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Inhibitory Effects of Termicin on Multidrug-Resistant *Bacillus cereus*: A Peptide Antibiotic Approach

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Abstract

The increasing prevalence of multidrug-resistant (MDR) *Bacillus cereus* in food systems poses a serious threat to public health and food safety, driven by its ability to cause foodborne illness, spoilage, and resistance to multiple classes of conventional antibiotics. This study evaluated the inhibitory effects of termicin, a termite-derived antimicrobial peptide, against MDR *B. cereus* strains isolated from commercial powdered soybean samples obtained from retail outlets in Awka Metropolis. Out of the samples analyzed, 33% were positive for *Bacillus* species. Phenotypic and biochemical characterization, followed by 16S rRNA gene sequencing, confirmed the isolates as *B. cereus* strains FORC60 (BCFOR), DQ01 (BCDQO), and CD3 (BCCD3), each showing 100% sequence identity with reference strains. Antibiotic susceptibility testing revealed substantial multidrug resistance, with an overall resistance rate of 40.74%; notably, BCCD3 exhibited the highest resistance level (75%), spanning multiple antibiotic classes. In contrast, termicin demonstrated strong, concentration-dependent inhibitory activity against all isolates, including the highly resistant BCCD3 strain. Increasing termicin concentrations (0.1–1.0%) resulted in a marked reduction in bacterial growth, with near-complete inhibition observed at the highest concentration. These findings indicate that termicin retains potent antimicrobial activity against MDR *B. cereus* despite resistance to conventional antibiotics, highlighting its promise as a peptide antibiotic candidate and a natural biocontrol agent for managing drug-resistant spore-forming pathogens in food and public health applications.

Keywords: Termicin, antimicrobial peptide, *Bacillus cereus*, multidrug resistance, food safety, powdered soybean.

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Introduction

The persistence and increasing resistance of *Bacillus cereus* in the global food supply chain represent a critical and complex challenge to food safety and public health (Glasset *et al.*, 2021; Idigo *et al.*, 2025a). As a Gram-positive, spore-forming bacterium ubiquitous in soil and agricultural environments, *B. cereus* readily contaminates raw ingredients and processed foods, where its heat-resistant spores can survive and subsequently germinate (Ehling-Schulz *et al.*, 2019; Okeke *et al.*, 2017; Dim *et al.*, 2025a). The pathogen is a leading cause of diarrheal and emetic foodborne illnesses worldwide, with its growing resistance to conventional antibiotics compounding the severity and difficulty of treating systemic infections (Scallan *et al.*, 2011; Miller *et al.*, 2022).

Powdered soybean, a nutrient-rich and globally consumed food ingredient, is a documented vehicle for *B. cereus* contamination (Kim *et al.*, 2018; Chude *et al.*, 2020; Dim *et al.*, 2025c). The desiccated nature of the product provides an environment conducive to spore survival, posing a significant risk for food spoilage and outbreaks of illness. This risk is amplified by the emergence of multidrug-resistant strains that may withstand first-line clinical treatments (Park *et al.*, 2019). Consequently, there is a pressing need for innovative antimicrobial strategies that can target resilient *B. cereus* strains.

Antimicrobial peptides (AMPs) represent a promising frontier, offering a mechanism of action that disrupts bacterial membranes—a target less prone to conventional resistance development compared to traditional antibiotics (Mahlapuu *et al.*, 2016). Terminicin, a cationic peptide isolated from the gut microbiome of *Macrotermes* termites, is one such candidate with documented activity against Gram-negative pathogens (Bulmer *et al.*, 2017). However, its efficacy against Gram-positive, spore-forming MDR pathogens like *B. cereus* remains largely unexplored.

Therefore, this study aimed to evaluate the *in vitro* inhibitory and bactericidal potential of terminicin against clinically significant, MDR *B. cereus* strains isolated from contaminated powdered soybean. By determining the minimum inhibitory and bactericidal concentrations, this research seeks to assess terminicin's dual potential: as a novel peptide antibiotic for therapeutic use and as a natural biocontrol agent to enhance food safety and mitigate the threat of resistant foodborne pathogens.

Materials and Methods

Sample Collection, Culture and Isolation of Enteric Bacteria

Powdered soybean samples were randomly collected from different shops in Awka Metropolis, and these were transported immediately to the laboratory for analysis. One-fifth gram (0.5 g) was weighed using an electronic weighing balance (MWP-600) and this was put into a test tube containing 5 ml of normal saline. This was turned thoroughly with sterile glass rod and a tenfold serial dilution was carried out to obtain different concentrations of the samples (10^{-1}). One milliliter of the prepared soil samples (10^{-1}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Bacillus ChromAgar medium (BCA). All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h. This was done using the method described in work published by Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Egbe *et al*. (2025a).

Characterization and Identification of the Isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018b), Iheukwumere *et al.* (2025f), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020a), Iheukwumere *et al.* (2025g) and molecular characterization as described in the study published by Gabriela *et al.* (2014), Ekesiobi *et al.* (2025), Ekechukwu *et al*. (2025a), Ekechukwu *et al*. (2025b), Ezedianafo *et al*. (2025a), and Ezedianafo *et al*. (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al.* (2020b), Idigo *et al*. (2025a), Idigo *et al*. (2025b), Idigo *et al.* (2025c), Idigo *et al*. (2025d), and Ezedianafo *et al*. (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al*., 2017; Iheukwumere *et al.*, 2017a; Iheukwumere *et al.*, 2017b; Iheukwumere *et al*., 2023a; Iheukwumere *et al.*, 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Iheukwumere *et al*. (2018c) Ike *et al*. (2025a), Iheukwumere *et al*. (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35±2^{0}C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al.* (2017d), Iheukwumere *et al*. (2022b), Iheukwumere *et al*. (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al*. (2025b) Ike *et al*. (2025c) Iheukwumere *et al*. (2022d), Idigo *et al*. (2025e), Obiefuna *et al*. (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al*. (2017), Obianom *et al*. (2024), Ekechukwu *et al*. (2025c), Obiefuna *et al*. (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al*. (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), Idigo *et al*. (2025e), Ezedianafo *et al*. (2025d), Ezedianafo *et al*. (2025e) and Iheukwumere *et al*. (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025d), Ike *et al*. (2025e), Idigo *et al*. (2025f), Idigo *et al*. (2025g) and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al*. (2017), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), and Idigo *et al*. (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al*. (2025h), Idigo *et al*. (2025i), Iheukwumere *et al*. (2025j) and Idigo *et al*. (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), Idigo *et al*. (2025k), Idigo *et al*. (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c), and Idigo *et al*. (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025l), Iheukwumere *et al*. (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2025n), and Iheukwumere *et al*. (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025p; Iheukwumere *et al*., 2025q; Chude *et al*., 2020).

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2025r; Iheukwumere *et al*., 2025s; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2025t; Nwike *et al*., 2017).

Susceptibility Patterns of the Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010). The isolates were aseptically subcultured into a broth culture and incubated at 35+ 2^0C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

***In vitro* activity of conventional antibiotics against the isolates using disc diffusion method:** The susceptibility of the isolates to the conventional antibiotics was done using disc diffusion method on Mueller Hinton agar. A sterile swab was used to inoculate the suspension of the isolate on the prepared and dried Mueller Hinton agar plate equally. It was then left to stay for 5 minutes. A sterile forceps was used to place the commercially prepared antibacterial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 37˚C for 24 h. Meter rule was used underside of the plates to determine the diameter zones of inhibition in millimeter as described in the study published by Iheukwumere *et al.* (2018), Egbe *et al.* (2025b), Egbe *et al.* (2025c), Iheukwumere *et al.* (2025d), and Iheukwumere *et al.* (2025e).

Sample Collection, Handling, Transportation of Macrotermes species: *Macrotermes* samples were collected from termiterium using hand picking and cleaned plastic containers. The samples were put into the perforated containers and the container was carefully covered. The covering of the containers deprived the termites from oxygen resulting in death. The containers were transported to the laboratory for analysis within 2 h of collection.

Extraction of termicin: Termicin, a peptide antibiotic, was extracted from the termite gut using a suitable solvent and thin layer chromatography (TLC). The process involved several steps. First, the termite guts were dissected and homogenized in a phosphate-buffered saline (PBS) solution to release the termicin peptide. The homogenate was then centrifuged to separate the supernatant, which contained the termicin peptide, from the cellular debris. The supernatant was then subjected to solvent extraction using a mixture of methanol and water (1:1, v/v). The methanol-water mixture was chosen as the solvent due to its ability to effectively solubilize the termicin peptide. The resulting extract was then applied to a TLC plate, which was developed using a solvent system consisting of n-butanol, acetic acid, and water (4:1:5, v/v/v). The TLC plate was visualized under ultraviolet (UV) light, and the band corresponding to termicin was identified based on its retention factor (Rf = 0.40 - 0.50) value. The termicin band was then scraped off the TLC plate and eluted with a small volume of methanol. The eluted termicin was then concentrated and purified using high-performance liquid chromatography (HPLC).

***In vitro* antibacterial susceptibility test:** This was ascertained using micro tube dilution method. Here, micro tube dilution plates was used. Different dilutions of the sample were prepared, 100 µL of each concentration was dropped in each well of the micro well, then 100 µL of the test isolate was added into the well. These were mixed and incubated at 37˚C for 24 h. The bacterial growth pattern was determined for the most potent minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) as described by Clinical and Laboratory Standards Institute/CLSI (2015), Iheukwumere *et al* (2025u), and Iheukwumere *et al.* (2025v).

Statistical Analysis: The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student "t" test as described in the study published by Iheukwumere *et al.* (2017e), Manasseh *et al.* (2025), Idigo *et al.* (2025n), Idigo *et al.* (2025o), Idigo *et al.* (2025p), Idigo *et al.* (2025q), Idigo *et al.* (2025r), Idigo *et al.* (2025s), Idigo *et al.* (2025t), Ugwu *et al.* (2025a) and Ugwu *et al.* (2025b).

Results

The characterization of the three bacterial isolates (C, D, and E) revealed a consistent profile typical of a common pathogen, with underlying strain-level variations that had significant implications for antibiotic resistance and treatment. Initial phenotypic analysis showed the isolates shared core morphological and biochemical traits. On nutrient agar, colonies appeared either cream-white or colourless, with a flat elevation and a rough surface edge. Microscopically, all were Gram-positive, motile rods capable of forming central endospores. Biochemically, they were catalase-positive, utilized citrate, hydrolyzed gelatin, and were negative for urease, indole production, and the methyl red test, while being positive for the Voges-Proskauer reaction and hydrogen sulfide production. A key differentiating feature was observed in the oxidase test, where isolates C and D were positive, while isolate E was negative. Further carbohydrate utilization tests revealed nuanced differences; for instance, isolate C fermented lactose, sucrose, and maltose, whereas isolate D utilized sucrose, maltose, and sorbitol (Table 1).

Definitive identification was achieved through molecular analysis. 16S rRNA gene sequencing confirmed all three isolates as strains of *Bacillus cereus* with exceptionally high confidence. Each isolate showed 100% query coverage and 100% percent identity to a reference genome in the database, with an E-value of 0.0. Isolate C was identified as *Bacillus cereus* strain FORC6, isolate D as strain DQ01, and isolate E as strain CD3 (Table 2).

Despite belonging to the same species, the isolates exhibited starkly different antibiotic resistance profiles. As detailed in Table 3, overall resistance among the 54 tested strains was high at 40.74%. However, resistance was not uniform. Isolate BCFOR (C) showed resistance in 37.93% of its strains, while BCDQO (D) was resistant in 29.41% of cases. Most alarmingly, isolate BCCD3 (E) demonstrated multi-drug resistance in 75% of its strains. The implicated antibiotics spanned several classes, including penicillins (APX, AM), cephalosporins (AU), fluoroquinolones (PEF, CPX), aminoglycosides (CN), macrolides (E, SP, Z), and others (SXT, ORF), indicating a broad-spectrum resistance mechanism.

The inhibitory activity of termicin against the isolates is shown in Table 4. Termicin exhibited potent, dose-dependent inhibitory activity against representative cultures of each strain (BCFOR, BCDQO, BCCD3). Bacterial growth, measured by optical density, decreased precipitously as the Termicin concentration increased from 0.1% to 1.0%. At the highest concentration, optical density readings approached zero, indicating near-complete inhibition of all tested isolates, including the highly antibiotic-resistant BCCD3 strain. This demonstrated that Termicin's mechanism of action remains effective against these pathogens despite their resistance to standard antibiotics.

Table 1: Characteristics of the bacterial isolates

Characteristics	C	D	E
Appearance on Nutrient agar	Cream-white	Colourless	Cream-white
Elevation	Flat	Flat	Flat
Surface edge	Rough	Rough	Rough
Molility	+	+	+
Endospore	+	+	+
Position	Central	Central	Central
Bulging	No	No	No
Gram reaction	+	+	+
Cell morphology	Rods	Rods	Rods
Catalase	+	+	+
Oxidase	+	+	-
Urease	-	-	-
Citrate	+	+	+
Gelatin	+	+/-	+
Casein	+	+/-	+/-
H_2S	+	+	+
Indole	-	-	-
MR	-	-	-
VP	+	+	+
Glucose	+	+	+
Maltose	+/-	+	+
Xylose	+/-	+/-	+/-
Galactose	+/-	+/-	+/-
Inositol	+/-	-	-
Sorbitol	-	-	+/-
Xylitol	+/-	+/-	+/-
Dulcitol	+/-	+/-	-

Table 2: Molecular characteristic of the isolates

Isolate code	Max score	Total score	Query cover (%)	E-value	Percent identity (%)	Accession Number	Description
C	7498	7498	100	0.0	100	CP020383	*Bacillus cereus* strain FORC6 (BC FOK) chromosome complete genome
D	11501	11501	100	0.0	100	CP097051	*Bacillus cereus* strain DQ01 (BCDQO) chromosome complete
E	6544	6544	100	0.0	100	CP040678	*Bacillus cereus* strain CD3 (BC CD3) chromosome complete

Table 3: Susceptibility of the isolates to conventional antibiotics

Isolate	N	Susceptible Strain (%)	Resistance Strain (%)	Implicated antibiotics
BCFOR	29	18 (62.07)	11 (37.93)	PEF, APX, SP, E, SXT, AU, CN, ORF, AM
BCDQO	17	12 (70.59)	5 (29.41)	PEF, APX, SP, E, SXT, AU, CPX, CN, ORF, AM
BCCD3	8	2 (25.00)	6 (75.00)	PEF, APX, SP, E, SXT, AU, Z, CN, ORF, AM
Total	54	32 (59.26)	22 (40.74)	

Table 4: Inhibitory activity of termicin against the test isolates

Conc. (%)	BCFOR	BCDQO	BCCD3
0.10	0.0000	0.0000	0.0000
0.20	0.5000	0.2500	0.5000
0.30	0.2500	0.1250	0.2500
0.40	0.1250	0.1250	0.1250
0.50	0.0625	0.0625	0.0625
0.60	0.0313	0.0313	0.0625
0.70	0.0156	0.0313	0.0625
0.80	0.0156	0.0156	0.0313
0.90	0.0078	0.0078	0.0313
1.00	0.0078	0.0039	0.0156

Discussion

The present study demonstrates the significant inhibitory potential of termicin, a termite-derived antimicrobial peptide, against multidrug-resistant (MDR) *Bacillus cereus* isolates. Molecular characterization confirmed the identity of the isolates as *B. cereus* strains FORC60 (BCFOR), DQ01 (BCDQO), and CD3 (BCCD3), each showing high sequence similarity (>99%) with reference strains, supporting their clinical and foodborne relevance.

Antibiotic susceptibility testing revealed considerable resistance among the isolates, particularly BCCD3, which exhibited the highest resistance profile, with 75% of strains resistant to multiple conventional antibiotics. Resistance was observed across several antibiotic classes, indicating the presence of MDR phenotypes. This finding aligns with global reports of increasing antimicrobial resistance in *B. cereus*, which complicates treatment and heightens public health concern (Ehling-Schulz et al., 2019; Kim et al., 2018).

In contrast to the reduced effectiveness of conventional antibiotics, termicin demonstrated strong and concentration-dependent inhibitory activity against all MDR isolates. Inhibition was first observed at 0.20% concentration, with progressive increases in activity up to 1.00%. Notably, BCDQO and BCFOR showed greater susceptibility to termicin, while BCCD3, despite its pronounced multidrug resistance, was still effectively inhibited at higher concentrations. This highlights the ability of termicin to overcome resistance mechanisms that limit the efficacy of standard antibiotics.

The observed antimicrobial activity of termicin is consistent with earlier studies on termite-derived antimicrobial peptides, which exert bactericidal effects primarily through membrane disruption rather than conventional intracellular targets (Bulmer et al., 2017; Dossey et al., 2018). Such mechanisms reduce the likelihood of resistance development and position peptide antibiotics as promising alternatives in the era of escalating antimicrobial resistance.

Overall, the findings underscore the therapeutic and biocontrol potential of termicin against MDR *B. cereus*. Its efficacy against strains resistant to multiple antibiotics suggests that termite-derived peptides may represent a novel class of antimicrobial agents suitable for controlling resistant foodborne and environmental pathogens.

Conclusion

This study confirms that termicin exhibits potent inhibitory activity against multidrug-resistant *Bacillus cereus* isolates, including strains with high resistance to conventional antibiotics. The concentration-dependent antimicrobial effect observed across all isolates highlights the promise of termicin as a peptide antibiotic candidate. These findings support further exploration of termite-derived antimicrobial peptides as natural alternatives or complements to conventional antibiotics, particularly for managing MDR bacterial infections in food safety and public health contexts.

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