UDC 614.446
DOI: 10.21668/health.risk/2025.2.08.eng

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Research article

# ISOLATION AND CHARACTERIZATION OF *BACILLUS CEREUS* STRAINS ISOLATED FROM A BEEF PIZZA FOOD POISONING INCIDENT IN HANOI

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*Bacillus cereus is one of the global causes of food poisoning. In this study, we isolated 10 strains of B. cereus from beef pizza samples identified as the cause of food poisoning among students at two kindergartens A and B in Vietnam in 2024.*

*Species identification was carried out using biochemical tests and MALDI-TOF technology; antibiotic resistance profile was constructed according to the M45 CLSI guidelines, and examined the presence of cytK, bceT, hbl (hblA, hblC, and hblD) and nhe (nheA, nheB, and nheC) toxin genes among these isolated strains. The antibiotic resistance testing results showed that isolated B. cereus strains were significantly resistant to several strong antibiotics, including penicillin (100 %), vancomycin (100 %), streptomycin (90 %), tetracycline (80 %), ampicillin (70 %), and erythomycin (70 %). In addition, 100 % of B. cereus strains (10/10) in the beef pizza sample were positive for the bceT toxin gene, 80 % of strains (8/10) were positive for the cytK toxin gene, and 60 % of strains (6/10) were positive for the nheA and nheC toxin genes, and negative for the NRPS emetic toxin gene. Our study contributes to the antibiotic resistance database for B. cereus associated with food poisoning in Vietnam and provides a valuable resource for developing reference materials aimed at the rapid diagnosis of food poisoning caused by diarrhea B. cereus type.*

***Keywords:*** *Bacillus cereus, food poisoning, antibiotic resistance, M45 CLSI, bceT, cytK, hbl, nhe.*

*B. cereus* is estimated to be responsible for nearly 12 % of total food poisoning incidents worldwide. The Centers for Disease Control and Prevention (CDC) in the United States reported that from 1998 to 2015, there were 619 food poisoning outbreaks caused by *Bacillus* spp., with 7,385 illnesses, 75 serious illnesses, and three deaths [1]. In China, a total of 419 food poisoning incidents caused by *B. cereus* were recorded from 2010 to 2020, including 7,892 people poisoned, 2,786 hospitalized and 5 fatalities [2]. Vietnam has also recorded numerous outbreaks involving *B. cereus*; for example, in 2020, 230 people were poisoned after eating food from a vegetarian restaurant in Da Nang contaminated with *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus),* and *B. cereus,* while in the same year dozens of preschool children in Can Tho were hospitalized after eating pho and yogurt contaminated with *B. cereus*. In 2022, more than 600 students of a school in Nha Trang were hospitalized after consuming fried chicken wings contaminated with *Salmonella*, *B. cereus* and *E. coli*, with one fatality reported.

*B. cereus* is a gram-positive, rod-shaped bacterium belonging to the Bacillaceae family and is one of the most common food poisoning agents. *B. cereus* is commonly found in soil, water, and food, especially rice products, processed foods and dairy[1]. Notably, *B. cereus* is heat-resistant and produces highly drug-



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[1] Ash C., Farrow J.A., Dorsch M., Stackebrandt E., Collins M.D. Comparative analysis of Bacillus anthracis, Bacillus cereus, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *Int. J. Syst. Bacteriol.*, 1991, vol. 41, no. 3, pp. 343–346. DOI: 10.1099/00207713-41-3-343

resistant spores, allowing this strain to survive in harsh environmental conditions. *B. cereus* is capable of producing toxins and causes two main types of poisoning: emetic toxin, which usually occurs when contaminated food is consumed, and diarrheal toxin, which is produced by the bacteria after the contaminated food has entered the gastrointestinal tract [3].

Widespread use of antibiotics has resulted in drug resistance in many bacterial strains, including *B. cereus*, which often produces β-lactamase enzymes that confer strong resistance to β-lactam antibiotics [4]. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, B. cereus strains are generally susceptible to aminoglycosides, clindamycin, chloramphenicol, erythromycin, and vancomycin. However, some studies indicate that B. cereus is resistant to tetracycline, streptomycin, ciprofloxacin, cloxacillin, erythromycin, and rifampicin [5].

Regarding diarrheal toxin-induced poisoning, *B. cereus* toxins associated with the diarrheal syndrome include non-hemolytic enterotoxin (*nhe*), hemolysin BL (*hbl*), cytotoxin K (*cytK*), and enterotoxin T (*bceT*)[2] [6–8]. However, the enterotoxic potential of *bceT* remains controversial in some studies [9, 10]. For emetic toxin-induced poisoning, the emetic toxin gene NRPS (Non-Ribosomal Peptide Synthetase) of *B. cereus* is responsible for producing toxic peptides such as Cereulide and isocereulides A-G. This gene functions independently of conventional protein synthesis and allows the bacteria to efficiently synthesize harmful compounds that increase the virulence of *B. cereus* in food poisoning cases [11].

In Hanoi, where traditional food preparation methods prevail, monitoring and characterizing B. cereus strains linked to outbreaks is essential to identify their origins, understand their epidemiological traits, and develop effective preventive measures.

**The aim of this study** is to construct antibiotic resistance profiles and detect virulence genes of *B. cereus* strains isolated from beef pizza food and related to the poisoning outbreak at two kindergartens A and B in Hanoi in 2024.

**Materials and methods.** Ten strains of *B. cereus* isolated from beef pizza samples were identified as the cause of food poisoning in students at two kindergartens A and B. Both preschool facilities are located in Hanoi and share a management system, with incidents occurring in 2024. Kindergarten A reported 135 students monitored for gastrointestinal disorders, while kindergarten B had 77 students in a similar condition. All relevant food samples were collected, preserved in a freezer, and sent to the National Institute for Food Control for testing and analysis.

***Isolation and identification of B. cereus in beef pizza sample.*** The detection method for *B. cereus* was performed according to ISO 7932:2004[3] (TCVN 4992:2005). Specifically, 10 g of each food sample was homogenized in 90 mL of peptone water (Merck). A dilution series up to $10^{-5}$ was prepared for each sample, and 100 µL was inoculated onto Mannitol Egg Yolk Polymyxin Agar (MYP; Merck) at each dilution. All plates were incubated overnight at 37°C. Colonies exhibiting typical morphology (flat, 2–3 mm diameter, serrated edges, pinkish color, surrounded by a clear zone) were selected for hemolysis testing on blood agar and biochemical tests using the API 50 Carbohydrate kit (bioMerieux, France). The typical *B. cereus* colonies that tested positive in biochemical tests were stored at -80 °C. For identification, these colonies were streaked onto Tryptone Soya Agar (TSA; Merck), incubated for 24 hours at 37 °C, and identified using MALDI-TOF Matrix-Assisted Laser Desorption Ionization - Time of Flight on the VITEK® MS system

[2] Agata N., Ohta M., Arakawa Y., Mori M. The bceT gene of Bacillus cereus encodes an enterotoxic protein. *Microbiology (Reading)*, 1995, vol. 141, pt 4, pp. 983–988. DOI: 10.1099/13500872-141-4-983

[3] ISO 7932:2004 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive Bacillus cereus – Colony-count technique at 30 degrees C, 3rd ed. *ISO*, 2004. Available at: https://www.iso.org/standard/38219.html (November 15, 2024).

(BioMérieux SA, Marcy l'Etoile, France). *E. coli* ATCC 8739 was used as a control strain during the run.

***Evaluation of antibiotic resistance characteristics of B. cereus in beef pizza sample.*** The antibiotic resistance of *B. cereus* strains was tested using the Kirby-Bauer disk diffusion method[4]. *B. cereus* was grown in 10 mL of sterile BHI medium until a cell density equivalent to a 0.5 McFarland standard was reached, approximately $1.5 \times 10^8$ CFU/mL. A sterile swab was used to spread the bacterial suspension on Muller-Hinton agar. A total of 11 types of antibiotics were tested according to M45 CLSI, including ampicillin (AMP, 10 µg/disk), chloramphenicol (C, 30 µg/disk), ciprofloxacin (CIP, 5 µg/disk), erythromycin (ERY, 15 µg/disk), imipenem (IPM, 10 µg/disk), meropenem (MRP, 10 µg/disk), ofloxacin (OFX, 5 µg/disk), penicillin (PRL, 10 IU/disk), streptomycin (S, 10 µg/disk), tetracycline (TE, 30 µg/disk), and vancomycin (VAN, 30 µg/disk). The disks were placed evenly on the agar surface using sterile forceps and incubated at 37 °C for 18 hours. The antibiotic susceptibility results were determined by measuring the diameter of the inhibition zone, which is the transparent area surrounding the antibiotic disk.

***Detection of toxin genes of B. cereus in beef pizza sample. DNA extraction method.*** Ten strains *of B. cereus* isolated from beef pizza samples were stored at -80 $^{o}$C. They were streaked on blood agar to capture pure strains, cultured in BHI (Brain Heart Infusion broth) broth, and incubated for 18–24 hours at 37 $^{o}$C. Total DNA of *B. cereus* was isolated according to the protocol for gram-positive bacteria of the GeneJET Genomic DNA Purification kit (Thermofisher; C5042). The total DNA concentration of *B. cereus* strains was quantified using a nanodrop spectrophotometer at 260 nm. The DNA solution was stored at -20 $^{o}$C until use.

***PCR and multiplex PCR reactions.*** Primer pairs used in this study for the detection of diarrheal and emetic toxin genes of *B. cereus* strains in beef pizza samples are listed in Table 1. PCR technique was applied for primer pairs bceT-F/R, EM1F/R, and *cytK*F/R, while the remaining primer pairs were subjected to multiplex PCR.

PCR mixture (25 µL) contained 12.5 µL of 2X PCR Master Mix (Thermo Scientific), 1 µL of forward primer (10 pmol), 1 µL of reverse primer (10 pmol), 3 µL of template DNA, and 7.5 µL of deionized water. The thermal cycling conditions for the primer pair EM1F/EM1R were set at 95 °C for 15 minutes; (95 °C for 30 seconds; 60 °C for 30 seconds; 72 °C for 60 seconds) for 30 cycles, followed by 72 °C for 5 minutes and holding at 4 °C. The thermal cycling conditions for the primer pair bceT-F/bceT-R were set at 94 °C for 5 minutes; (94 °C for 45 seconds; 55 °C for 45 seconds; 72 °C for 2 minutes) for 30 cycles, followed by 72 °C for 10 minutes and holding at 4 °C. The thermal cycling conditions for the primer pair cytKF/cytKR were set at 94 °C for 1 minute; (95 °C for 45 seconds; 54 °C for 1 minute; 72 °C for 2 minutes) for 35 cycles, followed by 72 °C for 5 minutes, and holding at 4 °C.

PCR mixture (25 µL) contained 12.5 µL of 2X PCR Master Mix (Thermo Scientific), 0.5 µL of each forward primer (20 pmol), 0.5 µL of each reverse primer (20 pmol), 3 µL of template DNA, and 3.5 µL of deionized water. The thermal cycling parameters for multiplex PCR amplification of the hblA, hblC, hblD, nheA, nheB, and nheC genes were as follows: 94 °C/2 min; (95 °C/15 s; 55 °C/45 s; 72 °C/2 min) x 35 cycles and 72 °C/5 min, and holding at 4 °C. PCR products were separated using a 1.5 % agarose gel made with 1X TAE buffer and Redsafe dye, subjected to electrophoresis at 110V for 50 minutes, followed by UV visualization, and were stored at -80 °C for future studies.

[4] Bauer A.W., Kirby W.M., Sherris J.C., Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 1966, vol. 45, no. 4, pp. 493–496.

Table 1

Primer pairs used for detection of toxin genes of *B. cereus*

| Name | Sequence (5'-3') | Gene | Size (bp) | Reference |
|---|---|---|---|---|
| bceT-F | CGT ATC GGT CGT TCA CTC GG | Enterotoxin *bceT* | 662 | [12] |
| bceT-R | GTT GAT TTT CCG TAG CCT GGG | | | |
| EM1F | GAC AAG AGA AAT TTC TAC GAG CAA GTA CAA T | NRPS | 635 | [13] |
| EM1R | GCA GCC TTC CAA TTA CTC CTT CTG CCA CAG T | | | |
| nheAF | TAC GCT AAG GAG GGG C | *nheA* | 499 | [14] |
| nheAR | GTT TTT ATT GCT TCA TCG GCT | | | |
| nheBF | CTA TCA GCA CTT ATG GCA G | *nheB* | 769 | |
| nheBR | ACT CCT AGC GGT GTT CC | | | |
| nhCF | CGG TAG TGA TTG CTG GG | *nheC* | 581 | |
| nhCR | CAG CAT TCG TAC TTG CCA A | | | |
| hblAF | GTG CAG ATG TTG ATG CCG AT | *hbl*A | 1154 | |
| hblAR | ATG CCA CTG CCT GGA CAT A | | | |
| HbICF | GAT ACT AAT GTG GCA ACT GC | *hbl*C | 740 | |
| HbICR | TTG AGA CTG CTC GTT AGT TG | | | |
| HbIDF | AAT CAA GAG CTG TCA CGA AT | *hbl*D | 829 | |
| HbIDR | CAC CAA TTG ACC ATG CTA AT | | | |
| *CytK*F | CGA CGT CAC AAG TTG TAA CA | Cytotoxin-K | 565 | |
| *cytK*R | CGT GTG TAA ATA CCC CAG TT | | | |

**Results and discussion.** Isolation, identification, and assessment of B. cereus contamination in beef pizza samples collected from kindergartens A and B. B. cereus was found in various dishes, with the highest concentration in beef pizza at $6.0 \cdot 10^5$ CFU/g in Kindergarten A and $6.8 \cdot 10^5$ CFU/g in Kindergarten B (Table 2).

It should be noted that both kindergartens sourced beef pizza from the same supplier. Currently, Vietnam does not have standards of microbial limits in pizza samples. When referring to microbial limits from other countries in the world, such as Law No. 329 of the Republic of Estonia in 2000 or the microbiological criteria established by the National Advisory Committee on Microbiological Standards for Foods under the U.S. Department of Agriculture, the maximum limit of *B. cereus* in pizza[5] or ready-to-eat food [15] is $10^3$ CFU/g. Consequently, the beef pizza consumed by students in kindergartens A and B exceeded the permissible limit by 600 times and 680 times, respectively. In cases where microbiological limits for ready-to-eat foods of the Australia-New Zealand Food Standards Code 2022[6] or the Microbiological Guide for Food in 2014 of the Hong Kong Centre for Food Safety[7] are applied, the acceptable limit for ready-to-eat foods is $10^5$ CFU/g. Consequently, the beef pizza from both kindergartens exceeded the limit by 6 and 6.8 times, respectively. In addition, the fried pork tenderloin, boiled mixed vegetables and seafood egg noodles of

[5] Governmental Regulation No. 166 of 2000 regarding validation of microbiological requirements for food groups. *FAOLEX Database*, 2000. Available at: https://www.fao.org/faolex/results/details/en/c/LEX-FAOC037807/ (September 19, 2024).

[6] Standard 1.6.1. Microbiological limits for food. *Food Standards Australia New Zealand*. Available at: https://www.foodstandards.gov.au/business/microbiological-limits (September 19, 2024).

[7] Food Legislation / Guidelines. *Centre for Food Safety*. Available at: https://www.cfs.gov.hk/english/food_leg/food_leg.html (September 19, 2024).

Table 2

Contamination levels of *B. cereus* (CFU/g) in food samples collected from kindergartens A and B

| | Kindergarten A | Kindergarten B |
|---|---|---|
| Fried pork tenderloin | ND | $5.0 \cdot 10^1$ |
| Boiled mixed vegetables | ND | $6.0 \cdot 10^1$ |
| Seafood egg noodles | ND | $8.0 \cdot 10^1$ |
| Beef pizza | $6.0 \cdot 10^5$ | $6.8 \cdot 10^5$ |

Note: ND, not detected.

kindergarten B (Table 2) also showed *B. cereus* concentrations of $5.0 \cdot 10^1$ CFU/g, $6.0 \cdot 10^1$ CFU/g and $8.0 \cdot 10^1$ CFU/g indicating potential transmission of *B. cereus* from beef pizza to other dishes. To better understand the epidemiological characteristics and develop effective preventive measures, *B. cereus* strains isolated from beef pizza samples in both Kindergartens A and B were evaluated for antibiotic resistance.

The results of the hemolysis tests showed that all isolated strains produced beta-hemolytic zones, a characteristic feature of *B. cereus* (Figure 1). The MALDI-TOF identification results of 10 colonies isolated from beef pizza samples of kindergartens A and B showed that all ten tested colonies achieved MS MALDI–TOF scores greater than 2.0, allowing identification at the species level as *B. cereus* (10/10; 100 %). Other biochemical characteristics of the strains were evaluated using the API 50 CHE kit, in which 100 % (10/10) of the *B. cereus* strains were positive for D-Ribose, D-Glucose, D-Fructose, N-Acetylglucosamine, Arbutin, Esculin/Ferric citrate, Salicin, D-Maltose, D-Trehalose, Starch (amidon) and Glycogen, 80 % (8/10) of the strains were positive for and D-Cellobiose; 70 % (7/10) of the strains were positive for D-Saccharose (sucrose); and 50 % (5/10) of the strains were positive for Gentiobiose (Table 3).

These results are consistent with the data published in Bergey's Manual of Systematic Bacteriology. Glycerol, glycogen and starch fermentation, and Esculin/Ferric citrate-reducing properties confirmed in this biochemical test are also the properties used to differentiate *B. cereus* from closely related *Bacillus* sp. [8] .

***Antibiotic resistance profile of B. cereus strains isolated from beef pizza samples.*** All strains were susceptible ($n$ = 10; 100 %) to meropenem and exhibited high sensitivity to ciprofloxacin ($n$ = 9; 90 %). The results also indicated an average resistance rate of *B. cereus* to ofloxacin ($n$ = 6; 60 %) and chloramphenicol ($n$ = 7; 70 %). *B. cereus* strains isolated from pizza samples had absolute resistance rates to penicillin ($n$ = 10; 100 %) and vancomycin ($n$ = 10; 100 %); they also showed high resistance rates to streptomycin ($n$ = 9; 90 %), tetracycline ($n$ = 8; 80 %), ampicillin ($n$ = 7; 70 %) and erythromycin ($n$ = 7; 70 %); and moderate resistance rates to imipenem ($n$ = 6; 60 %) (Figure 2).

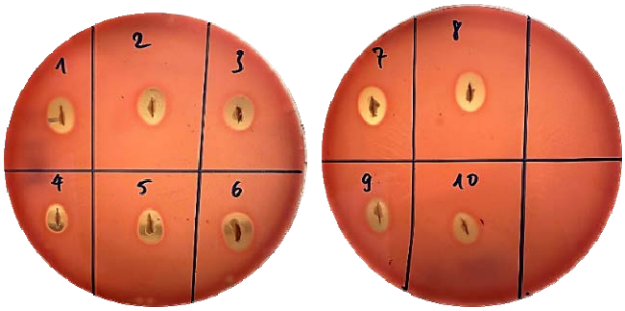


Figure 1. Hemolytic test results of 10 *B. cereus* strains isolated from beef pizza samples. 1–10: *B. cereus* strains isolated

[8] Bergey's Manual of Systematic Bacteriology. Volume One: The Archaea and the Deeply Branching and Phototrophic Bacteria, 2nd ed. In: D.R. Boone, R.W. Castenholz, G.M. Garrity eds. NY, Springer Publ., 2001, 722 p.

Table 3

Percentage of *B. cereus* strains ($n$ = 10) positive for tests in the API 50 CH kit

| No. | Test | Conc. (mg) | % | No. | Test | Conc. (mg) | % |
|---|---|---|---|---|---|---|---|
| 0 | Negative control | | 0 | 25 | Esculin / Ferric citrate | 1.16 / 0.152 | 100 |
| 1 | Glycerol | 1.64 | 100 | 26 | Salicin | 1.04 | 100 |
| 2 | Erythritol | 1.44 | 0 | 27 | D-Cellobiose | 1.32 | 80 |
| 3 | D-Arabinose | 1.4 | 0 | 28 | D-Maltose | 1.4 | 100 |
| 4 | L-Arabinose | 1.4 | 0 | 29 | D-Lactose | 1.4 | 0 |
| 5 | D-Ribose | 1.4 | 100 | 30 | D-Melibiose | 1.32 | 0 |
| 6 | D-Xylose | 1.4 | 0 | 31 | D-Saccharose | 1.32 | 70 |
| 7 | L-Xylose | 1.4 | 0 | 32 | D-Trehalose | 1.32 | 100 |
| 8 | D-Adonitol | 1.36 | 0 | 33 | Inulin | 1.28 | 0 |
| 9 | Methyl-ß-D-xylopyranoside | 1.28 | 0 | 34 | D-Melezitose | 1.32 | 0 |
| 10 | D-Galactose | 1.4 | 0 | 35 | D-Raffinose | 1.56 | 0 |
| 11 | D-Glucose | 1.56 | 100 | 36 | Starch | 1.28 | 100 |
| 12 | D-Fructose | 1.4 | 100 | 37 | Glycogen | 1.28 | 100 |
| 13 | D-Mannose | 1.4 | 0 | 38 | Xylitol | 1.4 | 0 |
| 14 | L-Sorbose | 1.4 | 0 | 39 | Gentiobiose | 0.5 | 50 |
| 15 | L-Rhamnose | 1.36 | 0 | 40 | D-Turanose | 1.32 | 0 |
| 16 | Dulcitol | 1.36 | 0 | 41 | D-Lyxose | 1.4 | 0 |
| 17 | Inositol | 1.4 | 0 | 42 | D-Tagatose | 1.4 | 0 |
| 18 | D-Mannitol | 1.36 | 0 | 43 | D-Fucose | 1.28 | 0 |
| 19 | D-Sorbitol | 1.36 | 0 | 44 | L-Fucose | 1.28 | 0 |
| 20 | Methyl-α-D-mannopyranoside | 1.28 | 0 | 45 | D-Arabitol | 1.4 | 0 |
| 21 | Methyl-α-D-glucopyranoside | 1.28 | 0 | 46 | L-Arabitol | 1.4 | 0 |
| 22 | N-Acetylglucosamine | 1.28 | 100 | 47 | Potassium gluconate | 1.84 | 0 |
| 23 | Amygdalin | 1.08 | 0 | 48 | Potassium 2-ketogluconate | 2.12 | 0 |
| 24 | Arbutin | 1.08 | 100 | 49 | Potassium 5-ketogluconate | 1.8 | 0 |

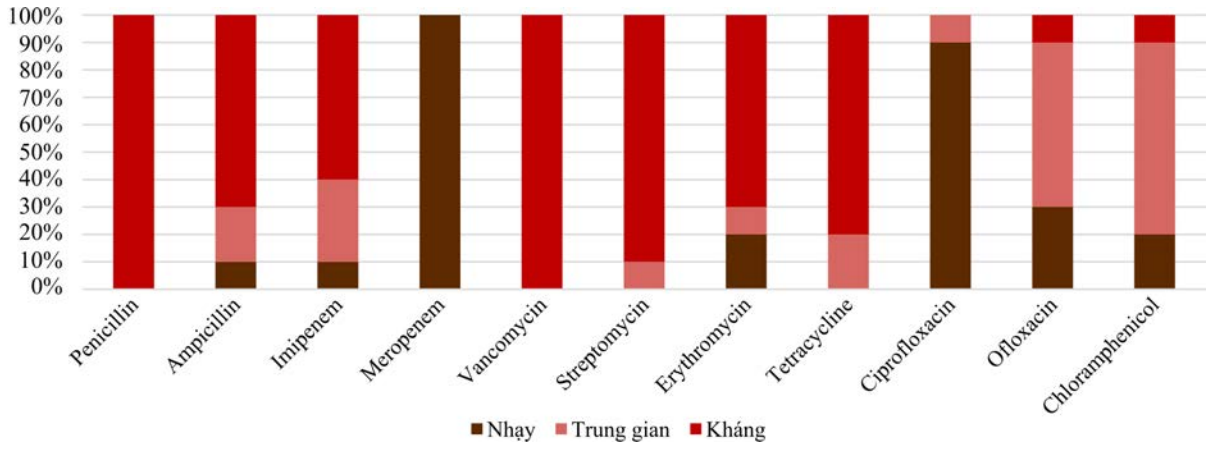


Figure 2. Antibiotic profile of *B. cereus* based on inhibition zone diameter (mm); (brown) resistant, (orange) intermediate, (red) susceptible

From the above results, meropenem and ciprofloxacin demonstrated promising antibacterial activity against the *B. cereus* strains in the study. In contrast to M45 CLSI guidelines, which indicate *B. cereus* is typically resistant to penicillin but often susceptible to vancomycin and macrolides, our study found that *B. cereus* strains from beef pizza samples exhibited 100 % resistance to vancomycin and 70 % resistance to erythromycin. Abdelaziz

et al. (2024) examined the antibiotic resistance patterns of *B. cereus* strains in food in Japan and reported high levels of resistance to vancomycin [16], which is similar to our study. Nakayama (2021) showed that *B. cereus* strains isolated from chicken in Ho Chi Minh City were resistant to ampicillin, ciprofloxacin, and tetracycline [17], while *B. cereus* strains analyzed in this study were sensitive to ciprofloxacin and had resistance rates to ampicillin and tetracycline. When comparing the antibiotic resistance profiles of 10 *B. cereus* strains isolated from beef pizza samples in the kindergarten poisoning case with *B. cereus* strains circulating globally, our results were similar to those of Algammal et al. (2022). *B. cereus* strains circulating in Egypt were susceptible to meropemen and exhibited resistance to several antibacterial agents such as erythromycin, streptomycin and tetracycline [18], similar to *B. cereus* strains in this study. Further investigations at the DNA level regarding virulence and antibiotic resistance genes are necessary to thoroughly explore the epidemiological characteristics and implement preventive measures against *B. cereus* in beef pizza samples.

***Presence of toxin genes of B. cereus in beef pizza sample.*** Ten strains *of B. cereus* isolated from beef pizza samples involved in the poisoning incidents at kindergartens A and B in Hanoi were analyzed for emetic (NRPS) and diarrheal (*hblA, hblC, hblD, nheA, nheB, nheC, bceT* and *cytK*) toxin gene using PCR (NRPS, *bceT* and *cytK)* and multiplex PCR *(hblA, hblC, hblD, nheA, nheB,* and *nheC).* The electrophoresis results (Figures 3–5) indicate that 100 % of the *B. cereus* strains (10/10) from the beef pizza sample were positive for the *bceT* gene, 80 % of the strains (8/10) were positive for the *cytK* gene and 60 % of the strains (6/10) were positive for the *nheA* and *nheC* genes. Additionally, 100 % of the strains in this study (10/10) were negative for NRPS gene (electrophoresis image not included in this paper).

Our findings are consistent with other studies in the world. In Iraq, B.M.S. Saeed et al. (2021) reported a very low detection rate of the emetic toxin gene in all food samples, approximately 7.69 %, indicating a low prevalence of emetic *B. cereus* strains in foods [14]. N. Jessberger et al. (2021) [19] and M. Bağcioğlu et al. (2019) [20] also reported that emetic *B. cereus* type was less commonly found in food compared to diarrheal type. Our study contributes to the database of *B. cereus* strains circulating in Vietnam; moreover, the *B. cereus* strains carrying the diarrheal toxin gene identified in this research provide material for developing a reference material to support rapid and accurate food poisoning diagnostics.

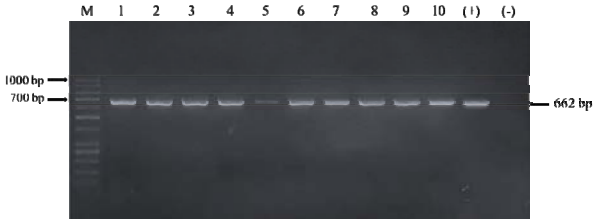


Figure 3. PCR product electrophoresis of *bceT* toxin gene. 1–10: *bceT* positive (662bp). M: DNA ladder 50bp; (+): Positive control; (-): Negative control

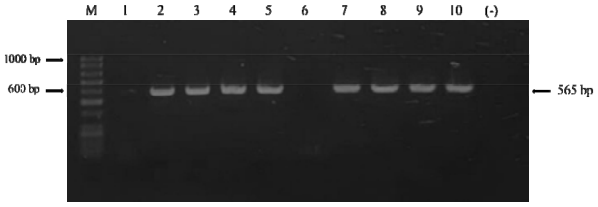


Figure 4. PCR product electrophoresis image of *cytK* toxin. 1, 6: Negative; 2–5 and 7–10: *cytK* positive (565bp). M: DNA ladder 50bp; (-): Negative control

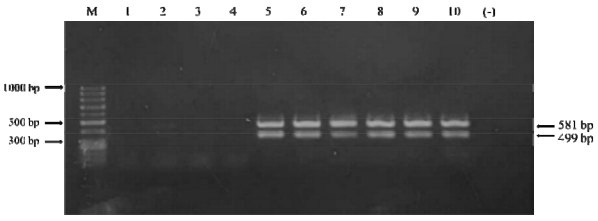


Figure 5. Electrophoresis images of multiplex PCR products of the *hbl* (*hblA*, *hblC*, and *hblD*) and *nhe* (*nheA*, *nheB*, and *nheC*) toxin genes: 1–4: Negative; 5–10: *nhA* and *nhC* positive (581bp, 499bp). M: DNA ladder 50bp; (-): Negative control

**Conclusion.** The study isolated 10 strains of *B. cereus* from beef pizza samples identified as the cause of poisoning among students at two kindergartens A and B, revealing *B. cereus* con-

centrations exceeding permissible limits set by current global regulations, specifically $6.0 \cdot 10^5$ CFU/g at Kindergarten A and $6.8 \cdot 10^5$ CFU/g at Kindergarten B. Among the 11 antibiotics used to evaluate the antibiotic resistance of isolated strains, *B. cereus* exhibited absolute resistance (100 %) to penicillin and vancomycin, high resistance rates to streptomycin (90 %), tetracycline (80 %), ampicillin (70 %) and erythromycin (70 %); moderate resistance to imipenem (60 %) and were susceptible to meropenem (100 %) and ciprofloxacin (90 %). Furthermore, 100 % of *B. cereus* strains (10/10) in the beef pizza sample were positive for *bceT* gene, 80 % of the strains (8/10) were positive for *cytK* gene and 60 % of the strains (6/10) were positive for *nheA* and *nheC* genes indicating that these *B. cereus* strains belong to the diarrhea-causing type. *B. cereus* remains a major pathogenic threat due to its rapid evolution, virulence genes, and antibiotic resistance, making continuous monitoring of the genetic profile and antibiotic resistance of circulating strains in Vietnam crucial for disease prevention and response.

**Funding.** The authors express their deepest gratitude for financial support provided by the National Institute for Food Control, Vietnam.

**Competing interests.** The authors declare no competing interests.

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*Pham Ngoc Ha, Ninh Thi Hanh, Vu Khanh Van, Tran Le Minh, Nguyen Thanh Trung. Isolation and characterization of Bacillus cereus strains isolated from a beef pizza food poisoning incident in Hanoi. Health Risk Analysis, 2025, no. 2, pp. 98–106. DOI: 10.21668/health.risk/2025.2.08.eng*

Received: 03.12.2024
Approved: 25.04.2025
Accepted for publication: 14.06.2025