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Apple cider vinegar exhibits promising antibiofilm activity against multidrug-resistant *Bacillus cereus* isolated from meat and their products

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ABSTRACT

Background: *Bacillus cereus* (*B. cereus*) biofilm is grown not only on medical devices but also on different substrata and is considered a potential hazard in the food industry. Quorum sensing plays a serious role in the synthesis of biofilm with its surrounding extracellular matrix enabling irreversible connection of the bacteria.

Aim: The goal of the current investigation was to ascertain the prevalence, patterns of antimicrobial resistance, and capacity for *B. cereus* biofilm formation in meat and meat products in Egypt.

Methods: In all, 150 meat and meat product samples were used in this study. For additional bacteriological analysis, the samples were moved to the Bacteriology Laboratory. Thereafter, the antimicrobial, antiquorum sensing, and antibiofilm potential of apple cider vinegar (ACV) on *B. cereus* were evaluated.

Results: Out of 150 samples, 34 (22.67%) tested positive for *B. cereus*. According to tests for antimicrobial susceptibility, every *B. cereus* isolates tested positive for colistin and ampicillin but negative for ciprofloxacin and imipenem. The ability to form biofilms was present in all 12 multidrug-resistant *B. cereus* isolates ($n = 12$); of these, 6 (50%), 3 (25%), and 3 (25%) isolates were weak, moderate, and strong biofilm producers, respectively. It is noteworthy that the ACV demonstrated significant inhibitory effects on *B. cereus* isolates, with minimum inhibitory concentrations varying between 2 and 8 µg/ml. Furthermore, after exposing biofilm-producing *B. cereus* isolates to the minimum biofilm inhibitory concentrations 50 of 4 µg/ml, it demonstrated good antibiofilm activity (>50% reduction of biofilm formation). Strong biofilm producers had down-regulated biofilm genes (*tasA* and *sipW*) and their regulator (*plcR*) compared to the control group, according to reverse transcriptase quantitative polymerase chain reaction analysis.

Conclusion: Our study is the first report, that spotlights the ACV activity against *B. cereus* biofilm and its consequence as a strong antibacterial and antibiofilm agent in the food industry and human health risk.

Keywords: Apple cider vinegar, *B. cereus*, Antimicrobial resistance, Biofilm.

Introduction

High biological value proteins, vitamins particularly B and certain minerals all essential for human development and well-being can be found in meat products. One of the most underappreciated foodborne illnesses worldwide is contaminated meat products containing toxic *Bacillus cereus* (*B. cereus*) (Ceuppens *et al.*, 2013; Ayako Kobashi *et al.*, 2023). Bacterial biofilms that form in the food matrix or on tools can cause foodborne infections (Adame-Gómez *et al.*, 2020). According to Boonyayatra *et al.* (2016), biofilm formation plays a significant role in the pathophysiology of numerous diseases in both humans and animals. Biofilm-forming bacteria have the ability to endure in unfavorable environments and, once inside an organism, to withstand the host immune system while developing resistance to the effects of antibiotics and disinfectants (Felipe *et al.*, 2017). Food production is thought to pose a potential health risk due to the formation of biofilms by *B. cereus* (Lindsay *et al.*, 2000). It is one of the leading causes of bacteria developing multidrug resistance (MDR) (Tewari *et al.*, 2012).

The current understanding of biofilm cell differentiation in species belonging to the *B. cereus* group is lacking. Nonetheless, a number of studies showed that the primary regulatory pathways supporting the formation of biofilms in species belonging to the *B. cereus* group are conserved. Through the peptide *PapR*,

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the phospholipase C regulator (*PlcR*) is in charge of detecting external signals such as population density and nutrition. Its regulon contains some virulent factors that initiate the necrotrophic factor, neutral protease regulator (*NprR*), which promotes the expression of kurstakin and initiates biofilm formation (Dubois *et al.*, 2012). According to Caro-Astorga *et al.* (2015), *TasA* has two paralogs: *calY*, which is located next to *sipW-tasA*, and *tasA*, which is a part of the *sipW-tasA* operon. Electron microscopy reveals that *TasA* and *CalY* are both involved in the production of fibers, and biofilm defects result from the eradication of their genes or *sipW* (Caro-Astorga *et al.*, 2015).

Antibiotics are recognized as one of the most important weapons in the fight against illness (Thomas *et al.*, 2015). Biofilm-forming bacteria are extremely adaptable and resistant to disinfectants and antibiotics. When treating both acute and chronic biofilm infections, high antibiotic resistance can be a barrier (Li and Lee, 2017). Many studies rely on mechanisms to disrupt bacterial quorum sensing by interfering with cell-cell communication to hinder the ability of *B. cereus* group strains, particularly *B. cereus* sensu stricto, to cause human infections. This process is known as quorum quenching (Waters and Bassler, 2005; Yehuda *et al.*, 2018). The prevention of bacterial growth through the use of antimicrobial agents is the most effective strategy for inhibiting the formation of biofilm, a topic that has received significant attention recently (Roy *et al.*, 2018). Consequently, a lot of research focused on nontraditional approaches such as biological products or herbal medicines as anti-biofilm agents (Schönborn *et al.*, 2017).

Apple cider vinegar (ACV) is a naturally occurring product of apple fermentation made of apple, sugar, and yeast. On Gram-positive bacteria, it exhibits antibacterial activity (Watson *et al.*, 2018). Its antioxidant and antibacterial properties against numerous pathogenic agents are attributed to a wide range of constituents, including vitamins, minerals, organic acids, polyphenols, and flavonoids (Xia *et al.*, 2020; Budak *et al.*, 2021). ACV had an antimicrobial effect on tested microorganisms such as *Vibrio cholerae*, *Candida tropicalis*, *C. albicans*, *Echerchia coli* O157:H7, and *Salmonella typhi*. Generally, a study has inspected the acetic acid effects, which is rich in vinegar, on the formation of biofilm and revealed that it reduced the biofilm formed by *Staphylococcus aureus* (Pedroso *et al.*, 2018). Therefore, it needs to discover recent antibiofilm and antiquorum agents preferable to the traditional treatment to eradicate the biofilm that affects the control of *B. cereus* infection. Herein, we investigated the antimicrobial susceptibilities and biofilm-producing abilities of *B. cereus* recovered from meat products. Thereafter, the *in vitro* antibiofilm and antiquorum sensing activities of ACV were assessed against MDR *B. cereus* isolates followed by evaluating the efficacy of ACV on the expression profile of biofilm-associated genes via reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).

Materials and Methods

Sampling

In all, 150 samples of meat and meat products including minced meat (40), shawarma (30), beef burger (25), beef kofta (25), beef luncheon (15), and sausage (15) were randomly collected from various supermarkets in the Sharkia Governorate of Egypt. As soon as possible, the samples were moved to the Bacteriology Laboratory, Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, for additional bacteriological analysis. They were aseptically placed into sterile containers, and stored in an icebox.

Isolation and identification of B. cereus group

The *B. cereus* group was isolated in compliance with ISO 21871 (2006). In summary, 90 ml of 0.1% buffered peptone water (Oxoid, UK) was used to suspend 20 g of each sample for a duration of 50 minutes at room temperature. To achieve a final dilution of 10^{-1}, an additional 90 ml of 0.1% peptone water was added. Homogenization was done for 30 seconds using the Stomacher 400 (Seward Pharma, Mfrs, and DistribUAC house, UK) for processing. *Bacillus cereus* selective agar base (Oxoid, UK) was used for selective plating, and it was then incubated for 24 hours at 37°C. New colonies from every pure culture were inspected for biotyping purposes catalase, gelatin liquefaction, citrate utilization, starch hydrolysis, motility, and hemolysis tests (Quinn *et al.*, 2002). The detection of para sporal protein toxin crystal and rhizoid growth was also carried out (Tallent *et al.*, 2012). Genotypic identification of the *B. cereus* group was applied using the genus (16S rRNA) and group-specific (*plcR)* primers in conventional PCR (cPCR) at the Animal Health Research Institute's Biotechnology Unite in Zagazig, Egypt. The used primer sequences, amplicon sizes, and thermo-cycling conditions are shown in Table 1.

Antimicrobial susceptibility testing of B. cereus isolates

Using the Kirby-Bauer disc diffusion method, the susceptibility of *B. cereus* isolates to different antimicrobials was investigated. Mueller Hinton agar media (MHA, Oxoid, UK) and eighteen standard antimicrobial discs (Oxoid, UK) representing fifteen antimicrobial groups were used in the experiment. The antimicrobial discs contained the following: ampicillin (AMP, 10 µg), cephalothin (kF, 30 µg), cefotaxime (CTX, 30 µg), imipenem (IPM, 10 µg), ciprofloxacin (CIP, 5 µg), ofloxacin (OX, 1 µg), trimethoprim-sulfamethoxazole (SXT, 1.25 µg/23.75 µg), doxycycline (DO, 30 µg), erythromycin (E, 15 µg), clindamycin (DA, 2 µg), vancomycin (VA, 30 µg), colistin (CT, 10 µg), tigecycline (TGC, 15 µg), fosfomycin (FF, 50 µg), and rifampicin (RA, 30 µg) (Bauer *et al.*, 1966). To determine the minimum inhibitory concentrations (MICs) of vancomycin and colistin (Sigma-Aldrich, Seelze, Germany), the broth

Table 1. Target genes, oligonucleotide primer sequences, amplicons, and cycling conditions used for PCR.

Target gene	Specificity	Primer sequence (5'–3')	Amplified product size (bp)	PCR cycling condition	Reference
16S rRNA	Housekeeping gene	F: ACTGGGACTGAGACACGG R: GATAACGCTTGCCACCTA	242	First, denaturation for 5 minutes at 94°C was followed by 35 cycles of 30 seconds at 94°C, 1 minute at 52°C, and 30 seconds at 55°C. Finally, elongation took place for 7 minutes at 72°C.	Huang *et al.*, 2021
plcR	Pleiotropic regulator	F: ACCCGACATTAAAATCGTTTG R: TAGTATGCCTTGCGCAGTTG	200	First, denaturation for 2 minutes at 94°C was followed by 30 cycles of 30 seconds at 94°C, 1 minute at 52°C, and 30 seconds at 72°C. Finally, elongation took place for 10 minutes at 72°C.	Oltuszak-Walczak, *et al.*, 2013
tasA	Amyloid like fiber	F: AGCAGCTTTAGTTGG TGG AG R: GTA ACT TAT CGC CTT GGA ATTG	488	First, denaturation for 5 minutes at 94°C was followed by 40 cycles of 30 seconds at 94°C, 45 seconds at 59°C, and 45 seconds at 72°C. Finally, elongation took place for 5 minutes at 72°C.	Caro-Astorga *et al.*, 2015
sipW	Signal peptidase	F: AGA TAA TTA GCA ACG CGA TCTC R: AGA AAT AGC GGA ATA ACC AAGC	488	First, denaturation for 5 minutes at 94°C was followed by 40 cycles of 30 seconds at 94°C, 45 seconds at 54°C, and 45 seconds at 72°C. Finally, elongation took place for 5 minutes at 72°C.	

microdilution assay (Rankin, 2005) was used. Due to the absence of *B. cereus* interpretative criteria in pertinent CLSI documents, the results of antimicrobial susceptibilities were interpreted in accordance with the *S. aureus* guidelines of the Clinical and Laboratory Standards Institute (Yu *et al.*, 2019, CLSI, 2010). As previously reported (Tambekar *et al.*, 2006), the multiple antimicrobial resistance (MAR) indices for every antibiotic and every isolate were computed. The strain of *B. cereus* ATCC®14579TM was employed as a quality control.

Apple cider vinegar

The ACV (5% acetic acid) used in this study was purchased commercially and kept chilled at 4°C until needed. It was provided by Gardens Company, Egypt. It has no fat or protein and is made up of 94% water, 5% acetic acid, and 1% carbohydrates.

Antimicrobial activity of ACV against B. cereus isolates

The antimicrobial activities of different ACV concentrations against MDR *B. cereus* isolates were determined using the agar well diffusion assay (Valgas *et al.*, 2007). The suspension turbidity of pure bacterial cell culture was adjusted to McFarland standard No. 0.5. (1–1.5 × 10^8 colony forming units/ml) using 0.85% physiological saline (Oxoid, UK). A bacterial suspension containing 100 µl was grown on MHA (Oxoid, UK) plates. Using a cork borer (7 mm in diameter), wells were made in the agar plate and then filled with 100 µl of prepared ACV at concentrations of 100%, 80%, 60%, 40%, and 20%. Controls were included, both positive (IPM, 10 µg) and negative (sterile distilled water). For 24 hours, the plates were incubated at 35°C. Millimeters were used to measure the diameters of the growth inhibition zones surrounding the wells. According to Choi *et al.* (2016), the isolates were categorized as resistant (0) for diameters less than

8 mm, moderately sensitive (+) for diameters between 8 and 20 mm, sensitive (++) for diameters between 20 and 30 mm, and very sensitive (+++) for diameters larger than 30 mm.

With the use of the broth microdilution assay, the MIC of ACV was ascertained (Rankin, 2005). *Bacillus cereus* inoculum size of approximately 5×10^5 CFU/ml was prepared in the appropriate broth culture media. Twofold serial dilutions of ACV were prepared in sterile distilled water from the stock solution (1,024 µg/ml). Aliquots (100 µl) of each ACV dilution were dispensed in sterile polystyrene, U-shaped bottom, 96-well culture plates (Techno Plastic Products, Switzerland). Each well received 100 µl of each bacterial suspension, which was then incubated for 24 hours at 37°C. After determining the ACV›s MIC and minimum bactericidal concentration (MBC) (Kwiecinski *et al.*, 2009), an orderly array method was used to calculate the MIC50 and MIC90 (Hamilton-Miller, 1991). The examined antibacterial agent has bacteriostatic activity when a ratio of MBC to MIC ≥ 4 where MBC is more than two dilutions of the MIC (Pankey and Sabath, 2004). Meanwhile, it is considered bacteriocidal if MBC/MIC = ≤4 where MBC is within two dilutions of the MIC.

Phenotypic detection of B. cereus biofilm

As previously mentioned, the Congo red agar method was used to qualitatively detect *B. cereus* biofilm (Reid, 1999). Biofilm production was shown by black colonies with a dry crystalline consistency. In addition, the microtiter plate method was used to quantitatively detect the formation of biofilms using flat-bottom microtiter plates (Techno Plastic Products, Switzerland) (Adame-Gómez *et al.*, 2020). In summary, isolates of *B. cereus* were cultured in tryptic soy broth (TSB; Oxoid, UK) at 37℃. Following a 10-minute pelleting process at 6,000 × g, the bacterial cells were dissolved in 5 ml of fresh medium. Using an ELISA reader (stat fax 2100, USA), the optical densities (ODs) of the bacterial suspensions were measured and normalized to an absorbance of 1.00 at 600 nm. 200 µl of 0.1% aqueous crystal violet solution (Al-Gomhorya Company, Egypt) was added to each well, and the plates were left to stand for 15 minutes to quantify the biofilm. After that, the excess crystal violet was removed from the wells by washing them three times with sterile phosphate buffer saline (PBS). 200 µl of an 80:20 (v/v) mixture of ethyl alcohol and acetone (Al-Gomhorya Company, Egypt) was used to extract the crystal violet bound to the biofilm. The absorbance of the extracted crystal violet was then measured at 545 nm using an ELISA reader. As negative and positive controls, respectively, wells containing a noninoculated medium and a biofilm-producing bacterium (*B. cereus* ATCC®14579TM) were employed. Every biofilm assay was run in triplicate. The biofilm production was interpreted using the standards outlined by Stepanovic *et al.* (2007). These parameters define the OD cut-off value (ODc) as the negative control's average OD plus three times its standard deviation (SD). The following standards were used to categorize the *B. cereus* isolate's capacity to form biofilm: ODc < OD ≤ 2× ODc = Weak biofilm producer, 2× ODc < OD ≤ 4× ODc = Moderate biofilm producer, and 4× ODc < OD = Strong biofilm producer. OD ≤ ODc = Not a biofilm producer.

Antibiofilm activities of ACV

The antibiofilm activities of different concentrations of ACV were applied using the crystal violet staining assay as a trial to control *B. cereus* biofilm formation and to prevent bacterial colonization. In brief, a 96-well polystyrene microtiter plate with a flat bottom was selected, and 100 µl of MHB was added to each well. Following a double-fold serial dilution of 100 µl of ACV, 100 µl of *B. cereus* suspension (10^6 cells/ml) was added. The plates were allowed to form a biofilm by being incubated for 24 hours at 37°C. The wells' contents were removed using an aspirator and three times cleaned in sterile PBS. The negative control (ACV well without *B. cereus* suspension) and the positive controls (wells containing *B. cereus* without ACV) were included. Three duplicates of each experiment were run. Biofilm inhibition % = (Control OD545 nm − Test OD545 nm)/(Control OD545 nm) × 100 was the formula used to determine the biofilm inhibition percentage (Raja *et al.*, 2011). In addition, the minimal antimicrobial concentration was defined as the minimal biofilm inhibitory concentration (MBIC), which makes inhibition of the biofilm formation. Moreover, MBIC50 and MBIC90 of the antibacterial agents which are the lower concentrations of the antibacterial agent showing 50% or 90% reduction in biofilm formation, respectively, were also calculated (Raja *et al.*, 2011).

The relative expression of biofilm biosynthesis genes and their regulator using SYPR Green RT-qPCR

Strong biofilm producers after being exposed to ACV's MBIC, *B. cereus* isolates were incubated for 12 hours at 37°C. Biofilm producers of *B. cereus* that are not treated are used as controls. After being collected, the biofilms were gently cleaned with PBS to remove any nonadherent cells. Following the manufacturer's instructions, total RNAs were extracted from the biofilms of both treated and untreated isolates using a QIAamp RNeasy Mini kit (Qiagen, Germany). By employing oligonucleotide primer sets and cycling conditions as specified in Table 1, the relative expression levels of the genes responsible for biofilm biosynthesis (*tasA* and *sipW*) and their regulator (*plcR*) were ascertained. Following the manufacturer's instructions, RT-qPCR was performed in triplicate in the real-time PCR thermal cycler (MX3005P, Stratagene, La Jolla, CA) using the QuantiTect SYBR Green Kit (Qiagen, Germany). Melting curves were created to confirm the reaction's specificity. The constitutive expression of the 16sRNA housekeeping gene served as a reference for normalizing the relative expression levels of the investigated genes. The comparative $2^{-\Delta\Delta CT}$ method (Livak Schmittgen, 2003) was used to determine the transcript levels fold changes of tested genes in treated

B. cereus biofilm producers in relation to their levels in the untreated ones.

Statistical analysis

The GraphPad Prism 8 software (GraphPad Software, San Diego, CA) was used to analyze the data. The statistical differences in the number of positive isolates and antimicrobial resistance in *B. cereus* isolates recovered from meat and meat products were assessed using Pearson's chi-square and Kruskal–Wallis tests. Analysis was conducted using the unpaired *t*-test for comparison of the fold change of gene expression between *plcR*, *tasA*, and *sipW* genes (in the presence of ACV) and the control ones (without ACV) (Yuan *et al.*, 2006). The numerical data are shown as means ± standard errors (SEs) (Duncan, 1955). At p-values < 0.05, significant differences were taken into account (Yuan *et al.*, 2006).

Results

Isolation and identification of B. cereus

Microbiological examination revealed 34 *B. cereus* isolates out of 150 (22.67%) examined meats and their derivatives. The higher recovery rate of *B. cereus* was reported in sausage (33.3%) followed by beef kofta (24%), and minced meat (22.5%) (Table 2). Nonsignificant differences were found in the amount of *B. cereus* recovered from different meat products by statistical analysis ($p > 0.05$).

Bacillus cereus isolates were identified on *B. cereus* selective agar media by their crenated colonial morphology. The isolates have a typical peacock-blue colour enclosed by strong precipitation of the egg yolk lecithin giving a hazy turquoise zone with no mannitol fermentation (positive Nagler's reaction). All the recovered isolates were hemolytic, could not grow at 6°C for 28 days, and did not show the rhizoid colonial appearance of *Bacillus mycoides* and *Bacillus pseudomycoide* as long hair-like colonies on nutrient agar plates. Biochemical identification revealed that isolates of *B. cereus* were motile and positive for citrate utilization, catalase, starch hydrolysis, and gelatin liquefaction. The isolates were free from protein crystals of *B. thuringiensis* after carbol fuchsin Ziehl–Neelsen staining. Within the *B. cereus* group, there is currently only one isolated and recognized species, *B. cereus*. All isolates of *B. cereus* with phenotypically suspicion were subsequently subjected to cPCR analysis, which relied on the identification of specific species (*plcR*) and genus (16S rRNA) primers that produced amplicons at 242 and 200 bp, respectively.

Table 2. Frequency of *B. cereus* in meat and meat products.

Sample type (no.)	No. of *B. cereus* isolates (%) *	*p*-value
Minced meat (40)	9 (22.5)	0.936
Shawarma (30)	6 (20)	
Beef burger (25)	5 (20)	
Beef kofta (25)	6 (24)	
Beef luncheon (15)	3 (20)	
Sausage (15)	5 (33.3)	
Total (150)	34 (22.67)	

* Number (%) of positive isolates relative to each source. p-values > 0.05 are statistically nonsignificant.

Antibiogram of B. cereus isolates

Thirty-four *B. cereus* isolates were tested for *in vitro* antimicrobial susceptibility to 18 different antimicrobial agents. The results showed that all of the isolates were sensitive to ciprofloxacin and imipenem (100%, each) followed by clindamycin (26.47%), tobramycin (20.59%), vancomycin (20.59%), rifampicin (17.60%), and gentamicin (14.70%). However, all of the *B. cereus* isolates were resistant to the following drugs: ampicillin and colistin (100%, each), cefotaxime (94.12%), sulfamethoxazole-trimethoprim (91.20%), cephalothin and fosfomycin (91.18% each), chloramphenicol, erythromycin and tigecycline (88.23% each), ofloxacin (85.29%) and doxycycline (82.35%). It is interesting to note that every *B. cereus* isolate had an MDR (resistant to at least three antimicrobial classes) MAR index, ranging from 0.55 to 0.77, which was significantly higher than 0.3 (Table 3). Statistical analysis exposed a significant difference ($p < 0.05$) in the resistance of *B. cereus* isolates to tested antimicrobials. The frequency of *B. cereus* susceptibility to selected antimicrobial agents and their resistance pattern is shown in Table 4.

Antimicrobial activity of ACV against B. cereus

As presented in Table 5, all ACV concentrations (100%, 80%, 60%, 40%, and 20%) exhibited marked inhibitory activities against *B. cereus* isolates while applying the agar well diffusion assay. The inhibitory zone diameters ranged from 44–48 mm/100%, 42–46 mm/80%, 38–44 mm/60%, 37–42 mm/40%, and 36–39 mm 20% concentrations. To determine precisely the antimicrobial properties of ACV, MICs, and MBCs were necessarily performed on 12 MDR *B. cereus* isolates. The MIC results showed that ACV exhibited strong antimicrobial activities against examined isolates with MIC ranging from 2 to 8 µg/ml. MIC50 and MIC90 of ACV against the examined isolates are 4 and 8 µg/ml, respectively. Some of the examined isolates showed MBC value at the same MIC value, considering the AVC as a bacteriocidal agent.

Biofilm production by B. cereus isolates

The qualitative recognition of biofilm on Congo red agar indicated that *B. cereus* biofilm producers (8/12; 66.67%) could alter transforming the media's red hue into black because of sucrose consumption. On trypticase soya broth, 12 *B. cereus* isolates were cultured for quantitative detection of biofilm production. Three *B. cereus* isolates (25%) were strong biofilm producers, 3 (25%) were moderate, and 6 (50%) produced weak

Table 3. Antibiogram of the *B. cereus* isolates recovered from meat and meat products.

Isolate No.	Source	Antimicrobial resistance pattern	MAR index
1	Shawarma	SXT, DO, AMP, VA, CT, E, OX, KF, C, CN, TCG, FF	0.667
2	Minced meat	SXT, DO, AMP, VA, CT, DA, E, CTX, OX, KF, C, CN, TCG, FF	0.778
3	Beef kofta	SXT, DO, AMP, CT, DA, E, CTX, KF, C, CN, TCG, FF, TOB, RA	0.778
4	Sausage	SXT, DO, AMP, VA, CT, CTX, OX, KF, C, CN, TCG, FF	0.667
5	Shawarma	AMP, VA, CT, DA, E, CTX, OX, KF, C, TCG, FF, TOB, RA	0.722
6	Beef kofta	SXT, AMP, CT, DA, E, CTX, KF, C, TCG, FF, TOB	0.611
7	Minced meat	SXT, DO, AMP, VA, CT, DA, E, CTX, OX, KF, C, TCG, FF, RA	0.778
8	Beef burger	SXT, DO, AMP, VA, CT, DA, E, OX, KF, C, TCG, FF	0.667
9	Minced meat	SXT, DO, AMP, CT, DA, E, CTX, OX, KF, C, TCG, FF	0.667
10	Shawarma	SXT, DO, AMP, VA, CT, E, CTX, KF, C, TCG, FF, RA	0.667
11	Beef burger	SXT, DO, AMP, VA, CT, DA, E, CTX, OX, KF, C, TCG, FF, RA	0.778
12	Minced meat	DO, AMP, CT, DA, E, CTX, OX, KF, C, TCG, FF, TOB	0.611
13	Sausage	SXT, DO, AMP, VA, CT, E, CTX, KF, C, TCG, FF	0.667
14	Sausage	SXT, DO, AMP, VA, CT, DA, CTX, OX, KF, C, TCG, FF	0.611
15	Beef kofta	SXT, DO, AMP, VA, CT, E, CTX, OX, KF, C, TCG	0.611
16	Shawarma	SXT, DO, AMP, CT, DA, E, CTX, OX, KF, C	0.556
17	Beef luncheon	SXT, AMP, VA, CT, DA, E, CTX, OX, KF, C, RA	0.611
18	Minced meat	DO, AMP, VA, CT, E, CTX, OX, KF, C, FF, RA	0.611
19	Sausage	SXT, AMP, VA, CT, DA, E, CTX, OX, KF, C, TCG, FF, RA	0.722
20	Beef burger	AMP, VA, CT, E, CTX, OX, KF, C, TCG, FF	0.556
21	Minced meat	SXT, DO, AMP, CT, DA, E, CTX, OX, KF, C, TCG, FF	0.667
22	Beef kofta	SXT, DO, AMP, VA, CT, CTX, OX, KF, C, TCG, FF	0.611
23	Beef kofta	SXT, AMP, CT, DA, E, CTX, OX, KF, C, FF	0.556
24	Beef burger	SXT, DO, AMP, VA, CT, E, CTX, OX, KF, C, TCG, FF	0.667
25	Minced meat	SXT, DO, AMP, VA, CT, DA, E, CTX, OX, KF, C, TCG, FF	0.722
26	Beef luncheon	SXT, DO, AMP, VA, CT, E, CTX, OX, KF, CN, TCG, FF	0.667
27	Minced meat	SXT, DO, AMP, VA, CT, E, CTX, OX, KF, CN, TCG, FF	0.667
28	Beef burger	SXT, DO, AMP, CT, E, CTX, OX, KF, CN, TCG, FF	0.611
29	Shawarma	SXT, DO, AMP, VA, CT, DA, E, CTX, OX, KF, C, CN, TCG, FF	0.778
30	Beef luncheon	SXT, DO, AMP, VA, CT, DA, E, CTX, OX, KF, C, CN, TCG, FF	0.778
31	Shawarma	SXT, DO, AMP, CT, E, CTX, OX, C, TCG, FF	0.556
32	Beef kofta	SXT, DO, AMP, VA, CT, DA, CTX, OX, KF, CN, TCG, FF	0.667
33	Sausage	SXT, DO, AMP, VA, CT, DA, E, CTX, OX, C, CN, TCG, FF	0.722
34	Minced meat	SXT, DO, AMP, VA, CT, E, CTX, OX, C, TCG, FF	0.611

SXT, Trimethoprim/sulfamethaxole; DO, Doxycycline; AMP, ampicillin; VA, Vancomycin; CT, Colistin; DA, Clindamycin; E, Erythromycin; CTX, Cefotaxime; OX, Ofloxacin; KF, Cephalothin; C, Chloramphenicol; CN, Gentamicin; TGC, Tigecycline; FF, Fosfomycin; TOB, Tobramycin and RA, Rifampicin. MAR, Multiple antibiotic resistance.

biofilm (Table 6). The results indicated that the quantitative detection of the biofilm is more accurate than the qualitative method using the Congo red agar.

Antibiofilm activities of ACV against MDR B. cereus isolates

The ability of ACV to stop the development of biofilms in isolated *B. cereus* was investigated and the findings were compared with the untreated (positive control) *B. cereus* biofilm producer in addition to the negative control (Table 7). The inhibition of biofilm development in every isolate was expressed as antibiofilm activity and reported as inhibition or reduction %. Increasing the concentrations of the tested agent resulted in a significant ($p < 0.05$) decrease in biofilm formation

Table 4. Frequency of antimicrobial resistance in *B. cereus* isolates (*n* = 34) recovered from meat and meat products.

Antimicrobial group	Antimicrobial agent	No. (%) of resistant *B. cereus*	MAR index	*p*-value
Penicillin	AMP	34 (100.00)	0.056	0.001
Cephalosporin	KF	31 (91.18)	0.051	
	CTX	32 (94.12)	0.052	
Carbapenem	IMP	0 (00.00)	0.00	
Fluoroquinolones	CIP	0 (00.00)	0.00	
	OX	29 (85.29)	0.047	
Sulphonamides	SXT	31 (91.18)	0.051	
Tetracyclines	DO	28 (82.35)	0.046	
Macrolides	E	30 (88.24)	0.049	
Lincosamides	DA	20 (58.82)	0.033	
Aminoglycosides	CN	11 (32.35)	0.018	
	TOB	4 (11.76)	0.007	
Glycopeptides	VA	25 (73.53)	0.041	
Phenicoles	C	30 (88.24)	0.049	
Polymyxins	CT	34 (100.00)	0.056	
Glycylcyclines	TGC	30 (88.24)	0.049	
Phosphonics	FF	31 (91.18)	0.051	
Ansamycins	RA	8 (23.53)	0.013	

AMP, ampicillin; KF, Cephalothin; CTX, Cefotaxime; IPM, Imipenem; CIP, Ciprofloxacin; OX, Ofloxacin; SXT, Trimethoprim/sulfamethaxole; DO, Doxycycline; E, Erythromycin; DA, Clindamycin; CN, Gentamicin; TOB, Tobramycin; VA, Vancomycin; C, Chloramphenicol; CT, Colistin; TGC, Tigecycline; FF, Fosfomycin and RA, Rifampicin; MAR, Multiple antibiotic resistance. *p-values* < 0.05 are statistically significant.

Table 5. Inhibitory zone diameters, MIC, and MBC of ACV against MDR *B. cereus* isolates (*n* = 12) recovered from meat and meat products.

Isolate no.	Source	Zone diameter (mm) against various ACV concentrations (%)					MIC (µg/ml)	MBC (µg/ml)
		100	80	60	40	20		
1	Mm 3	48	42	38	37	36	8	8
2	Bk 4	45	43	41	39	37	2	8
3	Sh 6	46	45	40	39	38	4	8
4	Mm 8	47	46	44	38	36	4	8
5	Bb 12	47	43	42	40	37	2	4
6	S 20	48	45	43	40	38	2	4
7	Mm 26	48	46	44	39	37	4	8
8	BL 27	45	43	41	38	36	4	4
9	Sh 30	44	43	42	40	38	2	4
10	Bl 31	47	45	41	39	36	2	4
11	Bk 33	47	46	43	42	37	4	8
12	S 34	48	45	44	42	39	8	8

Mm: Minced meat, Sh: Shawarma, Bb: Beef burger, Bk: Beef kofta, Bl: Beef Luncheon, S: Sausage. MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; ACV, apple cider vinegar.

Table 6. Biofilm production by *B. cereus* isolates.

Isolate No.	Code	Qualitative detection	Biofilm production (OD)	Negative control (ODC)	Average reading
1	Mm 3	Black	2.784	0.691	+++
2	Bk 4	Black	0.925	0.462	++
3	Sh 6	Red	0.709	0.589	+
4	Mm 8	Black	0.642	0.421	+
5	Bb 12	Black	1.301	0.649	++
6	S 20	Red	0.587	0.344	+
7	Mm 26	Black	1.054	0.524	++
8	BL 27	Red	0.455	0.396	+
9	Sh 30	Black	1.736	0.409	+++
10	Bl 31	Red	0.786	0.599	+
11	Bk 33	Black	0.629	0.437	+
12	S 34	Black	1.864	0.441	+++

Mm: Minced meat, Sh: Shawarma, Bb: Beef burger, Bk: Beef kofta, Bl: Beef Luncheon, S: Sausage. OD, optical density; ODC, Cut-off optical density (OD of negative control + 3×SD of negative control); +, weak; ++, moderate; +++, strong. The data represents ELISA reading at OD at 545 nm. Black indicates biofilm production, while red indicates no biofilm production.

in all cases. The antibiofilm effect of ACV against biofilms produced by *B. cereus* isolates was found to be good, with over 50% inhibition of biofilm development. The most common minimum biofilm inhibitory concentration (MBIC50) was 4 µg/ml (Table7).

Transcriptional analysis of biofilm genes using RT-qPCR

The relative expression of the pleiotropic regulator (*plcR*), amyloid such as fiber (*tasA*) and signal peptidase (*sipW*) genes were evaluated in MDR strong biofilm-producing *B. cereus* isolates by RT-qPCR. The relative expressions (fold-change of the expression levels) of the characteristic genes in MDR and adherent isolates after the addition of ACV as antibiofilm agent were matched with those in the control MDR and adherent isolates without the exposure to ACV. The 16S rRNA housekeeping gene was used for RT-qPCR normalization (Fig. 1). The results revealed down-regulation of biofilm genes in comparison to those of the control; this was highly significant (***) for *plcR* and *sipW* genes ($p < 0.0001$), while was significant (*) with *tasA* gene ($p < 0.05$) (Table 8).

Discussion

Biofilms can be produced by the *B. cereus* group of the genus *Bacillus* in a variety of beverage and food industry settings. Concerned about a significant source of post-process and recurrent cross-contamination in prepared foods that could lead to food poisoning or product degradation, biofilms are a major source of concern for the food trade. Vasudevan *et al.* (2003) state that biofilms are frequently to blame for recurrent infections. According to Wijman *et al.* (2007), control over biofilm formation is therefore dependent on providing mechanistic insight into the behavior of *B. cereus* in biofilms. Therefore, the main objectives of this study were to isolate, identify, and test for antibacterial susceptibility *B. cereus* that was isolated from samples of meat and meat products. The recovered *B. cereus* isolates were then used to detect biofilm formation both qualitatively and quantitatively. Furthermore, evaluation of ACV for a better understanding of its effect on the gene expression that is responsible for biofilm formation and its regulator, using RT-qPCR to reduce the drawbacks of antibiotic resistance.

The traditional identification methods were exposed as "presumptive *B. cereus*" because these methods could not distinguish *B. cereus* from other *Bacillus* group isolates (Vidic *et al.*, 2020). Therefore, many laboratories' stages should be performed to distinguish *B. cereus* from other *Bacillus* group species (Ramarao *et al.*, 2020). The selective media and biochemical tests that these methods were designed to use are labor-intensive, time-consuming, and require specialized personnel (Zhu *et al.*, 2016). However, molecular methods are more accurate for definitive identification. Herein, *B. cereus* was isolated from 34 out of 150 meat and meat product samples with an overall prevalence of 22.66%. *Our results were consistent with those stated by Guven et al. (2006) (22.4%) and* Amin and Tawfick (2021) *(*24%). *On the other hand, higher percentages of B. cereus recovered from meat product samples were detected by* Rather *et al.* (2011) (40%), Abd El Tawab *et al.* (2015) (38.33%), Yu *et al.* (2020) (35%), Tewari *et al.* (2015) (30.9%), Bashir *et al.* (2017) (29.33%), and Schlegelova *et al.* (2003) (28%). However, a lower prevalence rate

Table 7. Antibiofilm activity of ACV against *B. cereus* isolates.

Isolate No.	Isolate code	Control +ve (OD)	OD of *B. cereus* biofilm (inhibition %) against MICs of ACV (0.125–256 µg/ml)												
			256	128	64	32	16	8	4	2	1	0.5	0.25	0.125	*p*-value
1	Mm 3	2.784	0.006 (99.8) aA	0.0272 (90.22) bB	0.406 (85.41) cB	0.579 (79.2) cC	0.832 (70.1) aE	0.909 (67.35) aF	1.333 (**52.1**) bG	1.506 (45.9%) dH	1.0581 (43.21) cI	1.658 (40.44) eK	1.692 (39.22) bL	1.714 (38.43) aM	0.0001
2	Bk 4	0.925	0.007 (99.21) aA	0.097 (89.45) cB	0.124 (86.56) bB	0.227 (75.43) aD	0.275 (70.21) aE	0.356 (61.46) bF	0.416 (54.96) aG	0.447 (**51.62**) cG	0.478 (48.31) cH	0.518 (43.96) bK	0.552 (40.32) aL	0.569 (38.48) aM	0.0001
3	Sh 6	0.709	0.015 (97.83) bA	0.0678 (90.44) bB	0.131 (81. 56) aC	0.208 (70.66) cD	0.245 (65.42) cE	0.288 (59.37) cF	0.346 (**51.24**) cG	0.359 (49.33) aG	0.388 (45.27) bI	0.408 (42.45) dK	0.435 (38.64) cL	0.459 (35.26) dM	0.0001
4	Mm 8	0.642	0.011 (98.23) bA	0.073 (88.62) dB	0.133 (79. 28) cC	0.158 (75.38) aD	0.194 (69.78) abE	0.251 (60.90) beF	0.296 (53.89) abG	0.317 (**50.62**) aH	0.334 (47.97) cH	0.362 (43.61) bK	0.384 (40.19) aL	0.395 (38.47) aM	0.0001
5	Bb 12	1.301	0.003 (99.80) aA	0.096 (92.62) aA	0.248 (80. 94) bC	0.389 (70.10) cD	0.513 (60.57) dE	0.557 (57.19) dF	0.633 (51.35) cG	0.662 (**49.12**) bH	0.699 (46.27) aI	0.731 (43.81) bK	0.788 (39.43) bL	0.813 (37.51) bM	0.0001
6	S 20	0.587	0.005 (99.10) aA	0.038 (93.52) aA	0.109 (81. 43) aC	0.144 (75.47) aD	0.203 (65.42) bE	0.233 (60.31) beF	0.279 (**52.64**) bG	0.303 (48.38) beH	0.319 (45.66) bI	0.333 (43.27) bK	0.345 (41.13) aL	0.357 (39.18) cL	0.0001
7	Mm 26	1.054	0.015 (98.58%) aA	0.098 (90.70%) bB	0.211 (79. 98) cC	0.303 (71.25) bD	0.399 (62.14) eE	0.433 (58.92) dF	0.508 (**51.80**) cG	0.577 (47.15) cH	0.591 (43.93) cI	0.623 (40.89) eK	0.647 (38.61) cL	0.667 (36.72) cM	0.0001
8	Bl 27	0.455	0.009 (97.96%) bA	0.037 (91.87) abB	0.088 (80. 66) bC	0.139 (69.45) dD	0.174 (61.76) eE	0.202 (55.60) eF	0.227 (**50.11**) dG	0.244 (46.37) cdH	0.256 (43.73) cI	0.266 (41.53) dK	0.276 (39.34) bL	0.285 (37.36) bM	0.0001
9	Sh 30	1.736	0.002 (99.88) aA	0.169 (90.26) bB	0.361 (79. 21) cC	0.515 (70.33) cD	0.628 (63.82) eE	0.705 (59.39) cF	0.803 (53.74) abG	0.879 (**49.37**) bH	0.918 (47.12) aI	0.949 (45.33) cI	0.990 (42.97) eK	1.040 (40.10) bL	0.0001
10	Bl 31	0.786	0.004 (99.49) aA	0.082 (89.57) cB	0.167 (78. 75) dC	0.241 (69.34) dD	0.293 (62.72) dF	0.329 (58.14) cdF	0.358 (54.45) aG	0.392 (**50.13**) bcG	0.407 (48.22) cH	0.432 (45.04) cI	0.451 (42.62) eK	0.476 (39.44) cL	0.0001
11	Bk 33	0.629	0.004 (99.36) aA	0.071 (88.71) dB	0.128 (79. 49) cC	0.197 (68.68) dD	0.262 (58.34) eF	0.280 (55.48) eF	0.311 (**50.56**) dG	0.331 (47.38) cH	0.345 (45.15) bI	0.362 (42.45) cK	0.375 (40.38) aL	0.385 (38.79) aM	0.0001
12	S 34	1.864	0.010 (99.46) aA	0.174 (90.65) bB	0.344 (81. 52) aC	0.540 (70.98) cD	0.696 (62.60) eE	0.759 (59.22) cdF	0.862 (**53.67**) aG	0.962 (48.30) cdH	0.998 (46.37) abI	1.030 (44.65) aK	1.077 (42.12) eK	1.177 (39.98) cL	0.0001
p-value			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	

Mm: Minced meat, Sh: Shawarma, Bb: Beef burger, Bk: Beef kofta, Bl: Beef Luncheon, S: Sausage. OD: optical density. The data in the upper row of each isolate represent ELISA reading at OD 545 nm. The inhibition of biofilm formation of each isolate was stated as antibiofilm action (reduction or inhibition %) and was calculated as follows: (OD control-OD sample / OD control) *100; the inhibition % was indicated in the lower row of each isolate. The underlined OD numbers indicate nearly about 50% reduction in biofilm under MBIC50 of the antibacterial agents, which was defined as the lower concentration of the agent (µg/ml) which shows a 50% reduction in biofilm formation. Values in the same row carrying different capital superscripts or those that were defined in the same column carrying different small superscripts are significantly different ($p < 0.05$). ***p*-value < 0.05 is considered statistically different.

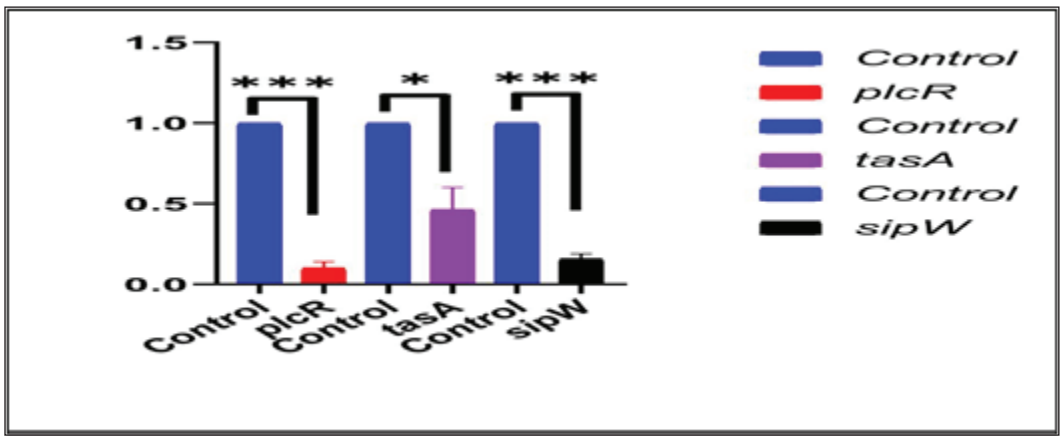

Fig. 1. Effect of ACV on the expression levels of *B. cereus plcR, tasA,* and *sipW* biofilm genes using RT-qPCR.

Table 8. Transcriptional analysis of biofilm genes using RT-qPCR after treatment with ACV.

Gene	Control	Fold change	*p*-value
plcR	1.00 ± 0.000 [b]	0.1029 ± 0.03685 [a***]	<0.0001
tasA	1.00 ± 0.000 [b]	0.4642 ± 0.1368 [a*]	0.0173
sipW	1.00 ± 0.000 [b]	0.1596 ± 0.03004 [a***]	<0.0001

Values bearing dissimilar superscripts (a,b) in the same row varied significantly ($p < 0.05$). Results were expressed as means ± SE.

was recorded by Konuma (1988) and Gharib *et al.* (2020) with percentages of 18.3% and 17.5%, respectively.

Bacillus cereus may be present in raw meat products because of unsanitary conditions during meat delivery, processing, or transportation. According to Floristean *et al.* (2007), improper storage temperatures of raw meat could potentially encourage the growth of bacteria. The processing of minced meat may be the reason for the high frequency of *B. cereus* isolation from edible meat products such as luncheons or the addition of expired additives, which can induce vegetation of *Bacillus* spores under insufficient heat treatment (Shawish and Tarabees, 2017).

Knowledge of antibiotic resistance patterns is of great alarm, as the major bacterial isolates may be highly resistant to frequently used antibacterial agents. Therefore, antimicrobial sensitivity testing is important to suggest suitable antibacterial agents for the treatment and prevention of resistance (Fiedler *et al.*, 2019).

In this study, the *B. cereus* isolates exhibited 100% resistance to both ampicillin and colistin, next cefotaxime (94.12%), sulfamethoxazole-trimethoprim (91.20%), cephalothin and fosfomycin (91.18%, each), chloramphenicol, erythromycin and tigecycline (88.23%, each), ofloxacin (85.29%), and doxycycline (82.35%). On the other side, the highest sensitivity rates of *B. cereus* were reported for ciprofloxacin and imipenem (100%, each). Similar antimicrobial susceptibility results against *B. cereus* isolates were previously documented (El-Sayed, 2019; Solanki *et al.*, 2019).

On the contrary, Fiedler *et al.* (2019) estimated the resistance of *B. cereus* to antibiotics and showed that it is highly resistant to penicillin G and cefotaxime (100%), ampicillin and amoxicillin/clavulanic acid combination (99.3%). However, these isolates were susceptible to ciprofloxacin, chloramphenicol, amikacin, imipenem, erythromycin, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole by 99.3%, 98.6%, 98.0%, 93.9%, 91.8%, 88.4%, 76.2%, and 52.4%, respectively. In addition, Merzougui *et al.* (2014) indicated that the isolates *B. cereus* were resistant to penicillin, oxacillin, and cefepime (100%, each), ampicillin (98.4%), and tetracycline (90.6%), but they were susceptible to gentamicin, erythromycin, and chloramphenicol by 100%, 84.4%, and 67.2%, respectively.

According to Shalini and Rameshwar (2005), food can be thought of as the primary means of antibiotic-resistant bacteria spreading from humans to animals. One of the main causes of antibacterial resistance is excessive antibiotic exposure. The overuse of antibiotics in hospitals, agriculture, animal husbandry, and the general public, along with the freedom to buy antibiotics without a prescription and use them carelessly, may be the cause of the rise in antibiotic resistance. Serious and setting are most likely the main contributing factors to the widespread spread of nosocomial infections that are resistant to antibiotics and are difficult to treat in the health service (Fiedler *et al.*, 2019).

The formation of biofilm is followed by significant alterations in the bacteria's physiology and genetic

makeup that reduce their susceptibility to practically all antibiotic classes (Melchior *et al.*, 2006). Microorganisms are shielded from external aggression by it, and certain bacteria that form biofilms are resistant to antibiotics. Thus, the high antibiotic resistance of bacteria that form biofilms is intended to encourage the use of antimicrobial medications (Takaine *et al.*, 2014) such as the use of substitute medicinal plants to treat diseases. Herein, 66.67% of *B. cereus* isolates (n = 12) were biofilm producers with black colonies on Congo red agar. Quantitatively, Six (50%), three (25%), and three (25%) produced weak, moderate, and strong biofilms, respectively, on tryptic soya broth in a microtitre plate. These findings came in parallel to those of several documents by JeeHoon and Beuchat (2005), Wijman *et al.* (2007), and Ozdemir and Arslan (2019) who reported *B. cereus* capability to form biofilms when examined either qualitatively or quantitatively.

A significant issue for food safety and human health is the formation of bacterial biofilms, which are produced by a variety of virulent bacteria. The most efficient way to prevent the formation of biofilm and stop bacterial growth when employing antimicrobial agents is to obstruct the formation of biofilm (Roy *et al.*, 2018). Therefore, finding novel agents that can serve as an unconventional and alternate means of treating infections brought on by bacteria resistant to conventional treatments is crucial.

In the present work, vinegar solution (5% acetic acid) has a double effect, where it prevented the progress and formation of biofilm of *B. cereus* at MIC range 2–8 µg/ml. The ability of vinegar to reduce biofilm formation is positively related to its acetic acid antibacterial activity in this study and the use of marketable ACV reduced the viability of *B. cereus* and its biofilm-forming ability. Our result corresponded with those of Pedroso *et al.* (2018) who reported that ACV 70% reduced *S. aureus* biofilm formation; they added that acetic acid, which is abundant when the vinegar ferments and is included in 3%–5% concentration, influenced the biofilm formation. In addition, Halstead *et al.* (2015) revealed that 0.31% acetic acid inhibited the formation of biofilm by *Pseudomonas aeruginosa*. According to Tsang *et al.* (2018), biofilm-associated methicillin-sensitive *S. aureus* was eliminated by 5% and 3% acetic acid, respectively.

The inhibition zone diameter by the agar well diffusion assay revealed that ACV exhibited marked inhibitory activities against *B. cereus* at different concentrations with zone diameters ranging from 36 to 48 mm. These findings matched with other previous studies by Yagnik *et al.* (2021), Gaber *et al.* (2020), and Baldas and Altuner (2018). In addition, ACV had an antimicrobial effect on tested microorganisms such as *V. cholerae*, *C. tropicalis*, *C. albicans*, *E. coli* O157:H7, and *S. typhi*.

Our relative expression data of *plcR*, *tasA,* and *sipW* genes by RT-qPCR in strong biofilm-producing MDR *B. cereus* isolates in the presence of ACV compared to those the control without ACV where the used ACV was considered as an antibiofilm agent. The results revealed the down-regulation of biofilm genes in comparison to those of the control. Caro-Astorga *et al.* (2015) have documented the involvement of various genes in the formation of biofilms, including the *sipW* and *tasA* genes. The production of *tasA* has been linked to the formation of amyloid-like fibrils, which in turn cause foliar biofilms in *Bacillus* species. When it comes to *sipW*, it codes for a protease that helps process *tasA*. Without a doubt, *B. cereus*'s persistence in food trade equipment is largely due to its presence in biofilms as the biofilm protects vegetative cells and spores from sanitizer's inactivation (Wijman *et al.*, 2007). This is the first report locally and internationally, where no studies were conducted on the ACV effect on biofilm gene expressions (*plcR, tasA*, and *sipW*), and limited studies only highlighted its effect on bacterial biofilm formation.

Conclusion

The high isolation rate of MDR *B. cereus* from meat and meat products in Egypt was highlighted by this study. Moreover, using ACV as an antibiofilm agent might offer a valuable method to lower the hazard of *B. cereus* infection and its persistence in the food industry either in public spaces or at home.

Author contributions

Rana M. Mahmoud, Ahlam A. Gharib, Norhan K Abd El-Aziz, and Ahmed M. Ammar designed the research. The microbiological techniques were performed by Rana M. Mahmoud. The molecular analyses and data analysis were performed by Rana M. Mahmoud, Ahlam A. Gharib, and Norhan K. Abd El-Aziz. The study was conceived and designed by Ahmed M. Ammar, El-Shaimaa Mesallam Ali, Aml Mokhtar, and Ghada A. Ibrahim. Norhan K. Abd El-Aziz, Ahlam A. Gharib, and Rana M. Mahmoud wrote the manuscript's first draft. The final manuscript was approved by all authors.

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Data availability

The manuscript contains the data that support the study's conclusions. Upon a reasonable request, the corresponding author will provide any additional data.

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