ORIGINAL RESEARCH

Newly Emerging MDR *B. cereus* in *Mugil seheli* as the First Report Commonly Harbor *nhe, hbl, cyt*K, and *pc-plc* Virulence Genes and *bla*1, *bla*2, *tet*A, and *erm*A Resistance Genes

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Background: *Bacillus cereus* is a common food poisoning pathogen in humans. This study aimed to investigate the prevalence, molecular typing, antibiogram profile, pathogenicity, dissemination of virulence and antibiotic resistance genes associated with natural *B. cereus* infection among *Mugil seheli*.

Methods: Consequently, 120 *M. seheli* (40 healthy and 80 diseased) were obtained from private fish farms in Port-said Governorate, Egypt. Afterward, samples were processed for clinical, post-mortem, and bacteriological examinations. The recovered isolates were tested for antimicrobial susceptibility, phenotypic assessment of virulence factors, pathogeneicity, and PCR-based detection of virulence and antibiotic resistance genes.

Results: *B. cereus* was isolated from 30 (25%) examined fish; the highest prevalence was noticed in the liver (50%). The phylogenetic and sequence analyses of the *gyr*B gene revealed that the tested *B. cereus* isolate displayed a high genetic similarity with other *B. cereus* strains from different origins. All the recovered *B. cereus* isolates (n =60, 100%) exhibited β-hemolytic and lecithinase activities, while 90% (54/60) of the tested isolates were biofilm producers. Using PCR, the tested *B. cereus* isolates harbor *nhe, hbl, cyt*K, *pc-plc*, and *ces* virulence genes with prevalence rates of 91.6%, 86.6%, 83.4%, 50%, and 33.4%, respectively. Moreover, 40% (24/60) of the tested *B. cereus* isolates were multidrug-resistant (MDR) to six antimicrobial classes and carried the *bla*1, *bla*2, *tet*A, and *erm*A genes. The experimentally infected fish with *B. cereus* showed variable mortality in direct proportion to the inoculated doses.

Conclusion: As far as we know, this is the first report that emphasized the existence of MDR *B. cereus* in *M. seheli* that reflects a threat to the public health and the aquaculture sector. Newly emerging MDR *B. cereus* in *M. seheli* commonly carried virulence genes *nhe, hbl, cyt*K, and *pc-plc*, as well as resistance genes *bla*1, *bla*2, *tet*A, and *erm*A.

Keywords: MDR *B. cereus*, virulence traits, antibiogram, virulence genes, antimicrobial resistance genes

Introduction

Aquaculture is an optimistic sector of food production, providing a source of animal protein to the world's population and bridging the gap in food shortages due to overpopulation.[1] In fact, about 17% of the world's animal protein sources come from fish, and more than 3.3 million people with 20% of their average per capita animal protein consumption

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substantially depending on aquatic organisms.[2] The world faces serious challenges to feed its growing population. This surpassing increase has been linked to overpopulation, income explosion and urbanization, and the structural development of the aquaculture sectors.[3] Therefore, the increase in fish demand must be supported by a concomitant rise in fish-producing sectors in line with the stagnation of fisheries.[4] Recently, there has been growing concern about the overuse of freshwater resources, which has prompted many authorities to make stately efforts to maximize fish productivity and profits through the expansion of mariculture.[5]

Mullets are a significant constituent of Egyptian fisheries and represent the main cash crops of artisanal fisheries in the country's lagoons and shores.[6] Mullets have been grown in the "Hosha" system along the Nile delta as part of a long-established custom for decades.[7] Egypt is currently a leading country in mullet farming with a record production of 129,000 million tons in 2015, which represents 10.5% of the aquaculture yield.[8] Due to the expansion and intensification of aquaculture, many synthetic chemicals and biologics have widely utilized to control and treat pond water and sediments, causing water and environmental pollution and the emergence of threatened emerging pathogens.[9] In fact, the frequent use of chemicals has adverse effects on fish health through impairment of water parameters, alteration of natural flora, and immunosuppression that are predisposing factors for emergence of bacterial diseases.[10]

Mullets, like other marine organisms, are susceptible to many bacterial diseases, including Gram-positive bacteria.[11] Among them, *Bacillus cereus* was mentioned earlier as a threatening fish pathogen.[12] *B. cereus* is a Gram-positive, motile, facultative anaerobes, and sporogenous ubiquitous bacteria frequently recovered from the water, soil, plants, and various food products.[13] It is an opportunistic foodborne pathogen of public health importance; it is initially considered the main cause of gastroenteritis and food poisoning in humans.[14] *B. cereus* possesses various toxins and other virulence traits encoded by several virulence genes, including hemolytic toxins and non-hemolytic enterotoxins, cytotoxin K (cytK), and hemolytic gene encoding phospholipase.[15]

The produced toxins are responsible for several human illnesses such as emesis and diarrheal syndromes, fulminant septicemia, nervous manifestations (brain abscesses and meningitis), and gas gangrene,[16] pneumonia,[17] eye infections (endophthalmitis), and neonates bacteremia.[18] *B. cereus* also harbored many antimicrobial-resistance genes and showed different resistance patterns to various antibiotic classes.[19] The bacteria are highly resistant to β-lactam antibiotics[20] and showed acquired resistance to some antimicrobial agents like erythromycin, ciprofloxacin, streptomycin, and tetracycline.[21]

A brief study of the whole genome sequence of *B. cereus* revealed that the bacteria harbored β-lactam resistant genes, macrolide-resistant genes, and tetracycline-resistant genes.[19] The regular and indiscriminate use of antibiotics to treat and/or control aquatic diseases, along with bad management, results in the existence of multidrug-resistant strains. Former studies have reported the occurrence of MDR bacterial pathogens in the aquaculture sector that could be transmitted to humans via the consumption of contaminated food.[22,23]

Bacillus species have been widely used in aquaculture practices as growth promoters, immunostimulants, and probiotics. However, under certain adverse conditions, the bacteria become pathogenic and serious systemic infections occur. *Bacillus* spp., particularly *B. cereus*, could induce diseases in several fish species, including common carp (*Cyprinus carpi*),[24] striped bass (*Morone saxatilis*),[12] European seabass (*Dicentrarchus labrax*),[25,26] white seabream (*Diplodus sargus*),[27] and stinging catfish (*Heteropneustes fossilis*).[28]

The pathogenicity of *B. cereus* varies greatly, with the strains being harmless or lethal. This variation may reveal constrains in detecting the dangers of bacteria and often leads to misbelief of the associated risks. Therefore, full awareness about the prospect pathogenicity of bacteria is a main challenge for the fish-producing industries. To our knowledge, this is the first study investigating the prevalence, sequence analysis, and antibiogram pattern of newly emerged *B. cereus* among cultured *Mugil seheli*. The study also provides new insights into virulence traits and antibiotic resistance genes typically inherited by bacteria, which contribute significantly to finding decisive solutions to treat or control the disease.

Methods
Animal Ethics
The present study was performed in compliance with the ARRIVE guidelines. All protocols were conducted according to relevant guidelines and regulations. Fish handling and all the experiments were approved by the Animal Ethics Review

Committee of Suez Canal University (AERC-SCU), Egypt. The authors have obtained a permission from the farm owners to conduct a research on their fish.

Fish Sampling

One hundred and twenty *Mugil seheli* (40 healthy and 80 diseased with an average weight 80±15 g) were obtained from private farms in Port-said Governorate, Egypt, from March 2021 to June 2021. The collected fish were transported to the laboratory in aerated sealed plastic bags for further clinical and bacteriological examinations.

Clinical and Post-Mortem Inspection

The collected fish were clinically inspected for detection of gross or internal lesions as previously described by Schäperclaus.[29]

Isolation and Identification of *B. cereus*

A 3–5 g of each examined (kidney, liver, and gills) sample was homogenized in 90 mL of buffered peptone water (BPW, Oxoid, UK) for 2 min using a stomacher. Moreover, the obtained samples were subjected to a 15-min heat treatment at 70 °C to eliminate vegetative cells and isolate the bacterial spores.[15] To prevent spore germination, the heated samples were immediately placed on ice. Subsequently, 100 μL were inoculated on Mannitol Egg-Yolk Polymyxin (MYP) agar plates and incubated at 37 °C for 24 h. The identification of *B. cereus* was carried out according to the colonial characters, morphological characteristics, endospore formation, and biochemical reactions: catalase and oxidase activity, H_2S test, methyl red Voges–Proskauer test, indole test, and carbohydrate fermentation ability, as described by Maturin and Peeler.[30] Furthermore, the isolates were phenotypically identified using the VITEK 2 compact system (bioMérieux, France),[31] whereas confirmed genetically using PCR-based detection of the *gyr*B gene as previously described by Yamada,[32] followed by gene sequencing.

*B. cereus gyr*B Gene Sequencing

The recovered *B. cereus* isolates were identical in their phenotypic profiles, so the PCR product of one randomly selected strain was sent for direct sequencing in both directions after the purification by QIAquick PCR-Product extraction kit (QIAGEN Sciences Inc., Germantown, MD, USA). The sequencing was performed using the Bigdye Terminator V3.1 cycle sequencing kit (Thermo Fisher Scientific, Waltham, MA, USA). The obtained sequences were deposited in the GenBank with accession number: MZ647998. The BLAST (Basic Local Alignment Search Tool) analyses were conducted to determine the sequence similarity to GenBank accessions. Besides, the phylogenetic analyses were conducted based upon the MegAlign module of Laser gene DNA Star version 12.1 using neighbor-joining in MEGA6.[33]

Phenotypic Assessment of Virulence Factors of *B. cereus* Isolated from *M. seheli*
Biofilm Production

As previously described by Osman,[34] the biofilm development was assessed using glass test tubes. Briefly, each *B. cereus* strain was inoculated into tryptic soy broth (Oxoid, Hampshire, UK) and incubated overnight without shaking at 37 °C. A sterile broth was used as a negative control. After broth removal, the incubated tubes were stained with 1% crystal violet and incubated for 15 min (to examine cells adhering to the test tube). Shortly, the tubes were cleaned thoroughly using sterile distilled water. Each strain was tested three times. The positive result was specified by the production of purple biofilm.

Hemolytic Activity

The tested isolates were streaked onto 5% sheep blood agar (Oxoid, UK) and incubated at 24 °C for 48 h. The appearance of β-hemolysis surrounding the colonies indicates a positive result, as previously described by Wiwat and Thiramanas.[35]

Lecithinase Production

The tested isolates were inoculated onto Mannitol Egg-Yolk Polymyxin (MYP) agar plates and incubated at 37 °C for 24 h. The lecithinase production was indicated by the formation of a zone of egg yolk precipitation surrounding the colonies, as previously described by Quinn.[36]

Antibiogram Pattern of the Recovered *B. cereus* Isolates

The antimicrobial resistance profiles of the retrieved *B. cereus* isolates were detected on Mueller-Hinton agar (Oxoid, Hampshire, UK) using the Kirby-Bauer disc diffusion method as previously described by Park.[37] The following antimicrobial agents (Oxoid, UK) were used (n=14); vancomycin (VAN) (30 μg), penicillin G (PEN) (10 U), amoxicillin (AMX) (30 μg), amoxicillin-clavulanic acid (AMC) (30 μg), meropenem (MEM) (10 μg), cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), nalidixic acid (NA) (30 μg), ciprofloxacin (CIP) (5 μg), sulfamethoxazole/trimethoprim (SXT) (25 μg), streptomycin (S) (10 μg), gentamycin (CN) (30 μg), doxycycline (DOX) (30 μg), and erythromycin (E) (15 μg). These antimicrobial agents were used frequently in the fish aquaculture sector in Egypt. The interpretation of results was performed according to the CLSI guidelines.[38] *B. cereus* ATCC 11778 strain was used as a reference strain. The tested *B. cereus* isolates were categorized according to their resistance patterns into MDR and XDR (Extensively drug-resistance means resistant to one or more antimicrobial agent in all except one or two antimicrobial classes) as previously described by Magiorakos.[39] The multiple antibiotic resistance (MAR) index was calculated as previously described by Krumperman[40] as the following formula: MAR = The number of antimicrobial agents to which the isolates are resistant/The total number of tested antimicrobial agents.

PCR Detection of Virulence, Enterotoxins and Antibiotic Resistance Genes in the Recovered *B. cereus*

PCR assay was used to determine the enterotoxins genes; *hbl, nhe, cyt*K, and *ces*, the hemolytic gene encoding phospholipases; *pc-plc*, and the antibiotic resistance genes; *bla*1, *bla*2, *tet*A, and *erm*A in the recovered *B. cereus* isolates. QIAamp DNA Mini Kit (QIAGEN Sciences Inc., Germantown, MD, USA/Cat. No. ID 51326) was used for genomic DNA extraction. Positive control strains (supplied by The AHRI, Dokki, Giza, Egypt) were used in the PCR assay, while a DNA-free reaction was involved as a negative control. The amplified PCR products were separated on agarose gel (1.5%) stained with ethidium bromide 0.5-μg mL^{-1}, and then photographed. The primers sequences (Thermo Fisher Scientific, Waltham, MA, USA), amplicon size, and the PCR conditions are illustrated in Table 1.

Pathogenicity Assay

Adaptation Period

Approximately 150 apparently healthy *Tilapia zillii* weighing 36 ± 5 g (with no history of previous infections) were collected from private farms in Ismailia Province, Egypt. The *T. zillii* was selected as a common model of marine fish due to its ease of handling, while *M. seheli* is difficult to be handled. To ensure that the collected fish are free from diseases, eight random samples (n=8) were harvested and subjected to bacteriological examination (conventional method of bacterial isolation and identification) as well as parasitological examination (wet mounting, squash preparation, macroscopic and microscopic examinations) as described by Meyers.[47] The fish were acclimated in 1000 L fibreglass tanks for 10 days before the experiment. All water parameters values were adjusted at the permissible limits. Only apparently healthy fish with normal responses were harvested for further study.

Inoculum Preparation

The bacteria were basically cultured on MYP agar and left incubated at 37 °C for 24 h. The characteristic pink, lecithinase-producing colony, was selected and streaked on Trypticase Soy Agar (TSA) (Oxoid, UK) at 37 °C for an extra 24 h. Subsequently, the bacterial suspension was adjusted at final concentrations (10^4–10^8 CFU mL^{-1}) using McFarland Turbidity Standard.[48]

Experimental Setup

About 100 acclimated *T. zillii* were distributed randomly into five groups, each holding two similar subgroups (n= 10). Each subgroup contains ten fish reared in a 120 L glass aquarium. Fish in the first group were injected intraperitoneally

Table 1 The Oligonucleotides Sequences Used in the PCR Assay

Primer	Sequence	Amplified Product	Amplification (35 Cycles)			References
			Denaturation	Annealing	Extension	
gyrB	F: GAA GTC ATC ATG ACC GTT CTG CAY GCN GGN GGN AAR TTY GA R: AGC AGG ATA CGG ATG TGC GAG CCR TCN ACR TCN GCR TCN GTC AT	1350 bp	94°C 1 min.	66°C 1 min.	72°C 1 min.	[32]
nhe	F:AAG CIG CTC TTC GIA TTC R: ITI GTT GAA ATA AGC TGT GG	766 bp	94°C 30 sec.	49°C 40 sec.	72°C 45 sec.	[41]
cytK	F: ACA GAT ATC GGI CAA AAT GC R: CAA GTI ACT TGA CCI GTT GC	421 bp	94°C 30 sec.	49°C 40 sec.	72°C 45 sec.	
ces	F: GGTGACACATTATCATATAAGGTG R: GTAAGCGAACCTGTCTGTAACAACA	1271 bp				
pc-plc	F: GAGTTAGAGAACGGTATTTATGCTGC R: CTACTGCCGCTCCATGAATCC	411 bp	94°C 1 min.	55°C 1 min.	72°C 1 min.	[42]
hbl	F: TGCACAAGAAACGACCGCTCA R: ATAATTTGCGCCCATTGTATTCCAT	987 bp	94°C 30 sec.	54°C 40 sec.	72°C 45 sec.	[43]
bla1	F: CATTGCAAGTTGAAGCGAAA R:TGTCCCGTAACTTCCAGCTC	680 bp	94°C 30 sec.	50°C 40 sec.	72°C 45 sec.	[44]
bla2	F: TTGTCGATTCTTCTTGGGATG R: CCCCTACTTCTCCATGACCA	483 bp				
tetA	F: GGCGGTCTTCTTCATCATGC R: CGGCAGGCAGAGCAAGTAGA	502 bp	95°C 1 min.	58°C 1 min.	72°C 1 min.	[45]
ermA	F:TCTAAAAAGCATGTAAAAGAA R: CTTCGATAGTTTATTAATATTAGT	645 bp	94°C 30 sec.	52°C 40 sec.	72°C 45 sec.	[46]

(IP) with 200 µL of sterile saline and considered as a negative control (C − ve). The other groups (T$_I$ -T$_V$), were injected IP with 200 µL of the overnight culture of *B. cereus* strain at different concentrations (10^4–10^8 CFU mL^{-1}, respectively) to detect the LD$_{50}$, as there is no published reference study. *B. cereus* isolate that harbored *nhe, hbl*, and *cytK* virulence genes were chosen as a reference virulent strain. The abnormal behaviour and pathological lesions of the treated fish were investigated for two weeks post-inoculation. Cumulative mortalities and LD$_{50}$ were also recorded and calculated. Moribund and dead fish were regularly harvested and bacteriologically examined to ensure the Koch's postulates.

Statistical Analyses

The obtained data were analyzed using the Chi-square test (SAS software, version 9.4, SAS Institute, Cary, NC, USA) (*p*-value< 0.05 indicates a significant difference among the measured parameters). Moreover, the correlation coefficient was performed using R-software (version 4.0.2; https//www.r-project.org/).

Results
Clinical and Post-Mortem Findings

The examined moribound *M. seheli* exhibited abnormal behavior, skin ulceration, abdominal distention, and hemorrhage on the body surface and gills (Figure 1A), and exophthalmia (Figure 1B). Moreover, the post-mortem examination showed hepatomegaly, congested kidney, and the accumulation of fluids in the abdominal cavity (Figure 2).
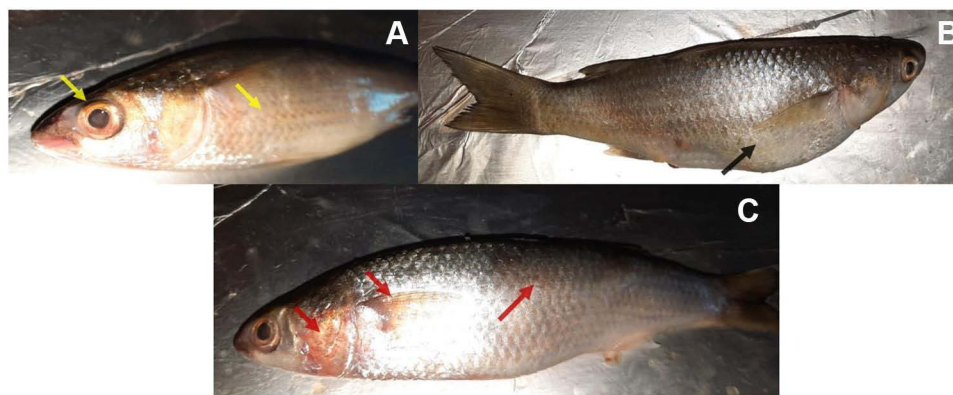
Figure 1 The Naturally infected *M. seheli* with *B. cereus* exhibited (**A**) slight exophthalmia, scales detachment (yellow arrows), (**B**) abdominal distension (black arrow), (**C**) hemorrhagic patches on the fin and operculum, and skin ulcerations (red arrows).

Figure 2 Naturally infected *M. seheli* with *B. cereus* showed enlargement and congestion of the kidney and liver (white arrows) with the accumulation of serous fluids in the body activity (S).

Phenotypic Characteristics and the Prevalence of *B. cereus* Among the Examined Fish

Morphologically, all the retrieved *B. cereus* isolates were Gram-positive, motile, short rods round which arranged in pairs or chains. The retrieved isolates have a single non-bulging endospore that may be central or terminal. The colonies on Mannitol Egg-Yolk Polymyxin agar plates were pink and surrounded by a zone of precipitation. Biochemically, all the recovered isolates were positive for catalase, citrate utilization, glucose fermentation, Voges-Proskauer, and nitrate reduction tests. Moreover, they were negative for indole, mannitol fermentation, H_2S production, oxidase, and methyl red tests. Moreover, the identification of all obtained isolates was confirmed using the VITEK2 compact apparatus and application of GP and BCL cards, where probability reached 100%. The results were estimated after comparing the results of the biochemical tests with the data carried in the VITEK2 software. The results of biochemical identification and VITEK2 were identical.

The prevalence of *B. cereus* among the examined fish was 25% (30/120). Concerning the distribution of *B. cereus* among different organs of the infected *M. seheli*, the highest prevalence was observed in the liver (50%), then kidney (35%), and gills (15%), as illustrated in Figure 3. Statistically, there is a significant difference in the prevalence of *B. cereus* between different internal organs of the examined *M. seheli* ($p < 0.05$).

Phylogenetic Analyses of the *gyr*B Gene

Phylogenetic and sequence analyses of the *gyr*B gene revealed that the tested *B. cereus* isolate (Accession No. MZ647998) displayed a remarkable genetic identity to other *B. cereus* strains from different origins. For example, *B. cereus* strain CS-17 (98.4%) of China (Accession No. KX346713), *B. cereus* strain H2 (95.7%) of isolated from rice in Japan (Accession No. AF136388), and *B. cereus* strain BC-AK (95.7%) of China (Accession No. CP020937), *B. cereus* strain XWH (95.5%) isolated from catfish in China (Accession No. KF022228), and *B. cereus* strain BC-Reem-1 (90.4%) isolated from raw milk in Egypt (Accession No. MT802303) as demonstrated in Figures 4 and 5. Besides, the nucleotide frequencies of adenine (A), thymine (T), cytosine (C), and guanine (G) were 36.85% (419), 26.65% (303), 15.57% (177), and 20.93% (238), respectively. Moreover, the nucleotide frequencies of A+T and C+G contents were 63.5% and 36.5%, respectively. The final alignments consisted of 1137 bp, where the sites 1108 and 29 were the most conserved sites and
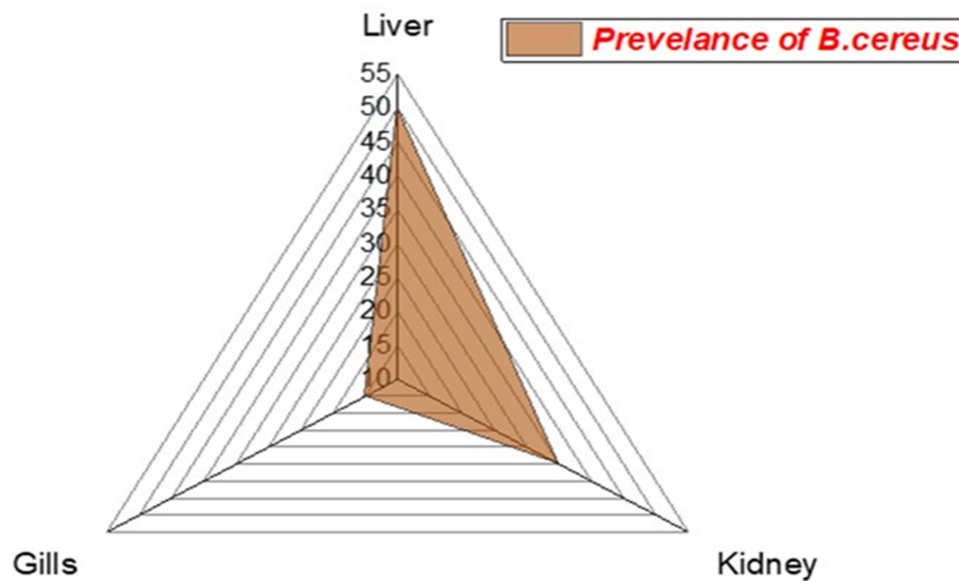
Figure 3 The radar illustrates the distribution of *B. cereus* among the internal organs of the examined *M. seheli*.
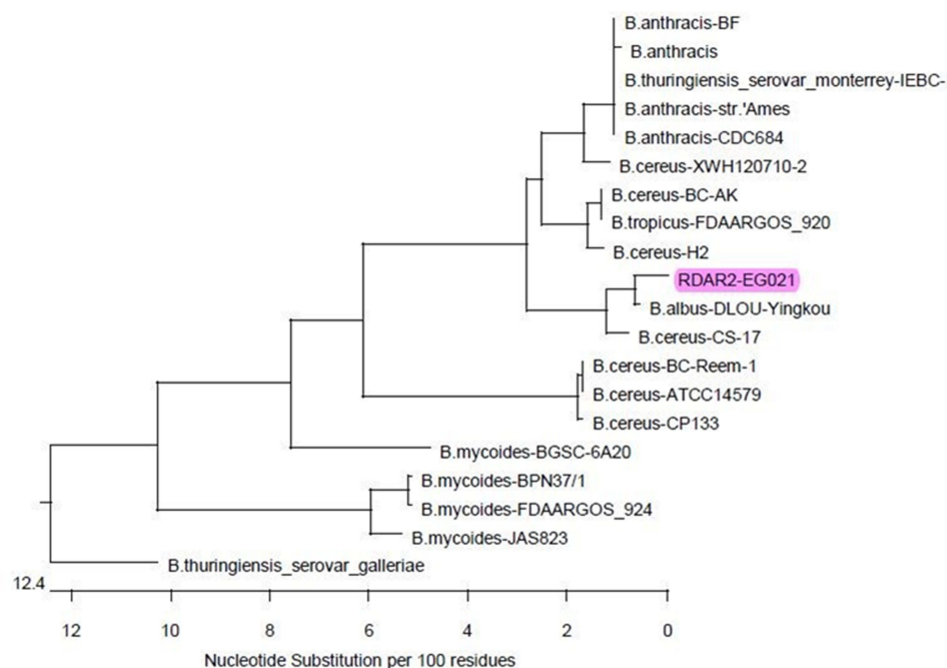
Figure 4 The figure illustrates the phylogenetic analysis of *B. cereus gyr*B gene sequencing. The tree clarifies the genetic relatedness of the tested *B. cereus* strain and other *B. cereus* strains from different origins submitted in the GenBank database. The tested strain in the current study is highlighted (RDAR2-EG021).

variable sites, respectively. Furthermore, this sequence was found to contain five open reading frames (ORFs): ORF1 (nt 412 to 519), ORF2 (nt 1063 to 1137), ORF3 (nt 237 to 1137), ORF4 (nt 447 to 334), and ORF5 (nt 252 to 172).

Phenotypic Assessment of Virulence Factors of *B. cereus* Isolated from *M. seheli*
Biofilm Production
Biofilm production was assessed among the recovered *B. cereus* isolates, where 90% (54/60) of the tested isolates were positive for biofilm formation. Among the biofilm producers (n=54), four isolates are weak biofilm producers (7.4%), seven isolates (12.96%) are moderate for biofilm producers, and forty-three isolates (79.6%) are strong biofilm producers (Figure 6).

Sequence pair distances of Bacillus gyrB nt.sequence

Percent Identity

Divergence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
1		90.4	99.3	98.4	95.7	95.7	95.7	95.5	95.5	95.5	95.5	95.5	95.4	90.5	90.4	90.4	87.4	86.6	86.4	86.4	1	RDAR2-EG021
2	10.4		91.1	90.6	91.6	91.6	91.6	91.1	91.5	91.1	91.1	91.1	91.0	90.0	100.0	99.8	90.6	89.0	88.7	88.8	2	B.cereus-BC-Reem-1
3	0.7	9.6		98.9	96.2	96.2	96.0	96.0	96.0	96.0	96.0	96.0	96.0	91.0	91.1	91.1	88.1	87.2	87.0	87.0	3	B.albus-DLOU-Yingkou
4	1.6	10.2	1.1		96.4	96.4	96.2	96.4	96.4	96.4	96.4	96.4	96.3	90.8	90.6	90.6	87.5	86.9	86.7	86.7	4	B.cereus-CS-17
5	4.5	9.0	3.9	3.7		100.0	99.5	97.4	97.4	97.4	97.4	97.4	97.3	92.1	91.6	91.6	87.8	87.1	86.8	86.8	5	B.cereus-BC-AK
6	4.5	9.0	3.9	3.7	0.0		99.5	97.4	97.4	97.4	97.4	97.4	97.3	92.1	91.6	91.6	87.8	87.1	86.8	86.8	6	B.tropicus-FDAARGOS_920
7	4.5	9.0	3.9	3.9	0.5	0.5		97.4	97.4	97.4	97.4	97.4	97.3	91.9	91.6	91.6	87.8	87.1	86.8	86.8	7	B.cereus-H2
8	4.7	9.6	4.1	3.7	2.7	2.7	2.7		98.9	100.0	100.0	100.0	99.9	91.5	91.1	91.1	87.7	86.9	86.7	86.7	8	B.anthracis-BF
9	4.7	9.2	4.1	3.7	2.6	2.6	2.6	1.2		98.9	98.9	98.9	98.8	91.6	91.5	91.5	87.6	87.2	87.0	87.0	9	B.cereus-XWH120710-2
10	4.7	9.6	4.1	3.7	2.7	2.7	2.7	0.0	1.2		100.0	100.0	99.9	91.5	91.1	91.1	87.7	86.9	86.7	86.7	10	B.anthracis-CDC684
11	4.7	9.6	4.1	3.7	2.7	2.7	2.7	0.0	1.2	0.0		100.0	99.9	91.5	91.1	91.1	87.7	86.9	86.7	86.7	11	B.thuringiensis_serovar_monterrey-IEBC-
12	4.7	9.6	4.1	3.7	2.7	2.7	2.7	0.0	1.2	0.0	0.0		99.9	91.5	91.1	91.1	87.7	86.9	86.7	86.7	12	B.anthracis-str.'Ames
13	4.8	9.7	4.2	3.8	2.8	2.8	2.8	0.1	1.2	0.1	0.1	0.1		91.4	91.0	91.0	87.6	86.8	86.6	86.6	13	B.anthracis
14	10.2	10.9	9.6	9.9	8.4	8.6	8.6	9.1	9.0	9.1	9.1	9.1	9.2		90.0	90.1	88.7	92.3	92.7	92.7	14	B.mycoides-BGSC-6A20
15	10.4	0.0	9.6	10.2	9.0	9.0	9.0	9.6	9.2	9.6	9.6	9.6	9.7	10.9		99.8	90.6	89.0	88.7	88.8	15	B.cereus-ATCC14579
16	10.4	0.2	9.6	10.2	9.0	9.0	9.0	9.6	9.2	9.6	9.6	9.6	9.7	10.8	0.2		90.6	89.1	88.8	88.9	16	B.cereus-CP133
17	13.9	10.1	13.2	13.8	13.5	13.5	13.5	13.6	13.7	13.6	13.6	13.6	13.7	12.4	10.1	10.1		91.2	91.4	91.3	17	B.thuringiensis_serovar_galleriae
18	14.8	12.0	14.2	14.5	14.3	14.3	14.3	14.5	14.2	14.5	14.5	14.5	14.6	8.1	12.0	11.9	9.5		98.7	98.6	18	B.mycoides-JAS823
19	15.1	12.3	14.4	14.7	14.7	14.7	14.7	14.4	14.4	14.4	14.8	14.8	14.9	7.7	12.3	12.2	9.2	1.3		99.9	19	B.mycoides-BPN37/1
20	15.1	12.2	14.4	14.7	14.7	14.7	14.7	14.8	14.4	14.8	14.8	14.8	14.9	7.7	12.2	12.1	9.3	1.4	0.1		20	B.mycoides-FDAARGOS_924
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		

Figure 5 The percentage of *B. cereus gyr*B nucleotide sequence identity.
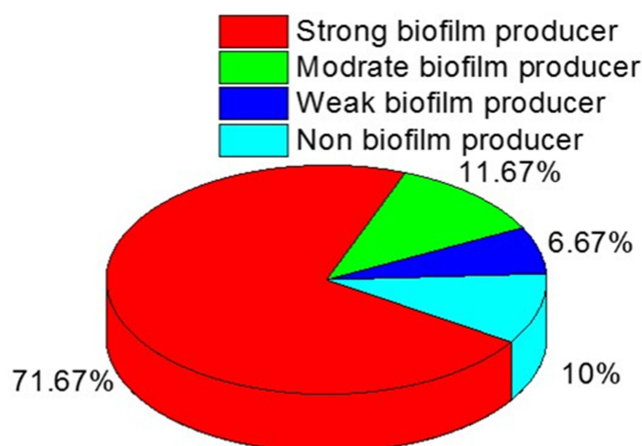
Figure 6 The distribution of biofilm formation among the isolated *B. cereus* strains.

Hemolytic Activity

In the present study, all the recovered *B. cereus* isolates (n=60, 100%) displayed β-hemolysis on sheep blood agar.

Lecithinase Reaction

The lecithinase activity is specified by the production of an opaque zone around colonies on egg yolk agar. All the tested isolates (n=60, 100%) were positive for lecithinase reaction. The statistical analyses revealed a non-significant difference in the phenotypic assessment of virulence factors in the recovered *B. cereus* strains ($p< 0.05$).

Antibiogram of the Recovered *B. cereus* Isolates

Using the disc diffusion method, vancomycin (98.3%), meropenem (96.6%), and ciprofloxacin (91.6%) exhibited a promising in-vitro antimicrobial activity against the recovered *B. cereus* isolates. Moreover, the retrieved *B. cereus* isolates displayed a significant resistance to various antimicrobial classes such as; Penicillins: penicillin and amoxicillin, (100% for each), β-Lactam-β-lactamase-inhibitor combination: amoxicillin-clavulanic acid (91.6%), Cephalosporins: cefotaxime and ceftazidime (83.3%), Sulfonamides: trimethoprim-sulfamethoxazole (100%), and Macrolides: erythromycin (66.6%). Furthermore, the obtained isolates exhibited intermediate resistance to nalidixic acid (78.4%) as

described in Table 2 and Figure 7. Statistically, the retrieved *B. cereus* isolates exhibited a significant difference in their sensitivity to various antibiotics ($p<0.05$). In addition, the correlation coefficient was determined among various tested antibiotics. Significant positive correlations were observed between PEN, DOX, AMX, and CAZ (r = 0.99); AMX, PEN, DOX, and CTX (r = 0.98); AMX, PEN, DOX, and SXT (r = 0.97); CAZ and AMC (r = 0.97); CTX and AMC (r = 0.96); SXT and E (r = 0.96); SXT and AMC (r = 0.95); CTX and E (r = 0.94); CAZ and E (r = 0.93); CN and CIP (r = 0.90); MEM and CN (r = 0.88); VAN and CN (r = 0.87) (as described in Figure 8).

Dissemination of Virulence and Antibiotic Resistance Genes in *B. cereus* Isolates

Using PCR, the tested *B. cereus* isolates commonly harbored the *nhe* (91.6%), *hbl* (86.6%), and *cyt*K (83.4%) virulence genes. Moreover, the prevalence of the *pc-plc* and *ces* virulence genes was 50% and 33.4%, respectively. Furthermore, the tested isolates harbored the *bla*1, *bla*2 (β-lactamase), *tet*A (tetracycline resistance), and *erm*A (erythromycin resistance) resistance genes were 100%, 83.3%, 100%, and 66.6%, respectively (Table 3 and Figure 9). The statistical analyses revealed a significant difference ($p <0.05$) in the existence of virulence genes in the tested *B. cereus* isolates. However, a non-significant difference ($p > 0.05$) was noticed in the dissemination of the antibiotic resistance genes among the retrieved *B. cereus* isolates.

Phenotypic and Genotypic Multidrug-Resistance Profiles in the Recovered *B. cereus* Isolates

Our findings proved that 40% (24/60) of the recovered *B. cereus*m isolates are multidrug-resistant (MDR: resistant to one or more antibiotics in three or more different classes) to six antimicrobial classes and carried *bla*1, *bla*2, *tet*A,

Table 2 Antimicrobial Susceptibility Patterns of the Recovered *B. cereus* Isolates (n=60)

Antimicrobial Classes	Tested Antimicrobial Agent	Interpretation					
		Sensitive		Intermediate		Resistance	
		n	%	n	%	n	%
Penicillins	Penicillin	0	0	0	0	60	100
	Amoxicillin	0	0	0	0	60	100
β -Lactam- β-lactamase inhibitor combination	Amoxicillin-Clavulanic acid	5	8.4	0	0	55	91.6
Cephalosporins	Cefotaxime	0	0	10	16.7	50	83.3
	Ceftazidime	1	1.7	9	15	50	83.3
Carbapenems	Meropenem	58	96.6	2	3.4	0	0
Aminoglycosides	Gentamycin	24	40	20	33.4	16	26.6
	Streptomycin	19	31.7	25	41.7	16	26.6
Fluoroquinolones	Ciprofloxacin	55	91.6	5	8.4	0	0
Quinolones	Nalidixic acid	13	21.6	47	78.4	0	0
Sulfonamides	Trimethoprim-Sulfamethoxazole	-	-	12	20	48	80
Tetracyclines	Doxycycline	0	0	0	0	60	100
Macrolides	Erythromycin	-	-	20	33.4	40	66.6
Glycopeptides	Vancomycin	59	98.3	1	1.7	0	0
Chi-square		424.24		218.84		260.11	
***p*-value**		<0.0001		<0.0001		<0.0001	
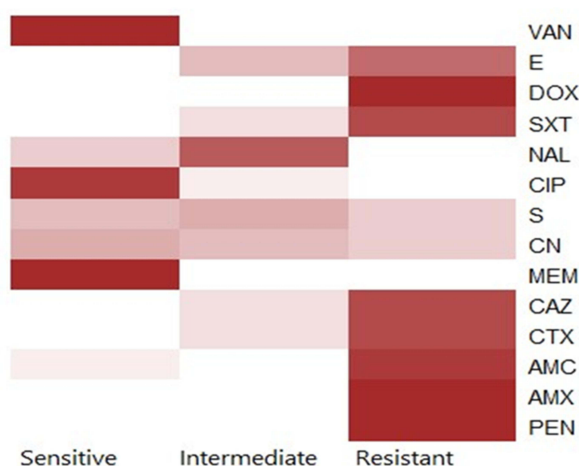
Figure 7 The heat-map clarifies the antimicrobial resistance profile of the obtained *B. cereus* isolates.
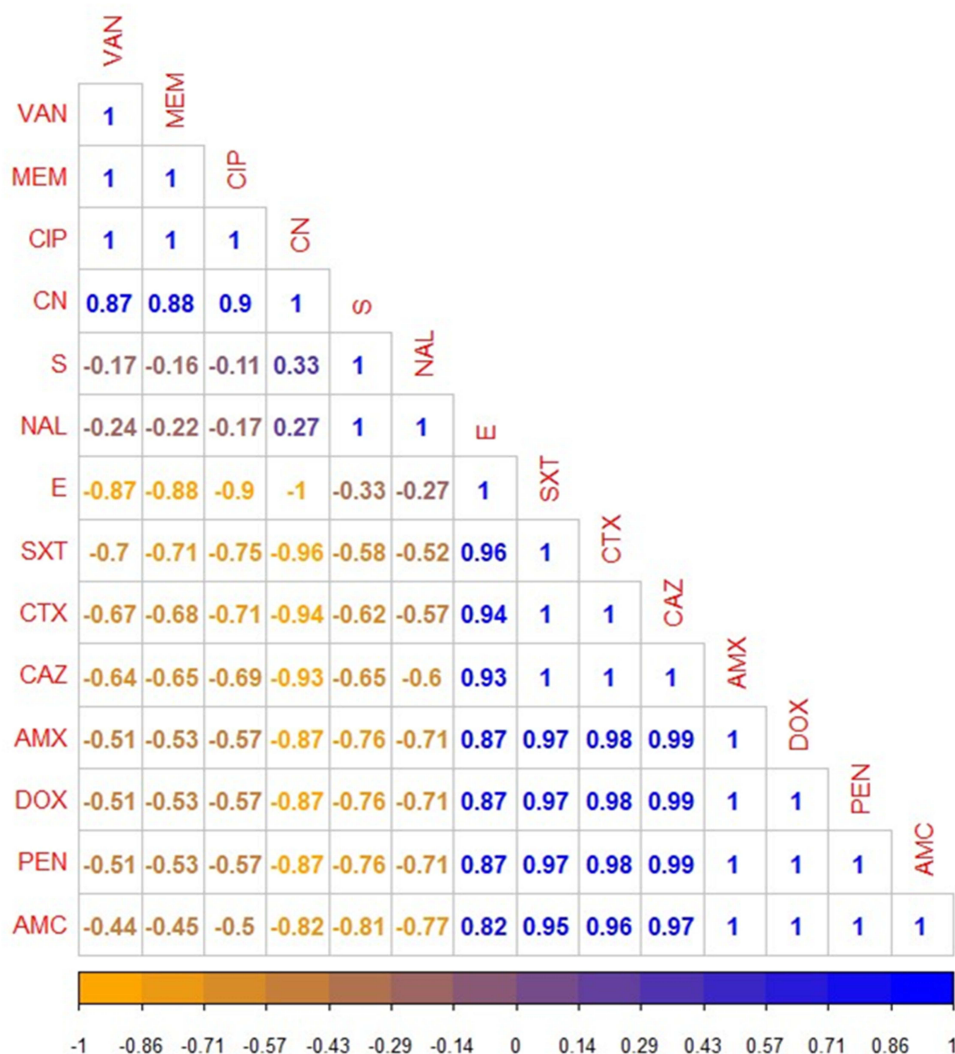
Figure 8 The heat-map clarifies the correlation coefficient (r) among various tested antibiotics in the present study. The intensity of colors indicates the numerical value of the correlation coefficient (r), Orange, and blue color refers to the negative and positive correlations, respectively.

Table 3 Dissemination of Virulence and Antibiotic Resistance Genes in the Isolated *B. cereus* (n=60)

Gene Function	Gene	No. of Positive	%	Chi-Square p-value
Virulence genes	*nhe*	55	91.6	23.169 0.0001171
	hbl	52	86.6	
	*cyt*K	50	83.4	
	pc-plc	30	50	
	ces	20	33.4	
Antibiotic-resistance genes	*bla*1	60	100	5.2381 0.1552
	*bla*2	50	83.3	
	*tet*A	60	100	
	*erm*A	40	66.6	

and *erm*A genes. Besides, 26.6% (16/60) of the obtained isolates expressed multidrug resistance to seven antimicrobial classes and harbored *bla*1, *bla*2, *tet*A, and *erm*A genes. In addition, 13.3% (8/60) of the retrieved *B. cereus* isolates are MDR to five antimicrobial classes and possessed *bla*1, *bla*2, and *tet*A genes, as illustrated in Table 4. The multiple antibiotic resistance (MAR) index values in this study ($\geq$ 0.2) showed multiple resistance patterns indicating that the retrieved isolates were originated from high-risk contamination, as illustrated in Table 4. The correlation coefficient (r) was assessed among the detected resistance genes in *B. cereus* isolates and various tested antibiotics. The results demonstrated significant positive correlations between: *bla*1 gene, AMX, PEN, and AMC (r=1); *bla*2 gene, ctx, and caz (r=1); *erm*A gene and E (r=1); *tet*A gene and DOX (r=1); *bla*1 gene and CAZ (r=0.99); *bla*1 gene and CTX (r=0.98); *bla*2 gene, AMX, and PEN (r=0.98); *bla*2 and AMC (r=0.96) as shown in Figure 10.

Pathogenicity Test

The cumulative mortality, morbidity, and pathological lesions were regularly observed in the challenged fish for 14 days post-inoculation. Herein, the challenged fish displayed dose-dependent mortalities and characteristic lesions of external hemorrhages, exophthalmia, and ascites, similar to those observed during the natural infection. Surprisingly, none of the control group fish showed any mortalities or pathological lesions. Moreover, the PM examination of the infected fish
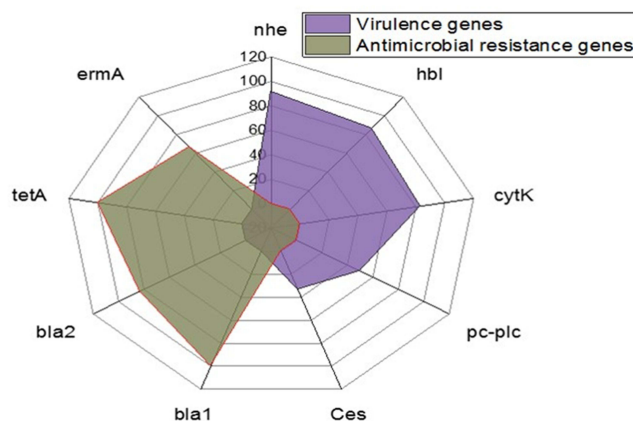
Figure 9 The occurrence of virulence and antibiotic resistance genes in the isolated *B. cereus* strains.

Table 4 The Phenotypic and Genotypic Resistance Profiles of the Isolated *B. cereus* Strains (n = 60)

| No. of Isolates	%	Type of Resistance	Phenotypic Resistance Profiles	Antibiotic Resistance Genes	MAR Index
24	40	MDR	**Penicillins**: PEN and AMX **β-Lactam- β-lactamase inhibitor combination**: AMC **Cephalosporins**: CTX and CAZ **Tetracyclines**: DOX **Macrolides**: E **Sulfonamides**: SXT	*bla*1, *bla*2, *tet*A, and *erm*A	0.57
16	26.6	MDR	**Penicillins**: PEN and AMX **β-Lactam- β-lactamase inhibitor combination**: AMC **Cephalosporins**: CTX and CAZ **Tetracyclines**: DOX **Macrolides**: E **Sulfonamides**: SXT **Aminoglycosides**: CN and S	*bla*1, *bla*2, *tet*A, and *erm*A	0.71
8	13.3	MDR	**Penicillins**: PEN and AMX **β-Lactam- β-lactamase inhibitor combination**: AMC **Cephalosporins**: CTX and CAZ **Tetracyclines**: DOX **Sulfonamides**: SXT	*bla*1, *bla*2, and *tet*A,	0.50
7	11.6	MDR	**Penicillins**: PEN and AMX **β-Lactam- β-lactamase inhibitor combination**: AMC **Tetracyclines**: DOX	*bla*1 and *tet*A,	0.28
3	5	Resistant	**Penicillins**: PEN and AMX **Tetracyclines**: DOX	*bla*1 and *tet*A,	0.21
2	3.33	MDR	**Penicillins**: PEN and AMP **Cephalosporins**: CTX and CAZ **Tetracyclines**: DOX	*bla*1, *bla*2, and *tet*A	0.35

Abbreviations: MDR, multi drug resistant; MAR, multiple antibiotic resistance.

displayed predictable signs of septicemia manifested by an enlarged liver, congested kidney, and serous fluid exudates in the abdominal cavity.

The results also clarified that cumulative deaths were relatively correlated with the inoculated doses and inherited virulence genes, as a higher mortality rate (75%) was recorded in the T_V group that received a bacterial dose of 10^8 CFU mL^{-1}, followed by T_{IV}-T_I groups that inoculated by 10^7–10^4 CFU mL^{-1}, respectively (Figure 11). The bacterial dose that induces ~50% mortality in the challenged *T. zillii* (LD$_{50}$) was 10^6 CFU mL^{-1}. Bacteriologically, *B. cereus* was successfully recovered from the pathognomonic lesions of moribund and freshly dead fish. The identification was confirmed based on its biochemical properties and molecular typing.

Discussion

Bacillus cereus is a ubiquitous bacterium of public health concern and has recently been categorized as a fish-threatening pathogen, causing huge economic losses in the aquaculture sector.[28] In the current study, the results of clinical and post-mortem inspections were congruent with Ali[27] who observed deep hemorrhagic skin ulcers, unilateral exophthalmia, tail
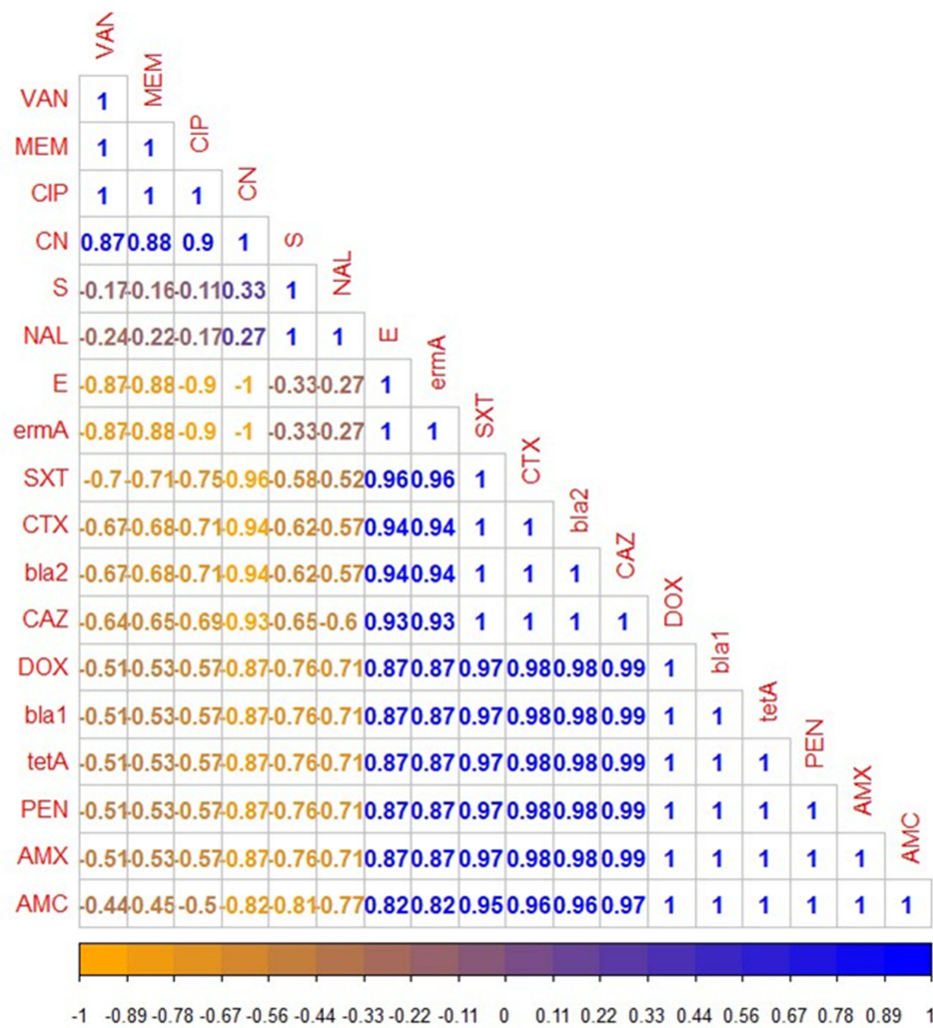
Figure 10 The heat-map illustrates the correlation coefficient (r) among the antibiotic resistance genes detected in the recovered *B. cereus* isolates and various tested antibiotics. The intensity of colors indicates the numerical value of the correlation coefficient (r), Orange, and blue color refers to the negative and positive correlations, respectively.
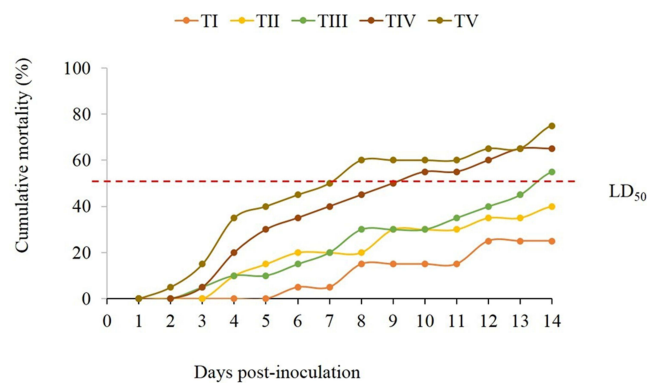
Figure 11 Cumulative mortality of *Tilapia zillii* subjected to intraperitoneal injection with different doses of *B. cereus* (T_I-T_V fish groups received a bacterial dose of 10^4–10^8 CFU mL^{-1}, respectively). LD_{50} is a bacterial dose (10^6 CFU mL^{-1}) that induces ~50% (exactly 55%) mortality in the challenged fish.

rot, and congested parenchymatic organs in white seabream (*Diplodus sargus*) suffered from a mixed infection with *Staphylococcus epidermidis* and *B. cereus*. Similar results have been reported in infected stinging catfish,[28] and seabass with *B. cereus*.[26] Consistent with the present findings, diffuse necrotizing dermatitis was observed in the naturally infected fish with *B. cereus*.[49]

The recorded ante- and post-mortem lesions are a pre-determined consequence of the fish's immune response to the invading pathogen and may refer to successful colonization and reproduction of the isolated bacteria, in agreement with Aboyadak.[26] The variety of lesions also indicates the severity of the current infection and is primarily attributable to the virulence traits encoded by bacteria. *B. cereus* has been found to be related to the Epizootic Ulcerative Syndrome in *Datnioides microlepsis*.[50] *B. cereus* possessed several virulence determinants like cytotoxin K, hemolysins, enterotoxins, and phospholipase, which together may lead to disease induction.[51] These toxic substances have noxious effects on host tissues through hemagglutination, impaired vascular permeability, apoptosis, and skin necrosis.[52]

Regarding the bacteriological examination, all recovered isolates displayed the typical phenotypic characteristics of *B. cereus*, consistent with Osman[15] and Gdoura-Ben.[53] The isolates also showed harmony in their morphology with identical biochemical profiles. Moreover, the results obtained by the VITEK2 GP and BCL cards were highly comparable and nearly similar to those obtained by conventional biochemical assays. Herein, the VITEK 2 compact system showed a high probability of identifying *B. cereus* up to 100%. The system was widely acceptable for the detection and distribution of these bacteria and other foodborne pathogens in fish and ready-to-eat products.[54]

In this study, *B. cereus* was not isolated from the substantially healthy *M. seheli*, but the bacteria were recovered from moribund diseased fish with a total prevalence of 25%. Our findings were comparable to those reported by Ali,[27] who reported increased mortality (38.33%) in *D. sargus* broodstock suffered from mixed infection with *S. epidermidis* and *B. cereus*. A previous bacteriological study of Chandra[28] suggested that *B. cereus* infection is responsible for 97% of stinging catfish mass mortalities at Burdwan farms in West Bengal, India. *B. cereus* is occasionally referred to as a pathogen of fish.[12] It is a ubiquitous environmental pathogen capable of causing disease in immunocompromised hosts. It is systematically accused of causing severe infection and tissue degeneration in affected fish species.[26]

Regarding the intensity of *B. cereus* among different examined organs, the liver was the main affected organ, then the kidney and gills, with no significant difference ($p < 0.05$). These results are in agreement with those reported by Younes[55] who mentioned that hepatopancreas, kidney, and gills were the most predominant infected organs with *B. cereus*. Differences in prevalence could be attributed to geographic variation, environmental stressors, fish species, and sampling time during the study.

In the current study, the PCR results showed that all the examined isolates harbored a conserved *gyr*B gene of *B. cereus*, in agreement with Yamada[32] who stated that *gyr*B was a reliable diagnostic marker and had superior use over the *16S rRNA* gene to distinguish *B. cereus* from other closely related species. Phylogenetic analyses of the amplified amplicon displayed a remarkable genetic identity and cross-lineage to other *B. cereus* strains from different origins, which may indicate the possible influence of global trade in infection transmission, particularly in the absence of restriction measures for import and export and non-compliance with guidelines on aquaculture advisory services and transport of live fish seeds.[56] Herein, the final alignments consisted of 1137 bp, where the sites 1108 and 29 were the most conserved sites and variable sites, respectively. Besides, this sequence was found to contain five open reading frames (ORFs 1–5), which may distinguish these bacteria from other relevant species within the same family.

Concerning the phenotypic assessment of *B. cereus* virulence factors, 90% of the tested isolates showed different patterns (week-moderate-strong) of biofilm formation, in line with Osman[15] who observed the same phenomena in 84.7% of *Bacillus* species recovered from various meat products. Indeed, most members of the genus *Bacillus* are firmly attached to the biotic and non-biotic surfaces by forming biofilms.[57] Therefore, the reported biofilm activity of the tested isolates indicates their strong association with the host tissue and thus increased disease prevalence and incidence among cultured groups. It also reflects the hazard impact and public health concerns of *B. cereus* as a foodborne pathogen that may cause severe illness, particularly in people who eat undercooked or raw infected fish.[58] Biofilm is most likely to be important because it persists in food processing equipment and protects spores and vegetative fragile cells from inactivation by biocides.[59]

Surprisingly, all tested isolates of *B. cereus* showed strong hemolytic activity on sheep blood agar. The present investigation is consistent with the previous findings of Beecher and Wong[60] and Hwang and Park.[61] Accordingly, the surge in hemolytic activity in this study may be concomitant with some related-virulence genes inherited by the bacteria,[62] which still requires further investigation. It was reported that most of the foodborne isolates showed strong hemolytic activity and that most isolates of *B. cereus* retrieved from different food sources showed hemolysis.[63] Likewise, all tested isolates were positive for lecithinase reaction and showed opaque zone around colonies on egg yolk agar, in agreement with Hwang and Park,[61] who reported that 26% and 10% of *B. cereus* isolates retrieved from infant foods showed low and high lecithinase activities, respectively. Lecithinase has been proposed as a presumptive virulence tool in systemic infection with enterotoxin,[64] and could often be entangled in hemolysis.[65] *Bacillus* species are known to produce various exoenzymes like hemolysin, gelatinase, lecithinase, enterotoxins, and phospholipase that are responsible for food poisoning and serious human illness.[66]

Regarding the antibiogram pattern, vancomycin, meropenem, and ciprofloxacin exhibited promising antimicrobial activity against the tested *B. cereus* isolates. Our results are consistent with those reported by Chandra[28] who observed a higher sensitivity of *B. cereus* isolates to ciprofloxacin. In contrast, the tested *B. cereus* isolates were resistant to penicillins, sulfonamides, β-Lactam-β-lactamase-inhibitor combination, cephalosporins, nalidixic acid, and macrolides. Nearly similar findings were reported by Savić[67] and Mousa.[68] The present findings affirm the emergence of MDR isolates of *B. cereus* in aquaculture sectors and set the fact that the food chain can be a major route of transmission of MDR bacteria between animals and humans.[69] The random use of antibiotics to treat emerging diseases has dire consequences in the aquaculture industry, as it may lead to the existence of MDR strains.[70–72] Hence, the regimen use of an antibiotic susceptibility testing system is important for selecting specific effective antibiotics and overcoming such problems.[22,73]

As for the dissemination of virulence genes between *B. cereus* isolates, there was a significant variation ($p < 0.05$) in the existence of virulence genes, where the *nhe*, *hbl*, and *cyt*K genes were the most prevalent. These results are consistent with those reported by Osman,[15] who detected several genes encoding hemolytic (*hbl*) and non-hemolytic (*nhe*) enterotoxins and cytotoxin K (*cytK*) in *B. cereus* isolates recovered from some poultry and beef meat. The *nhe* and *cytK* genes are primarily virulence genes that induce enterotoxins production in *B. cereus*.[74] High prevalence of the *nhe* and *hbl* genes complex has been previously reported in *B. cereus* isolates from environmental and food origins.[75] In the current study, all tested isolates of *B. cereus* contained one or more enteric genes reflecting their public health importance and their potential role in inducing diarrheal disease in humans, in agreement with Smith.[76] About 73% of food poisoning strains contained one or more HBL complex genes.[77] Interestingly, the *nhe* gene was the most dominant HBL complex gene inconsistent with previous findings of Tewari.[78]

Herein, the *cyt*K enterotoxin gene was detected in 83.4% of the recovered *B. cereus* isolates, in agreement with Ngamwongsatit,[79] while others found it in a smaller proportion of their isolates.[80] Likewise, the *ces* virulence gene was determined in 33.4% of *B. cereus* isolates, which differed greatly from the previous findings of Abdeen[81] who detected *ces* genes in 50% of tested isolates. The *ces* gene, found on the megaplasmid of *B. cereus,* is accountable for the production of the cereulide toxin that causes foodborne illness and emesis in humans.[13] This toxin is thermostable, active within a wide range of pH (2–11), and abundantly produced during long-term storage of contaminated food.[82] Meanwhile, the prevalence of *pc-plc* gene in this study was 50%, which is quite similar to the results of Abdeen.[81] The *pc-plc* gene is a major virulence determinant for *B. cereus* toxins which embraces the cytolytic unit cereolysin AB.[83] The gene has hemolytic activity and a vital role in toxin production.[84] The predominance of both *ces* and *pc-plc* genes among the tested isolates emphasizes their virulence and reflects the public health significance of this study.

Concerning the correlation between the genotypic and phenotypic multidrug resistance, most of the retrieved isolates showed multidrug resistance to six or more antimicrobial classes and carried *bla*1, *bla*2, *tet*A, and *erm*A resistance genes; these results came in harmony with Fiedler[19] and Bianco.[85] Intriguingly, the MAR index values in this study were ≥ 0.2, alarmingly reflecting that the recovered isolates derived from high-risk origins of contamination. *bla*1 and *bla*2 are chromosomal genes of *Bacillus* species that encoding the penicillinase 2a group and metalloenzyme functional group 3.[86] The *bla*1 possesses penicillinase activity, while *bla*2 seems to be a cephalosporinase, penicillinase and carbapenem-hydrolysis.[87] Herein, both *bla*1 and *bla*2 genes are entirely expressed in all isolates, which is nearly similar to those

reported by Tahmasebi.[88] The resistance of the recovered isolates to amoxicillin-clavulanic acid may be attributed to the synergism of *bla*1 and *bla*2 genes. Moreover, several previous studies emphasized the resistance of *B. cereus* to β-Lactam -β-lactamase-inhibitor combination.[19,89,90] In contrast to our findings, Chen[91] reported an impairment of *bla*1 transcription in *B. cereus* group that harbored bla promoter-lacZ transcriptional fusions and attributed that to the beta-galactosidase activity of the group.

Foreseeably, all recovered strains that harbored *tet*A and *erm*A genes showed extensive inhibition regions for tetracycline and erythromycin, proposing a close relationship between *tet*A resistance gene and phenotypic resistance against tetracycline. The present investigations are congruent with the previous results of Park.[37] Overall, antibiotic resistance (AMR) is an inescapable problem worldwide and is closely associated with high mortality and medical crisis.[92] AMR is usually caused by the transmission of an antibiotic resistance gene among bacteria, even distantly related ones.[93] Since there is no obvious MAR_{index} standard for *B. cereus*, the assessment of human health risks has become unfeasible due to the newly emergence of MDR isolates of *B. cereus* in aquaculture sectors. These facts maximize the proper use of antibiotics for aquatic diseases control and reflect the significance of routine antimicrobial susceptibility testing in limiting the spread of MDR isolates.

With respect to the pathogenicity assay, fish infected with *B. cereus* presented variable mortality in direct proportion to the inoculated doses and displayed the same clinical signs that were found in the naturally infected fish. Our findings have almost resembled a previous study of Younes[55] that reported obvious skin lesions, popeyes, abdominal distension, and hemorrhagic patches on the skin and gills of experimentally infected *C. gariepinus* with *B. cereus*. Clinical and post-mortem signs observed here were almost similar to the previous findings in white seabream experimentally infected with *B. cereus*.[27] Herein, the mean lethal dose (LD_{50}) for the isolated *B. cereus* was 10^6 CFU mL^{-1}, in parallel with a previous study of Younes,[55] which emphasized that the LD_{50} of *B. cereus* was about 2.7×10^6 CFU mL^{-1}. Our results were relatively high compared to Chandra[28] who demonstrated that a dose of 3.6×10^5 CFU $fish^{-1}$ was adjacent to LD_{50} value and considered suitable for the experimental infection of stinging catfish. Accordingly, affected tissue dissociation could be either related to inherited virulence genes or to the lethal and degenerative effect of bacterial exotoxins and active enzymes, which boost tissue necrosis and cell damage.[50,94]

In conclusion, as far as we know, this is the first study that highlighted the new emergence of MDR *B. cereus* in *M. seheli* that alarmingly reveals a significant threat to both human health and the aquaculture sector. Newly emerging MDR *B. cereus* in *M. seheli* commonly harbor *nhe*, *hbl*, *cyt*K, and *pc-plc* virulence genes and *bla*1, *bla*2, *tet*A, and *erm*A resistance genes. The consistent application of antimicrobial susceptibility testing is essential for screening the newly emerging MDR strains and detecting the antibiotic of choice. Vancomycin and meropenem displayed a hopeful in-vitro antimicrobial activity against the recovered MDR *B. cereus* isolates from fish. The combination of phenotypic and molecular-based detection techniques is a reliable epidemiological tool for the detection of newly emerging MDR *B. cereus* strains in fish.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflict of interest.

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