

A One Health Perspective on Bacillus Cereus in Animal-Origin Food Chains

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Abstract

The most common foodborne pathogen that have important role in the food chains of the animal origin is *Bacillus cereus*. Because numerous presumptive isolates of the *Bacillus* do not accurately represent true *B. cereus*, relying solely on culture-based identification can be misleading. The aim of this study was to make a clear assessment of its true prevalence via molecular confirmation, while also investigating the distribution of the *hblA* and *blaZ* genes among that verified isolates. These samples were recently obtained from workers, environmental sources, and poultry in the city of the Al-Qadisiyah, Iraq. To achieve this, a total of the 200 samples were initially cultivated by researchers on nutrient agar and Mannitol Egg Yolk Polymyxin (MYP). After this, we personally selected 40 presumptive isolates and amplified them using PCR targeting the 16S rRNA gene (594 bp) for the precise identification. The presence of the resistance and virulence-associated genes was then checked exclusively in the confirmed *B. cereus* isolates. We evaluated their phenotypic resistance to ampicillin using the disk diffusion method. Molecular confirmation ultimately verified that merely 15 (37.5%) of the 40 isolates initially recognized as presumptive *Bacillus* via phenotypic evaluation were truly *B. cereus*. Ampicillin resistance was observed in the all confirmed isolates that contain *hblA* and *blaZ* genes. With 100% of the all tested isolates confirmed, the rates of the confirmation peaked in the workers' hand swabs and washing water. A uniformly distributed presence was indicated because there was no difference across various sample sources according to the statistical analysis ($P > 0.05$). An overestimation of the *B. cereus* prevalence is the main cause of the limitations of the culture-based techniques, as demonstrated by these findings. From a public health perspective, the fact that the verified isolates were uniformly toxigenic and ampicillin-resistant is deeply concerning. The important need for the harmonized surveillance approaches that depending on one Health, specifically within poultry production systems, is highlighted by several observations such as this.

Keywords: antimicrobial resistance; *Bacillus cereus*; One Health; virulence genes.

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Introduction:

Both global and local agriculture heavily produce poultry, making it one of the most vital products. Economic growth and food security are underpinned by this low-cost, high-quality source of the animal protein, especially in the low- and middle-income countries, that have important implications for the global food systems (Food and Agricultural Organisation (FAO) 2013; Mottet and Tempio 2017). It is expected by experts that no fewer than 9.7 billion people will populate the planet by 2050. That cause a 45% rise in the poultry product consumption (United Nations, 2019).

Critical concerns regarding antimicrobial use, public health, and food safety have been raised by the rapid expansion of the poultry production, despite these advantages. Scientists recognize *Bacillus cereus* as an important foodborne pathogen. That may be because of the its ability to form highly resistant spores and produce enterotoxins such as the non-hemolytic enterotoxin (Nhe), enterotoxin bceT, and hemolytic enterotoxin (Hbl) (Ehling-Schulz et al., 2019; Carroll et al., 2022; Stenfors Arnesen et al., 2008).

Diarrheal food poisoning is primarily associated with these toxins. That depending on their level of the expression and mode of the action (Jessberger et al., 2020). Recently, growth has been witnessed by the poultry industry in the Iraq, with several hundred thousand tons now being reached by annual production. While this expansion supports economic activity and food supply, pressing challenges have been simultaneously introduced by it. There are many broader public health risks, food safety issues, and antimicrobial usage concerns that refer to this growth. Increasing attention has been gained by *Bacillus cereus* as a significant foodborne microorganism among the pathogens of the concern. Its pathogenic potential is substantially contributed to by its capacity to form highly resilient spores, coupled with its ability to produce a range of the enterotoxins. The non-hemolytic enterotoxin (Nhe), enterotoxin bceT, and hemolytic enterotoxin (Hbl) form these toxins that referred by many previous studies (Ehling-Schulz et al., 2019; Carroll et al., 2022; Stenfors Arnesen et al., 2008).

These toxins are commonly linked to diarrheal foodborne illness. Also, the severity and manifestation of the symptoms dictate by levels of their mechanisms of the action and expression (Jessberger et al., 2020). Another complicates the situation of strains further is the growing emergence of the multidrug-resistant (MDR). bla and tet genes is considered important cause that increase in the antimicrobial resistance. Another determinants elements are often associated such as mobile genetics with these. That can help their spread across bacterial populations (Rossi et al., 2018; Park et al., 2018). When they use antibiotics indiscriminately in the animal production systems usually farmers intensify the selective pressure, particularly without firm veterinary supervision. That couse resistance development and dissemination, as results elevated risk to public health (WHO, 2021; Mulchandani et al., 2023).

Environmental pathways also play an important role in widespread contamination that can be caused by poultry waste improper handling and disposal. Then, the resistant bacteria and their associated genes by these practices effectively turned into a persistent reservoir by environment. Recognition as a comprehensive and the practical framework has been gained by one Health approach within the this complex landscape. A more integrated response to the antimicrobial resistance is offered by it. That depending on linking with human, animal, and the environmental health to establish a strong relationship, antimicrobial resistance has been classified as a major global priority by World Health Organization, reflecting urgency of the issue (WHO, 2021; WHO, 2023).

Food and Agriculture Organization Strategic introduced action plans that aimed at encouraging the responsible and the sustainable use of the antimicrobials in the animal of production systems (FAO, 2023), international standards for the prudent use of the antimicrobials in the veterinary medicine have been established by World Organisation for the Animal Health (WOAH, 2023).By adopting an integrated strategy the enhancement of the biosecurity protocols, the optimization of the surveillance systems, and the strict regulation of the antimicrobial usage are necessitated. Probiotics and essential oils also encourage natural interventions by researchers (Gadde et al., 2017; Callaway et al., 2021). The aim of current study is to examine the prevalence, virulence determinants, and antimicrobial resistance profiles

of the *Bacillus cereus* across animal-derived food chains.

Materials and Methods:

Sample Collection:

Randomly collected 200 samples from Al-Qadisiyah Province, Iraq, in the September 2025. There are many sources that classified to three groups. Poultry (n = 125), environmental items (n = 50), and workers' hand swabs (n = 25) were comprised by these specimens. For the example, the poultry portions that consist of the liver, lungs, cecum, thigh muscle, and intestines. Also, washing water, drinking water, and cutting tool swabs made up the environmental group. The team aseptically gathered and transported all items under refrigerated conditions. After that, the Microbiology Laboratory at the College of the Veterinary Medicine, University of the Al-Qadisiyah, processed them to illustrate the important results.

Isolation and Phenotypic Identification:

The specimens were inoculated into nutrient agar to make clear observations. Good growth was exhibited by *Bacillus cereus* isolates, forming structures that consist of the irregular margins and dull surfaces. There are many large and spreading colonies. After that, the team streaked these cultures onto Mannitol Egg Yolk Polymyxin (MYP) agar. We identified presumptive *Bacillus cereus* as pink to red colonies. That may be because lecithinase activity is the main cause of the precipitation zone surrounding them. Then, researchers subcultured typical colonies to obtain pure isolates. The VITEK® 2 Compact system (bioMérieux, France) confirmed the phenotypic identification. Also, BCL cards specific for the Gram-positive bacteria were utilized. These cards perform 64 automated biochemical tests to illustrate the important profile of the isolates.

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing was performed. That depending on the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to CLSI (2018) guidelines. Researchers prepared a bacterial suspension adjusted to a 0.5 McFarland standard. Then, they uniformly inoculated it onto the agar surface. To make the distribution even. There are many antibiotic disks such as Ampicillin (25 µg) and Erythromycin (10 µg) that we utilized. Also, the remaining disks form from Tetracycline (10 µg), Vancomycin (30 µg), Cefotaxime (30 µg), and Ofloxacin (5 µg). After the incubation of the plates at 37°C for 24 hours, we measured the inhibition zone diameters in the millimeters.

Molecular Detection:

Genomic DNA Extraction:

According to the manufacturer's instructions, researchers extracted the genomic DNA using the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). First, we transferred 1 mL of the overnight bacterial culture ($\sim 1 \times 10^9$ cells) into a 1.5 mL microcentrifuge tube. There are many cells that called bacteria in the this volume. Then, we centrifuged the mixture at 10,000 rpm for 1 minute. After that, we discarded the supernatant. We resuspended the pellet in the 200 µL of the lysozyme buffer and 180 µL of the GT buffer. To make the suspension uniform, we vortexed it. We incubated the tubes at 60°C for 10 minutes with intermittent mixing. The important step is this. Next, we added 200 µL of the GB buffer, vortexed it, and incubated it again at 60°C for 10 minutes. We immediately mixed 200 µL of the absolute ethanol into the sample. All this is necessary. We transferred the mixture to a GD column. We centrifuged it at 10,000 rpm for 1 minute. Also, we washed the column with 400 µL of the W1 buffer and 600 µL of the wash buffer. That cause the removal of the impurities. We dried the column matrix by centrifuging it at 12,000 rpm for 2 minutes. Recently, we eluted the DNA with 100 µL of the preheated elution buffer. Then, centrifuged the final product at 10,000 rpm for 1 minute to collect the extract.

Assessment of the DNA Purity and Concentration:

The DNA concentration was determined in the (ng/µL) and its purity (A260/A280 ratio) using a

NanoDrop spectrophotometer (Thermo Scientific NanoDrop Lite, USA). The team calibrated the instrument using nuclease-free water. Then, we analyzed 1 μ L of the each DNA sample. All this was done to make highly precise measurements about the extracts.

Primers for the Identification and Virulence Genes:

For molecular confirmation of the *Bacillus cereus*, PCR amplification targeting the 16S rRNA gene (GenBank accession no. NR_074540.1). The PCR primers for the detection of the *Bacillus cereus* based on 16S ribosomal RNA gene and antibiotic resistance genes were designed in the this study using NCBI-Genbank database and primer3 plus designer server. The virulence factor and enterotoxin genes primers were designed by (Hansen et al., 2001). These provided by ScientificResercher.Co.Ltd. Ltd in the Iraq as following table (1):

Primer	Sequence (5'-3')		Product Size	Genbank surce
16S rRNA gene	F	CATGCAAGTCGAGCGAATGG	594bp	NR_074540.1
	R	CAATGACCCTCCACGGTTGA		
hblA gene	F	GTGCAGATGTTGATGCCGAT	320 bp	Hansen et al., 2001
	R	ATGCCACTGCGTGGACATAT		
blaz	F	AAGTTAGGTGGTCCATCCGC	570 bp	M15195.1
	R	CCCCCACTGAAACGCTTCAT		

Additionally, seven virulence genes (bceT, hblA, hblD, hblC, nheA, nheB, and nheC) were detected using primers described by (Hansen *et al.* 2001).

PCR Master Mix and Thermocycling Conditions:

PCR reactions were carried out in the a total volume of the 25 μ L containing:

PCR Master mix	Volume
DNA template 5-50ng	5 μ L
Forward primer (10pmol)	2 μ L
Reveres primer (10pmol)	2 μ L
GoTaq ®Green PCR master	25 μ L
PCR water	3.5 μ L
Total volume	25 μ L

Amplification was performed using a T100 Thermal Cycler (Bio-Rad, USA) under the following conditions:

PCR step	Temp.	Time	repeat
Initial Denaturation	95°C	5min	1
Denaturation	95°C	30sec.	35 cycle
Annealing	58°C	30sec	
Extension	72°C	1 min	
Final extension	72°C	5min	1
Hold	4°C	Stop	-

Agarose Gel Electrophoresis:

PCR products were analyzed by electrophoresis on a 1–2% agarose gel prepared in the 0.5 $\times$ TBE buffer and stained with ethidium bromide (0.5 μ g/mL). A volume of the 10 μ L of the each PCR product was mixed with loading dye and loaded into the wells.

Electrophoresis was performed at 90 V for the 60 min. A 100–2000 bp DNA ladder (Bioneer, Korea) was used as a molecular marker. Bands were visualized under UV light using a transilluminator (Wised, Korea) and photographed with a digital camera.

4. Results:

4.1. Cultural Characteristics on Nutrient Agar and MYP Agar:

Robust growth was exhibited by *Bacillus cereus* isolates on nutrient agar, and large widely spreading colonies were produced by them. A distinctive outline was given to each colony by irregular margins, while a dull surface was presented. There are many dry to slightly granular textures. The characteristic “ground-glass” appearance was clearly displayed by the colonies under close observation. The important preliminary identification of the bacterium under non-selective conditions was enabled by these morphological traits. All this provided a visual foundation.



Figure (1): Cultural characteristics on Nutrient Agar (NA) for *Bacillus cereus* isolate. The colonies display large, irregular margin and a dull, “ground-glass” appearance, then obtained isolated colonies clearly using the streaking method after 24 hours of the incubation at 37°C.

Distinctive pink to red colonies were formed by the isolates when they were cultivated on MYP agar, which is a selective and differential medium for the *B. cereus*. That may be because their inability to ferment mannitol was reflected by these features. The important diagnostic trait of this species is lecithinase activity. This specific activity was clearly indicated by a precipitation zone surrounding the colonies. Also, the growth of the Gram-negative bacteria was inhibited by the presence of the polymyxin B in the medium.



Figure (2): *B. cereus* isolates on Mannitol Egg Yolk Polymyxin (MYP) agar for illustrate the morphological appearance that show colonies as a distinct pink-red color, that indicate to non-mannitol fermenting status (lecithinase activity).

The highest isolation rates on this medium were recorded in the drinking water and washing water samples (26.66% each), whereas the lowest rate was observed in the cecal samples (12%). No statistically significant differences were detected among the different sample sources ($P = 0.951$).

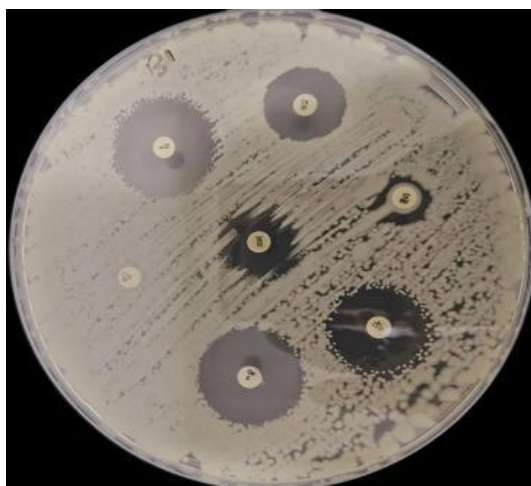


Figure (3): Antibiotic susceptibility patterns of the *B. cereus* isolates using Mueller-Hinton agar with Kirby-Bauer disk diffusion method.

4.2. Molecular Confirmation of the *Bacillus cereus* Using 16S rRNA Gene:

All isolates ($n = 40$) were molecularly confirmed as *Bacillus cereus* using PCR targeting the 16S rRNA gene, which yielded a specific amplification product of the 594 bp, as illustrated in the agarose gel electrophoresis (Figures 4.5.6).

The confirmation rates reached 100% in the washing water samples and workers' hand swabs, while lower detection rates were observed in the other sources, ranging from 16.66% in the cecal samples to 50% in the intestinal samples. Fisher's exact test revealed no statistically significant differences among

sample sources ($P = 0.234$), indicating a widespread distribution of the organism across different components of the food chain.



Figure (4): Agarose gel electrophoresis image that shows the PCR product analysis of the 16S rRNA gene, for the detection *Bacillus cereus* isolates, the Marker ladder (2000-100bp), The positive samples (S1-S10) were showed positive PCR amplification at 594bp PCR product size.

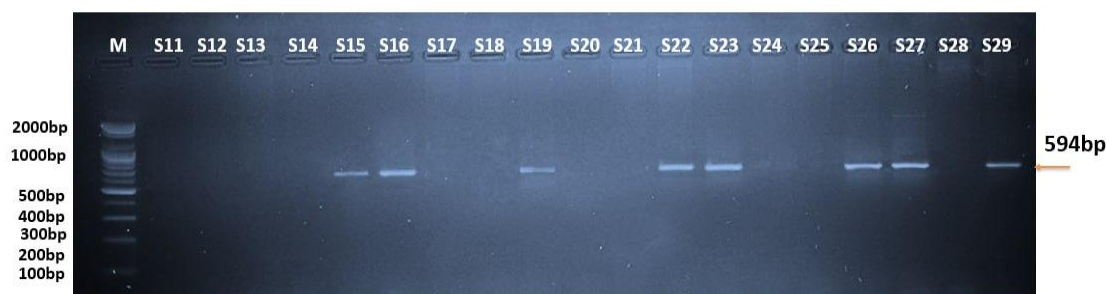


Figure (5): Agarose gel electrophoresis image that shows the PCR product analysis of the 16S rRNA gene, for the detection of *Bacillus cereus* isolates, the marker ladder (2000-100bp), The positive samples (S11-S29) were shown positive band at 594bp PCR product size.



Figure (6): Agarose gel electrophoresis image that shows the PCR product analysis of the 16S rRNA gene for the detection of *Bacillus cereus* isolates, the marker ladder (2000-100bp), The positive samples (S30-S40) were shown band at 594bp PCR product size.

4.3. Detection of the Virulence Gene hblA:

The presence of the virulence gene *hblA*, encoding a component of the hemolysin BL (Hbl) complex, was investigated in the all tested isolates ($n = 15$). The results demonstrated that 100% of the isolates (15/15) were positive for the *hblA*, with clear amplification bands observed at 320 bp in the all samples (Figure 7).

A potential to produce Hbl toxin is carried by most *B. cereus* isolates. That refer to the high prevalence we recently observed in the current study. There are many factors causing diarrheal food poisoning, but this is the most critical. Researchers consistently identified the *hblA* gene alongside other virulence genes, such as *hblC*, *hblD*, and *nhe*. While their frequencies varied. All tested isolates contained *hblA*. That mean its occurrence is uniform. A central role in the pathogenic potential is suggested by all this. A notable variation in the distribution of the virulence gene profiles among the isolates was highlighted by

the statistical analysis ($P = 0.018$). Some genes appeared more frequently. Also, certain combinations were rare. Considerable diversity in the virulence patterns is pointed to by this uneven distribution. That not all *B. cereus* strains carry that same pathogenic potential is clearly suggested by these findings.

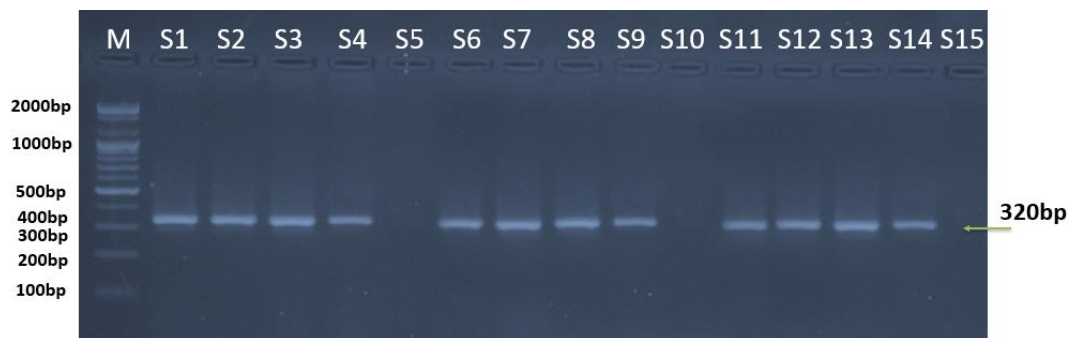


Figure (7): Agarose gel electrophoresis showing the PCR amplification of the *hblA* virulence gene, in the *Bacillus cereus* isolates. The marker ladder (2000–100 bp) is shown for the reference. Positive samples (S1–S15) exhibited clear bands that refer to PCR products at the expected size of the 320 bp, confirming the presence of the *hblA* gene.

4.4. Detection of the Antimicrobial Resistance Gene (*blaZ*) and Corresponding Phenotypic Expression:

The identified antimicrobial resistance gene *blaZ* in the all tested isolates (15/15). A clear amplification product of the 570 bp was yielded by it (Figure 8). Beta-lactamase production and resistance to beta-lactam antibiotics are the main cause of the this gene's activity. The disk diffusion results were phenotypically matched by this genetic finding. Complete resistance to ampicillin was shown by every isolate. That mean either no inhibition zones or smaller zones than the CLSI cutoff were produced by them. The isolates' ability to inactivate beta-lactam antibiotics is underscored by this strong concordance. That cause potential therapeutic options to be severely limited. Interestingly, the *blaZ* gene was consistently accompanied by the *tet* gene. Tetracycline resistance is conferred by this combination to make survival easier. A widespread multidrug resistance profile is pointed to by all this co-existence. There are many *B. cereus* strains such as those sourced from environmental samples, workers, and poultry that have important multidrug resistance traits.

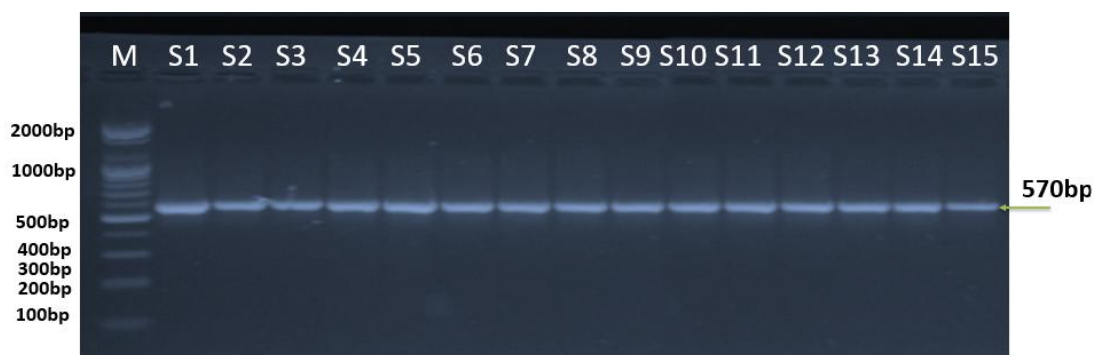


Figure (8): Agarose gel electrophoresis illustrating the bands of PCR amplification of the *blaZ* that are responsible for antibiotic resistance in the *Bacillus cereus* isolates. DNA ladder (2000–100 bp) used as a molecular size source. All samples (S1–S15) exhibited distinct amplification bands at the expected size of the 570 bp, that uniform presence of the this band across all isolates confirms the consistent detection of the *blaZ* gene.

4.5. One Health Implications:

The detection of the virulence gene *hblA* alongside the antimicrobial resistance gene *blaZ* concurrently. These isolates form from various sources such as workers' hand swabs, processing tools, washing and drinking water, and poultry tissues. A shared contamination network is clearly pointed to by this

concurrent detection. It is unlikely that this genetic uniformity across diverse sources is incidental. Rather, active cross-transmission of the pathogenic and resistant *Bacillus cereus* strains is strongly suggested by it. There are many strains spreading along the food production continuum and into the surrounding environment. That may be because of the poor hygiene practices. A pressing need for the a more integrated control framework is underscored by these observations. Isolated interventions cannot be relied upon by authorities to address the spread of the these strains.

5. Discussion:

The successfully isolated and identified of the *Bacillus cereus* from workers' hand swabs, environmental sources, and poultry samples. There are many swabs collected in the Al-Qadisiyah Province, Iraq. Both selective and non-selective media were utilized by researchers to base the initial identification on cultural characteristics. The typical pink to red coloration was exhibited by colonies on MYP agar. That mean a distinct zone of the lecithinase precipitation was clearly shown. Meanwhile, the characteristic "ground-glass" appearance was shown by growth on nutrient agar. All this supported the important preliminary identification of the isolates. Previous descriptions of the *B. cereus* are consistent with these findings. That refer to confirming the reliability of the these media for the initial isolation (Ehling-Schulz et al., 2019; Carroll et al., 2022).

The dependability of the MYP and nutrient agar as primary isolation media is reinforced by these observations. That may be because they align closely with established descriptions (Ehling-Schulz et al., 2019; Carroll et al., 2022). An extra layer of the certainty was provided by molecular verification. Researchers confirmed that all 15 isolates belonged to *B. cereus* by using PCR amplification targeting the 16S rRNA gene (594 bp). To make the results clear, this left little room for the ambiguity. The strength of the molecular tools is underscored by such precision. That like when dealing with members of the *Bacillus cereus sensu lato* group. Phenotypic differentiation alone often proves inadequate here (Stenfors Arnesen et al., 2008).

Also detected no statistically significant variation in the isolation rates among the most different sample sources ($P > 0.05$). That the organism is not confined to a single niche is suggested by this uniformity. Rather, it is broadly distributed across poultry-associated environments. The remarkable ecological resilience of the *B. cereus* is described by earlier reports that are echoed by this finding. The formation of the durable endospores largely drives its persistence. There are many structures capable of the withstanding adverse conditions. These can remain viable throughout the processing environment, in the water, and on equipment surfaces (EFSA, 2016; Alali & Ricke, 2019). That may be the main cause of the contamination.

With respect to virulence determinants, the *hblA* gene was carried by every examined isolate (100%). A key component of the hemolysin BL (Hbl) enterotoxin complex is encoded by it. Also, we identified the companion genes, *hblC* and *hblD*. While they appeared at lower frequencies of the 80% and 66.66%, respectively. Findings from earlier investigations are mirrored by this pattern. Enterotoxin-associated genes are frequently detected by scientists among *Bacillus cereus* strains circulating in the food chains (Jessberger et al., 2020). That cause diarrheal illness. Functionally, pathogenicity is strong associated with the presence of the *hblA*. Pore formation in the intestinal epithelial cells is contributed to by it. That cause fluid secretion and inflammation ultimately (Ehling-Schulz et al., 2019). Concern is raised by its detection across workers' hand swabs, environmental sources, and poultry samples. A plausible route for the dissemination of the virulent strains throughout the food production continuum and into human exposure settings is pointed to by this.

When researchers considered antimicrobial resistance, a similar uniformity emerged. The *blaZ* gene was harbored by all isolates. Beta-lactamase encoding and resistance to beta-lactam antibiotics are mediated by it. Phenotypic observations were entirely consistent with this genotypic profile. Complete resistance to ampicillin was recorded by us across the dataset. Such concordance is not unexpected. Resistance to beta-lactams in the *B. cereus* is widely documented by experts. It is often regarded as an intrinsic trait.

Chromosomally encoded beta-lactamase enzymes largely drive this (Rossi et al., 2018; Park et al., 2018). A pronounced multidrug-resistant profile is pointed to by the simultaneous presence of the *blaZ* and *tet* genes in the all examined isolates (100%). It is unlikely that such complete co-occurrence is incidental. Rather, sustained selective pressure is reflected by it. The extensive and often unregulated use of the antimicrobials in the poultry production systems most plausibly drives this. Comparable patterns have been documented by researchers in the poultry-associated *Bacillus cereus* isolates. Resistance traits tend to accumulate and persist under antibiotic exposure here (Mulchandani et al., 2023; WHO, 2021). From a clinical standpoint, concern is raised by this. Poor responsiveness to routinely prescribed agents, including ampicillin and tetracycline, may be exhibited by infections caused by these strains. That cause therapeutic management to be complicated.

When viewed through a One Health lens, a tightly interconnected transmission network is suggested by the repeated detection of the identical virulence (*hblA*) and resistance (*blaZ*) determinants. These isolates form from workers' hands, processing equipment, water sources, and poultry tissues. The boundaries between environmental reservoirs, humans, and animals appear increasingly blurred. That have important implications. Global reports are resonated with by this observation. The spread of the resistant and pathogenic bacteria is attributed to factors such as the misuse of the antimicrobials in the animal husbandry and inadequate waste disposal practices (FAO, 2023; WOA, 2023; UNEP, 2023). Notably, the notion of the widespread environmental dissemination rather than localized contamination events is reinforced by the absence of the significant variation in the distribution of the *B. cereus* across sampled sources. Coordinated intervention is called for the by such a scenario. There are many needs such as the exploration of the effective antibiotic alternatives, stricter veterinary regulation, and strengthened biosecurity measures (Gadde et al., 2017; Callaway et al., 2021).

In summary, a broad distribution of the *Bacillus cereus* within poultry production systems in the Al-Qadisiyah, Iraq, is revealed by the findings of the current study. A tangible public health concern is underscored by the consistent detection of the key virulence and resistance genes. More than isolated efforts will be required by authorities to address this issue.

Conclusion:

A broad distribution of the *Bacillus cereus* across poultry production chains in the Al-Qadisiyah Province, Iraq, is revealed by the current study. There are many matrices that consist of the processing equipment, water, workers' hand swabs, and chicken tissues. Primary screening was reliably supported by initial identification. That depending on phenotypic traits that called cultural characteristics observed on nutrient agar and MYP. However, researchers provided a more definitive classification through PCR confirmation targeting the 16S rRNA gene (594 bp). To make precise identification of the *B. cereus* isolates ensured, all this was necessary.

The inherent toxigenic potential of the isolates was highlighted by the uniform detection of the *hblA* virulence gene among all of the them (100%). That have important implications. Diarrheal foodborne illness is strong associated with this gene. That may be because it acts as a critical component of the hemolysin BL (Hbl) enterotoxin complex. Also, the *blaZ* resistance gene was carried by every isolate. Phenotypic resistance to ampicillin was demonstrated by them simultaneously. The pervasive presence of the beta-lactam-resistant strains across human-associated, environmental, and animal sources is pointed to by these results. Such as those we tested.

The recurrence of the identical resistance and virulence determinants in the isolates obtained from such diverse origins is considered the most crucial finding. More than coincidental contamination is suggested by this pattern. Rather, interconnected systems experience active cross-sector transmission, which is implied here. That mean a tight relationship. The One Health perspective, where environmental, animal, and human health are inextricably linked, is aligned closely with these findings. Therefore, coordinated action is clearly and pressing needed. There are many strategies that can be applied, for the example, the exploration of the alternative control strategies, judicious antimicrobial use, strengthened biosecurity

practices, and integrated surveillance programs. The risks posed by toxigenic and multidrug-resistant *B. cereus* within animal-derived food chains must be curbed.

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