GTT GC							
Ces	F.GGTGACACATTATCATATAAGGTG	1271			49°C	72°C for 60 second		
	R.GTAAGCGAACCTGTCTGTAACAACA							

mg/L of benzocaine solution, and serum was recovered by centrifugation of clotted blood for fifteen minutes at 3000rpm.

For the following assays, the supernatant was collected and promptly stored in a refrigerator at -20 °C. Some blood parameters and enzymes were measured by enzymatic methods using an automated analyzer.

Analytical statistics

The IBM SPSS22 (2012) software program was used to evaluate the results (USA, Chicago, IL, IBM SPSS Inc.). (P =0.05) was used to conduct statistical analyses of the data.

RESULTS AND DISCUSSION

In the present study, out of 400 samples, 44/200 from Tilapia (22%), 32/200 from Mullet (16 %), and a total of 76/400 (19%) were recorded**.** Colonies of *B. cereus* formed uniform individual pink-orange colonies (Figure 1), and lecithinase positive. Gram-positive rods were found in all of these isolates. Catalase, citrate utilization, nitrate reduction, Voges-Proskauer, and motility tests were all positive in the 76 isolates. On sheep blood agar, beta-hemolysis is produced, oxidase test was negative.

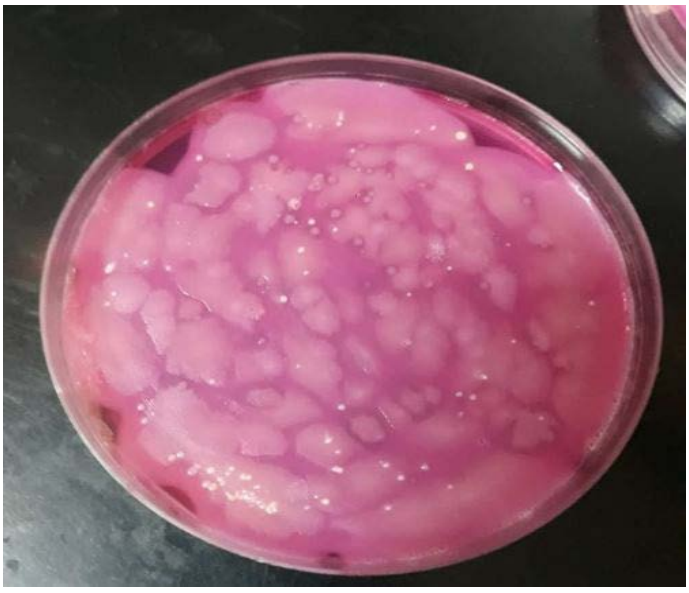

Figure 1: Colonies of *B. cereus* on MYP media.

The *B. cereus* isolates were identified via *groEL* gene sequencing. The *groEL* PCR produced from 28 reference *B. cereus* groups was sequenced. Each amplified product yielded a 533-base-pair sequence. Our strain groups were found to be identical to each other (Figures 2, 3 and 4), with the similarity of nucleotide sequence ranging between 98 % and 100 %. The *B. cereus* strains in our study were comparable by the topology of the *gro*EL- based on the phylogenetic trees, we could identify three subclusters.

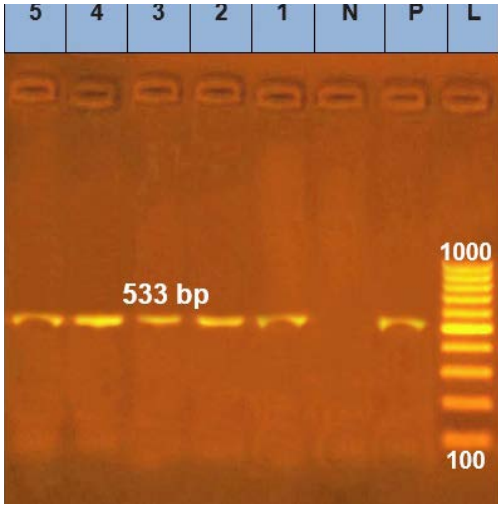

Figure 2: Detection of *groEL*at 533 bp by agarose gel electrophoresis. Lane L: marker of DNA (100 bp), (control positive: P, control negative: N), (Lane 1, 2, 3, 4 and 5): positive samples.

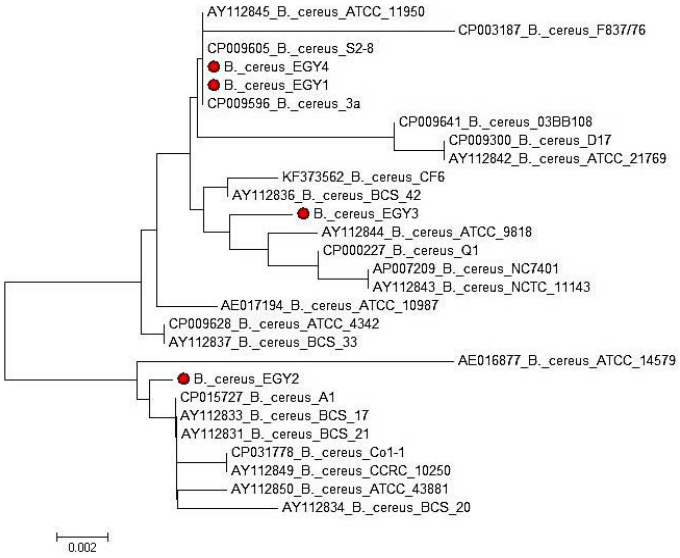

Figure 3: Phylogenetic relatedness of the *groEL* gene. Subclustering of the analysed strains into 3 subclusters could be indicated by maximum-likelihood unrooted tree generated.

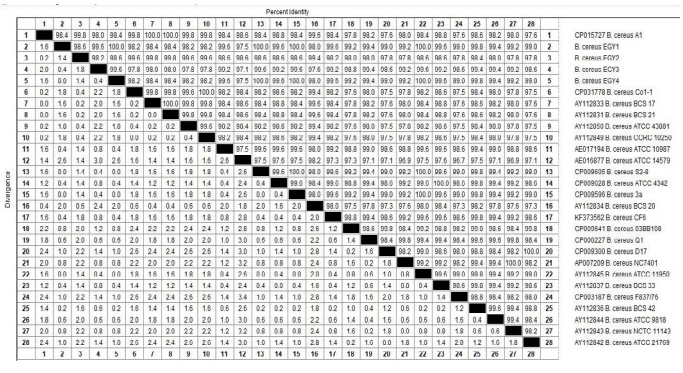

Figure 4: Sequence distance of the *groEL* gene of the tested *B. cereus* strains (generated by lasergene software) showing identity range of 97.1-100% with different *B. cereus* strains and ours in this research.

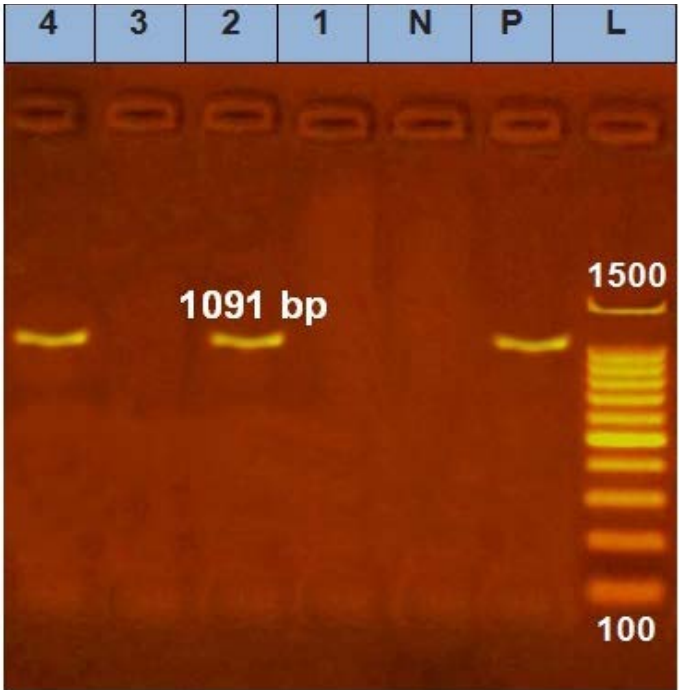

Figure 5: Detection of *hbl* at 1091 bp by agarose gel electrophoresis. Lane L: marker of DNA (100 bp), (control positive: P, control negative: N), Lane 2 and 4: positive samples, Lane 1 and 3: negative samples.

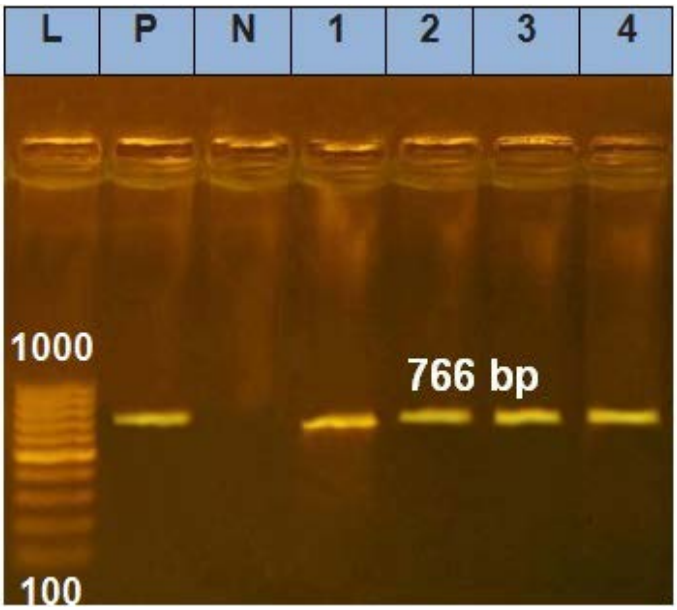

Figure 6: Detection of *nhe* at 766 bp by agarose gel electrophoresis. Lane L: marker of DNA (100 bp), (control positive: P, control negative: N), Lane 1, 2, 3 and 4: positive samples.

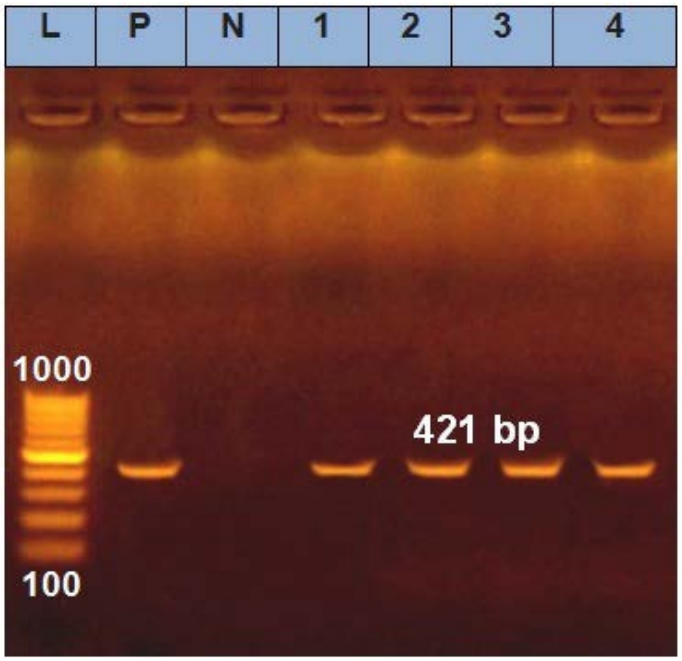

Figure 7: Detection of *Cyt k* at 421 bp by agarose gel electrophoresis. Lane L: marker of DNA (100 bp), (control positive: P, control negative: N), Lane 1, 2, 3 and 4: positive samples.

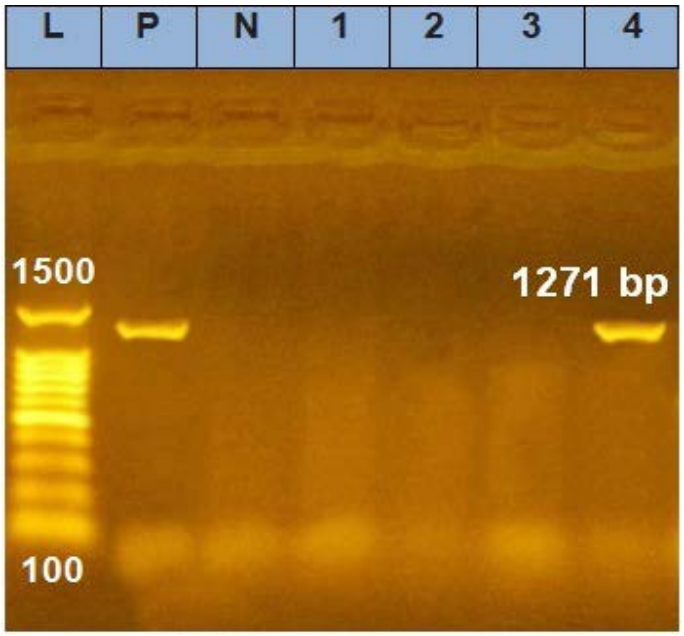

Figure 8: Detection of *ces* at 1271 bp by agarose gel electrophoresis. Lane L: marker of DNA (100 bp), (control positive: P, control negative: N), Lane 1, 2 and 3: negative, only lane 4: positive.

In this study, *B. cereus* isolates were highly sensitive to erythromycin, amikacin, ciprofloxacin, and gentamycin. Our isolates were sensitive to vancomycin, (rifampicin and chloramphenicol), enrofloxacin, oxytetracycline and streptomycin (98%), (96.6%), (95.4%), (85%) and (92%), respectively. In contrast, ampicillin and cephalosporin resistance were found in most of the isolates (98.8%) and (89%), respectively.

In experimentally infected fish, clinical symptoms like those caused by a natural infection in a farm, externally (darkness in color, abnormal behavior, tail and fin erosion, skin ulceration, distended abdomen with vent protrusion). Internally, they showed congestion and enlargement of the liver and kidney with ascites). Some signs were observed, such as: off food, lethargy, darkness in color, hemorrhagic spots and patches of skin and gills, tail and fin erosion,

ascitis with vent protrusion. The skin hemorrhagic patches led gradually to skin ulceration, which extended to involve the underlying musculature (Figures 9, 10, 11). Internally, the lesions included ascites, edema, liver congestion, enlargement of the liver, kidney, and spleen (Figure 12). Table 2 reveals cumulative mortalities of Tilapia fish during the experimental period were 3 fish (15%) in a group (G1) that fed on the basal diet only. The high mortality rate (80%) occurred in a group (G2), which was infected with *B. cereus* without treatment, and the mortalities were rare, with a percentage of 20% in a group (G3) which was treated with erythromycin and infected with *B. cereus* on the first day of the experiment.

Figure 9: Experimentally infected with *O. niloticus* showing tail and fin rot, darkness in color, loss of scales and ulceration.

Figure 10: Experimentally infected with *O. niloticus* showing deep ulceration.

Figure 11: Experimentally infected with *O. niloticus* showing deep ulceration.

The results are shown in Table 3 explains the reisolation of *B. cereus* in G2 (positive control), incidence from first and second week respectively. While the incidence of reisolation of *B.cereus* in G3 (infected with *B. cereus* on the first day and treated with erythromycin on day 5 for 10 days) from muscles, spleen, liver, and gills muscle, spleen, liver, and gills was 83.3% and 91.6% on were 16.6% and 8.3% on the first and second week, respectively. Selected blood parameters and enzymes in *O. niloticus* infected with *B. cereus* are shown in Table 4.

Figure 12: Experimentally infected with *O. niloticus* showing deep ulceration.

Table 2: Mortality rate of examined fishes during the experimental period.

Group No	No of dead fish						Total		
	W1 (For 6 day)						W2	NO	%
	Zero day	First day	Second day	Third day	Fourth day	Fifth day			
G_1	0	0	1	0	1	0	1	3	15
G_2	0	1	0	3	2	1	9	16	80
G_3	1	0	1	1	1	0	0	4	20

Zero day: day of infection with *B.cereus*. The percentage was calculated according to total number of each group (n=20).

Table 3: Reisolation of *B.cereus* from infected fish.

Time of collection	Group No.	No. of examined fish	Positive samples of *B. cereus* Total					
			Muscle	Spleen	Liver	Gills		
			No	No	No	No	No	%
Week1	G1	3	0	0	0	0	0	0
	G2	3	1	3	3	3	10	83.3
	G3	3	0	0	1	1	2	16.6
Week2	G1	3	0	0	0	0	0	0
	G2	3	2	3	3	3	11	91.6
	G3	3	0	0	0	1	1	8.3

The percentage was calculated according to the total number of each group organs (n = 12).

The *B. cereus* is a bacteria that causes two special kinds of food poisoning diarrhea and vomiting. The former induces diarrhea 6 to 15 hours after intake, while the latter induces vomiting with nausea half an hour to 6 hours after ingestion (Schoeni and Wong, 2005), and also fatal meningitis (Evreux et al., 2007). These bacteria can be transmitted through heat-treated and manufactured food products due to *B. cereus* is a spore former organism. At high temperatures, spores can persist. This organism is remarkable for its ability to withstand the most extreme conditions, even the pasteurization process (Novak et al., 2005). *B. cereus* has been found in a variety of foods, including fish (Kamat et al., 1989), and certain isolates can thrive at low temperatures (Te Giffel et al., 1997). Out of 400 samples, 44/200 from Tilapia (22%) 32/200 from Mullet (16 %), totally 76/400 (19%).

Table 4: Selected blood parameters and enzymes in *O. niloticus* infected with *Bacillus cereus.*

Inf. treated	Inf. untreated	Control	Blood parameter
3.7±2.13[a]	2.66±1.54[b]	4.25±2.45[a]	Total protein (g/dl)
1.12±0.64[a]	0.84±0.48[b]	1.35±0.78[a]	Albumin (g/dl)
2.36±1.37[a]	2.13±1.23[b]	2.5±1.46[a]	Globulin (g/dl)
0.47±1.0[a]	0.39±0.8[b]	0.54 ±1.1[a]	Albumin:globulin (g/dl)
8.66±5.01[b]	8.3±4.79[b]	9.0±5.2[a]	Urea (mg/dl)
1.47±0.85[a]	0.65±0.37[b]	0.67±0.38[b]	Creatinine (mg/dl)
22.0±12.72[c]	32.0±1849[a]	16.67±9.63[b]	ALP activity (u/l)
67.67±39.11[b]	74.0±42.77[c]	48.3±27.94[a]	AST activity (u/l)
69.67±40.26[a]	74.0±42.77[b]	67.67±39.11[a]	ALT activity (u/l)

Inf. (infected) - [a,b]The means of the different superscripts within a row differ considerably ($P< 0.05$).

In this regard, some previous studies such as those reported by (Hassanien et al., 2018; Rasool, 2017; Sanjoy et al., 2009) from different countries varied from 24 to 36.7% have shown a higher prevalence rate of *B. cereus* than the mentioned rate. *Bacillus cereus* isolates were identified according to *groEL* gene sequences. Following *16S rRNA* sequence comparisons, phylogenetic studies based on sequences of *the groEL* gene have revealed relationships that were previously obscure and contentious (Viale et al., 1994). Phylogenetic research on eubacteria has benefited greatly from gene sequences generated from amplicons, such as the *groEL* gene, which has proven to be useful in phylogenetic investigations (Poyart et al., 1995; Viale et al., 1994). Due to their similarities, several studies have found difficulty in differentiating and phylogenetic relationships of certain *Bacillus* taxa according to sequences of *16S rRNA* gene in prior investigations (Liu et al., 2013).

Recently, to identify between species, housekeeping genes were used (Durak et al., 2006). The *groEL* PCR results through 28 references of *B. cereus* strains were sequenced. Each amplified product yielded a 533-base-pair sequence. By comparison of sequences,our strain groups were found to be identical to each other, due to the similarity of the nucleotide sequence. These strains in our research were comparable by the topology of the *gro*EL which based on the phylogenetic tree, we could identify three subclusters. The first included two strains (*B. cereus EGY1* and *B. cereus EGY4*) which were closely identical and found in the same very short branch as they were closely related to each other and that indicate identical sequences. On the other hand, they were showed identity percentage of 100% (*CP009596 B. cereus 3a*), (*CP009605 B. cereus S2-8)* and *(AY112845 B. cereus ATCC 11950)*, reduced activity of mitochondrial dehydrogenase, detachment, necrosis, and activity of hemolytic are among the biological actions of these strains (Minnaard et al., 2007). *Cap*A, B, and C genes act as crucial role in its virulence in *(CP009641 B. cereus 03BB108)* which was compared on gene bank with our strains and it was not identical to any of them and it was identical by 99.2% with *Bacillus anthracis* in this research (Hoffmaster et al., 2006), 99% (*CP003187 B. cereus F837/76), (CP009300 B. cereus D17*) and *(AY112842 B. cereus ATCC 21769),* that are capable of producing both hemolytic and nonhemolytic enterotoxins (Lund and Granum, 1997), clinical infections, including deadly pneumonia, have been linked to these strains (Bottone, 2010).

The second included *(B. cereus EGY2)* was also identical to that of 7 *B. cereus* nucleotide sequence similarity ranged from 98.6% (*AE016877 B. cereus ATCC 14579),* 99.4%(*AY112834 B. cereus BCS 20),* 99.6% *(AY112849 B. cereus CCRC 10250, AY112850 B. cereus ATCC 43881 and CP031778 B. cereus Co1-1)* to 99.8% (*AY112831 B. cereus BCS 21*and *AY112833 B. cereus BCS 17),* seasoning *(BcS)*, food borne diarrhea, characterised as the the "diarrheic syndrome"as a result of enterotoxins production by *B. cereus* (Mckillip, 2000). Within third group, the sequence of *B. cereus EGY3* was also nucleotide sequence similarity to that of 9 *B. cereus*. The identity ranged from 99.2% (*KF373562*

B. cereus CF6, AP007209 B. cereus NC7401, AY112843 B. cereus NCTC 11143, AE017194 Bacillus cereus ATCC 10987, CP009628 Bacillus cereus ATCC 4342 , *AY112837 B. cereus BCS 33*) to 99.4% (*AY112836 B. cereus BCS 42, AY112844 B. cereus ATCC 9818, CP000227 B. cereus Q1*). *AY112844 B. cereus ATCC 9818* is an example of *B. cereus* spores that can withstand extreme heat (Montville et al., 2005).

Furthermore, relying on the study of genes as new molecular characters has had different degrees of success. The similarities of sequences among *Bacillus* isolates in our investigation were 99% to 100%, 98.6% to 99.8%, and 99.2% to 99.4%, respectively. In previous studies, sequence similarity ratios for 16S rRNA gene in *Bacillus* varry from 92% to 99.8% (Caama˜no-Antelo et al., 2015; Liu et al., 2013). Emesis-inducing strains *NCTC 11143* and *B. cereus ATCC 11950* were located in the same subcluster of the *groEL* phylogenetic tree. Similarity ratio between this strain (*NCTC 11143*) and (sample 3) was high (99.2%) and higher (100%) between *B. cereus ATCC 11950*) and (samples 1 and 4). Yu et al. (2003) reported high rate of similarity (99.3%). The existence of *Bacillus* species are known to cause food poisoning is a serious concern for human health. Principal virulence factors driving diarrheal illness are enterotoxin genes (*HBL* and *NHE* complexes), as well as *cyt* K generated by *Bacillus* spp. (Lund and Granum, 1997; Lund et al., 2000). The fourth isolate is the most harmful, which has *(hbl), (nhe), (cytK)* and *(ces)* virulence genes. Tewari et al. (2015) reported that 89.7 % (26 /29), 55.2 % (16/29) and 41.4 % (12/29) of *B. cereus* strains possess the *nhe*, *hbl* and *cyt* K, respectively. Ehling-Schulz et al. (2006) detected *nhe, hbl, cyt* K and *ces* toxins genes from *B. cereus* food isolates and clinical isolates. According to Fatma and Seza (2019), production of the *HBL* and *NHE* in *Bacillus* isolates were 23.1% and 15.4%, respectively, while (41.7%) of enterotoxin, *ces* and *cyt*K1 genes was not found in any of the isolates.The obtained results of Hassanien et al. (2018) using PCR indicated that, hemolytic toxin*(hbl)* was 75% (9/12), 62% (8/13) and 60% (12/20), non-hemolytic toxin*(nhe)*, was 25% (3/12), 38% (5/13) and 40% (8/20), cytotoxin K (*cytK*) was 16.5% (2/12), 15% (2/13) and Cereulide (*ces)* was 16.5% (2/12), 7.5% (1/13) and 5% (1/20) in positive samples of *B. cereus* in raw Tilapia, Mackerel and Sardine, respectively.

The *Cyt* K was first recognised in a strain that caused the spread of diarrheal syndrome linked to food poisoning (Lund et al., 2000). Also, Fagerlund et al. (2004) detected *cyt*K in a strain that causes the fatal food poisoning. According to earlier research, *Bacillus cereus* and its toxins have been found in a number of foods, including fish (Das et al., 2009). As a result of PCR, the lack of detection of one or more *hbl* or *nhe* genes is due to the high polymorphism of *hbl* and *nhe* gene sequences (Guinebretiere et al., 2002). These results agree with our findings regarding the low level of the reveal of the *hbl* genes. According to Tewari et al. (2015), the location and source of origin affect the presence of enterotoxin genes. Low *cyt*K and *ces* genes have been observed in *Bacillus* species involving *B. cereus* (Ahaotu et al., 2013). Also, Yim et al. (2015) recorded that the incidence of the (*ces*) gene was between 1.5% and 17.2% which partially agrees with our results. *B. cereus* strains evaluated in our investigation showed varying degrees of portability to the antibacterial drugs.

For ampicillin, almost all tested bacteria were resistant. That is consistent with earlier research revealing this group's strong resistance to ß-lactam antimicrobials (Andrews and Wise, 2002). This resistance could be attributed to the germs' ability to synthesize ß-lactamase, enzymes involved in the antibiotic's breakdown (Park et al., 2009). Synthesis of -lactamases can result in resistance to cephalosporins up to the third generation (Ozcelik and Citak, 2009). The uncontrolled use of antibiotics has contributed to the formation and spread of resistant bacteria, which can then be spread to humans by the food chain, posing major public health risks (Apata, 2009). Moreover, Oladipo and Adejumobi (2010) recorded that all isolates were susceptible to ciprofloxacin (100%). The antibacterial portability of *B. cereus* to various antimicrobial drugs was assessed, allowing for improved management of the bacteria. This research found that all strains were sensitive to vancomycin and erythromycin, which is consistent with (Dejana et al., 2015). They found that all *B. cereus* were susceptible to imipenem, vancomycin, and erythromycin in their study. Clinical manifestations and postmortem looked like those seen in *B. cereus* infections (Ali et al., 2019; Younes et al., 2021). Reisolation of *B. cereus* in G2, the incidence of *B. cereus* from muscle, spleen, liver, and gills were 83.3% and 91.6% at first and second week respectively. While the incidence of reisolation of *B. cereus* in G3 (infected with *B. cereus* at 1st day and treated with erythromycin on day 5 for day 10) from muscles, spleen, liver, and gills was 16.6% and 8.3% on the first and second week, respectively.

On days 10 to 15, erythromycin-treated fish gradually recovered, becoming active and resuming food intake and activity. Selected blood parameters and enzymes in *O. niloticus* infected with *B. cereus* proved that the infection results in hepatorenal damage. This may be due to the evolution of oxidative stress. Infected fish exhibit high levels of AST, ALT, ALP, and creatinine, decreased total protein, albumin, globulin, albumin: globulin ratio. While within the treated group, reversible alterations occurred, resulting in near-normalization.Total protein, albumin, and globulin levels in "untreated" fish were considerably lower than in control and treated fish. Because they are the most major components of blood serum, they are thought

to be efficient indicators of humoral immunity and well-being in fish (Abdel-Daim et al., 2019).

Vascular leakage, as well as a failure to manufacture and proteolyze serum, results in a drop in serum protein levels (Ellis et al., 1981). Low levels of albumin fractions, which are involved in hypoproteinaemia, are given top priority. Low levels of albumin may be due to loss from skin lesions, catabolism is elevated in acute inflammation, and synthesis is inhibited due to hepatopathy or renal injury (Chandra et al., 2015). In comparison to their respective normal values, the serum total protein content of the untreated fishes decreased by up to 37.4 percent, albumin by up to 38 percent, and globulin level by up to 15 percent. Because albumin possesses antihaemolytic and antibacterial properties, and alpha-globulins are immunoglobulin transporters, their decrease encourages infection. The increased protein levels induced by renal failure caused by the infection could explain the rise in blood creatinine with lower total protein, globulin, and albumin. It has been shown to increase considerably in creatinine due to fatigue in renal glutathione.

This results in a lower filtration rate of the glomerular which affects the efficiency of the kidney (Abdelkhalek et al., 2015). Two essential enzymes found in all tissues are AST and ALT. They catalyze the transition of glutamic acid's amino group (-NH2) to oxaloacetic acid or pyruvic acid (Chandra et al., 2015). Following *B. cereus* infection in *O. niloticus*, data revealed a significant rise in both enzymes. The increase in serum AST, ALT, and ALP activity could indicate significant clinical damage and histological alterations in the liver as a result of the infection, so the activities of these enzymes in fish serum are well-known as indicators of liver function (Shakoori et al., 1994).

CONCLUSIONS AND RECOMMENDATIONS

In this research, detecting virulence genes and antimicrobial susceptibility are considered the best way to assess the health risk of *B. cereus*. Using phylogenetic markers can help distinguish bacteria from the *B. cereus* group. We presented a strategy for using sequences of the *groEL* gene to classify *B. cereus* group bacteria. These findings of this study will aid in the knowledge of genetic variation among this microbe, which is important in the food sector. As a result, this retail fish study could be useful for human health and epidemiological techniques. Therefore, our findings provide primary data that can be used to make risk evaluations to avoid food poisoning.

NOVELTY STATEMENT

This research article is novel. The main objective of this paper is to find out the incidence of B. cereus in fish, depending on molecular methods with gene sequencing, determining the toxin gene profiles of isolates and assessing their antimicrobial resistance patterns to selected antibiotics, with the study of the effect of infection with this microbe on Tilapia fish and some of its blood parameters and enzymes. Even though many researchers were worked on the gene technology of B. cereus, very few researchers were reported about the similarities in genetic sequence among strains that cause disease states and poisoning in both fish and humans The knowledge of genetic variation among this microbe is the main criteria for determining the type of microbe, its virulence, and how to eliminate it. These data are very useful in the human health and epidemiological techniques. Many researchers compared these data in their work. Though there are similar researches, but in the present work, the relationship between the genetic sequence of the Egyptian isolates and those on the gene bank, where they are similar to each other in a large percentage and also with their counterparts on the gene bank are studied exclusively. From this study we conclude that, our findings provide primary data that can be used to make risk evaluations to avoid food poisoning.

AUTHOR'S CONTRIBUTION

AMR is contributed in chemicals, materials, and research methods in the manuscript. MRB, EAK, and NAA shared in the strategy and sample analysis. All authors took part in the analysis. They drafted, revised, and approved the manuscript.

Conflict of interest

The authors confirmed that there were no conflicts of interest.

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