

## **Bacillus cereus in meat products: Prevalence, toxins profile, antibiogram profile, and antimicrobial activity of Apple cider vinegar**

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### **Abstract**

*Bacillus cereus* is a significant foodborne bacterium that is prevalent in a variety of dietary products. This study aimed to assess the contamination rate, enterotoxin genes, and antibacterial susceptibility of *B. cereus* detected in 20 samples each of minced beef, beef shawarma, beef burger, beef kofta, beef sausage, chicken shawarma, chicken kofta, chicken kabab, and chicken sausage that were acquired from a variety of markets in the Aswan Governorate, Egypt. In addition, the antimicrobial impact of Apple cider vinegar (ACV) on *B. cereus* was investigated. The highest *B. cereus* levels were found in beef kofta samples ( $2.44 \times 10^3 \pm 0.16 \times 10^2$  CFU/g), followed by beef burger ( $2.02 \times 10^3 \pm 0.18 \times 10^2$  CFU/g) and beef sausage ( $1.88 \times 10^3 \pm 0.12 \times 10^2$  CFU/g). On an average, 30% of the samples were contaminated with *B. cereus*. All of the putative isolates showed *B. cereus* DNA according to PCR findings of the *gyrB* gene. Most of the strains (16/54) had the *hblA* gene, which was substantially more abundant than *hblC* (7/54) and *hblD* (5/54). However, *nheA* was detected in 10/54 samples and was substantially more prevalent than *nheB* (5/54) and *nheC* (3/54). Of the strains, 10 out of 54 have *cytK*. By comparison, the *cesB* detection rate was just 6/54, indicating that emetic strains are less frequent in meat products than diarrhea strains. Most strains were resistant to ampicillin, cefoxitin, and colistin (100% each), while they were entirely sensitive to imipenem, nalidixic acid, and vancomycin, rendering them the most significant antibiotics. By the agar well diffusion technique, all concentrations of ACV (10%, 30%, 70%, and 100%) were confirmed to have significant inhibitory activities against *B. cereus*, suggesting that ACV could be employed as a natural antimicrobial preservative in meat products.

Nevertheless, more research is necessary to find other traits of *B. cereus* in meat products and the actions of other natural antibacterials.

**Keywords:** Antimicrobial resistance, Apple cider vinegar, *Bacillus cereus*, natural preservative, toxigenic genes

## Introduction

Food safety is a pertinent issue globally that affects international trade and human health. Foodborne infections pose serious risks to consumer health and place a financial strain on healthcare systems around the world, making them a major global public health concern (El-Hawary *et al.*, 2025). A wide range of microbial pathogens that contaminate different kinds of meat products are responsible for the millions of foodborne illnesses that are reported each year. Among these pathogens, *Bacillus cereus* has maintained its significance as a result of its association with foodborne outbreaks and the ability to cause serious illness (Foxcroft *et al.*, 2024). *B. cereus* is a rod-shaped, aerobic, or facultatively anaerobic, gram-positive, motile, spore-forming bacterium that is common in nature and can also be found in food. Vegetative cells of *B. cereus* can live and replicate in a pH range of 5 to 10. They can also develop in a moderately broad variety of temperatures and are highly resistant to salting. Alternatively, spores are exceptionally resilient to a variety of extreme conditions, including chilling, drying, high temperatures, and gamma- and UV-irradiation. This enables *B. cereus* to persist on a variety of surfaces and in the environment (Tirloni *et al.*, 2022).

*B. cereus* is capable of producing a variety of virulence influences and can infiltrate the gastrointestinal mucosa through digestion, resulting in diarrhea and vomiting (Song *et al.*, 2019). Diarrhea is related to four distinct enterotoxins: hemolysin BL (HBL, encoded by *hblA*, *hblC*, and *hblD*), cytotoxin K (CytK, encoded by *cytK*), enterotoxin FM (EntFM, encoded by *entFM*), and non-hemolytic enterotoxin (NHE, encoded by *nheA*, *nheB*, and *nheC*). However, emesis, or vomiting, is related to a tiny, acid- and heat-stable toxin produced by *cesB* genes (Ehling-Schulz *et al.*, 2015). Other than food-related illness, *B. cereus* is linked to severe sicknesses like pneumonia, endocarditis, osteomyelitis, endophthalmitis, and necrotizing fasciitis (Ikeda *et al.*, 2015).

The public health relevance of *B. cereus* has already been established worldwide, but its specific survival capabilities and possible environmental adaptations may become more significant for food safety authorities and the food industry when considering our changing climate. *B. cereus* presents a substantial public health concern because of the quantity of meat products consumed in Egypt and the nature of their processing. A rise in its abundance in these foods increases the danger

of exposure and the propagation of antibiotic-resistant bacteria. The significance of *B. cereus* for public health is highlighted by theories on its environmental and temperature range adaptability (Foxcroft *et al.*, 2024). Antibiotics are still the best way to treat microbial illness, involving those produced by *B. cereus*. Conversely, the prevalent application of antibacterials has caused the development of antibacterial-resistant strains, linking strains that are resistant to more than one antibiotic (Friedman *et al.*, 2016). Thus, it is imperative to discover the profile of antibiotic resistance of *B. cereus* to select the appropriate medications for therapy schemes.

Numerous investigations have been carried out to find novel techniques to prolong the duration of protection of meat and its products without using chemical supplements, as there is growing concern about the present developments regarding applying different common substitutes to improve the duration of keeping meat and its products safe and improve its shelf life. This is particularly relevant given the substantial rise in the manufacturing of meat products and their role in supplying the desired flavor and taste (Nady *et al.*, 2024).

Apple cider vinegar (ACV) is an organic by-product of apple fermentation, consisting of apple, sugar, and yeast. It shows antibacterial efficacy against gram-positive microbes. Chemicals in plants, such as organic acids, minerals, flavonoids, vitamins, and polyphenols, work together to fight bacteria and other harmful substances (Mahmoud *et al.*, 2024). The long-term tracking and trending of *B. cereus* in meat products can provide information on any significant changes in their prevalence, including specific food types. Such information could provide an early indication of changing factors in the environment, food chain, and food handling, and potentially impact food safety practices and standards. This research looked into the incidence, enterotoxin genes, and antibacterial resistance patterns of *B. cereus* strains in some meat products in Aswan, Egypt, as well as the effectiveness of ACV as a natural preservative against *B. cereus*.

## Materials and methods

### Samples

Between March and May 2023, 180 meat product samples—20 samples each of minced beef, beef shawarma, beef burger, beef kofta, beef sausage, chicken

shawarma, chicken kofta, and chicken kabab—were collected from retail markets in the Aswan Governorate, Egypt. Every sample was stored under 4°C after being transferred to the lab from separate sterile bags in an icebox.

#### Enumeration, isolation, and identification of *B. cereus*

Every sample (25 g) was mixed with peptone water 0.1% (225 mL) (Oxoid, CM0009B) to make sequential dilutions and homogenized in a stomacher (Seward®400) (ISO 21871, 2006). One milliliter of the initial dilution was then placed on Mannitol Egg Yolk-Polymyxin agar (MYP) (Oxoid, CM09) and incubated for 24 h at 30°C. The colony counter was used to enumerate the lecithinase activity of a typical *B. cereus* colony, which was pink in color and surrounded by a precipitation zone. Then, a single colony was spread on chromogenic *B. cereus* agar plates (Huankai). Based on Quinn *et al.* (2002), various apparent colonies on chromogenic *B. cereus* agar plates were selected and incubated for 24 h at 37°C for additional biochemical characterization (Gram stain, starch hydrolysis, catalase, nitrate reduction, Voges–Proskauer, citrate utilization, lysozyme resistance, and anaerobic fermentation of glucose). In addition, the recognition of parasporal protein toxin crystal and rhizoid proliferation were conducted (Tallent *et al.*, 2012).

#### PCR for detection of *B. cereus* toxins profile

GeneJET™ Genomic DNA Purification Kit (Thermo Fisher, K0722) assisted in extracting genomic DNA from the positively identified *B. cereus* culture, and the DNA is preserved at –20°C. After using *gyrB* gene primers to identify the *B. cereus* genotype, multiplex PCR was employed to recognize eight virulence factors (*hblA*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, *cytK*, and *cesB*). The 25 µL PCR reaction consists of 12.5 µL COSMO PCR RED Master Mix, 1 µL each of reverse and forward primers (20 pmol), 6 µL DNA, and 4.5 µL free nuclease water. The amplification was conducted by the methods previously described (Ehling-Schulz *et al.*, 2005; Hansen and Hendriksen, 2001; Tewari *et al.*, 2015). Table 1 displays the thorough sequence of data used. The PCR product was analyzed by gel electrophoresis in a 1% agarose stained with SYBR SAFE (0.6 g/100 mL) at 100 V for 30 m (Elbarbary *et al.*, 2024) and captured using a UV LED (BioRad).

#### Examining the antibiotic sensitivity of *B. cereus* strains

The Kirby–Bauer disk diffusion susceptibility technique, as described by Khairy *et al.* (2024), was employed to

assess sensitivity to antimicrobials of all *B. cereus* isolates. Picked fresh isolate colonies were placed in 2 mL of sterile saline, combined, and then incubated at 37°C for 24 h. After that, the turbidity of the suspension was set by matching it to the 0.5 McFarland standard solution. On Muller-Hinton agar (MH) (Oxoid, CM0337), an immersed swab (HiMedia, PW009) from an inoculum tube was streaked three times. The antibacterial disks were put and distributed superficially on the MH agar with sterile forceps. After 24 h of incubation at 37°C, the inhibition area was finally assessed. The data were interpreted under Clinical Laboratory Standards Institute (CLSI 2017), and the strains were grouped as susceptible (S), intermediate (I), or resistant (R) following Magiorakos *et al.* (2012). Twenty antibiotics (Oxoid, UK) were tested, including ampicillin (AMP, 10 µg), quinupristin (QD, 15 mg), cefoxitin (FOX, 30 mg), cephalothin (kF, 30 µg), ciprofloxacin (CIP, 5 µg), cefotaxime (CTX, 30 µg), imipenem (IPM, 10 µg), vancomycin (VA, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25 µg/23.75 µg), chloramphenicol (C, 30 mg), nalidixic acid (NL, 30 µg), clindamycin (DA, 2 µg), doxycycline (DO, 30 µg), erythromycin (E, 15 µg), colistin (CT, 10 µg), tetracycline (TE, 30 mg), gentamicin (CN, 10 mg), nitrofurantoin (FD, 300 mg), rifampicin (RA, 30 µg), and kanamycin (K, 30 mg). The antimicrobial agents that were analyzed are frequently employed in the veterinary and health sectors of Egypt. For each antibiotic and isolate, the multiple antimicrobial resistance (MAR) indices were assessed. A MAR rate of < 0.2 shows that the isolates developed from a polluted source with a low risk. On the other hand, isolates with a MAR > 0.2 have been from high-risk sources of pollution (Lozano *et al.*, 2020).

#### *In vitro* assessment of antibacterial action of ACV

The ACV utilized in this investigation was obtained from Bragg Co., USA, via Amazon. eg. It was organic raw ACV, unfiltered, 5% acidic, unpasteurized, unheated, and had the amazing mother of vinegar.

The antibacterial effect of ACV was assessed using the agar well-diffusion technique against *B. cereus* isolates, as earlier reported by Balouiri *et al.* (2016). Using 0.9% sterile saline solution, the suspension turbidity of purified bacterial culture (10<sup>6</sup> CFU/mL) was under 0.5 McFarland. Subsequently, a sterile cotton swab was employed for spreading 100 µL of the sample onto Mueller-Hinton agar (Hi-Media) plates. Using a sterilized cork borer, holes (7 mm) were created in the plates. These were then filled with 100 µL of produced ACV of 10%, 30%, 70%, and 100%, and incubated at 37°C for 24 h. A negative control was recognized by sterile demineralized water, although a positive control was established via antibiotic discs (ampicillin, 10 µg). The inhibitory halo diameter was measured

Table 1. Oligonucleotide primer sequences used for PCR.

Primer	Primer sequence 5'- 3'	Annealing temp (°C)	Amplicon size (bp)	Reference
<i>gyrB</i>	F- TCATGAAGACCC TGTGTACG R- CGACGTGTCAATTC ACGCGC	63	475	Tewari et al. (2015)
<i>HblA</i>	F- GTGCAGATGTTGATGCCGAT R- ATGCCACTGCGTGGACATAT	55	320	
<i>HblC</i>	F- AATGGTCATCGGAACCTCTAT R- CTCGCTGTTCTGCTGTTAAT	55	750	
<i>HblD</i>	F- AATCAAGAGCTGTCACGAAT R- CACCAATTGACCATGCTAAT	55	430	Hansen and Hendriksen (2001)
<i>NheA</i>	F- TACGCTAAGGAGGGGCA R- GTTTTATTGCTTCATCGGCT	55	500	
<i>NheB</i>	F- CTATCAGCACTTATGGCAG R- ACTCCTAGCGGTGTTC	55	770	
<i>NheC</i>	F- CGGTAGTGATTGCTGGG R- CAGCATTCTGACTTGCCAA	55	583	
<i>cytK</i>	F- AAAATGTTTAGCATTATCCGCTGT R- ACCAGTTGTATTAATAACGGCAATC	55	238	
<i>cesB</i>	F- GGTGACACATTATCATATAAGGTG R- GTAAGCGAACCTGTCTGTAACAACA	53	1271	Ehling-Schulz et al. (2005)

using a gauge (mm). Assessments were taken in triplicate to establish the mean and standard deviations of the inhibition zone, which were determined. The strains were classified as resistant (0) for diameters < 8 mm, moderately sensitive (+) for 8–20 mm, sensitive (++) for 20–30 mm, and very sensitive (+++) for diameters >30 mm.

#### Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assessments

Following CLSI (2012) references, MIC and MBC were assessed via a standard broth microdilution procedure. Fresh Mueller-Hinton broth was employed to produce the bacterial suspensions for the experiment, with the concentration of bacteria adjusted to  $10^6$  CFU/mL. Twofold serial dilutions of ACV from the standard solution (1,016 µg/mL) were made in sterile distilled water. A volume of each ACV dilution (100 µL) was poured on U-shaped bottom, sterile polystyrene, 96-well culture plates (Techno Plastic Products, Switzerland). Every well got 100 µL of every bacterial suspension, kept at 37°C for 24 h. The MIC was found to be the smallest amount of antibacterial agent that completely stopped visual growth (CLSI, 2012). This means that there was no growth in the well that was related to the positive and negative growth wells. The MBC was recognized as the lowest dose that produced no observable growth following the incubation period (Andrews, 2001). The MBC was identified by subculturing 10 µL of the suspension from every well on MHA. The plates were subsequently left at 37°C for 24 h, or until growth was detected in the positive growth control. Every test was approved in triplicate, and the mean

± standard error of the mean was used to present the findings.

#### Statistical analysis

All of the data were examined using GraphPad Prism 9.0 under one-way analysis of variance (ANOVA). Outcomes were presented as mean ± SEM with a significance value of  $p < 0.05$ .

## Results

#### Occurrence of *B. cereus*

From Table 2, it is evident that the incidence of *B. cereus* counts in the studied samples was the highest in beef kofta samples ( $2.44 \times 10^3 \pm 0.16 \times 10^2$  CFU/g), followed by beef burger ( $2.02 \times 10^3 \pm 0.18 \times 10^2$  CFU/g) and beef sausage ( $1.88 \times 10^3 \pm 0.12 \times 10^2$  CFU/g), with no significant variations between them while there are significant differences observed between other samples. Chicken Kabab ( $0.17 \times 10^2 \pm 0.02 \times 10^2$  CFU/g) and chicken shawarma reported the lowest count ( $0.24 \times 10^2 \pm 0.01 \times 10^2$  CFU/g).

Of the 180 RTE samples evaluated, 54 (30%) samples have *B. cereus*. According to the findings, the incidence of *B. cereus* was 45% in beef kofta and beef burgers; 35% in minced beef, beef sausage, and chicken burgers; 25% in beef shawarma; and 10% in chicken shawarma and chicken kabab (Figure 1). *B. cereus* was recognized on

**Table 2.** The mean values of *Bacillus cereus* count (CFU/g) in the examined samples (n = 20 each).

Sample	Min	Max	Mean±SE
Minced beef	0.36×10	6.42×10 <sup>3</sup>	0.86×10 <sup>2</sup> ±0.13×10 <sup>2b</sup>
Beef kofta	0.88×10	8.62×10 <sup>4</sup>	2.44×10 <sup>3</sup> ±0.16×10 <sup>2a</sup>
Beef burger	0.67×10	7.58×10 <sup>4</sup>	2.02×10 <sup>3</sup> ±0.18×10 <sup>2a</sup>
Beef shawarma	0.074×10	2.76×10 <sup>2</sup>	0.47×10 <sup>2</sup> ±0.05×10 <sup>2c</sup>
Beef sausage	0.64×10	4.33×10 <sup>4</sup>	1.88×10 <sup>3</sup> ±0.12×10 <sup>2a</sup>
Chicken shawarma	0.058×10	0.78×10 <sup>2</sup>	0.17×10 <sup>2</sup> ±0.01×10 <sup>2c</sup>
Chicken kofta	0.48×10	5.63×10 <sup>2</sup>	1.52×10 <sup>2</sup> ±0.11×10 <sup>2b</sup>
Chicken burger	0.73×10	7.48×10 <sup>3</sup>	1.72×10 <sup>2</sup> ±0.16×10 <sup>2b</sup>
Chicken Kabab	0.033×10	0.43×10 <sup>2</sup>	0.24×10 <sup>2</sup> ±0.02×10 <sup>2c</sup>

Data followed by different superscript letters (a–c) is significant at  $p < 0.05$ .

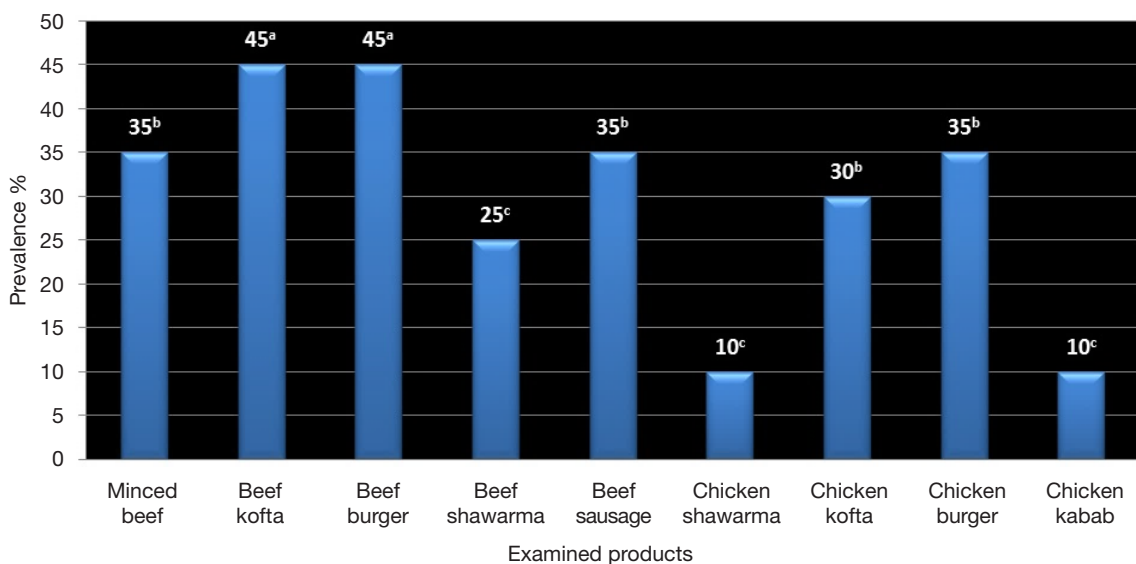
special agar plates by their distinct wavy colony shapes. Standard *B. cereus* colonies appear pink in color and have a surrounding area that shows precipitation, which indicates lecithinase action, and they do not ferment mannitol (this shows a positive Nagler response). Tests showed that *B. cereus* samples had mobility and were able to use citrate, produce Voges-Proskauer, have catalase activity, ferment glucose, and break down gelatin. Similarly, the isolates were negative for oxidase, H<sub>2</sub>S generation, methyl red, and indole. The samples did not have any protein crystals from *Bacillus thuringiensis* after being stained with carbol fuchsin using the Ziehl–Neelsen method.

*Toxins profile of B. cereus by multiplex PCR*

Following phenotypically suspicious isolates of *B. cereus*, PCR analysis—relying on the identification of a specific species—*gyrB*—was then performed. On agarose gel, all isolates generated 475 bp PCR products that were specifically *B. cereus* (Figure 2). The toxins’ profile established in this research using multiplex PCR and its distribution was assessed and categorized in Figure 3. The virulence genes of *B. cereus* are categorized into two groups based on their pathogenic properties: enterotoxin genes (*hblA*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, and *cytK*) and cereulide synthetase genes (*cesB*). In hemolysin enterotoxin genes, the *hblA* gene occurs in most of the strains (16/54) and was considerably higher than *hblC* (7/54) and *hblD* (5/54). Nonhemolytic enterotoxin, *nheA*, was detected in 10/54 and was significantly higher than *nheB* (5/54) and *nheC* (3/54). Cytotoxin K (*cytK*) was detected in 10/54 of the strains. However, only 6 out of 54 samples tested positive for the cereulide synthetase gene (*cesB*), suggesting that emetic bacteria are less abundant in meat products compared to diarrheal ones (Figures 3 and 4). The incidence of toxigenic factors in the obtained strains showed statistically a notable variation ( $p < 0.05$ ).

*Antibiogram profile of B. cereus*

Every *B. cereus* isolate underwent testing for antibacterial sensitivity to 20 chosen antibiotics. Tables 3 and 4 displayed that most of the isolates were resistant to ampicillin, cefoxitin, and colistin (100% each), while being completely susceptible to imipenem, nalidixic acid, and



**Figure 1.** Prevalence of *Bacillus cereus* in the examined products. Data with different superscript letters (a–c) are significant at  $p < 0.05$ .

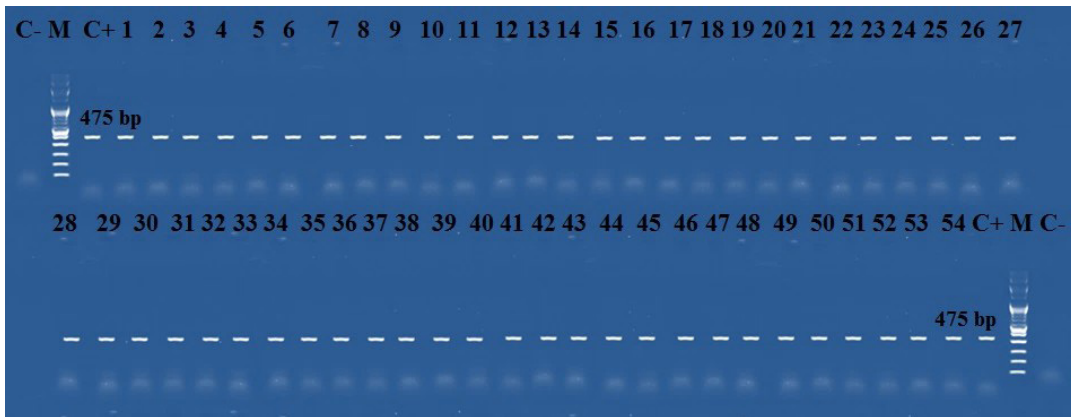


Figure 2. Electrophoretic profile of amplification products of the confirmed *gyrB* *B. cereus* gene at 475 bp. Lanes 1–7: minced beef, lanes 8–16: beef kofta, lanes 17–25: beef burger, lanes 26–30: beef shawarma, lanes 31–37: beef sausage, lanes 38–39: chicken shawarma, lanes 40–45: chicken kofta, lanes 46–52: chicken burger, and lanes 53–54: chicken Kabab. M: marker (50 bp), C+: positive control, C-: negative control.

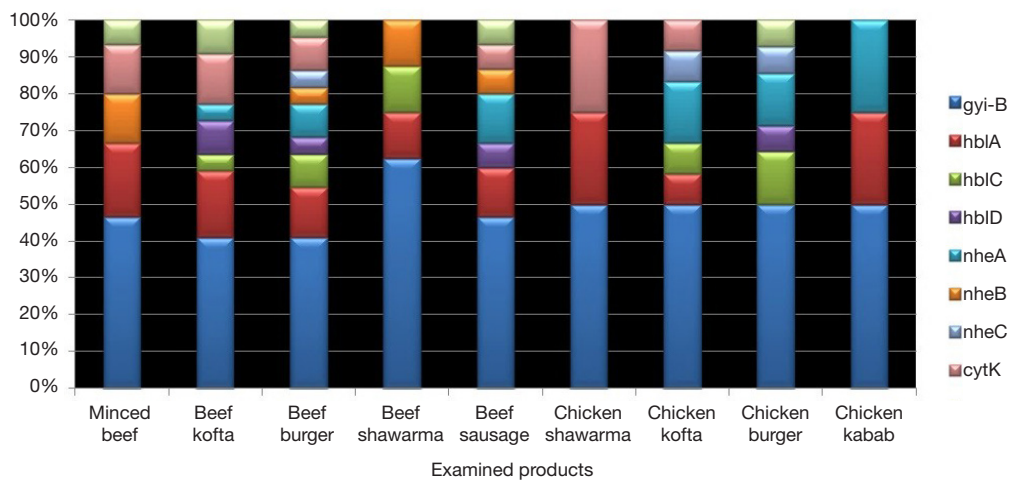


Figure 3. Prevalence of virulence enterotoxin and emetic genes of *Bacillus cereus* isolates. There is a significant variance at  $p < 0.0001$ .

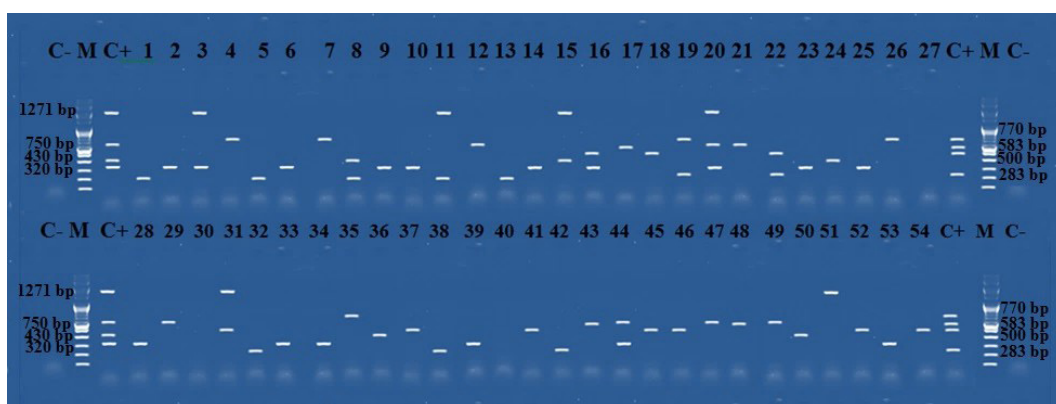


Figure 4. Electrophoretic profile of amplification products of enterotoxin and emetic genes in *Bacillus cereus*: *hblA* at 320 bp, *hblC* at 750bp, *hblD* at 430bp, *nheA* at 500 bp, *nheB* at 770 bp, *nheC* at 583bp, *cytK* at 238bp, and *cesB* at 1271bp. Lanes 1–7: minced beef, lanes 8–16: beef kofta, lanes 17–25: beef burger, lanes 26–30: beef shawarma, lanes 31–37: beef sausage, lanes 38–39: chicken shawarma, lanes 40–45: chicken kofta, lanes 46–52: chicken burger, and lanes 53–54: chicken Kabab. M: marker (50 bp), C+: positive control, C-: negative control.

**Table 3.** The interpretation of antimicrobial resistance of *Bacillus cereus* isolates (n = 54).

Antimicrobial agents	Sensitive		Intermediate		Resistance	
	No.	%	No.	%	No.	%
AMP	0	0	0	0	54	100
kF	7	13	0	0	47	87
QD	29	53.7	5	9.3	24	44.4
FOX	0	0	0	0	54	100
CTX	11	20.4	4	7.4	39	72.2
IPM	54	100	0	0	0	0
NL	54	100	0	0	0	0
CIP	23	42.6	6	11.1	25	46.3
SXT	7	13	7	13	40	74.1
DO	17	31.5	9	16.7	28	51.8
E	12	22.2	5	9.3	37	68.5
DA	41	76	0	0	13	24
VA	54	100	0	0	0	0
CT	0	0	0	0	45	100
TE	27	50	5	9.3	23	42.6
C	30	55.6	3	5.6	21	38.8
FD	31	57.4	7	13	16	29.6
RA	28	51.8	0	0	26	48.1
GN	36	66.7	11	20.4	7	13
K	42	77.8	0	0	12	7.6
p value	p < 0.0014		p < 0.0001		p < 0.0001	

AMP: ampicillin, kF: cephalothin, QD: quinupristin, FOX: cefoxitin, CTX: cefotaxime, IPM: imipenem, NL: nalidixic acid, CIP: ciprofloxacin, SXT: trimethoprim-sulfamethoxazole, DO: doxycycline, E: erythromycin, DA: clindamycin, VA: vancomycin, CT: colistin, TE: tetracycline, C: chloramphenicol, FD: nitrofurantoin, RA: rifampicin, GN: gentamicin, and K: kanamycin.

vancomycin, making them the most significant antibiotics. The *B. cereus* strain showed multidrug resistance (resistance to at least three types of antibiotics) between 0.15 and 0.85, with an average of 0.517. The investigated *B. cereus* strains showed statistically a notable variation in their sensitivity to different antibacterials ( $p < 0.05$ ).

*Antimicrobial action of ACV against B. cereus*

By the agar well diffusion experiment, all ACV doses (10%, 30%, 70%, and 100%) confirmed substantial inhibitory influence against *B. cereus* isolates, as shown in Table 5. More research was done on the inhibition zone ranges (mm) of the several antibiotics employed in the current investigation at their concentrations. The inhibitory zone diameter was between 11.5±0.6 and 16.4±0.3 mm (10% ACV), 13.6±0.4 and 19.4±0.8 mm (30% ACV), 17.2±0.2 and 24.7±0.5 mm (70% ACV), and 19.8±0.6 and 31.6±0.8 mm (100% ACV). Using 15 *B. cereus* isolates,

**Table 4.** Antibiogram profile of *Bacillus cereus* isolates (n = 54).

Isolates No.	Antimicrobial resistance profile	No. of antibiotics	MAR index
16	AMP, FOX, CT, kF, CTX, QD, CIP, SXT, DO, E, DA, C, TE, FD, RA, GN, K	17	0.85
13	AMP, FOX, CT, CTX, QD, E, CIP, DA, C, TE, FD, RA, GN, K	14	0.70
11	AMP, FOX, CT, CTX, kF, CIP, SXT, DO, E, DA, RA, K	12	0.60
8	AMP, FOX, CT, CTX, CIP, FD, RA, GN, K	9	0.45
4	AMP, FOX, CT, SXT, DO, TE, FD	7	0.35
2	AMP, FOX, CT,	3	0.15
54	MAR average		0.517

MAR: multiple antibiotic resistant, AMP: ampicillin, kF: cephalothin, QD: quinupristin, FOX: cefoxitin, CTX: cefotaxime, IPM: imipenem, NL: nalidixic acid, CIP: ciprofloxacin, SXT: trimethoprim-sulfamethoxazole, DO: doxycycline, E: erythromycin, DA: clindamycin, VA: vancomycin, CT: colistin, TE: tetracycline, C: chloramphenicol, FD: nitrofurantoin, RA: rifampicin, GN: gentamicin, and K: kanamycin.

MIC and MBC were inevitably carried out to ascertain exactly the antibacterial qualities of ACV. The MIC data revealed that ACV had high antibacterial action contrary to the tested isolates, with MICs ranging from 0.14 to 1.25 mg/mL. In the context of the AVC as a bacteriocidal agent, certain isolates that were investigated exhibited an MBC rate similar to the MIC rate.

**Discussion**

*B. cereus* is one of the most prevalent foodborne bacteria, causing serious food poisoning. The condition is primarily characterized by vomiting, diarrhea, liver failure, necrotic enteritis, and abdominal discomfort. Furthermore, *B. cereus* is commonly described to be the most common bacteria present in a variety of meat products. It is considered a public health concern (Algammal *et al.*, 2024) following ingestion of a contaminated meal that has more than 10<sup>4</sup>–10<sup>5</sup> *B. cereus* spores or vegetative cells/g (Gao *et al.*, 2018). The study found that there was a potential danger of *B. cereus*, with counts ranging from 0.17×10<sup>2</sup> ± 0.02×10<sup>2</sup> in chicken kabab to 2.44×10<sup>2</sup> ± 0.16×10<sup>2</sup> in beef kofta. Alarmingly, *B. cereus* counts in certain of the investigated samples (beef kofta, beef burger, and beef sausage) surpassed the allowed limits (<10<sup>3</sup> CFU/g) indicated by the Health Protection Agency (2009) in England. Furthermore, Stenfors *et al.* (2008) verified that low *B. cereus* levels in food could cause major occurrences of food poisoning among consumers. In addition, the product is not appropriate for human consumption if the count of *B. cereus* exceeds 10<sup>4</sup> CFU/g or mL (FSANZ, 2001). In Hong Kong, ready-to-eat foods

Table 5. Inhibitory zone diameters, MIC, and MBC of ACV against *Bacillus cereus* isolates ( $n = 15$ ).

Isolate no.	Zone diameter (mm) against various ACV concentrations (%)				MIC (mg/mL)	MBC (mg/mL)
	10	30	70	100		
1	14.3±0.7	16.4±0.2	19.5±0.2	22.7±0.1	0.14	0.28
2	12.5±0.3	13.6±0.4	17.2±0.2	20.4±0.2	0.16	0.32
3	15.2±0.6	18.2±0.6	21.7±0.2	26.5±0.6	0.16	0.32
4	14.8±0.3	18.2±0.5	22.6±0.3	28.4±0.2	0.14	0.14
5	11.5±0.6	14.3±0.5	18.5±0.3	21.7±0.3	0.16	0.64
6	12.7±0.3	14.9±0.3	20.0±0.4	24.3±0.6	0.14	0.28
7	16.2±0.7	19.4±0.8	24.7±0.5	31.2±0.8	1.25	2.5
8	12.9±0.3	15.5±0.6	19.5±0.4	25.7±0.3	0.14	0.28
9	15.4±0.8	19.2±0.3	24.3±0.7	30.5±0.7	0.14	0.56
10	15.8±0.3	16.7±0.3	18.5±0.3	19.8±0.6	0.16	0.64
11	13.7±0.4	17.4±0.6	18.7±0.4	21.5±0.4	0.14	0.14
12	16.4±0.3	18.6±0.4	22.5±0.2	29.7±0.3	0.14	0.28
13	14.4±0.2	17.8±0.3	23.3±0.7	31.6±0.8	0.56	0.56
14	15.2±0.6	19.2±0.7	23.5±0.4	31.4±0.7	0.16	0.64
15	12.8±0.5	16.6±0.3	20.4±0.3	28.5±0.8	1.25	2.5

ACV: Apple cider vinegar, MIC: Minimal inhibitory concentration, MBC: minimal bactericidal concentration.

are sorted into three groups based on the amount of *B. cereus* they contain: satisfactory ( $<10^3$  CFU/g), acceptable ( $10^3$ – $10^5$  CFU/g), and unsatisfactory ( $>10^5$  CFU/g). It is illegal to sell “unsatisfactory” ready-to-eat foods (Centre for Food Safety, 2014). Therefore, regulations, directives, and decisions represent the main regulatory acts applicable to veterinary, sanitary, and food safety for the protection of consumers. All regulations are centered on the protection of the agro-alimentary line, food safety, and the protection of consumer interests. This is one of the main reasons why the involvement of civil society and consumers in debating and passing veterinary, sanitary, and food safety legislation, particularly food-related legislation, is increasingly obvious (Bondoc, 2016a, b).

Consequently, it is imperative to maintain appropriate temperature control, even throughout food preparation. Cold foodstuffs must be preserved at a temperature below 4°C, while hot foods must be preserved at a temperature above 60°C to prevent food with *B. cereus* (Mostafa et al., 2022). All of the isolates (30%) that were obtained for this study showed the distinctive phenotypic characteristics of *B. cereus* and showed agreement in their biochemical reactions. The higher occurrence of beef kofta and beef burgers compared to chicken kabab and chicken shawarma may be related to the preparation methods used for each type of meat and the inclusion of intestinal parts. In addition, adding spices and vegetables to meat may enhance the risk of *B. cereus* contamination and serve as another cause of contamination (Shawish and Al-Humam, 2016). Our findings matched those informed

by Bashir et al. (2017), Tewari et al. (2015), and Yu et al. (2020), with respective percentages of 29.3%, 30.9%, and 35%. Higher ratios of *B. cereus* from meat products were noted by Abd El Tawab et al. (2015), Hwang and Park (2015), and Owusu-Kwarteng et al. (2017), who reported 38.3%, 47%, and 50.5%, respectively. Low percentages were recorded by Algammal et al. (2024), Amin and Tawfik (2021), Mahmoud et al. (2024), and Mostafa et al. (2022), who found *B. cereus* in 21%, 24%, 22.7%, and 11.1%, respectively, of the examined samples. In addition, common risk factors that contribute to the spread of *B. cereus* foodborne poisoning include ambient pollution, improper food temperature processing, and improper cleaning of food production equipment and preparation surfaces (Yu et al., 2020).

*B. cereus* has been linked to meat additives such as rice and flour that are used in the production of meat products (Giffel et al., 1996). Meat products were most likely contaminated during handling and preparation or after they had been processed. Furthermore, leaving the items out of the refrigerator for many hours promotes *B. cereus* proliferation and thus enterotoxin release (Shawish and Tarabees, 2017). Moreover, incorrect management of meat products next to cooking permits *B. cereus* spores to produce vegetative cells that grow and cause food poisoning (Hassan et al., 2019). Furthermore, additives, seasonings, and spices are added, which are regarded as a potential hazard since they increase the quantity of *Bacillus* spores and thus increase the chance of food illness (Shawish and Tarabees, 2017).

Molecular approaches are more precise for making conclusive identifications. As shown in Figures 2 and 3, the housekeeping gene *gyrB* of *B. cereus*, a molecular diagnostic marker, was positive in all detected phenotypic isolates of *B. cereus*. The public health significance of *B. cereus* strains as a reason for severe food illness in humans is highlighted by the fact that all of the strains obtained in this research inherited one or more enterotoxigenic genes. This matches the findings of Algammal *et al.* (2024), Amin and Tawfick, (2021), Fracalvieri *et al.* (2022), Mahmoud *et al.* (2024), Owusu-Kwarteng *et al.* (2017), and Tewari *et al.* (2015). The pathogenicity is primarily supported by numerous virulence factors and toxins expressed by the appropriate genes. The consumption of *B. cereus*-polluted food causes illness. *B. cereus* cells adhere to the human intestinal mucosa, colonize, and produce enterotoxins (Algammal *et al.*, 2024). The primary virulence determinants associated with food poisoning produced by *B. cereus* are the *nheABC*, *hblABCD*, *cytK*, and *cesB* genes (Berthold-Pluta *et al.*, 2019). Many foodborne *B. cereus* outbreaks have been identified globally, and the sickness manifests in emetic and diarrheal forms. Cytotoxin K, a powerful heat-labile enterotoxin, is regarded as the primary virulence factor implicated in severe diarrhea, while emetic sickness is credited primarily to the cereulide toxin (ECDC, 2019). Furthermore, *B. cereus* has been linked to serious human diseases such as pneumonia, neonatal bacteremia, gas gangrene, bacterial meningitis, and ocular infections (Algammal *et al.*, 2024).

Antibiotic therapy is the principal management for *B. cereus* infection. However, the failure of antibacterial treatment occurs from the development of antibacterial-resistant *B. cereus* strains, mostly from drug misuse or the gaining of resistance genes by horizontal gene transfer (Gao *et al.*, 2018). Thus, the detection of the antibacterial resistance outline of *B. cereus* is of paramount importance to public well-being. In this investigation, imipenem, nalidixic acid, and vancomycin showed significant antibacterial activity against *B. cereus* strains from the various items tested. Furthermore, the retrieved strains were the consequence of high-risk contamination, as shown by the concerning MAR value of 0.517 (>0.2) (Qenawy *et al.*, 2024). These outcomes coincide with those validated by Algammal *et al.* (2024) and Ikeda *et al.* (2015), who found that every acquired *B. cereus* strain was quite sensitive to vancomycin. In addition, the *B. cereus* isolates that were obtained were entirely resistant to ampicillin, cefoxitin, and colistin, and they exhibited exceptional resistance to almost all of the antibiotics that underwent testing, involving erythromycin, trimethoprim-sulfamethoxazole, doxycycline, and cefotaxime. Our results complement those emphasized by Mahmoud *et al.* (2024), Savić *et al.* (2016), and Yu *et al.* (2020). According to these findings, multidrug-resistant (MDR) *B. cereus* has been occurring in a diversity of

meat products, indicating that it may be a major way for human consumers to contract foodborne MDR *B. cereus* (Bhunia, 2018). According to Algammal *et al.* (2024), the careless application of antibiotics in the medical and agricultural fields and the general public, as well as the ability to obtain medicines without a prescription and use them recklessly, promotes the emergence of MDR strains.

Various food preservation methods, particularly chemical antimicrobial agents, have long been applied industrially to stop the growth of bacteria in food products (Tropea, 2022), thereby improving the safety and extending the shelf life of the products. The scientific community and food companies have been motivated to look for efficient substitutes for the chemical antibacterial agents frequently employed in food preservation in recent years because of the growing understanding of the impact of diet on human well-being. Consumers are indeed skeptical of the use of these compounds, despite their stringent regulation, as a result of their potential long-term health risks (Primavilla *et al.*, 2023). ACV is a fermented product that is categorized as a functional food because of its constituents and nutrients, including vitamins and minerals, as well as its ability to improve its production characteristics and have an inhibitory impact on a variety of bacteria by stopping the movement of nutrients via their cell membrane (Nady *et al.*, 2024). The finding of this research displayed that ACV showed significant inhibitory effects against *B. cereus* at varying concentrations, as evidenced by the inhibition zone diameter determined by the agar well diffusion test. The zone diameter was between 11.5±0.6 and 16.4±0.3 mm (10% ACV), 13.6±0.4 and 19.4±0.8 mm (30% ACV), 17.2±0.2 and 24.7±0.5 mm (70% ACV), and 19.8±0.6 and 31.6±0.8 mm (100% ACV). Herein, with MIC ranging from 0.14 to 1.25 mg/mL, the MIC data revealed that ACV showed high antibacterial activity against the investigated isolates. Considering the ACV to be a bacteriocidal agent, some of the isolates under examination displayed MBC values at the same MIC value. These results matched those of earlier investigations by Gaber *et al.* (2020), Mahmoud *et al.* (2024), and Yagnik *et al.* (2021). The present findings are indicative of the efficacy of ACV as a natural preservative, as it holds a variety of active components, including antibacterial antagonists, as well as organic acids like malic acid, acetic acid, and phenolic mixtures like cresols, phenol, and ketone constituents (Nady *et al.*, 2024). Organic acids work to stop bacteria from growing in several ways, such as by destroying the bacteria's outer membrane, consuming the energy of the microbes, and increasing osmotic pressure, which breaks down the cell membrane and encourages the manufacture of antibacterial peptides in the host cells. This force the host cells to discharge many vital nutrients, like glutamic and acid ions, to equilibrate the osmotic pressure inside the cells, which stops bacteria from growing normally (Al-Hadidy

et al., 2023). Minimizing the application of extra ingredients in organic manufacturing is often a substantial technological challenge, but it is being promoted by the usage of ACV in organic meat handling.

## Conclusion

According to this investigation, the presence of MDR *B. cereus* that harbored one or more enterotoxin genes in meat products poses a significant threat to public health. As a result, stricter sanitation regulations must be implemented at all production, handling, and storage stages. Furthermore, employing ACV as a natural antibacterial agent may be a useful way to reduce the risk of *B. cereus* illness and its occurrence in the food sector, whether in public places or at home, suggesting that it could be a useful natural substitute for traditional preservatives. Future research must assess potential uses in food production for a sustainable strategy to safeguard the health of customers. Testing these substances against bacteria resistant to several drugs is crucial for developing several approaches to handle the rising issue of drug resistance. Furthermore, ACV derivatives can be used as preservatives in several industries, including healthcare (e.g., cosmetics and medications).

## Ethics statement

The Scientific Research Ethics Committee, Aswan University, Faculty of Veterinary Medicine (Approval No.: 15-02-2023) approved all the tests and procedures.

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## Data availability statement

The entire data have been offered in the publication

## Authors contributions

Nady Elbarbary, Mohamed Dandrawy, and Maha Abdelhaseib were in charge of conceptualization,

data curation, validation, and methodology. Nasreddin Rhouma and Mostafa Abdelhafeez did formal analysis and investigation. Mounir Bekhit, Ahmed Ezzat, and Wageh Darwish were responsible for investigation, visualization, and supervision. Nady Elbarbary, Layla Al mutairi, Sohaila El-Hawary, and Amin Al-Doaiss were responsible for writing – original draft, revision, and editing of the paper. The authors shared evenly and approved the whole manuscript.

## Conflicts of Interest

The authors' interests do not conflict with one another.

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